Background and Purpose

Multiple approaches are being studied to enhance the rate of thrombolysis for acute ischemic stroke. Treatment of myocardial infarction with a combination of a reduced-dose fibrinolytic agent and a glycoprotein (GP) IIb/IIIa receptor antagonist has been shown to improve the rate of recanalization over fibrinolysis alone. The combined approach to lysis utilizing eptifibatide and rt-PA (CLEAR) stroke trial assessed the safety of treating acute ischemic stroke patients within three hours of symptom onset with this combination.

Methods

The CLEAR trial was an NIH/NINDS-funded multi-center, double-blind, randomized, dose-escalation and safety study. Patients were randomized 3:1 to either low-dose rt-PA (Tier 1: 0.3 mg/kg, Tier 2: 0.45 mg/kg) plus eptifibatide (75 mcg/kg bolus followed by 0.75 mcg/kg/min infusion for 2 hours), or standard dose rt-PA (0.9 mg/kg). The primary safety endpoint was the incidence of symptomatic intracranial hemorrhage (ICH) within 36 hours. Secondary analyses were performed regarding clinical efficacy.

Results

Ninety-four patients (40 in Tier 1 and 54 in Tier 2) were enrolled. Combining the two dose tiers, the combination group (N=69) had a median age of 71 and a median baseline NIHSS score of 14, and the standard rt-PA group (N=25) had a median age of 61 and a median baseline NIHSS score of 10 (p=0.01 for NIHSS score). Fifty two (75%) of the combination group and 24 (96%) of the control group had a baseline modified Rankin scale of 0 (p=0.04). There was 1 (1.4%, 95%CI: 0-4.3%) symptomatic ICH in the combination group and 2 (8.0%, 95%CI: 0-19.2%) in the rt-PA only arm (p=0.17). During randomization in Tier 2, the independent Data Safety Monitoring Board review demonstrated that the safety profile of the combination therapy at the Tier 2 doses was such that further enrollment was statistically unlikely to indicate inadequate safety for the combination group, the ultimate outcome of the study. Thus the study was halted. There was a trend towards increased clinical efficacy of standard rt-PA compared to the combination group.

Conclusion

The safety of the combination of reduced dose rt-PA plus eptifibatide justifies further dose ranging trials in acute ischemic stroke.

Introduction

Recombinant tissue Plasminogen Activator (rt-PA) is the only FDA-approved pharmacological therapy for acute ischemic stroke.[1] Despite the demonstrated efficacy of rt-PA in the NINDS rt-PA stroke trials, only 30-40% of occluded major vessels are recanalized in the first hour after treatment with rt-PA.[2] In addition, recent studies have documented appreciable risk of reocclusion.[3] Methods that may enhance thrombolysis warrant clinical exploration. Glycoprotein (GP) IIb/IIIa inhibitors block the final common pathway of platelet aggregation and thus have considerable therapeutic potential in the treatment of thrombotic diseases such as acute MI and acute ischemic stroke. GP IIb/IIIa inhibitors are commonly used to treat acute MI and to prevent ischemic complications associated with percutaneous coronary interventions. For acute ischemic stroke treatment, experimental and clinical studies suggest that combination therapy (fibrinolytic plus GP IIB/IIIA inhibitors) could provide faster and more complete lysis than fibrinolytics alone.[4]

Eptifibatide is a cyclic heptapeptide GP IIb/IIIa inhibitor that is commercially available and is indicated the treatment of patients with acute coronary syndrome, including patients who are to be managed medically and those undergoing percutaneous coronary intervention. Eptifibatide alone, without a fibrinolytic agent, has been found to decrease both the incidence of acute MI and mortality in patients with unstable angina.[5] Eptifibatide alone has also been shown to reduce ischemic events and arterial re-occlusions in patients undergoing percutaneous coronary interventions.[6-8] Notably, the platelet aggregation inhibition that prevents the ischemic events after PTCA has not been associated with higher rates of ICH.[9] While not available at the time of this study's design, there is now data that full cardiac dose GP IIb/IIIa antagonists alone increase the rate of ICH over placebo in stroke patients. Notably, the

dosing of the GP IIb/IIIa antagonist in the CLEAR trial is significantly lower than the full cardiac dosing. [10] The eptifibatide dosing in the CLEAR trial was chosen to be slightly less than one half of the cardiac dose in this safety and dose escalation trial.

Based on the potential to improve upon the efficacy of fibrinolysis for acute ischemic stroke, we hypothesized that combination therapy could be explored as an adjunct to standard thrombolytic therapy. Thus we sought to execute a dose escalation trial to assess the safety profile of combination therapy as the basis for potential further clinical exploration.

Methods

The CLEAR trial was a multi-center, randomized, double-blind, sequential, dose-escalation safety study of low dose rt-PA in combination with eptifibatide versus standard dose rt-PA given to patients diagnosed with acute ischemic stroke and treated within 3 hours of symptom onset. An Investigational New Drug (IND) application was approved by the FDA prior to trial initiation and is held by the principal investigator (AP). The Institutional Review Board of each site approved the study protocol, and written informed consent was obtained prior to entry for each patient. The trial was sponsored by the National Institutes of Neurological Diseases and Stroke (NINDS) as a Specialized Programs of Translational Research In Acute Stroke (SPOTRIAS) program project. Study drugs were supplied at no charge by the manufacturers. The trial design, execution and the data analysis were accomplished independent of the manufacturers. Eligible patients were 18 to 80 years of age with a clinical diagnosis of acute ischemic stroke and an NIHSS score > 5. The protocol required that study drug be initiated within 3 hours from the time the patient was last seen normal. Inclusion and exclusion criteria are listed in Table 1. Note that these criteria differ from those used for the NINDS rt-PA stroke trials. The upper age limit was added as there was some concern for the potential for increased ICH in older patients treated with combination therapy in the cardiac literature.[11] The NIHSS score > 5 was added because 81% of placebo-treated patients with an NIHSS score ≤ 5 in the NINDS rt-PA trials had a 90 day modified Rankin Score of 0-1 making this group's

potential benefit too small to include in a safety trial.[12] The upper limit for the creatinine is based on the renal excretion of eptifibatide.

From July 2003 to April 2007, nine US centers (19 hospitals) enrolled 94 patients. Patients were enrolled into two sequential dosing tiers via a centralized randomization telephone at the clinical coordinating center at the University of Cincinnati. Patients were randomized in a 3:1 ratio to receive the combination of low dose rt-PA and eptifibatide or standard rt-PA per the NINDS protocol. Dose tier one was designed to have 40 patients. After completion of dose tier one and a blinded review by the DSMB, the study progressed. Dose tier two was designed to have a total of 60 patients. Drug dosing regimens in the two tiers were as follows. The dose of rt-PA was 0.3 mg/kg in tier 1 and 0.45 mg/kg in tier 2 with a maximum weight of 100 kg. The dose of eptifibatide that was slightly less than one half of the cardiac dose which and was the same in both tiers: a 75 mcg/kg bolus followed by a two hour infusion of 0.75 mcg/kg/min.

In order to reach a therapeutic serum concentration of rt-PA using a lower total dose in the combination arm, the rate of infusion of rt-PA had to be greater than the standard rt-PA infusion. Blinding of the rt-PA dosing was maintained through careful double blind/double dummy pattern. All patients received a bolus of rt-PA (either 15% of the low dose in the combination arm or 10% of 0.9 mg/kg in the control arm.) These were prepared in the investigational pharmacies to a volume of 10 ml to avoid unblinding. All patients then received two sequential infusion bags that were *either* the remainder of the low dose rt-PA over 30 minutes followed by a placebo over 30 minutes *or* the remainder of the 0.9 mg/kg standard dose divided between two bags each running over 30 minutes in the control group. The infusion bags were made to a volume of 50 ml to avoid unblinding. (See Figure 1 for method of drug delivery / blinding) All study drugs were colorless and compatible in infusion lines so there was no potential unblinding due to mixing of agents in the IV lines.

Patients were monitored clinically throughout the infusion by study personnel, and then admitted to an intensive care unit for continued monitoring for at least 24 hours. Patients had standardized clinical evaluations at 2 hours, 24 hours and 5 days or discharge, followed by telephone follow-up at 7 days and a final in-person standardized evaluation at 90 days. Outcomes measured were the NIHSS, Glasgow Outcome Scale, Barthel Index, EuroQOL, Stroke Specific Quality of Life and Stroke-Free Status questionnaire. Radiological outcome measures included a 24-hour safety CT to evaluate for ICH, and a 24-hour MRI with MRA to evaluate infarct volume and arterial patency.

The primary safety endpoint was the incidence of symptomatic ICH within 36 hours of onset. Secondary safety endpoints included asymptomatic ICH, mortality and clinically significant extracranial bleeding. While this was a safety trial, clinical outcomes were also collected to assess for potential efficacy. The primary outcome measures for this pilot study were evidence of early neurological improvement, as measured by the incidence of NIHSSS of ≤ 2 at 24 hours, and late improvement, as measured by the incidence of modified Rankin (mRS) of 0-1 or return to baseline at 3 months. Secondary functional endpoints at 90 days included a Barthel Index of ≥ 95, Glasgow Outcome Scale of 1, and a NIHSS score of 0 or 1. In addition, the EuroQOL quality of life index and the Stroke Specific Quality of Life Scale were administered at three months. Ninety-day outcome was assessed by study investigators not directly involved with acute treatment of the patient. All investigators were certified in the NIHSSS and received standardized training regarding the modified Rankin, Barthel, Glasgow, and EuroQol assessments.

Data were all managed and analyzed using SAS® Version 9.1 (SAS Institute Inc, Cary, NC). The case report forms were all double-entered into a custom SAS database. Univariate analysis included range checking, in particular against enrollment criteria. Bivariate analysis to compare baseline descriptors between the experimental and control group consisted of Wilcoxon rank sum, and Fisher's exact test as appropriate based on the nature of the data being analyzed. Analysis of the efficacy outcome variables

was done using multiple logistic regression to control for appropriate covariates. Variables considered for inclusion as potential covariates were age, baseline NIHSS score, history of prior stroke, history of diabetes, baseline mRS, time to treatment, and the interaction of age and baseline NIHSS score. These variables were chosen based on the findings of the NINDS rt-PA trials.[12] Dose tier was also considered as a potential covariate. Multivariable analyses were repeated for each dose tier.

Information was available for all subjects regarding the safety endpoint. However, even though every effort was made to obtain 90-day follow-ups, there were 4 subjects for whom information was unavailable. For these subjects the worst case scenario was assumed, as consistent with other stroke trials.

Results

The study enrolled 94 subjects; 40 in dose tier one and 54 in dose tier two. Combining the two dose tiers, the experimental group (N=69) was older, had greater stroke severity at baseline, and was less likely to have a pre-Stroke modified Rankin of 0 at baseline compared to the standard rt-PA group (N=25); median age of 71 vs. 61 (p=0.09), median baseline NIHSS score of 14 vs. 10 (p=0.04), 52 (75%) with a baseline modified Rankin scale of 0 vs. 24 (96%), (p=0.04). (Table 2) There were no other significant demographic or medical history differences between the two groups, and the median time to treatment was very similar at 2.5 and 2.6 hours, respectively.

Dose tier one consisted of 40 patients, including 29 patients randomized to combination therapy and 11 randomized to standard IV rt-PA. One symptomatic ICH did occur in the experimental group during the first dose tier, and there were no safety concerns after that tier was completed. Dose tier two was initiated after approval from the DSMB and FDA. The trial was held briefly for DSMB review during dose tier two after the release of the negative findings from the AbESTT trial due to concerns about GP IIb/IIIa safety in acute ischemic stroke.[10] The DSMB review found no safety concerns and trial recruitment was

continued. Dose tier two ultimately enrolled 54 of the planned 60 patients. At this point in the trial, an unblinded DSMB review demonstrated that the safety profile of the combination therapy at the current doses was such that further enrollment was statistically unlikely to indicate inadequate safety for the combination group, the ultimate outcome of the study. In fact, a futility analysis assuming the worst possible scenario for the six subjects remaining to be randomized resulted in a p-value equal to 1.0 for the safety outcome. Thus the study was halted.

The primary safety outcome of symptomatic ICH was found in 1 (1.4%, 95% CI: 0-4.3%) of 69 patients in the combination group and 2 (8.0%, 95%CI: 0-19.2%) of 25 patients in the standard treatment arm (p=0.17). Figure 2 shows the CT images of all ICHs, both symptomatic and asymptomatic, from the trial. Thus, despite the older age and higher baseline stroke severity in the combination treated group, both factors previously associated with increased hemorrhagic risk, the symptomatic ICH rate for combination treatment was not higher. Similarly, asymptomatic ICH was less frequent in the experimental arm (n=7, 10.3%) than in the control arm (n=3, 12.0%).

Outcome data comparing standard IV rtPA and combination therapy in the total group, as well as by dose tier, are shown in Tables 3 and 4. Prior to a review of efficacy outcome data it must be emphasized that the two groups in this safety study were very different with regard to variables that are known to predict outcome including age, NIHSSS and pretreatment mRS. In the unadjusted analysis, there were no differences between the treatment groups with regards to primary measure of functional outcome, case-fatality, symptomatic hemorrhage, or improvement in stroke severity. One secondary measure of functional outcome (the Barthel Index) was statistically significant in favor of standard therapy. After controlling for covariates of age, baseline NIHSS score, pre-Stroke baseline mRS, time to treatment, history of diabetes, and age by NIHSS score interaction, there was no signal of efficacy compared to rt-PA found for the combination therapy group.

Tables 5 and 6 offer a comparison of the combination group to patients from the NINDS rt-PA trials that were similar to the CLEAR patients in that they have a baseline NIHSSS >5 and Age 18-80 inclusive. In addition, only the patients from the 91-180 min group of the NINDS trials were selected as the median time to treatment in the CLEAR trial was 2.55 hours, and patients treated at less than 90 min did not have an appropriate comparator. As can be seen, the only demographic difference was age, the NINDS control patients being younger, 66.7 vs. 71.4 years (p=0.02). The only outcome variables that approached significance were the lower rate of symptomatic ICH in the combination patients compared to rt-PA patients 1% vs. 8% (p=0.08), and a trend toward better Glasgow Outcome Score and greater likelihood of NIHSS <= 2 at 24 hours for combination patients compared to NINDS control patients (p=0.06 for both after adjustment for baseline variables). Thus, the combination patients had a trend toward lower hemorrhage rate compared to rt-PA, no obvious difference in clinical outcome compared to rt-PA, and a signal of efficacy compared to control in NINDS.

The median time from onset to treatment for the combination patients was 2.53 hours (IQR 2.25, 2.83) and for the rt-PA patients was 2.62 hours (IQR 2.41, 2.83). The median time from symptom onset to ED arrival was 0.92 hours and 0.97 hours respectively. The median times from ED arrival to CT were 0.23 hours for combination patients and 0.30 hours for the rt-PA patients, and the median times from CT to treatment were 1.34 hours and 1.37 hours respectively.

Discussion

The CLEAR trial was the first randomized, blinded, safety trial of the combination of low dose rt-PA and a glycoprotein IIb/IIIa antagonist for acute ischemic stroke. The primary goal was to assess the safety of combination therapy initiated within three hours of symptom onset. As a baseline for ICH rate for reference, we analyzed the patients from the NINDS rt-PA trials that had an NIHSS > 5, were within the age range of 18-80 and were treated after 90 minutes. In that group the symptomatic ICH rate was 8.1%. This mirrors what was found in the 25 control patients treated with rt-PA in the CLEAR trial. Thus the

occurrence of only 1 symptomatic ICH in the 69 combination patients (1.4%) was indeed encouraging safety data for the combination therapy. In addition to the primary safety outcome, other serious adverse events (SAEs) were carefully evaluated. Similar to ICH rates, no other SAE rate was noted to be different between the two groups.

The CLEAR trial safety results must be considered in the context of the marked disparity in age and baseline NIHSS score between the combination and control groups. It is well-known that both older age and higher baseline NIHSS score are significant predictors of symptomatic ICH after rt-PA treatment of acute ischemic stroke.[13] Despite the marked disparity favoring the standard IV rt-PA group (younger age and lower stroke severity and more zero baseline mRS), the combination therapy group did not show a higher rate of symptomatic or asymptomatic ICH. The fact that the study combination was safe despite these increased risk factors for ICH further suggests that the combination of eptifibitide and rt-PA for treatment of ischemic stroke within three hours of symptom onset is safe enough to consider further exploration.

From the first grant application submission, the plan of the CLEAR Investigators was to proceed with a follow-up trial if the safety results of CLEAR were within pre-specified limits. The safety stopping rules for each tier specified that if the observed sICH rate exceeded the expected rate for comparable patients treated with rt-PA in the NINDS rt-PA stroke trials the trial would have been placed on hold with a mandatory DSMB review. The trial was neither designed nor powered for efficacy. Notably, the trial design ultimately resulted in a large disparity in age and stroke severity between the two groups, due to the play of random chance and small numbers of patients in the randomly allocated groups. For future smaller phase II trials adaptive or stratified randomization may be a necessary strategy to minimize group disparities.

This safety data allows for continued exploration of the combination of fibrinolytics and GP IIb/IIIa

antagonists in acute stroke. Combination therapy of fibrinolytics with GPIIb/IIIa blockers has been an important focus of research in cardiac patients. The combination approach provides two simultaneous mechanisms for thrombolysis via disruption of the clot's fibrin meshwork coupled with disaggregation of platelets. The result is improved arterial patency with the combination of GPIIb/IIIa blockade and fibrinolytic agents versus fibrinolytic agents alone.[4]

Of particular importance to acute ischemic stroke treatment, the rate of recanalization in acute MI occurring with the combination of a fibrinolytic and GP IIb/IIIa inhibitor has consistently been faster and more effectively than with a fibrinolytic alone. This has been demonstrated using the continuous measurement of ST segment elevation in patients with AMI as a surrogate marker for recanalization.[14]

Animal research demonstrates that significant microvascular platelet and fibrin accumulation occurs in the setting of MCA occlusion in primates and rodents.[15-18] Microvascular occlusions by platelet-fibrin aggregates have been shown to be preventable with the use of an experimental GPIIb/IIIa inhibitor given just prior to MCA occlusion.[19] In another study, infusion of an experimental GPIIb/IIIa inhibitor was associated with a significant reduction in microvascular platelet accumulation and was associated with a 70% reduction in cerebral infarct size.[16] The data suggest an important role for post-occlusive distal platelet deposition in the evolution of an acute ischemic stroke.

Further preclinical data utilizing an embolic model in mice have demonstrated that the combination of a fibrinolytic and GP IIb/IIIa inhibitor, increases the efficiency of dissolving an embolus, decreases infarct volume, and improves microvascular patency as compared to fibrinolytic agent alone. In this research, treatment with rt-PA and a GP IIb/IIIa antagonist reduced the perfusion deficits and significantly enhanced cortical perfusion.[20]

Thus, there is considerable cardiac and preclinical literature to support the hypothesis that the combination may, at appropriate dosages, prove efficacious in acute ischemic stroke. The CLEAR trial had demonstrated, despite significant imbalances in randomization, that there is adequate safety of the combination at the studied dosages to pursue a higher dose regimen.

Study Limitations

This was a pilot safety trial and any efficacy conclusions beyond hypothesis generation are unwarranted. The disparities in baseline characteristics confound analysis as described above. This problem could be prevented with an adaptive randomization technique in a future trial.

There were also significant logistical limitations that became apparent during the execution of the trial. The greatest limitation of the CLEAR trial was the cumbersome design of the double blind drug dosing. In order to reach likely effective serum concentrations of rt-PA with lower dosing, the time interval for the low dose infusion had to be shorter than the one hour infusion of standard dose rt-PA. Thus, the rt-PA infusion was split into two bags; for the low dose arm, the entire rt-PA dose was in the first bag. The investigators were forced to wait while the investigational pharmacies prepared a total of two boluses and 3 infusion bags prior to initiation of drug. While the investigators' practice was to inform the pharmacy as soon as a possible patient was identified, delays in treatment due to pharmacy preparation time did occur. As a surrogate marker for the delay, median time from onset to treatment in these patients was 2.55 hours but the median time from CT to initiation of study drug was 1.35 hours which is likely longer than the median time for open label at most experienced centers. As a comparison, in the NINDS rt-PA stroke trials the median (IQR) time from CT to initiation of drug therapy was 0.58 hours (0.38, 0.83) for all patients in the trial. Breaking this down by groups, the median times from CT to treatment were 0.43 hours (0.27, 0.58) for patients treated in less than 90 minutes and 0.75 hours (0.55, 1.08) for patients

treated between 91 and 180 minutes. Any future trial should consider starting open label rt-PA as soon as clinically possible.

Conclusions

The CLEAR trial was a dose escalation and safety study of the combination of low dose rt-PA combined with a GP IIb/IIIa antagonist eptifibatide in acute ischemic stroke patients that were treated within 3 hours of symptom onset. The safety profile of the combination regimens was excellent. The investigational team believes that the combination of reduced dose rt-PA and eptifibatide appears safe enough for consideration of further dose ranging trials in acute ischemic stroke. The combined approach to lysis utilizing eptifibatide and rt-PA – enhanced regimen (CLEARER) trial will be a randomized trial of rt-PA 0.6 mg/kg plus eptifibatide vs. standard dose rt-PA. The trial is designed to evaluate safety of this higher dosage combination as well as to determine whether the estimated signal of efficacy warrants proceeding to a Phase III clinical trial.

References

1. Tissue plasminogen activator for acute ischemic stroke. The national institute of neurological disorders and stroke rt-pa stroke study group. *N Engl J Med*. 1995;333:1581-1587
2. Wolpert SM, Bruckmann H, Greenlee R, Wechsler L, Pessin MS, del Zoppo GJ. Neuroradiologic evaluation of patients with acute stroke treated with recombinant tissue plasminogen activator. The rt-pa acute stroke study group. *AJNR Am J Neuroradiol*. 1993;14:3-13
3. Alexandrov AV, Grotta JC. Arterial reocclusion in stroke patients treated with intravenous tissue plasminogen activator. *Neurology*. 2002;59:862-867
4. Pancioli AM, Brott TG. Therapeutic potential of platelet glycoprotein iib/iiia receptor antagonists in acute ischaemic stroke: Scientific rationale and available evidence. *CNS Drugs*. 2004;18:981-988
5. Inhibition of platelet glycoprotein iib/iiia with eptifibatide in patients with acute coronary syndromes. The pursuit trial investigators. Platelet glycoprotein iib/iiia in unstable angina: Receptor suppression using integrilin therapy. *N Engl J Med*. 1998;339:436-443
6. Randomised placebo-controlled trial of effect of eptifibatide on complications of percutaneous coronary intervention: Impact-ii. Integrilin to minimise platelet aggregation and coronary thrombosis-ii. *Lancet*. 1997;349:1422-1428
7. O'Shea JC, Hafley GE, Greenberg S, Hasselblad V, Lorenz TJ, Kitt MM, Strony J, Tcheng JE. Platelet glycoprotein iib/iiia integrin blockade with eptifibatide in coronary stent intervention: The esprit trial: A randomized controlled trial. *Jama*. 2001;285:2468-2473
8. Tcheng JE, Harrington RA, Kottke-Marchant K, Kleiman NS, Ellis SG, Kereiakes DJ, Mick MJ, Navetta FI, Smith JE, Worley SJ, et al. Multicenter, randomized, double-blind, placebo-controlled trial of the platelet integrin glycoprotein iib/iiia blocker integrelin in elective coronary intervention. Impact investigators. *Circulation*. 1995;91:2151-2157
9. Mahaffey KW, Harrington RA, Simoons ML, Granger CB, Graffagnino C, Alberts MJ, Laskowitz DT, Miller JM, Sloan MA, Berdan LG, MacAulay CM, Lincoff AM, Deckers J, Topol EJ, Califf RM. Stroke in patients with acute coronary syndromes: Incidence and outcomes in the platelet glycoprotein iib/iiia in unstable angina. Receptor suppression using integrilin therapy (pursuit) trial. The pursuit investigators. *Circulation*. 1999;99:2371-2377
10. Adams HP, Jr., Effron MB, Torner J, Davalos A, Frayne J, Teal P, Leclerc J, Oemar B, Padgett L, Barnathan ES, Hacke W. Emergency administration of abciximab for treatment of patients with acute ischemic stroke: Results of an international phase iii trial. Abciximab in emergency treatment of stroke trial (abestt-ii). *Stroke*. 2007
11. Topol EJ. Reperfusion therapy for acute myocardial infarction with fibrinolytic therapy or combination reduced fibrinolytic therapy and platelet glycoprotein iib/iiia inhibition: The gusto v randomised trial. *Lancet*. 2001;357:1905-1914
12. Ingall TJ, O'Fallon WM, Asplund K, Goldfrank LR, Hertzberg VS, Louis TA, Christianson TJ. Findings from the reanalysis of the ninds tissue plasminogen activator for acute ischemic stroke treatment trial. *Stroke*. 2004
13. Khatri P, Wechsler LR, Broderick JP. Intracranial hemorrhage associated with revascularization therapies. *Stroke*. 2007;38:431-440

14. Rebeiz AG, Johanson P, Green CL, Crater SW, Roe MT, Langer A, Giugliano RP, Lincoff AM, Newby LK, Harrington RA, Topol EJ, Califf RM, Wagner GS, Krucoff MW. Comparison of st-segment resolution with combined fibrinolytic and glycoprotein iib/iiia inhibitor therapy versus fibrinolytic alone (data from four clinical trials). *Am J Cardiol*. 2005;95:611-614
15. Del Zoppo GJ, Copeland BR, Harker LA, Waltz TA, Zyroff J, Hanson SR, Battenberg E. Experimental acute thrombotic stroke in baboons. *Stroke*. 1986;17:1254-1265
16. Choudhri TF, Hoh BL, Zerwes HG, Prestigiacomo CJ, Kim SC, Connolly ES, Jr., Kottirsch G, Pinsky DJ. Reduced microvascular thrombosis and improved outcome in acute murine stroke by inhibiting gp iib/iiia receptor-mediated platelet aggregation. *J Clin Invest*. 1998;102:1301-1310
17. Garcia JH, Liu KF, Yoshida Y, Lian J, Chen S, del Zoppo GJ. Influx of leukocytes and platelets in an evolving brain infarct (wistar rat). *Am J Pathol*. 1994;144:188-199
18. Zhang ZG, Zhang L, Tsang W, Goussev A, Powers C, Ho KL, Morris D, Smyth SS, Coller BS, Chopp M. Dynamic platelet accumulation at the site of the occluded middle cerebral artery and in downstream microvessels is associated with loss of microvascular integrity after embolic middle cerebral artery occlusion. *Brain Res*. 2001;912:181-194
19. Abumiya T, Fitridge R, Mazur C, Copeland BR, Koziol JA, Tschopp JF, Pierschbacher MD, del Zoppo GJ. Integrin alpha(iib)beta(3) inhibitor preserves microvascular patency in experimental acute focal cerebral ischemia. *Stroke*. 2000;31:1402-1409; discussion 1409-1410
20. Ding G, Jiang Q, Zhang L, Zhang ZG, Li L, Knight RA, Ewing JR, Wang Y, Chopp M. Analysis of combined treatment of embolic stroke in rat with r-tpa and a gpiib/iiia inhibitor. *J Cereb Blood Flow Metab*. 2005;25:87-97

Table 1 Inclusion and Exclusion Criteria

Inclusion Criteria

Patients must have a serious measurable neurological deficit on the NIH Stroke Scale due to focal brain ischemia.

An NIH Stroke Scale score >5 at the time that intravenous study drug is begun.

Age: 18 through 80 years (i.e. candidates must have had their 18th birthday, but not had their 81st birthday).

Intravenous therapy must be initiated within 3 hours of onset of stroke symptoms.

Exclusion Criteria

Clinical

History of stroke in the past 3 months

Previous intra-cranial hemorrhage, neoplasm, subarachnoid hemorrhage, or arterial venous malformation

Clinical presentation suggests a subarachnoid hemorrhage, even if initial CT scan is normal

Hypertension at time of treatment; systolic BP > 185 or diastolic > 110 mmHg or aggressive measures to lower blood pressure to below these limits are needed.

Presumed septic embolus

Presumed pericarditis including pericarditis after acute myocardial infarction

Recent (within 30 days) surgery or biopsy of parenchymal organ

Recent (within 30 days) trauma, with internal injuries or ulcerative wounds

Recent (within 90 days) severe head trauma or head trauma with loss of consciousness

Any active or recent (within 30 days) serious systemic hemorrhage

Known hereditary or acquired hemorrhagic diathesis, coagulation factor deficiency; or oral anticoagulant therapy with prothrombin time greater than 15 or INR > 1.4

Baseline lab values: positive urine pregnancy test, glucose < 50 or > 400 mg/dl, platelets <100,000 /mm^3, Hct < 25 %, or creatinine > 4 mg/dl

Ongoing renal dialysis, regardless of creatinine

If heparin has been administered within 48 hours, the patient must have a normal partial thromboplastin time (PTT)

Arterial puncture at a non-compressible site or a lumbar puncture in the previous 7 days

Seizure at onset of stroke

Pre-existing neurological or psychiatric disease that would confound the neurological or functional evaluations

Other serious, advanced, or terminal illness or any other condition that the investigator feels would pose a significant hazard to the patient if rt-PA or eptifibatide therapy were initiated

Patients whose peripheral venous access is so poor that they are unable to have two standard peripheral intravenous lines started.

Current participation in another research drug treatment protocol. Patient cannot start another experimental agent until after 90 days

Informed consent is not or cannot be obtained

Any known history of amyloid angiopathy.

CT Scan Exclusions

High density lesion consistent with hemorrhage of any degree.

Significant mass effect with midline shift.

Large (more than 1/3 of the middle cerebral artery) regions of clear hypodensity on the baseline CT scan. Sulcal effacement and/or loss of grey-white differentiation alone are not contraindications for treatment.

Figure 1.

This figure represents the bolus and infusion methodology for the two arms of the CLEAR trial. The Eptifibatide or placebo is given as a bolus followed by a two hour infusion. The rt-PA is given as a bolus followed by two sequential infusion bags.

Bag B#1: rt-PA, either 85% of lower dose experimental regimens, or 45% of standard dose infused over 30 minutes

Bag B#2: (started after bag B#1 finished) Normal saline (lower dosing experimental regimens) or rt-PA (45% of standard dose) infused over remaining 30 minutes

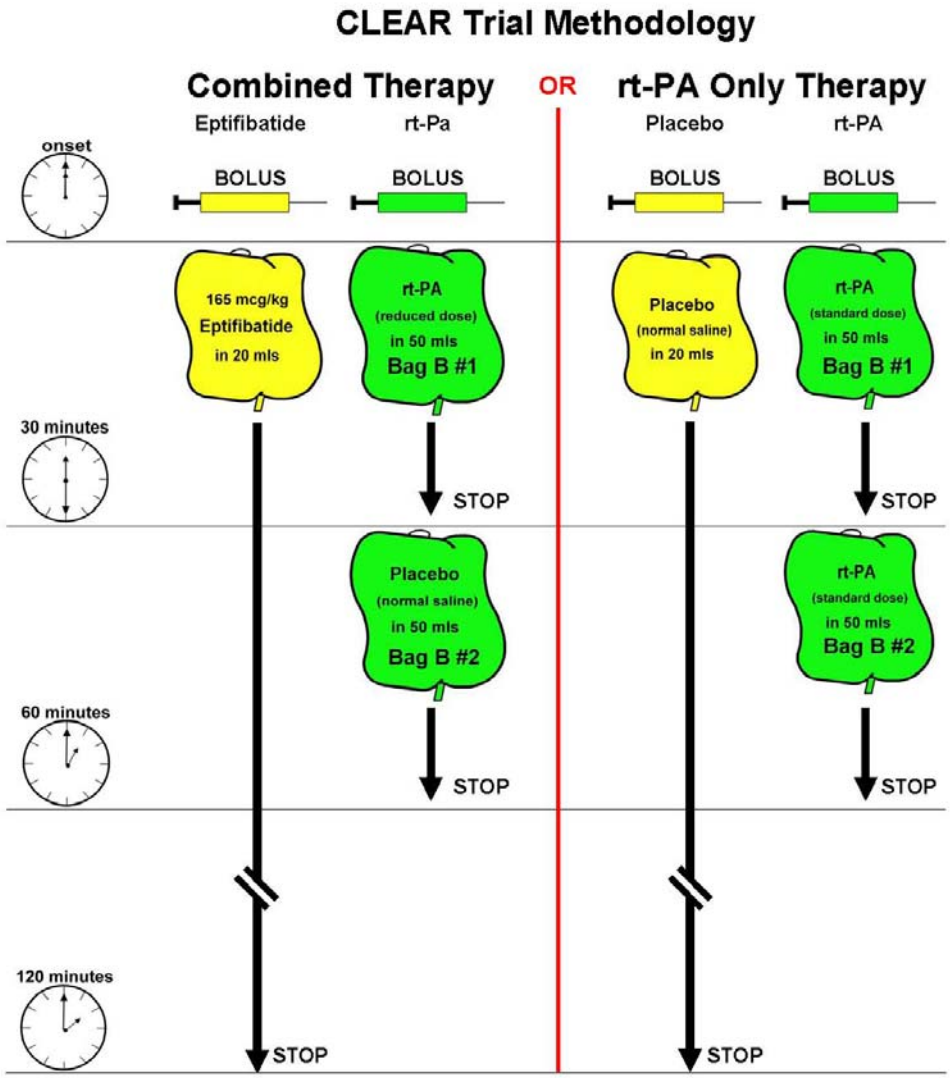

Figure 2 – Picture of ALL Hemorrhages

Table 2

	Tier 1			Tier 2			Overall			Total
	Combined	Control	p-value	Combined	Control	p-value	Combined	Control	p-value	
N	29	11		40	14		69	25		94
Prior stroke	4 (14%)	1 (9%)	1.0	9 (22%)	3 (21%)	1.0	13 (19%)	4 (16%)	1.0	17 (18%)
Hx diabetes	8 (28%)	1 (9%)	0.40	9 (22%)	3 (21%)	1.0	17 (25%)	4 (16%)	0.58	21 (22%)
Hx hypertension	23 (79%)	6 (54%)	0.12	26 (65%)	9 (64%)	0.96	49 (71%)	15 (60%)	0.31	64 (68%)
Base mRS=0	19 (66%)	11 (100%)	0.04	33 (82%)	13 (93%)	0.66	52 (75%)	24 (96%)	0.04	76 (81%)
Base mRS 0-1	24 (83%)	11 (100%)	0.30	37 (92%)	14 (100%)	0.56	61 (88%)	25 (100%)	0.10	86 (91%)
*Age (yrs)	72.7 (67, 77)	60.1 (52, 74)	0.03	68.0 (52, 77)	61.5 (56, 77)	0.79	71.4 (62, 77)	61.2 (55, 74)	0.09	68.8 (60, 77)
*Baseline NIHSS	14.0 (10, 20)	14.0 (9, 16)	0.23	13.5 (8, 17)	8.5 (6, 12)	0.02	14.0 (10, 20)	10.0 (6, 14)	0.01	13.0 (8, 17)

*Time to Treat (min)	2.5 (2.2, 2.8)	2.6 (2.3, 2.8)	0.61	2.5 (2.2, 2.8)	2.6 (2.5, 2.9)	0.27	2.5 (2.2, 2.8)	2.6 (2.4, 2.8)	0.28	2.6 (2.2, 2.8)
Glucose (mg/dL)	115 (101, 139)	121 (99, 145)	0.90	117 (99, 146)	114 (96, 158)	0.88	117 (100,141)	116 (99, 146)	0.90	117 (99, 146)
Systolic BP (mmHg)	157 (141, 186)	140 (122, 183)	0.20	150 (138, 166)	158 (137, 178)	0.37	153 (139, 175)	154 (137, 178)	0.75	154 (137, 176)
Diastolic BP (mmHg)	82 (71, 95)	82 (74, 91)	0.87	84 (74, 90)	88 (77, 101)	0.26	83 (74, 93)	82 (77, 101)	0.28	82 (74, 95)
MAP	112 (96, 124)	103 (90, 122)	0.63	108 (94, 116)	113 (97, 129)	0.16	108 (95, 117)	106 (97, 124)	0.55	108 (96, 120)

Data expressed as n (%) or *median and (25^{th}, 75^{th} percentile)

Table 3 Clinical Outcomes At Three Months for Tier 1, Tier 2 and The Overall Trial comparing combined and rt-PA only groups

	Tier 1			Tier 2			Overall		
	Combined	Control	p-value	Combined	Control	p-value	Combined	Control	p-value
N	29	11		40	14		69	25	
*mRS (0-1 or return to baseline)	9 (31%)	5 (45%)	0.47	12 (30%)	7 (50%)	0.21	21 (30%)	12 (48%)	0.14
*Barthel (<=95)	12 (41%)	8 (73%)	0.16	20 (50%	10 (71%)	0.22	32 (46%)	18 (72%)	0.04
*Glasgow Outcome Score (0)	13 (45%)	5 (45%)	1.0	15 (38%)	8 (57%)	0.23	28 (41%)	13 (52%)	0.35
SICH	1 (3%)	1 (9%)	0.48	0 (0%)	1 (7%)	0.26	1 (1%)	2 (8%)	0.17
SICH or ASICH	5 (17%)	3 (27%)	0.66	3 (8%)	2 (14%)	0.60	8 (12%)	5 (20%)	0.32
Death (90 days)	8 (28%)	1 (9%)	0.40	7 (18%)	2 (14%)	1.0	15 (22%)	3 (12%)	0.38
Death (7 days)	4 (14%)	1 (9%)	1.0	5 (12%)	0 (0%)	0.31	9 (13%)	1 (4%)	0.28
NIHSS (decreased >=4 in 24 hours)	14 (48%)	6 (54%)	1.0	15 (38%)	5 (36%)	1.0	29 (42%)	11 (44%)	1.0
NIHSS (<=2 at 24 hours)	2 (7%)	1 (9%)	1.0	7 (18%)	4 (29%)	0.45	9 (13%)	5 (20%)	0.51

Data expressed as n (%), comparisons made using Fisher's exact test due to small numbers. *Deaths (n=18) and missing (n=4) coded as "bad" outcome.

Table 4 Adjusted Odds Ratios and associated 95% confidence intervals for Clinical Outcome At Three Months**

	Tier 1		Tier 2		Overall	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
*mRS (0-1 or return to baseline)	0.19 (0.02, 1.84)	0.15	0.64 (0.13, 3.30)	0.60	0.56 (0.19, 1.63)	0.29
*Barthel (<=95)	0.24 (0.01, 4.60)	0.34	0.28 (0.04, 1.75)	0.17	0.46 (0.14, 1.53)	0.20
*Glasgow Outcome Score (0)	1.90 (0.25, 14.76)	0.54	0.55 (0.11, 2.91)	0.48	1.03 (0.36, 3.00)	0.95
Survival at 90 days	0.56 (0.01, 33.3)	0.78	0.35 (0.02, 6.41)	0.48	0.95 (0.19, 4.81)	0.95
Survival at 7 days	Not estimable		Not estimable		0.41 (0.04, 4.05)	0.45
SICH or ASICH	0.18 (0.02, 1.97)	0.16	0.23 (0.01, 5.30)	0.36	0.28 (0.06, 1.23)	0.09
NIHSS (decreased >=4 in 24 hours)	0.51 (0.06, 4.18)	0.53	0.77 (0.16, 3.69)	0.74	0.61 (0.20, 1.86)	0.38

NIHSS (<=2 at 24 hours)	0.20	0.50	0.34	0.27	0.70	0.62
	(0.01, 20.8)		(0.05, 2.34)		(0.17, 2.87)	

*Deaths (n=18) and missing (n=4) coded as "bad" outcome.

**Controlling for covariates of age, baseline NIHSS, baseline mRS, time to treatment, history of diabetes, age by NIHSS interaction, baseline glucose and baseline systolic and diastolic blood pressure

Table 5 Characteristics

	CLEAR	NINDS rt-PA	p-value	CLEAR	NINDS Control	p-value
N	69	115		69	151	
Prior stroke	13 (19%)	21 (18%)	0.92	13 (19%)	23 (15%)	0.50
Hx diabetes	17 (25%)	25 (22%)	0.65	17 (25%)	34 (22%)	0.73
Base mRS=0	52 (75%)	97 (84%)	0.13	52 (75%)	126 (83%)	0.16
Base mRS 0-1	61 (88%)	104 (90%)	0.66	61 (88%)	137 (91%)	0.59
*Age (yrs)	71.4 (62, 77)	69.3 (59, 75)	0.32	71.4 (62, 77)	66.7 (56, 74)	0.02
*Baseline NIHSS	14.0 (10, 20)	15.0 (9, 20)	0.45	14.0 (10, 20)	15.0 (10, 22)	0.23
*Time to Treat (min)	2.5 (2.2, 2.8)	2.6 (2.2, 2.9)	0.37	2.5 (2.2, 2.8)	2.5 (2.1, 2.8)	0.78

Data expressed as n (%) or *median and (25^{th}, 75^{th} percentile)

Table 6 Outcome variables

	CLEAR	NINDS rt-PA	p-value	CLEAR	NINDS Control	p-value
N	69	115		69	151	
*mRS (0-1 or return to baseline)	21 (30%)	46 (40%)	0.19	21 (30%)	36 (24%)	0.30
*Barthel (<=95)	32 (46%)	53 (46%)	0.97	32 (46%)	57 (38%)	0.23
*Glasgow Outcome Score (0)	28 (41%)	47 (41%)	0.97	28 (41%)	44 (29%)	0.09
SICH	1 (1%)	9 (8%)	0.08	1 (1%)	2 (1%)	1.0
SICH or ASICH	8 (12%)	13 (11%)	0.95	8 (12%)	8 (5%)	0.10
Death (90 days)	15 (22%)	21 (18%)	0.56	15 (22%)	31 (20%)	0.84
Death (7 days)	9 (13%)	9 (8%)	0.25	9 (13%)	14 (9%)	0.40
*NIHSS (decreased >=4 in 24 hours)	29 (42%)	47 (41%)	0.88	29 (42%)	57 (38%)	0.55
*NIHSS (<=2 at 24 hours)	9 (13%)	15 (13%)	1.0	9 (13%)	8 (5%)	0.04

Data expressed as n (%). *Deaths and missing coded as “bad” outcome.