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The Neuroprotection with Statin Therapy for Acute Recovery Trial (NeuSTART): an adaptive design phase I dose-escalation study of high-dose lovastatin in acute ischemic stroke

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Abstract

There is growing experimental and clinical evidence that by reducing downstream products of the mevalonate pathway other than cholesterol, HMG-CoA reductase inhibitors ('statins') have beneficial effects on endothelial function, coronary and cerebral blood flow, inflammation, and hemostasis. Statins have been shown in rodent models of acute ischemic stroke to reduce neuronal injury and infarct size in a dose-dependent fashion. The objective of this early phase trial will be to determine the maximal-tolerated dose of lovastatin for short-term acute stroke therapy. In this multicenter phase 1B dose-escalation and dose-finding study, 33 patients with acute ischemic stroke will be administered lovastatin in increasing doses from one to 10 mg/kg daily for 3 days beginning within 24 hours after symptom onset. The primary safety outcome will be occurrence of myotoxicity or hepatotoxicity, defined by clinical and laboratory criteria, and the study is designed to determine the highest dose of lovastatin that can be administered with <10% risk of myotoxicity

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or hepatotoxicity. The statistical design of the study utilizes an adaptive design, the Continual Reassessment Method, which is novel to stroke trials, to find the optimal dosage. The dose–toxicity model is calibrated such that the method will eventually select a dose that causes 7–13% dose-limiting toxicity (within 3% of target). A sample size of 33 will ensure that estimates of any binary variables will have a 95% confidence interval of width 0.34, and enable us to detect any unexpected toxicity that occurs at 5% rate (in a non-dose-dependent fashion) with probability 0.82. The probability of choosing a dose for further trials with 25% or higher likelihood of toxicity is no more than 23%. The presently described trial represents a new approach for treatment of acute ischemic stroke, as well as a novel way of conducting a phase I trial, evaluating safety and determining an optimal dose of a potential neuroprotectant drug.

Keywords

stroke; neuroprotection; statins; clinical trial

Introduction

Hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or ‘statins,’ are of tremendous benefit in prophylaxis against myocardial infarction (MI), stroke, and other vascular events (1, 2). The benefit of statins in prevention may be at least partly independent of their effects on lowering LDL cholesterol (3-5). There is growing experimental and clinical evidence that by reducing downstream products of the mevalonate pathway other than cholesterol, the statins have beneficial effects on endothelial function, coronary and cerebral blood flow, inflammation, and hemostasis (4, 5). More recently, several statins have been shown in rodent models of acute ischemic stroke to reduce neuronal injury and infarct size (6-13). In these models, statins have been administered both before experimental stroke, and as acute treatment early (3 hours) after stroke. The mechanism of this neuroprotection is also independent of the effect on cholesterol, and appears primarily related to improved endothelial function, increased cerebral blood flow, and reduced inflammation, rather than on a direct neuronal cytoprotective benefit. In these studies, there was a dose–response effect of statins, with the greatest neuroprotective effect at highest doses. There is also evidence from animal models that the statins may have prorestorative effects on neuronal recovery after stroke when used up to 24 hours after stroke, including angiogenesis, synaptogenesis and neurogenesis (14).

Observational studies and preliminary clinical trials in humans provide some support for a benefit of statins in acute ischemic stroke when administered at traditional doses. Several investigators have reported that patients already taking statins at the time of stroke have a better functional outcome and reduced mortality compared to patients not taking statins (15-18). In a pilot study of patients with acute ischemic stroke (19), 60 patients were randomly assigned to simvastatin 40 mg daily for 7 days after stroke followed by 20 mg daily up to 90 days vs. placebo. Statistically significant improvement in the NIH Stroke Scale was seen at 90 days, but also as early as 3 days after treatment was initiated. Retrospective analysis of data from 852 patients in a clinical trial of citicoline demonstrated a beneficial effect of statins taken after stroke on functional outcome at 3 months; 14.4% of

patients began statin therapy within 4-week of stroke onset (20). Despite improved outcomes, however, it is not clear that prior statin use is associated with less severe strokes at presentation in humans (21).

Preliminary evidence from early phase clinical trials in cancer patients further suggest that the high doses found to be of benefit in animal models may be well tolerated for short periods of time in human beings. Because statins have an effect on a number of cell cycle and signal transduction pathways due to their ability to block the mevalonate pathway, they have been considered as potential anti-neoplastic agents. Lovastatin in particular, has been tested in humans at doses similar to and higher than those that have been shown to be neuroprotective in rodent models (22-25).

Despite their role in vascular prophylaxis, no translational studies have been performed to test the role of high-dose statins administered as early therapy in stroke patients. The Neuroprotection with Statin Therapy for Acute Recovery Trial (NeuSTART) drug development program was developed to address this deficiency in our knowledge. This protocol describes the rationale and design of an initial phase 1B dose-escalation and dose-finding study designed to test the hypothesis that short-term statin therapy, at doses that are maximally effective in animal studies of neuroprotection, is feasible and safe in patients with acute ischemic stroke.

Rationale for choice of lovastatin, dose, timing and duration

Lovastatin has many features that recommend its use in the present dose-escalation study. First, human safety data at high doses already exist for lovastatin in cancer clinical trials (22-24). Second, lovastatin has been found to be neuroprotective in rodent acute stroke models (6-12). Third, lovastatin has many pharmacokinetic properties that make it desirable in a study of neuroprotection at high doses. It is relatively lipophilic, and it is able to cross the blood-brain barrier.

The rationale for using escalating doses of lovastatin above standard approved doses is based on safety results in humans, as well as animal model data regarding neuroprotection. Preclinical pharmacological and toxicological data in multiple species (mouse, rat, rabbit, and dog) demonstrated linear pharmacokinetics of statins (26). The therapeutic dose for vascular prophylaxis and cholesterol reduction is approximately 1 mg/kg/day, which yields concentrations of 0.1 μM . In all animal species tested, chronic administration of doses that achieved concentrations of 2–4 μM (i.e., 20–40 times than currently recommended in humans) was tolerated well for months. Concentrations of >20 μM , an order of magnitude higher, were associated with progressive anorexia and death. In the cancer clinical trials, doses of up to 10 mg/kg/day were tolerated for up to 7 days on a monthly cycle (22-24). The doses in animals that achieved neuroprotection were within the safe range. Animal models generally used doses of 0.2–20 mg/kg of lovastatin or simvastatin, with a linear dose-response effect without a threshold in most models. The doses proposed for this trial should achieve serum levels of about 2 μM , well within the safe range, and within the range neuroprotective in animals. Lovastatin, like most statins is poorly soluble, and there is no

approved parenteral statin at present. Therefore, the feasibility of administering large oral doses of statins will also be tested in this phase 1B trial.

The ideal timing and duration of statin therapy for neuroprotection in humans is difficult to determine. In most animal studies, statin therapy was started before stroke (5-11). In animal models of statin therapy administered after stroke, drug was given starting at 3 hours (12) and 24 hours (14). When administered at 24 hours, there was no definite effect on infarct size, although there was an improvement in functional outcomes. Taken together, these data indicate that high-dose therapy must be started early to be effective at reducing infarct size, although it is uncertain where within the 24-hour period the upper limit of the time window lies. Because the phase 1B study is focused on safety and dose finding rather than efficacy or surrogates for efficacy, a time window of 24 hours will be used. Most models of ischemic injury, moreover, suggest that the injury from inflammatory mechanisms, on which statins are thought to have some of their effect, may peak at 48 hours and continue for at least 72 hours after stroke. We therefore chose to administer high-dose lovastatin for 3 days in this phase 1B study.

The suggestion that higher doses of statins become less effective for neurological recovery when administered after 24 hours (14), when combined with the data on acute effects of the statins, indicate that there may be a role for early use of high-dose statins followed by a return to lower, more conventional doses, post stroke. This is in accordance with our plan to have all patients take 20 mg lovastatin daily after 72 hours.

Study design

Overall plan

NeuSTART is a phase 1B dose-escalation and dose-finding study in which ischemic stroke patients will be treated within 24 hours of symptom onset at escalating dosage levels of short-term high-dose lovastatin (1, 3, 6, 8, or 10 mg/kg/day for 3 days). The statistical design will utilize the two-stage Continual Reassessment Method (CRM) used in cancer clinical trials to find the optimal dosage of chemotherapeutic agents (27, 28). In Stage 1, three patients will be treated at the lowest dose level, and patients will continue to be treated according to the schedule outlined in Table 1. Once a primary safety outcome is observed, dose escalation will be dictated by the CRM and the Data and Safety Monitoring Committee (DSMC) (Stage 2). The primary outcome of this phase 1B study will be musculoskeletal and hepatic toxicity, defined by clinical and laboratory criteria, with a 1-month follow-up. Pharmacokinetic parameters will also be assessed. If the results of this study demonstrate safety, then a phase 2A safety and surrogate outcome trial will be performed.

Inclusion and exclusion criteria

Patients with acute ischemic stroke who can be treated within 24 hours of symptom onset are eligible. A time window of 24 hours was chosen because the primary goal of the study is to assess safety rather than efficacy. Full inclusion and exclusion criteria are listed in Table 2. If preliminary data indicate that a patient meets eligibility criteria the patient (or legally authorized representative) is approached to participate in the study, and consent is obtained.

Study interventions, administration, and duration

The time of first dose is considered time 0. Patients are administered the total daily dose in four daily-divided doses, to be consistent with the cancer trials that demonstrated safety at high doses in that patient population (22-24). Study drug will be administered orally; for dysphagic patients, medication will be administered via nasogastric tube. After the initial 3 days of acute dosage, all patients receive lovastatin 20 mg daily for 27 days, unless they have experienced toxicity. All patients who experience a primary safety event will stop drug.

Handling of study intervention

Doses will be prepared and dispensed as an integer number of either 10, 20, or 40 mg tablets, according to the patient's weight. According to this scheme, patients receive an actual dose within 6% of the target-calculated dose on a milligram per kilogram (mg/kg) basis. The maximum daily dose for any individual patient, however, will be based on a maximum weight of 100 kg; thus the maximum daily dose at each dose level will be 100, 300, 600, 800, and 1000 mg. If the patient is unable to swallow tablets, crushed lovastatin tablets will be administered via nasogastric tube. For study days 4–30, tablets will be provided in a similar manner, using lovastatin 20 mg tablets, and will be dispensed on either an outpatient or inpatient basis, as needed.

Concomitant interventions

Because of the risk of statin myopathy, patients will be prohibited from taking other medications that might increase the risk of developing myopathy. The following medications are prohibited: cyclosporine, itraconazole, ketoconazole, erythromycin, azithromycin, clarithromycin, nefazodone, gemfibrozil, other fibrates, niacin, amiodarone, and verapamil. Patients already taking statins can be enrolled as long as the patient and treating physician are willing to discontinue the current statin in favor of the study medication.

Clinical and laboratory investigations

The schedule of evaluations is shown in Table 3. Demographics, medical history, medications, complete physical and neurological examination, NIH Stroke Scale (NIHSS), modified Rankin Scale (mRS), brain CTscan, laboratory evaluation, and standard ECG recording are performed before dosing. Electrolytes (sodium, potassium, chloride, bicarbonate), renal function (BUN, creatinine), and glucose and CBC will be measured at baselines, 5 and 7, and as clinically indicated.

Safety laboratory tests, including liver function tests [alanine aminotransferase (AST)] and aspartate aminotransferase (ALT) levels, albumin, alkaline phosphatase, direct and total bilirubin, LDH), and creatine phosphokinase (CK), will be measured at baseline (on day 0 before drug), and on days 1, 2, 3, 5, 7, and 30. A muscle and liver clinical assessment for pain, strength, jaundice, and bleeding is also completed at baseline, and days 1, 2, 3, 5, 7, and 30. This schedule permits measurement of these safety labs and clinical assessment at intervals between doses; if significant elevations are detected, the investigator will be able to

stop further treatment. Muscle pain, jaundice, and bleeding will be categorized as not present, mild, moderate, and severe; strength will be categorized using the standard Medical Research Council grading system from 0 to 5. In addition, platelet aggregation studies will be performed at 24 hours, or when available, by a specialized coagulation laboratory. To evaluate the effects of high-dose lovastatin on platelet function, turbidometric platelet aggregation studies will be conducted using adenosine diphosphate (ADP) (20 μ m), collagen (100 μ g/ml), and ristocetin. No patients in this study will receive intravenous tissue plasminogen activator (t-PA), but to investigate the potential for high dose lovastatin to alter the thrombolytic potential of t-PA, blood samples from patients receiving high-dose lovastatin will be tested for whole blood clot lysis in the absence and presence of t-PA.

Pharmacokinetic analyses (lovastatin levels) will be performed 30 min before and 2 hours after doses one and 12, as well as on the mornings of days 4 and 5 to ensure excretion. To assess potential anti-inflammatory mechanisms of highdose lovastatin, inflammatory biomarkers (high-sensitivity C-reactive protein, interleukin-6, tumor necrosis factor- α) will also be measured at baseline, and on days 1, 2, 3, 7, and 30. Lipid levels (total cholesterol, HDL, triglycerides, LDL) will be performed at baseline, and on days 1, 2, 3, 7, and 30, to provide another indirect measure of the activity of lovastatin.

Pulse rate and blood pressure will be measured three times predose (at least 10-min apart), approximately every 4 hours during the first 72 hours post dose, and on days 7 and 30. Standard 12-lead ECGs will be recorded predose, on days 2, 3, and 7. The NIHSS (29, 30) will be performed immediately prior to enrollment, at 72 hours post dose, and then on days 7 (or at hospital discharge if before day 7), and 30. The results of physical examination will be registered at baseline and days 1, 2, 3, 7, and 30. Disability and handicap will be measured using standard measures in stroke trials (31), the Barthel Index (BI) (32, 33), and mRS (34).

Adverse events, use of concomitant medication, and compliance with study procedures will be assessed throughout the study. Patients who discontinue prematurely; either voluntarily or due to laboratory abnormality, will be asked to return for a final follow-up safety assessment at 30 days.

Safety outcomes

Significant muscle or hepatic toxicity was chosen as the primary endpoint for the study based on well-established understanding of the likely toxicity expected with statin agents gained from multiple prior clinical trials in patients with cardiovascular disease, as well as the results in smaller early-phase studies in cancer patients (22-24). The primary safety outcome is the development of clinical or laboratory evidence of major hepatic or muscle toxicity. Previous studies typically defined statin-associated liver function abnormalities as elevations in AST and/or ALT levels greater than three times the upper limit of normal (ULN) (35, 36). Myopathy is a rare but more worrisome potential complication. In its most severe form CK elevations are dramatic ($> 100 \times$ ULN) and rhabdomyolysis may occur, leading to renal failure and even death. The FDA has defined rhabdomyolysis as requiring evidence of organ damage in addition to CK elevation $> 10\,000$ U/l. The definition of the primary safety outcome, in keeping with other statin trials, is thus defined as either:

1. *liver toxicity*: liver function test (ALT/AST) increase at any time point $> 3 \times \text{ULN}$; or development of jaundice, otherwise unexplained coagulopathy, or other clinical evidence of hepatitis or liver failure; or
2. *muscle toxicity*: increase in CK at any time point $> 10 \times \text{ULN}$; or clinical evidence of muscle pain or weakness not related to the stroke and associated with $\text{CK} > 5 \times \text{ULN}$.

In addition, other unexpected adverse events will be recorded. Screening for adverse events occurs daily for the first 7 days of the study and at the 30-day follow-up. Data regarding seriousness, severity, resolution, and relationship to study drug will be determined, and a preliminary narrative summary recorded. When a primary safety outcome occurs, the patient will discontinue treatment. Follow-up will continue to the end of the study period (30 days), and include all regularly scheduled assessments.

The safety data will be available within 24 h to the study statistician (K. C.), who will make recommendations to continue or change the dose level for the next patient based on the data obtained to that point using the CRM. The Chairman of the Data and Safety Monitoring Committee will call a meeting of the DSMC shortly after the conclusion of the 7th day after treatment initiation in the last patient treated in any given dosage group. After the DSMC has met and reviewed the data, they will then authorize the escalation to the next dose level if appropriate according to the CRM. The DSMC will also have discretion to determine whether or not study drug should be discontinued in a patient with an unexpected event (which may or may not be related to study drug). There will thus be parallel systems in place to prevent premature dose escalation (i.e., the CRM and the DSMC).

Management of adverse experiences

Development of hepatic or musculoskeletal toxicity will be managed by the clinical team as appropriate, using the following guidelines. Drug treatment will stop as soon as the clinical or laboratory abnormality (as defined above) is reached. Liver function and CK tests will continue to be monitored daily until normal. In the case of hepatic toxicity, coagulation tests and bilirubin will be monitored. In the case of CK elevation, patients will be hydrated, and ubiquinone (coenzyme Q10, administered as Q-Gel 60mg Softgels, Tishcon Corp, Westbury, NY) will be administered at a dose of 60 mg p.o. q.i.d. (22), where appropriate clinical consultation will be obtained from a clinical hepatologist or muscle specialist.

Statistical analysis and sample size calculations

This phase 1B study is a dose-finding study of the maximum-tolerated dose (MTD) of short-term high-dose lovastatin in stroke patients treated within 24 hours of symptom onset. Each participant receives high-dose lovastatin, 1–10 mg/kg/ day, on days 1–3 post onset, and receives 20 mg/day for another 27 days (Table 1). Toxicity will be evaluated continuously up to day 30. The objective of this study is to determine the MTD in this patient population. We define the MTD to be a dose that is associated with a 10% rate of occurrence of the primary safety endpoint or the dose-limiting toxicity (DLT) within the 30-day treatment

period. The study design tolerates 10% toxicity in stroke patients in order to be able to treat patients at a higher and thus potentially efficacious dose.

Patients are scheduled to be enrolled in groups of size three. The first three patients receive dose level 1, and dose escalation for subsequent groups before any DLT is observed is given in Table 1. Once a primary safety event is observed in a patient, the CRM (27) and the time-to-event CRM (27), will be used to facilitate dose escalation. After every patient, the laboratory values and vital summary for the currently enrolled patients will be reported to the statistician (K. C.), who will estimate the MTD with a dose–toxicity probability model based on currently available observations. The next patients will then be treated at the model-based MTD estimate. The CRM has been shown to be efficient at finding the MTD with limited sample size. One limitation of the traditional CRM is the potential delay in patient accrual because complete information on the enrolled patients is required before enrolment of the next block of patients. Thus, we will use the modified time-to-event CRM (37) to allow staggered patient entry. This method may be distinguished from the CRM by also using toxicity information collected on days 1, 2, 3, and 7. Thus, if a new patient arrives before the current group has completed its 30-day evaluation, partial information is still available for estimating the MTD without suspending the trial and turning away potential subjects.

We plan to accrue 33 evaluable patients using the CRM with an empiric dose–toxicity model (38). The term ‘evaluable’ will be defined for this phase 1B safety and dose-finding study as a patient with either:

1. primary safety outcome during the 30 days of the study, or
2. muscle and liver tests on day 30 after treatment.

Patients who are either lost to follow-up at 30 days or die of an event unrelated to either muscle or liver disease will be replaced for the primary analysis. The DSMC will make the final determination whether an event is related to the study therapy; should the DSMC determine that patient withdrawal or death is related to the study therapy, such an event will be counted as DLT. However, all patients including those who are considered nonevaluable, will be included in analyses during the trial (for dose-escalation purposes) and after the trial.

The dose–toxicity model is calibrated such that the CRM will eventually select a dose that causes 7–13% DLT, i.e., within 3% of our target (39). Generally, a sample size of 33 will ensure that estimates of any binary variable will have a 95% confidence interval of width no > 0.34 , and enable us to detect any unexpected toxicity that occurs at 5% rate (in a non-dose-dependent fashion) with a 0.82 probability. This design has the operating characteristics shown in Table 4, with P (select) denoting the probability that a given dose level is selected as the MTD. The calculations of the operating characteristics are based on 5000 simulated trials with the following assumptions:

- a. Patients arrive according to a Poisson process with rate two patients per month.
- b. A DLT, if it occurs, occurs randomly and uniformly before days 1, 2, 3, 7, and 30. TITE–CRM is shown to be robust to the underlying time-to-event distribution (37).

This design with 33 patients selects the correct MTD with probabilities over 50% in all five scenarios in Table 4. In particular, we have 66% probability to select the 10 mg/kg dose under scenario 5, which we deem to be the most probable scenario. More importantly, given our primary focus on safety in this study, the probability of choosing a dose with 25% or higher likelihood of toxicity is no more than 18%.

Source of funding

This trial is being conducted as part of an NIH/NINDS-funded program project focused on the therapy of acute stroke [the New York-Columbia Collaborative Specialized Program of Translational Research in Acute Stroke (SPOTRIAS)]. Participating centers include the initiating center at Columbia University Medical Center, and a second center at NY Hospital – Cornell.

Discussion

The presently described trial represents a new approach to treatment of acute ischemic stroke, as well as a novel way of conducting a phase I trial evaluating safety and determining an optimal dose of a potential neuroprotectant drug. Currently, there are limited therapies available for the treatment of acute ischemic stroke. The only currently FDA-approved medication for this condition is intravenous tissue plasminogen activator (r-tPA), when administered within 3 hours of stroke symptom onset. No neuroprotectant agent has yet been approved for the treatment of acute ischemic stroke in the United States, although citicholine has been approved for use in other countries (40). Numerous other agents have been tested in human clinical trials without success. Identification of neuroprotectant medications for the treatment of stroke remains an important objective in clinical stroke research, and success could have important implications for treatment of other neurological disorders, as well.

HMG-CoA reductase inhibitors, or statins, are a rational choice for a neuroprotectant medication. First, there are several mechanisms by which statins may exert a neuroprotective effect. Statins upregulate eNOS production, which increases cerebral blood flow, have anti-inflammatory effects, and promote synaptogenesis and neurogenesis after stroke. This pleiotropy, importantly, may have advantages compared with other medications with only single focused mechanisms of action.

Second, statins have been widely used for prevention of cardiac disease and stroke, and there is thus an extensive experience available documenting their safety and efficacy in vascular disease patients. In particular, the SPARCL trial (2) has demonstrated the benefit in secondary stroke prevention of atorvastatin when administered to patients without cardiac disease or hyperlipidemia (only patients with LDL levels < 100mg/dl were excluded). Importantly, in SPARCL, patients began treatment starting at least 30 days after stroke. Stroke prevention guidelines continue to advise the use of statins after stroke based on patients' overall vascular risk (41), and SPARCL does not provide evidence that statins need to be started immediately after stroke, or that they provide any neuroprotective benefits.

Third, there is consistent evidence of benefit of statins when administered in rodent models of acute ischemic stroke. These have included studies in which statins were given before stroke, up to 24 hours after stroke, and with thrombolytic agents (6-12, 14). When administered at 24 hours, however, there was no definite effect on infarct size, although there was an improvement in functional outcomes (14). In these studies, there has been evidence of a dose-response effect on neuroprotection, as well, although there is also evidence that higher doses may have less benefit when given at later timepoints after stroke. These data may be interpreted as providing evidence that high-dose therapy must be started early to be effective at reducing infarct size, although it is uncertain when the benefits of high-dose therapy vanish. They further provide evidence of a pre-emptive effect even when given after an acute neuroprotectant is likely to be no longer operative.

Fourth, there is evidence that the high doses that are likely to provide the greatest benefit for neuroprotection may be tolerated by human beings. Because high doses of statins were thought to have antineoplastic properties, they have been tested in cancer trials (22-24) and shown to be relatively safe and well tolerated for short periods of time, akin to their likely use in the setting of stroke, as well. The present trial, therefore, provides an opportunity to test the safety of high-dose statin therapy in a stroke patient population. It is anticipated that the results will allow the further development of this approach in phase two safety and ultimately phase three efficacy trials.

Our study design has several strengths. First, our design incorporates a novel, adaptive method of dose escalation that has been used extensively in cancer treatment trials, but infrequently in stroke trials, although it has been recommended for trials in patients with other neurological disorders (42). Traditional dose-escalation schemes would have fixed numbers of patients receiving prespecified doses, and would utilize prespecified rules for dose escalation or stopping. According to the design used in our study, however, the prespecified dosage scheme (Table 1) will be switched to a model-based scheme (i.e., the time-to-event CRM) if a primary safety outcome occurs. Upon observing DLT at a dose level, a model-based scheme may require additional patients to be entered at the same dose level, or recommend de-escalation back to the previous dose level, until more patients have been treated at that lower level. Thus the number of patients who need to be treated at a given dose is constantly being adjusted based on the number of actual occurrences of the primary safety outcomes. This allows great responsiveness in the study design to the occurrence of adverse events, and it ensures that the study minimizes the likelihood that any patient will receive a toxic dose of medication. The CRM and the time-to-event CRM are particularly efficient at finding the MTD with a limited sample size.

We prespecified that it would be reasonable to use a dose of lovastatin that causes up to 10% toxicity that occurs within the 30-day treatment period, if there is a reasonable expectation of some potential benefit. It should be noted as well that a relatively conservative definition of a safety outcome, based primarily on laboratory values alone, was chosen. Thus even a lab abnormality alone, without associated clinical features, will be counted as an outcome. Because our primary focus was on safety in this study, the probability of choosing a dose with 25% or higher likelihood of toxicity is limited to no more than 23%.

One limitation of the traditional CRM is the potential delay in patient accrual because complete information on the enrolled patients is required before enrolment of the next block of patients. Our method, the modified time-to-event CRM (37), is even more efficient in allowing staggered patient entry and using toxicity information collected on days 1, 2, 3, and 7. Thus, when new patients arrive before previously treated patients complete their 30-day evaluation, partial information is still available for estimating the dose without suspending the trial and turning away potential subjects. This is particularly important in stroke trials, in which recruitment may be slow and missed opportunities for recruitment are particularly disappointing. Using the CRM and time-to-event CRM requires extensive calibration of the dose–toxicity model. Computer simulation is an indispensable tool to validate a dose–escalation design to ensure that it yields satisfactory operating characteristics (cf. Table 4). In addition, numerical techniques can be used to fine-tune a dose–toxicity model, and public software is available for model calibration in the CRM (43).

There are also limitations to our study. Because statins are in general poorly soluble, there are no parenteral formulations of statin available for human use. For this reason, we are limited to using oral statins and therefore will need to use a large number of pills. Our study is thus also a test of the feasibility of orally administering a large number of these pills, and we are further addressing this limitation by using four daily doses of pills. We will also have a limited ability to test the mechanism of action of statin therapy as a potential neuroprotectant. We will however measure levels of several inflammatory markers at different timepoints after administration, and we will thus be able to assess whether there is an early dose–response effect related to the anti-inflammatory effects of the statins. While use of MRI or other imaging modalities to assess cerebral blood flow, representing an effect on eNOS, would have been ideal, funding was not available for imaging in this primarily safety-oriented study. Finally, this study will not provide information about statin efficacy, but only safety. Further studies will be required to assess efficacy and potential mechanisms of action.

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Table 1

Starting dose-escalation plan

Phase 1B cohort #	Cohort size (N)	Lovastatin dose: q6 hours for 72 hours (mg/kg/day)	Maximum dose (based on dose calculated for weight of 100 kg) (mg)	Dose days 3–30 (mg/day)
1	3	1	100	20
2	3	3	300	20
3	3	6	600	20
4	3	6	600	20
5	3	8	800	20
6	3	8	800	20
7	3	8	800	20
8	3	10	1000	20
9	3	10	1000	20
10	3	10	1000	20
11	3	10	1000	20
Total	33			

The Safety Monitoring Committee will meet and discuss continuation to the next cohort after every cohort of three patients. These dose levels apply before any dose-limiting toxicity (DLT) is observed. Dose (de-) escalation will be conducted according to the time-to-event Continual Reassessment Method (see text) once a DLT is observed.

Table 2**Inclusion and exclusion criteria**

<i>Inclusion criteria</i>	
1	Age 18
2	Satisfies the criteria for ischemic stroke: acute focal neurological deficit of likely ischemic vascular origin
3	Patient or legally authorized representative has provided written informed consent before study entry
4	Patient can receive the first treatment dose within 0–24 hours of stroke onset. For patients found with stroke on awakening, it will be assumed that the stroke occurred the last time that the patient was known to be normal
5	Patient has pretreatment brain CT scan compatible with ischemic stroke and excludes hemorrhagic and nonvascular etiologies of symptoms
6	Patients taking statins at time of stroke may be included
<i>Exclusion criteria</i>	
1	Brain imaging study shows a lesion other than ischemic stroke that could explain patient's symptoms (intracranial or subarachnoid hemorrhage, arteriovenous malformation, aneurysm, multiple sclerosis, tumor, abscess or other). Asymptomatic meningiomas are allowed
2	Mild stroke, defined as NIH Stroke Scale <2
3	Patient has received or is expected to receive intravenous rt-PA within 3 hours or intra-arterial rt-PA within 6 hours of stroke onset, according to our institutional standard of care
4	Receipt of intravenous rt-PA after 3 hours or intra-arterial rt-PA after 6 hours poststroke onset
5	Patient is comatose, regardless of etiology (>4 points on the first three items of the NIHSS)
6	History of intolerance or allergic reaction to any statins (myotoxicity, hepatic dysfunction, rash, etc.)
7	Use of drugs within past 30 days that utilize the cytochrome CYP3A pathway (cyclosporine, itraconazole, ketoconazole, erythromycin, azithromycin, clarithromycin, nefazodone)
8	Use of drugs within past 30 days that increase risk of myotoxicity with statins (gemfibrozil, other fibrates, niacin, amiodarone, verapamil)
9	Baseline major electrolyte disturbances (sodium <125 or >150, potassium and <3.0 or 4.5)
10	Recent major trauma (<3 months).
11	Hypothermia (body temperature <96°F).
12	Baseline hypoxia (defined as oxygen saturation <92% on room air)
13	History of likely or proven systemic viral infection within 30 days
14	Known HIV infection or use of protease inhibitors
15	Endocarditis likely as cause of stroke
16	Mitochondrial disorder likely as cause of stroke
17	Pregnancy or lactation
18	History of rhabdomyolysis, myopathy, or other severe muscle disease
19	History of hepatitis, decompensated liver disease (ascites, bleeding varices or encephalopathy), or liver failure
20	Liver function tests (ALT, AST) 2 × upper limit of normal
21	Unstable cardiovascular (includes uncontrolled hypertension), pulmonary, gastrointestinal, hepatic or musculoskeletal disease
22	Patient has evidence of congestive heart failure or has history of end-stage cardiovascular disease (e.g., CHF NYHA Class III or IV or unstable angina)
23	Abnormal ECG showing: hemodynamically significant arrhythmia or frequent PVCs (>5/minute) (controlled atrial arrhythmia will not be an exclusion); evidence of acute myocardial infarction; Mobitz Type II second-degree AV block or third-degree AV block; ventricular tachycardia or ventricular fibrillation
24	Significant renal insufficiency, indicated by serum creatinine >2.0 mg/dl
25	Hypoglycemia (glucose <60 mg/dl) or diabetic ketoacidosis unresponsive to therapy
26	Any of these hematologic abnormalities: Hb <10 g/dl; WBC <3.0 × 10 ³ /mm ³ ; platelet count <50,000/mm ³

- 27 Received an investigational drug within 30 days
- 28 Severe behavioral or social problems that may interfere with the conduct of clinical study procedures
- 29 Patient unlikely, in the investigator's opinion, to complete the study and return for follow-up visits for any reason

Table 3

Schedule of assessments

Procedure	Baseline 0*	High-dose treatment phase				Follow-up phase day			
		Day 1	Day 2	Day 3	Day 4	Day 5	7±1	30±2	
Informed consent	X								
Medical history/ weight	X								
Prior medications/doses	X								
Head CT	SOC	Only as needed clinically							
ECG	SOC	X		X			X		
Dose		Administered four times daily (q6 hours)				20 mg lovastatin daily			
Blood pressure/pulse rate	X (x3) [†]	Every 4 hours					X	X	X
Temperature/respiratory rate	X	Every 4 hours					X	X	X
Physical exam	X	X	X	X	X	X	X	X	X
NIHSS	X	X	X	X	X	X	X	X	X
Muscle/liver assessment		X	X	X	X	X	X	X	X
Liver function/CK tests	SOC	X	X	X	X	X	X	X	X
Chemistry, hematology, glucose	SOC	SOC		SOC		X	X	X	X
Lipid tests	SOC	X		X		X	X	X	X
Coagulation (INR/PTT)	SOC	SOC		SOC		X	X	X	X
Inflammatory markers CRP/IL6/TNF	X	X		X		X	X	X	X
Platelet function/clot lysis studies		X ^{//}							
urinalysis	SOC								
β-HCG	X								X
Pharmacokinetics [§]	X	X		X (X2)	X	X			
Concomitant Meds	X	X	X	X		X	X	X	X
Barthel Index [¶]	X						X	X	X
Modified Rankin score [¶]	X						X	X	X
Assess AEs		X	X	X	X	X	X	X	X

* Time 0 refers to time of first administration of study drug.

[†] The baseline blood pressure and pulse will be measured three times before dose one, at least 10 min apart. β-HCG will be measured only in women of child-bearing age.

§ The pharmacokinetic assays will be drawn at 30 min before and 2 hours after first dose and 12th dose, and also on days 4 and 5.

¶ Modified Rankin Scale (mRS) and Barthel Index (BI): retrospective mRS and BI at study entry, thereafter administered to assess evolution.

// Platelet aggregation studies will be administered on day 1 unless laboratory not available on day 1; in these cases, it will be administered on first day laboratory available.

X: performed as part of protocol. SOC: Standard of Care.

Table 4

Operating characteristics of study

Initial Dose	1 mg/kg	3 mg/kg	6 mg/kg	8 mg/kg	10 mg/kg	Average #DLT
Toxicity rate	10%	25%	30%	35%	40%	
<i>P</i> (select)	0.87	0.12	0.01	0.00	0.00	4.6
Toxicity rate	4%	10%	25%	30%	35%	
<i>P</i> (select)	0.30	0.55	0.14	0.01	0.00	3.7
Toxicity rate	1%	4%	10%	25%	30%	
<i>P</i> (select)	0.02	0.27	0.55	0.15	0.01	3.1
Toxicity rate	1%	1%	4%	10%	25%	
<i>P</i> (select)	0.00	0.03	0.26	0.54	0.18	2.4
Toxicity rate	1%	1%	1%	4%	10%	
<i>P</i> (select)	0.00	0.00	0.05	0.28	0.66	1.4

DLT, Dose-limiting toxicity. Numbers in bold indicate the pre-specified target toxicity (10%) and probability of selecting that dose as the correct dose.