The NINDS t-PA Stroke Trial Protocol

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Protocol

December 23, 1993

12/23/93 Page 28, first paragraph, clarified the distinction between the Protocols for Parts A and B.
Page 28, second paragraph, changed "acute stroke" to "acute ischemic stroke."
Page 28, third paragraph, clarified that primary outcome is as specified in Section IIIA.
Page 29, first paragraph, added "290 patients were recruited to Part A"
Page 30, first paragraph, added "This impact is expected even though the primary outcome for Part A was activity measured by the 24-hour stroke scale."
Page 30, last paragraph, changed the t-PA investigators to the Project Officer and Clinical t-PA Trial Investigators.
Page 32, corrected secondary outcomes to be consistent with secondary outcomes in Part A.

- # 4 changed from within 180 minutes to within 90 minutes.
- # 9 added and 3 months.
- #10 added
- #12 added
- #13 added

Page 33, C1.2 defined standardized.
Page 35, clarified that either serum or fingerstick blood glucose is allowed, and the CT scan is of the brain.
Page 38, added "Principal Investigator at the Coordinating Center and the biostatisticians at the Coordinating Center"
Page 38, C1.1, changed "at the first" to "Pending"
Page 40, #5, changed "if worsening" to "If clinical worsening"
Page 42, clarified analyses plan
Page 57, deleted old references 30 (O'brien), 31 (Pocock)
Page 57, added new references 30-32

01/14/93

Page 4 - **OVERVIEW**

This protocol describes Parts A and B of the NINDS t-PA Stroke Trial, a collaborative clinical trial of intravenous rt-PA in patients with acute ischemic stroke. The trial compares rt-PA and placebo using a randomized double blind design. There are eight Clinical Centers.

For Part A of the trial activity as measured by the stroke scale at 24 hours is the primary outcome. Part A ended 11/2/92. For Part B of the trial efficacy as measured by the Barthel Index at three months is the primary outcome. Part B began 11/3/92. More detail is provided in Parts A and B to follow.

Page 4, Last paragraph - changed follow-up to "one year" from "until the end of the trial".

Page 12, 3rd paragraph - changed follow-up to "one year" from "until the end of the trial".

Page 17, 3rd paragraph - changed follow-up to "6 months and one year" from every 6 months "until the end of the trial".

Page 28, under "Introductory Summary" - added first paragraph to read "Initially the trial was designed to address only short term outcomes. However because recruitment progressed so rapidly for Part A it appeared to be possible to extend recruitment and address three month outcome as a primary hypothesis. At the time the decision was made there had been no formal analysis of the three month outcome data and the clinical investigators had not seen any data related to the estimated treatment effect."

Page 28, Deleted paragraph beginning "A research issue parallel to the . . ."

Page 28, Changed paragraph to read "All patients will be followed for one year. The primary outcome measure is the Barthel Index at 3 months. Secondary outcome measures include functional scale scores, the NIH stroke scale score at 24 hours and 7-10 days, and volume of infarcted brain as measured by computerized tomography (CT) at 7-10 days and at three months after onset of stroke. Another secondary outcome measure will be the NIH stroke scale score at 2 hours after initiation of therapy. It is understood that the 24 hour outcome of this study is considered a clinical measure of rt-PA activity, while the 3 month outcome is considered a measure of efficacy. We anticipate that the primary outcome measure will correlate with the secondary measurements of infarct volume and other functional outcome measures at three months. An analysis will be done to examine the relationship between the primary and secondary outcome measures. The adverse effects of rt-PA and the mortality and morbidity within both groups will also be described."

Page 29, under "Background and Significance" - rewritten to clarify and separate Parts A & B of the Protocol.

Pages 31, 32 and 32, added new Primary Hypothesis, and Secondary Hypotheses 1 and 2.

Page 34, changed paragraph to now read: "After completion of baseline examination, required laboratory tests, CT scan, informed consent, and randomization, rt-PA treatment will be initiated in eligible patients. Patients will be followed for one year."

Page 37, added new paragraph under "5. Unblinding" - "All except the Data and Safety Monitoring Committee will be kept blinded to the analysis of Part A of the trial. Also, in order to maintain blinding with respect to outcome the investigators will not be given the specifics of the sample size calculations for Part B.

Page 38, last paragraph under "3. Follow-up" changed paragraph to read: "After the 3 month visit, all patients will continue to be followed and a health history questionnaire will be completed at six months and one year.

Page 41, paragraph under "A. Training and Orientation", changed first sentence from ". . .information meetings will be held . . ." to ". . .information meetings were held . . ." and added to end of paragraph "in Part A."

Page 42, under "Data Analysis" made multiple changes to address Part B of the trial.

Page 43, deleted paragraph beginning - "Numerous secondary. . ."

Page 45, deleted first paragraph under "3. Sub-committees" - beginning "Planning Committees . . ."

Page 46, number 6, changed "Patient Coordinators' Committee" to "Recruitment Coordinators' Committee"

Page 47, under "Sample Size" made multiple changes to address Part B of the trial.

11/23/92
Distinction between Part A and Part B of the trial clarified.

11/03/92
Primary outcome changed from evidence of early activity to long term efficacy.

3/30/92
Page 9, IV. DESIGN, A, first sentence, was changed from " The target population for the t-PA trial includes men and women ages 18 through 80 admitted...." to "The target population for t-PA trial includes men and women aged 18 and older admitted ...."

Page 11, number 1 was changed from "1. Age 18 through 80 years." to "Age 18 or older."

2/8/91

Page 15, letter J, was changed from "evaluation of a cardinal sign . . . (the cardinal sign is that feature of the Stroke Scale that best reflects the patient's neurological deficit). to "evaluation of level of consciousness and weakness in the arms and legs . . (using methods for motor arm and leg described in the NIH Stroke Scale).

11/19/90

Page 10, second paragraph, the last sentence was added which reads "Patients for whom the drug was mixed but not administered will be followed to determine hospital discharge status (dead, alive and discharged to home, nursing home, rehabilitate unit, etc.)."

Page 12, number 6 was changed from "Patient has had major surgery or serious trauma including head trauma in the previous 14 days." It now reads "Patient has had major surgery or serious trauma excluding head trauma in the previous 14 days or serious head trauma in the previous 3 months."

Page 12, number 9, was changed from "On repeated measurement, patient has mean blood pressure $\geq$ 135 torr at the time treatment is to begin [mean is diastolic + (systolic-diastolic)/3], or patient requires aggressive treatment to reduce blood pressure to within these limits." to "On repeated measurement, patient has a systolic blood pressure > 185 or diastolic blood pressure >110 or patient requires aggressive treatment to reduce blood pressure to within these limits."

THE NINDS T-PA STROKE TRIAL
2/8/91

Overview

This protocol describes Parts A and B of the NINDS t-PA Stroke Trial, a collaborative clinical trial of IV rt-PA in patients with acute ischemic stroke. The trial compares rt-PA and placebo using a randomized double blind design. There are eight Clinical Centers.

For Part A of the trial activity as measured by the stroke scale at 24 hours is the primary outcome. For Part B of the trial efficacy as measured by the Barthel Index at three months is the primary outcome. More detail is provided in Parts A and B to follow.

The NINDS t-PA STROKE TRIAL PROTOCOL - PART A

I. Introductory Summary

This protocol describes the essential aspects of the NINDS t-PA Stroke Trial, a collaborative, Phase IIB clinical trial of intravenous recombinant tissue-plasminogen activator (rt-PA) in patients with acute stroke. This trial compares rt-PA with placebo using a randomized, double-blind design. The trial will involve nine participating clinical centers, recruiting approximately 280 patients over a 27-month period. A key feature of this study will be the emphasis on rapid mobilization in order to begin treatment within 180 minutes of stroke onset, both to increase the chance of benefits and to reduce the risk of hemorrhage. Patients who are randomized to receive rt-PA will be treated with a dose of 0.90 mg per kg (90mg maximum dose) infused over 60 minutes, with 10% of the total dose given as an initial bolus.

A research issue parallel to the question of efficacy is the importance of the timing of treatment. 140 patients will be included in the trial for whom treatment will begin 90 minutes or less from onset of symptoms. These patients may be more likely to benefit from treatment and may be less likely to have serious intracerebral hemorrhage. However, limiting treatment to within 90 minutes is very restrictive. For that reason, the trial will include 140 stroke patients who can be treated 90 to 180 minutes from onset of symptoms. The inclusion of these patients will help to determine if there may be some benefit from later treatment.

All patients will be followed for one year. The primary outcome measure is neurologic function as measured by the NIH stroke scale at 24 hours after onset of stroke. Secondary outcome measures include functional scale scores, the NIH stroke scale score, and volume of infarcted brain as measured by computerized tomography

(CT) at 7-10 days and at three months after onset of stroke. Another secondary outcome measure will be the NIH stroke scale score at 2 hours after initiation of therapy. It is understood that the primary endpoint of this study is a clinical measure of early outcome. We anticipate, however, that the outcome measure will correlate with the secondary measurements of infarct volume and functional outcome at three months. An analysis will be done to examine the relationship between the primary and secondary outcome measures. The adverse effects of rt-PA and the mortality and morbidity within both groups will also be described.

General policies with regard to design, conduct, organization and administration of the NINDS t-PA Trial are presented in this protocol. Sample size estimation and a listing of participating institutions and principal investigators are included in the appendix. A manual of procedures (MOP) will specify the operations and procedures needed for implementation and execution of the trial.

II. Background and Significance

Stroke is a serious health problem worldwide and is the third leading cause of death in the United States. Nearly 500,000 Americans have strokes each year and approximately 30% of this number die. Stroke related health care costs are now estimated to be $12.8 billion annually. In addition to its financial costs, stroke is a tragic occurrence in the lives of its victims: patients lose the capacity to walk, express themselves, and care for themselves, even though in many instances their other systemic functions are relatively preserved.

Although progress has been made in stroke prevention over the past several decades, limiting the devastation of acute stroke remains a key goal for the nearly 500,000 Americans who suffer a stroke each year. The major cause of these strokes is cerebral infarction, which is the end result of a complex series of cellular metabolic events that occur rapidly after interruption of nutrient blood flow to a region of the brain (1). Both duration and severity of focal cerebral ischemia are important in determining whether or how much brain infarction occurs (2). Acute thrombus formation or migration is the principal cause of blood flow interruption in at least 75% of cerebral infarctions (3). In several animal models of focal cerebral ischemia, restoration of cerebral blood flow within two to six hours after initial occlusion has been associated with smaller volumes of cerebral infarction and improved functional outcomes (4,5,6).

Use of Thrombolytic Agents for Acute Stroke

Efforts to restore blood flow have included emergency operative revascularization and dissolution of thrombi by thrombolytic agents. Thrombolytic agents dissolve thrombi that occlude blood vessels by activating the proteolytic enzyme plasmin. From 1958 to 1979, streptokinase, urokinase, thrombolysin, fibrinolysin, and plasmin were studied as potential therapy for patients with acute ischemic stroke (5,7,8). However, the safety and efficacy of these agents in these early studies is difficult to evaluate since computed tomographic (CT) scanning was not yet in use and treatment was given always after several hours and sometimes several days following onset of symptoms.

The introduction of CT in the mid-1970's enabled investigators to insure that patients did not have brain hemorrhage prior to treatment with a thrombolytic agent. Descriptions of more than 400 acute stroke patients treated with urokinase, streptokinase, or rt-PA have been published (7). However, because no randomized trials of thrombolytic therapy for acute cerebral infarction have been completed since the introduction of CT scanning, clinical efficacy cannot be estimated. In addition, historical controls in whom neurological examinations were performed within the first hours after stroke onset are not available for comparison. Recognizing these limitations, the pooled clinical results are considerably more encouraging than the results prior to the availability of CT. Of the 342 patients reported for whom clinical information is provided, 124 (36%) showed major improvement (7). Many of the responses could have been secondary to thrombolysis. Early clot lysis was documented for 50% of the stroke patients treated with intraarterial streptokinase or urokinase (7) following a variety of dosage regimens. In the angiographic pilot study of del Zoppo and colleagues (9), patients treated early were more likely to have successful thrombolysis. For patients for whom clinical and angiographic information was provided, major improvement occurred in 26 of 51 with recanalization (51%) but occurred in only 3 of 49 without recanalization (6%) ($p < 0.001$, Chi-square test). With regard to safety, five studies have been performed in which post-treatment CT scans were required, allowing for detection of symptomatic or asymptomatic intracerebral hemorrhage (ICH). ICH occurred in 17 of the 192 patients treated (9%) (7). Ten of the hemorrhages were symptomatic, and eight were fatal. However, intracerebral hematomas have been reported to occur in patients with cerebral infarction who were not treated with thrombolytic agents. In a prospective study of stroke patients treated with dextran or hydroxyethylstarch infusions and either low dose heparin (2 x 5000 units/day) or aspirin, Hornig et al (10) reported that 9 (14%) of 65 patients developed an intracerebral hematoma within two weeks of onset of cerebral infarction.

Use of Thrombolytic Agents in Animal Models of Focal Cerebral Ischemia

A number of workers have developed various experimental models and protocols to test the hypothesis that timely administration of thrombolytic therapy will be effective

in recanalizing occluded cerebral vessels and reducing cerebral infarct size (11). Recent experiments have utilized rt-PA as the thrombolytic agent. Arterial recanalization with rt-PA treatment has been reported as long as 24 hours after embolization, particularly with higher dosage levels (5 mg/kg) (12). The experimentally effective dose for recanalization ranged from 0.35 to 5.0 mg/kg administered as multiple boluses or intravenous infusion over 30-90 minutes (11), without an apparent increased risk of hemorrhagic complications when compared with controls (12). A beneficial effect on neurologic outcome or infarct size has been noted in several different animal models if thrombolytic treatment was begun within 30 minutes to three hours of arterial occlusion (11).

Use of rt-PA in Humans

Recombinant human tissue-type plasminogen activator (Activase TM or rt-PA), which was first used in humans in 1984 (13), is a thrombolytic agent with properties that make it relatively clot specific, i.e. the activation of plasmin is concentrated at the site of clot formation rather than throughout the vascular tree. This clot specificity should theoretically minimize the risk of hemorrhage. The drug can be given intravenously with rapid achievement of therapeutic levels, and clot lysis can be effected within minutes.

In humans, rt-PA infusion can induce rapid lysis of pathologic thrombi in the setting of acute myocardial infarction (14), unstable angina, (15), acute pulmonary embolism (16), and peripheral arterial occlusion (17). The drug was approved for use by the FDA for acute myocardial infarction in November of 1987 and for pulmonary embolization in June of 1990.

Use of rt-PA in patients with acute myocardial infarction leads to a higher patency of the infarct-related artery, limitation of infarct size and improved survival (14). There is some recent evidence that an initial bolus of rt-PA is associated with higher patency rates of the infarct-related artery (14). The most devastating complication of thrombolytic therapy, intracerebral hemorrhage, has been reported in the larger studies to range from 0.3 to 1.5% (14). During the early phase of the TIMI study, it was recognized that a 150-mg dose of rt-PA was associated with a 1.5% incidence of intracranial hemorrhage (18). As a result, the dose of rt-PA was reduced to 100 mg and the overall rate of ICH was 0.6% subsequently (18).

The success of rt-PA and other thrombolytic agents in acute myocardial infarction and the results of animal studies of rt-PA for acute focal cerebral ischemia, gave rise to the hope that rt-PA, if given within the first hours following stroke onset, could be effective in treating human ischemic stroke. As a first step in assessing rt-PA in humans, a pilot study was begun in September, 1986. This study was funded by the Division of

Stroke and Trauma (DST), National Institute of Neurological Disorders and Stroke (NINDS). The study objectives were 1) to accomplish patient evaluation and treatment within 90 minutes from symptom-onset, attempting to maximize safety and potential efficacy, 2) to estimate the highest dose of rt-PA that can be safely administered to patients with acute stroke, and 3) to estimate potential therapeutic efficacy.

In this trial, 74 patients received intravenous rt-PA within 90 minutes of symptom-onset (dose range = 10-87 mg) (19). Major neurological improvement occurred during the rt-PA infusion in 20 (27%) of the 74 patients. At 24 hours, 34 (46%) of the 74 patients had at least a four-point improvement in the NIH Stroke Scale Score. A four-point change could correspond to a change from a severe (no anti-gravity strength) in the face, arm, and leg, to a mild facial paresis and drift of the outstretched arm and leg. Intracerebral hematomas occurred in three patients. One was fatal and another occurred outside of the region of cerebral infarction. All three hemorrhages occurred among the 26 patients treated at the higher dose tiers (37-42 mg/meter^2). No hemorrhages occurred in those patients treated at a dose less than 37 mg/meter^2.

Since completion of the initial study, 18 additional patients have been treated at a dose of 32 or 37 mg/meter^2 without subsequent hemorrhage (unpublished data). In addition, 20 patients were subsequently treated at 91-180 minutes from symptom onset (Haley C: Written communication July 30, 1990). Of the 20 patients, four had at least a four-point improvement on the NIH Stroke Scale Score at 24 hours. There were two intracerebral hemorrhages, one at a dose of 32 mg/meter^2 and another at 37 mg/meter^2 respectively. The proposed dose of rt-PA for the present study, 0.9 mg/kg, is roughly equivalent to the dose tier 32 mg/meter^2 in the pilot study. At this dose, 13 (48%) of 27 patients treated within 180 minutes had at least a four-point improvement on the NIH Stroke Scale Score at 24 hours and one developed an intracerebral hemorrhage.

Preliminary interim results of another dose-escalation safety study of rt-PA for acute cerebral infarction have been reported in abstract form (20). Patients who had an acute cerebral infarction and angiographic documentation of an appropriate arterial occlusion were treated with a 60-minute infusion of rt-PA within eight hours of symptom onset. Upper dose levels exceeded the highest dose tiers of the NINDS pilot study. The primary positive efficacy response was angiographically documented reperfusion of the previous occlusion. The primary negative safety response was intracerebral hematoma. Of the first 57 patients, 22 (39%) patients had complete or partial arterial recanalization at 60 minutes. Intracerebral hematoma occurred in four patients, three of which were fatal. Data concerning neurologic outcome have not yet been reported.

The NINDS pilot study indicates that ischemic stroke patients can be treated with intravenous rt-PA within 90 minutes of symptom-onset with relative safety and with

significant early neurologic improvement in over 40%. Data on patients treated from 91-180 minutes from symptom onset are more limited. However, because of the lack of controls, either randomized or historical, convincing evidence of any effect attributable to rt-PA is lacking. Therefore, before a larger trial to test efficacy can be justified, a smaller Phase II-B study with controls must be completed.

III. Objectives

The goals of the NINDS t-PA Stroke Trial are stated both as primary and secondary hypotheses to be addressed through analyses of the experience in the two treatment groups: 0-90 minutes and 91-180 minutes from stroke onset. Also specified are sub-group hypotheses to be tested by comparison of outcomes in particular strata within the study population.

A. Primary Hypotheses

1. There is a difference between the rt-PA treated group and placebo group enrolled within 90 minutes of stroke onset in the proportion showing significant, early improvement. Significant early improvement is defined as a decrease from baseline of 4 or more points in the NIH stroke scale or a complete resolution of neurological deficit within 24 hours from onset of stroke. The expected outcome is improvement in the rt-PA treatment group. It is also of interest to detect an improvement in the placebo as compared to the rt-PA group. Thus a two sided alternative was chosen. For the same reasons, the secondary hypotheses also have been formulated as two sided alternatives. Onset of stroke is defined as the beginning of symptoms related to the stroke.

2. There is a difference between the rt-PA treated group and placebo group enrolled between 90 and 180 minutes of stroke onset in the proportion showing significant early improvement.

3. There is a difference between the rt-PA treated group and placebo group enrolled within 180 minutes of stroke onset in the proportion showing significant early improvement.

B. Secondary Hypotheses

Because of the pilot nature of this trial, there are numerous secondary hypotheses of interest.

1. There is a difference between the rt-PA and placebo groups in the proportion showing on the table improvement two hours from the start of treatment. On the table improvement is defined as a decrease from baseline of 2 or more points in the NIH stroke scale or complete resolution of the neurological deficit.

2. There is a difference between the rt-PA and placebo groups in the proportion showing improvement at 7-10 days post stroke onset. Improvement is defined as a decrease from baseline of 5 or more points in the NIH stroke scale or a complete resolution of the neurological deficit.

3. There is a difference in median standardized change score between the rt-PA and placebo groups at 3 months post stroke onset. (Based on the NIH Stroke Scale)

4. There is a difference between the rt-PA and placebo groups in mean brain volume, mean lesion volume, and the mean lesion/brain volume ratio at 24 hours, 7-10 days, and three months post stroke onset.

5. There is a difference between the rt-PA and placebo groups in median Barthel index at 7-10 days and 3 months.

6. There is a difference between the rt-PA and placebo groups in the proportion with a Glasgow outcome scale score of one at 3 months.

7. There is a difference between the rt-PA and placebo groups in the median modified Rankin score at 7-10 days, and 3 months post stroke onset.

8. There is a difference between the rt-PA and placebo groups in the proportion showing deterioration between 2 and 24 hours post stroke onset. A deterioration is defined as a four point increase in NIH stroke scale score or death.

9. There are significant correlations between the NIH Stroke Scale at 24 hours post stroke onset and lesion volume NIH Stroke Scale, Barthel Index, modified Rankin and Glasgow Outcome Scale at 3 months post stroke onset.

C. Additional Objectives

Additional objectives are to:

1. Evaluate the possible adverse effects of rt-PA use in stroke patients.

2. Describe the natural history of stroke in the placebo group.

3. Sensitize the medical community to the need for rapid action in managing patients with acute stroke and to describe various measures developed for speeding treatment to these patients.

D. Sub-group Hypotheses

Because of the reduced sample in the sub-groups the power of the tests for these sub-groups is reduced below the power of the study as a whole. The following sub-group hypotheses will be tested:

1. The proportion of patients showing improvement at 24 hours from onset of stroke will differ between patients receiving rt-PA within 90 minutes of onset of stroke and patients receiving rt-PA between 90 and 180 minutes from onset of symptoms. Improvement is defined as a four point decrease in the NIH stroke scale or a complete resolution of the neurological deficit.

2. Patients with and without on the table improvement will differ in median standardized NIH stroke scale change score function at 3 months post stroke onset. On the table improvement is defined as a decrease of 2 or more points in the NIH stroke scale or a complete resolution of neurological deficit at 2 hours after onset of treatment.

3. Patients taking Acetylsalicylic Acid (ASA) prior to randomization
   a. and treated with rt-PA will be more likely to show improvement at 24 hours from onset of stroke than patients treated with rt-PA with no previous usage of ASA due to ASA's effect on platelets and reocclusion problems;
   b. will have a higher rate of symptomatic intracranial bleeding defined as either hemorrhagic conversion of cerebral infarction, or intracerebral hematoma in the area of the brain separate from the infarct, or subarachnoid hemorrhage, or subdural hematoma.

4. Patients who have a greater activation of the fibrinolytic system, as evidenced by a lower fibrinogen and higher FDP level will have a greater risk of symptomatic intracerebral hemorrhage as defined in 3b above.

IV. Design

A. Introduction

The target population for the t-PA trial includes men and women aged 18 and older admitted to a hospital emergency room with a confirmed ischemic stroke and able to begin treatment within 180 minutes of onset of symptoms. Every effort will be made to include women and minorities in the trial. Also included in the target population are patients in the same age group who have a stroke while already hospitalized. Patients who have contraindications to the use of rt-PA or other conditions thought likely to impair follow-up to three months will not be eligible for the study.

After completion of baseline examination, required laboratory tests, CT scan, informed consent, and randomization, rt-PA treatment will be initiated in eligible patients. Patients will be followed for one year.

Specifications for the estimation of sample size for the study are given in Appendix A. In general, the estimate of 140 patients for each time group is based on several assumptions: the expected percent of patients showing improvement based on the pilot trial (40%), low mortality in the first 24 hours, a two-tailed critical level of 0.05 and power of 0.95.

B. Enrollment

1. Recruitment

All centers will have a recruitment coordinator who will be responsible for enhancing efforts to recruit stroke patients soon after onset of symptoms. This person will be responsible for coordinating education of local communities and physicians. The goal of the educators program will be to encourage early presentation to the emergency department following the onset of stroke. There will also be education of prehospital care providers about the nature of the t-PA trial and their role in providing early assessment and transport.

2. Eligibility Assessment

a. Patient Log

For the purpose of documenting the emergency room and hospital population from which the patients in this trial are drawn, each clinical center will maintain a log of all patients with ischemic and hemorrhagic stroke presenting within 24 hours of the onset of their symptoms. This information will be helpful in planning the recruitment of patients if there is a future Phase III trial. Included in the log will be information regarding the time of onset of stroke symptoms, circumstances at the time of onset, the means by which the patient was transported to the emergency department, date and time of admission to the emergency department, age, sex, race and zip code. For those patients who are not randomized, the primary reason should also be recorded. Also, if trial medication was mixed but not used this should be recorded along with the medication packaging number. Patients, for whom the drug was mixed but not administered, will be followed to determine hospital discharge status (dead, alive and discharged to home, nursing home, rehabilitate unit, etc.)

b. Eligibility Assessment

Prior to treatment blood must be drawn for:

Complete blood count (CBC) and platelet count
Prothrombin time
Partial thromboplastin time
Blood glucose
Serum electrolytes and creatinine

A 12 lead electrocardiogram must be done but can be started as soon as possible after treatment has been initiated.

Prior to treatment the investigator must know the results of the following:

CBC and platelet count
Prothrombin time if the patient has a history of oral anticoagulant therapy in the week prior to treatment initiation. (Those currently on oral anticoagulants are excluded, see exclusion criteria 5).
Partial Thromboplastin Time if patient has received heparin within 48 hours of treatment initiation.

Blood Glucose
CT Scan (non-contrast)

The beginning of treatment will not be delayed because test results other than those listed are not available. After randomization and beginning of treatment, if the results of tests exclude the patient, treatment will be stopped and the patient will continue to be followed in the group to which they are randomized.

To be eligible the following inclusion conditions must be met:

1. Age 18 or older.
2. Clinical diagnosis of ischemic stroke causing a measurable neurological deficit defined as impairment of language, motor function, cognition and/or gaze, vision, or neglect. Ischemic stroke is defined as an event characterized by the sudden onset of an acute focal neurologic deficit presumed to be due to cerebral ischemia after CT excludes hemorrhage.
3. Time of onset well-established to be less than 90 minutes before treatment would begin, or
   time of onset well-established to be less than 180 minutes before treatment would begin and the total number of patients at the Clinical Center that have been treated within 180 minutes from onset does not exceed by more than 2 the number of patients treated within 90 minutes.

c. Exclusion Criteria

Patients are not eligible when any of the following exclusion conditions apply:

1. Patient has (a) only minor stroke symptoms or (b) major symptoms which are rapidly improving by the time of randomization.
2. There is evidence of intracranial hemorrhage on CT scan.
3. Patient has a clinical presentation that suggests subarachnoid hemorrhage, even if the initial CT scan is normal.
4. Patient is a female and lactating or known or suspected to be pregnant.
5. Patient has platelet count less than 100,000, prothrombin time greater than 15, patient has received heparin within 48 hours and has an elevated partial thromboplastin time (> upper limit of normal for each laboratory) or patient is currently taking oral anticoagulants.
6. Patient has had major surgery or serious trauma excluding head trauma in the previous 14 days or serious head trauma in the previous 3 months.
7. Patient has history of gastrointestinal or urinary tract hemorrhage in previous 21 days.

8. Patient has had arterial puncture at a non-compressible site or a lumbar puncture in the previous 7 days.
9. On repeated measurement, patient has a systolic blood pressure > 185 or diastolic blood pressure > 110 at the time treatment is to begin or patient requires aggressive treatment to reduce blood pressure to within these limits.
10. Patient has a history of stroke in the previous 3 months or has ever had a intracranial hemorrhage considered to put the individual at an increased risk for intracranial hemorrhage.
11. Patient has a serious medical illness that is likely to interfere with this trial.
12. Patient has abnormal blood glucose (< 50 or > 400 mg/dl).
13. Patient has clinical presentation consistent with acute myocardial infarction or patient has a clinical presentation suggesting post-myocardial infarction pericarditis.
14. Patient who in the judgement of the investigator cannot be followed for three months.
15. Patient had a seizure at onset of stroke.

d. Informed Consent

Informed consent will be obtained prior to randomization.

3. Baseline

Prior to randomization blood must be drawn for later analysis of fibrinogen and fibrinogen degradation products. Also a baseline NIH stroke scale should be obtained. As soon as possible after randomization, additional data should be obtained as described in the manual of procedures.

4. Randomization

A stratified blocked randomization will be used to assign patients to rt-PA or placebo. The strata will include clinical center and time from onset of symptoms ($\leq$ 90, >90 minutes to $\leq$ 180 minutes). Block size will be varied. Prior to randomization the treating physician must confirm that the patient meets eligibility criteria.

Because of the limited time available for randomization, randomization will take place at the hospital where treatment will be given. The medication, rt-PA must be obtainable 24 hours a day in order to guarantee drug availability. A patient is considered randomized and in the trial at the time infusion of trial medication begins.

Within the first two hours of administration of the trial medication, the Coordinating Center must be called and given the time, date, location, study ID number, date of birth, sex and race of the patient. The Clinical Center must also keep a careful inventory of drugs for the trial and complete the drug accountability form.

5. Unblinding

The trial medication, rt-PA, will be provided by Genentech along with an identically packaged placebo. The Coordinating Center will know the medication assignment for a patient. Each medication package will have a removable label to be opened at the Clinical Center if unblinding is necessary. Conditions requiring unblinding are generally those occurring within a few days of treatment. To unblind, the patient's physician must require knowledge of the drug in order to provide appropriate patient care. Additionally, because the clinical centers will be carrying out laboratory analyses which may provide information which will unblind, procedures for storing this information as described in the manual of procedures must be followed.

For some conditions such as myocardial infarction or surgery, unblinding the specialist rather than informing the trial investigator should be considered. The trial investigator would remain blinded.

The Executive Committee consists of the Coordinating Center Principal Investigator and Co-principal Investigator and the Project Officer. The Executive Committee of the Steering Committee will review all unblindings. Where the decision to unblind is not related to an emergency situation, the clinical investigator should discuss the need to unmask with the Executive Committee prior to unmasking. Two months after the drug is administered, the unopened drug label should be mailed to the Coordinating Center. If the label has been opened, an unblinding form must be completed and mailed to the Coordinating Center. The Coordinating Center should be notified by telephone on the first working day after any unblinding.

C. Treatment Protocol

1. Dosage Schedule

The dose was based primarily on data from the results of the Phase I portion of this trial. The chosen dose represents the dose that appears to offer the best potential for benefit while maintaining an acceptably low risk of hemorrhage. The dosage will be 0.90 mg/kg (90 mg maximal dose), 10% to be given as an initial bolus over one minute,

followed by continuous IV infusion over sixty minutes. After the first Safety and Data Monitoring Committee review, dosage adjustments may be made.

2. Follow-up

Upon notification that a patient has been enrolled in the study, the Coordinating Center will transmit a patient follow-up schedule to the Clinical Center. The patient follow-up schedule is given in the manual of procedures. The NIH stroke scale will be completed at 2 hours after initiation of treatment, and at 24 hours (± 20 minutes), 7-10 days and 3 months (± 2 weeks) after onset of stroke. Also a Glasgow outcome score will be completed at 3 months. The Barthel Index should be completed at 7-10 days and 3 months. A modified Rankin will be completed to describe the patient's condition prior to stroke and at 7-10 days and 3 months. Follow up CT scans will be performed at 24 hours, 7-10 days and 3 months after onset of symptoms. In addition to baseline, blood will be drawn for analysis of fibrinogen and fibrinogen degradation products at 2 hours after treatment initiation and 24 hours post stroke onset. Other follow-up data to be collected are discussed in the manual of procedures.

After the 3 month visit, all patients will continue to be followed and a health history questionnaire will be completed at 6 months and one year.

3. General Patient Management Guidelines and Other Therapy

For all patients:

a. Admission to a Neurology Special Care area or Intensive Care Unit.
b. Therapy for acute stroke other than rt-PA (including heparin or antiplatelet drugs) will not be initiated during the infusion or for 24 hours following the onset of symptoms (see C.4).
c. Supplemental oxygen as indicated.
d. Initially NPO; later, diet as tolerated and no nasogastric tube (NGT) for at least 24 hours except when clinically indicated and no other less invasive option is available.
e. IV fluids to maintain adequate urine output during the first 24 hours.
f. Cardiac Monitoring.
g. Blood pressure monitoring and appropriate measures to control BP within acceptable limits.
h. Central venous pressures, pulmonary artery pressure, and cardiac output monitored as indicated but not prior to, during the 60 minute rt-PA infusion,

or for 30 minutes following the end of rt-PA infusion unless an emergency situation intervenes. Pre-existing peripheral lines may be maintained but will be closely watched and compressed if removed.

i. As already noted, uncontrolled hypertension would pose a potentially serious risk of bleeding to these patients; but, rather than continuously monitoring blood pressure with an arterial cannula, which would pose its own set of risks, we prefer to rely on frequent (every 15 minute) blood pressure monitoring during the first two hours and every 30 minute monitoring for 6 hours thereafter before relaxing the monitoring to every 60 minutes for the next 16 hours.

j. Neurological check by evaluation of level of consciousness and weakness in the arms and legs performed every 15 minutes for the first 2 hours, every 30 minutes for the next 6 hours, and hourly thereafter for the next 16 hours (using methods for motor arm and leg described in the NIH Stroke Scale).

k. Urinalysis (results to be reported when obtained for medical indications). Urinary catheterization shall be avoided if possible, especially until 30 minutes following completion of rt-PA treatment.

l. Stool guaiac and NGT guaiac (results to be reported when obtained for medical indications).

m. The patient will be under the immediate supervision of one of the named trial investigators until after the 2 hour post treatment exam is completed.

n. If the patient is admitted to the emergency department, the goal will be to begin treatment within 55 minutes from the time of admission. If the patient is hospitalized at the time of stroke onset, the goal is to begin treatment within 55 minutes of the time of notification of a trial investigator. In other words, the only acceptable delays are those which delay the arrival of the patient to the hospital.

o. Physical, occupational and speech therapy should be started as soon as possible when appropriate.

If, during or after infusion, sudden worsening occurs in any patient, (a) a neurological evaluation will be performed and reported, (b) a CT scan will be done as soon as possible, and, (c) if hemorrhage is discovered, the Coordinating Center will be notified within two hours. If worsening occurs after infusion, clinical management will proceed at the discretion of the investigator.

4. Adjunctive Stroke Therapy

Although heparin and to a lesser extent aspirin are conventionally used in patients with coronary thrombosis to prevent re-occlusion and maintain arterial patency after

rt-PA therapy, no adjunctive antithrombotic therapy will be given in this trial during the first 24 hours after the onset of symptoms, until after the patient has had their 24 hour exam and the CT scan has been evaluated. At that time, standard medical therapy can be given. Standard medical therapy may include aspirin (325-1300 mg daily), or heparin (for example mini-dose for prevention of deep vein thrombosis or full dose for prevention of recurrent cerebral embolism). If full dose heparin is used, the 24 hour CT scan should be reviewed and if clinically indicated a new CT scan obtained to exclude cerebral hemorrhage. No heparin bolus should be given, and the PTT should be maintained no more than 2 x control. If full dose of heparin is used, the PTT should be monitored daily and platelet count every other day, and these results recorded on the case report form. A form will be completed documenting any aspirin or other antithrombotic therapy used by any patients during the two weeks prior to entry in the trial. IV crystalloid therapy should be given to maintain euvolemia, but hypervolemic therapy with either colloid or blood substitutes should not be used. Standard antihypertensive therapy may be used as needed. If calcium channel blockers are used, their use should be carefully recorded. Osmotic agents such as mannitol or glycerol, intubation with hyperventilation, muscular paralysis, and sedation can be used in the setting of uncontrolled elevation of intracranial pressure. Corticosteroids should be avoided unless specifically indicated for a systemic condition. Central nervous system active drugs such as sedatives and hypnotics should be avoided. Antidepressant drugs can be given if clinically indicated.

These limitations on adjunctive therapy will minimize any risk of cerebral hemorrhage associated with combined rt-PA and antithrombotic therapy. They will also prevent the introduction of a second form of therapy which would confound the interpretation of data.

5. Management of Hemorrhage

If the worsening occurs during the infusion and intracerebral hemorrhage is suspected, the rt-PA infusion will be discontinued. Each center will be responsible for having personnel trained and available at all times to treat intracerebral hemorrhage in patients receiving rt-PA. A suggested management plan approved by the Steering Committee is given in the Manual of Procedures.

6. Mortality and Adverse Effects

Although mortality is not an outcome for this trial, mortality will be evaluated. The Clinical Center will call the Coordinating Center within two hours after a patient's death is ascertained. Calls will be monitored regularly by Coordinating Center staff to insure

notification of the FDA within the required time frame. The Clinical Center will obtain the death certificate and other documentation (information from witnesses, relatives, autopsy information, hospital or physicians records, if possible), and complete the relevant study forms. This documentation should be forwarded to the Coordinating Center. All names should be removed and a study I.D. should be written on each document. Deaths will be coded by a nosologist retained by the Coordinating Center using the death certificates and other relevant medical information. Should there be a need for a more detailed review in the future, the information will be available in the patient's file. Data on mortality will be provided to the Safety and Data Monitoring Committee and to the FDA.

A similar procedure should be followed for any life threatening/serious adverse effects (see manual of procedures for definition). The Coordinating Center should be called within 2 hours of the Clinical Center's notification that a life threatening adverse effect occurred. For any other adverse effects, an adverse experience form should be completed and forwarded to the Coordinating Center as soon as possible. The Coordinating Center will notify Genentech within 72 hours of any adverse event or death reported to the FDA.

V. Conduct Of The Trial

A. Training and Orientation

Initial training and information meetings will be held for patient coordinators, clinical coordinators, neurologists, laboratory staff and neuroradiologists from each clinical center prior to the start of data collection for the trial.

B. Patient Safety and Confidentiality

Patients participating in a clinical trial have the right to privacy. Thus, stringent policies relating to confidentiality will be adopted for this trial. Name, social security numbers or medical record numbers will not be included on forms sent to the Coordinating Center. A stringent password protection program is in effect for the data files. Additionally, any forms collected for the study will be stored in locked file cabinets. As further protection study staff will sign a confidentiality policy.

On monitoring visits Coordinating Center staff will need to review some patient charts. However, no names or medical record numbers will be recorded on any

monitoring visit reports. Coordinating Center staff will be bound by the same confidentiality policies as noted previously.

With regard to patient safety, interim analyses will be carried out prior to Safety and Data Monitoring Committee meetings. Tabulations of all side effects, by treatment group, will be prepared and presented to the Data and Safety Monitoring Committee as requested.

C. Data Monitoring and Quality Control

Once a year a team from the Coordinating Center will visit each clinical center to monitor clinical performance. At some centers, the Coordinating Center team may need to visit more frequently. Additionally a clinical research nurse (CRN) will make frequent unscheduled visits to the centers to review and compare case report forms and medical records.

The Study Coordinator at each clinical center has responsibility for all data collected in that center. Information gathering is standardized at the clinical centers through the manual of procedures and training mentioned previously.

At the Coordinating Center quality assurance procedures will be carried out for data entry, data coding, laboratory analysis and CT readings.

D. Data Analysis

For each time group (≤ 90, > 90 to ≤ 180 minutes from stroke onset) and for the two time groups combined, the primary outcome variable is defined as an improvement from admission of four points in the Stroke Scale within 24 hours from stroke onset or complete resolution of the neurological deficit. Separate Mantel-Haenszel analyses will be used to test for a treatment effect within a time group. Clinical centers will be the strata. A Mantel-Haenszel analysis will also be used to test for a treatment effect in the two time interval groups combined. In this analysis strata include clinical center and time from onset of symptoms (≤ 90, > 90 minutes). Interim analyses for the primary endpoint will be carried out every 6 months just prior to each Safety and Data Monitoring Committee meeting with adjustment of critical levels as appropriate using methods of Fleming and O'Brien (21). Symmetric boundaries will be used.

Although this is a Phase II trial with an intermediate endpoint, status at 24 hours post treatment, the secondary endpoint, status at 3 to 6 months is also of interest. An opportunity would be lost to gather longer term clinical information on outcome at three

to six months if the trial was terminated early based on efficacy at 24 hours. For this reason, median standardized NIH Stroke Scale change score at 3 months, also will be included in the interim analyses every 6 months. The test statistic will be based on a stratified nonparametric analysis. Any decision to stop the trial based on efficacy would require consideration of both the primary endpoint at 24 hours and secondary endpoint at 3 months and would not be based solely on statistical considerations. A decision to stop for efficacy would take into consideration the potential for gathering further data on toxicity and on the natural history of the placebo group. This decision would also be balanced by consideration of the effect of withholding treatment from future stroke patients.

Multivariate analyses will be carried out as appropriate. Interim analyses will also be carried out for hemorrhage and mortality. Mantel-Haenszel and logrank tests will be used. For these analyses the upper boundary will be developed using the method of Fleming and O'Brien. The lower boundary will be set arbitrarily at -2.0 as suggested by DeMets and Ware (22-23). This will allow stopping of the trial if a strong, albeit non-significant trend in an adverse direction is observed. Because of safety concerns, the interim analyses of adverse events will be performed on a schedule related to the number of events. After every three symptomatic hemorrhagic conversions or hematomas an interim analysis will be done. The number three is based on the data from the previous pilot study (Background) study. Also, after every ten deaths there will be an interim analysis.

Because this is a Phase IIB trial, numerous secondary hypotheses and subgroup analyses have also been proposed.

E. Authorship of Papers

As previously agreed any publications or presentations of data on trial patients must have the permission of the NINDS. Also centers are not to separately present or publish data relating to the objectives of the trial. The NINDS t-PA Stroke Trial Group will be listed as author on major trial publications. Investigators, the NINDS project officer, and committees will be listed at the end of the paper.

VI. Organizational Structure

A. Introduction

The participating units in this collaborative clinical trial include: nine individual clinical centers, a Coordinating Center which includes a laboratory and CT scan reading center, the NINDS Division of Stroke and Trauma.

B. Participating Sites

1. Clinical Centers

Nine clinical centers will be responsible for recruiting the required number of patients, administering the study drugs, coordinating patient care, and collecting the information required by the trial protocol. The Principal Investigator is responsible for the overall conduct and performance of the clinical center. The organization of each clinical center will differ, but each clinical center will have one person specifically identified as the clinical coordinator and one person identified as the patient recruitment coordinator. The clinical coordinator will be responsible for such critical matters as checking Emergency Room logs, appointment scheduling, checking the completeness of forms, and arranging the shipment of blood specimens and CT data to the Coordinating Center.

2. Coordinating Center

The Coordinating Center will provide overall guidance and coordination of the data management, quality control, data analysis for the study, centralized measurement of fibrin and fibrin-fibrinogen degradation products and monitoring for safety. This will include preparation of the procedure manual, data collection forms and the various reports. Although the primary responsibility for early recruitment is at the clinical centers, the Coordinating Center will assist in this effort, not only by monitoring the degree of success at the various centers, but also by holding meetings of the patient recruitment coordinators to develop recruitment strategies for each of the clinical centers and then monitoring the success of the implementation of these plans.

a. Coagulation Laboratory

Frozen plasma and serum will be sent from the clinical centers to the Coordinating Center coagulation laboratory. The coagulation laboratory at Henry Ford Hospital will

receive and analyze frozen plasma samples for fibrinogen and frozen serum for the fibrinogen degradation products.

b. CT Reading Center

The clinical centers will send CT scan data to the Coordinating Center for reading. Although centers will have read the baseline scan to determine eligibility, these scans should also be sent to the Coordinating Center to be read. All clinical center sites will be asked to send data tapes and hard copies of each CT of the brain.

The initial interpretation will include:

A description of the quality of the study
Presence or absence of an infarct
The arterial or venous distribution of the infarct.
The infarct and brain volume.
Any additional findings on the CT scan that can affect the management of the patient.
The presence or absence of hemorrhage

3. The NINDS Division of Stroke and Trauma

The NINDS provides research funding for the trial, monitors the trial with respect to safety, monitors performance, and gives scientific direction to the trial.

C. Study Administration

1. Steering Committee

The Steering Committee includes principal investigators from each clinical center, the NINDS Project Officer and the Coordinating Center principal investigator and co-principal investigator. The Steering Committee will prepare the final protocol and provide scientific direction for the study at the operational level. The Steering Committee will advise and assist the Coordinating Center on operational matters, monitor the performance of the clinical centers and receive requests for any proposed ancillary studies. The Steering Committee will also report major problems and recommend changes in the protocol to the Project Officer and the Safety and Data Monitoring Committee. Beyond providing study guidance, the Steering Committee also provides the forum for Principal Investigators to serve as study collaborators.

2. Executive Committee

The Executive Committee consists of the Coordinating Center Principal Investigator and Co-principal Investigator and the Project Officer. The Executive Committee of the Steering Committee will review all unblindings. Where the decision to unblind is not related to an emergency situation, the clinical investigator should discuss the need to unblind with the Executive Committee prior to unblinding.

3. Sub-committees

Planning Committees
During the planning stages of the trial subcommittees will be formed to provide input in areas specific to the protocol or patient management.

The Steering Committee will appoint the following subcommittees:

The **Publications Subcommittee** will establish a procedure for reviewing every scientific paper or abstract using unpublished t-PA trial data (including ancillary study data) prior to forwarding the paper for approval by the NINDS. This subcommittee will also review every paper using published t-PA trial data which purports to represent official t-PA trial views or policy. This policy applies to papers prepared for publication or oral presentation. The subcommittee will also review ancillary studies to ensure patient safety, trial design and scientific integrity are not compromised prior to submitting the study to the Steering Committee for review and the NINDS for approval.

The **Quality Assurance Subcommittee** will monitor the performance of the clinical centers, Coordinating Center, central laboratory and CT reading center. Outcome data is not reviewed by this subcommittee. The NINDS will appoint an independent board to monitor all aspects of the trial.

The **Stroke Scale Subcommittee** will address issues relating to the reliability, validity, and redundancy in the NIH Stroke Scale and other measures of neurologic function. The subcommittee will also develop definitions for the manual of procedures and address procedural questions relating to the scales throughout the trial.

4. Safety and Data Monitoring Committee

The Safety and Data Monitoring Committee will act in an advisory capacity to the NINDS on policy matters throughout the trial. This group also periodically reviews trial

results and reports of adverse effects. The Board is selected by the NINDS and is comprised of members who are not trial investigators. Board meetings are attended, when appropriate, by senior investigators from the Coordinating Center including the principal investigator, co-investigator and other Coordinating Center investigators as necessary. The Board will review the final protocol, review any subsequent changes to the protocol including any necessary changes to the dose schedule, and make recommendations on early termination of the trial (if appropriate). The Board will also review clinical center performance, make recommendations and assist in resolution of problems reported by the Steering Committee.

5. Clinical Coordinators' Committee

The Clinical Coordinator's Committee will discuss issues relevant to patient management and data reporting. This committee is composed of all the clinical coordinators from the clinical centers and the clinical coordinator from the Coordinating Center.

6. Patient Coordinators' Committee

The Patient Coordinators' Committee will discuss issues relevant to community education and patient recruitment. This committee is composed of all the patient recruitment coordinators from the clinical centers and the coordinator and health educators from the Coordinating Center.

Appendix

Sample Size

Power estimates for the analysis are based on a two-sample test of proportions. Based on the pilot study at least 40% of the rt-PA group will show a 4 point decrease in the NIH Stroke Scale score or complete resolution of neurological deficit at 24 hours from onset of stroke. For the overall comparison of the two time groups combined there will be a sample size of 280 (140 per group). With 280, setting the critical level at 0.05, two-sided test, and absolute difference of 20% from control can be detected with a power of 0.95. (40% in rt-PA group versus 20% in controls). Within a time group ($\leq$ 90 minutes or $>$ 90 to $\leq$ 180 minutes) with a sample size of 140 (70 per group), setting the critical level at 0.05, two-sided test, an absolute difference of 24% from control can be detected with a power of 0.90. (40% in rt-PA group versus 16% in controls). Because of the risk of hemorrhage, the difference between rt-PA treated and placebo groups must be large to be clinically meaningful.

Calculations were based on the arc sine approximation to the binomial. Using a stratified analysis may slightly reduce the power of the analyses. The critical level for testing was not adjusted for multiple comparisons as each analysis is considered to be a separate experiment addressing a prespecified hypothesis.

The NINDS t-PA STROKE TRIAL PROTOCOL - PART B

I. Introductory Summary

Initially the trial was designed to address only short term outcomes. However, because recruitment progressed so rapidly for Part A it appeared to be possible to extend recruitment and address three month outcome as a primary hypothesis. At the time the decision was made there had been no formal analysis of the three month outcome data and the clinical investigators had not seen any data related to the estimated treatment effect. The protocols for Parts A and B are the same except for the objectives, background and data analysis plan. There were 290 patients in Part A and there will be 320 in Part B for a total of 610 in the entire Trial.

This protocol describes the essential aspects of Part B of the NINDS t-PA Stroke Trial, a collaborative, clinical trial of intravenous recombinant tissue-plasminogen activator (rt-PA) in patients with acute ischemic stroke. This trial compares rt-PA with placebo using a randomized, double-blind design. The trial will involve eight participating clinical centers. A key feature of this study will be the emphasis on rapid mobilization in order to begin treatment within 180 minutes of stroke onset, both to increase the chance of benefits and to reduce the risk of hemorrhage. Patients who are randomized to receive rt-PA will be treated with a dose of 0.90 mg per kg (90mg maximum dose) infused over 60 minutes, with 10% of the total dose given as an initial bolus.

All patients will be followed for one year. The primary outcome is a global improvement in function at three months that is described in detail in Section IIIA. Secondary outcome measures include functional scale scores, the NIH stroke scale score at 24 hours and 7-10 days, and volume of infarcted brain as measured by computerized tomography (CT) at 7-10 days and at three months after onset of stroke. Another secondary outcome measure will be the NIH stroke scale score at 2 hours after initiation of therapy. It is understood that the 24 hour outcome of this study is considered a clinical measure of rt-PA activity, while the 3 month outcome is considered a measure of efficacy. We anticipate that the primary outcome measure will correlate with the secondary measurements of infarct volume and other functional outcome measures at three months. An analysis will be done to examine the relationship between the primary and secondary outcome measures. The adverse effects of rt-PA and the mortality and morbidity within both groups will also be described.

General policies with regard to design, conduct, organization and administration of the NINDS t-PA Trial are presented in this protocol. Sample size estimation and a listing of participating institutions and principal investigators are included in the appendix. A manual of procedures (MOP) will specify the operations and procedures needed for implementation and execution of the trial.

II. Background and Significance

Recruitment of patients in Part A of the NINDS t-PA stroke trial began in February, 1991. Within 18 months of starting recruitment, many significant changes occurred. When the trial began, serious doubts remained that the stringent requirements for early patient recruitment within 90 and 180 minutes could be satisfied. The unexpected result has been that recruitment has been more rapid than original projections; 290 patients were recruited to Part A, seven months ahead of schedule. The rapid recruitment has done much to convince some that early thrombolytic therapy can be accomplished, if it should be shown to be effective. The method of balancing patients between the two time groups has been effective.

Despite a complicated protocol and lengthy data collection form, data collection has been proceeding on schedule. Baseline data, centralized CT scan quantification, and three-month outcome data have all been collected accurately and completely. A major effort to train and evaluate investigators in the use of the NIH stroke scale has been completed. A classification scheme for intracranial hemorrhage has been developed; the scheme combines CT scan data and clinical observations so that the hemorrhagic risk of thrombolytic therapy can be assessed. An intricate but highly reliable method for distribution of the study drug (either active or inactive rt-PA) has been developed. Extremely effective patient recruitment teams have been developed in eight cities involving nearly 40 different community and academic hospitals. The capability to recruit stroke patients in sufficient quantity to carry out a high quality larger efficacy trial has developed sooner than expected.

During the same 18 months, many significant changes occurred in the U.S. and European stroke research communities. In February, 1991, no results of clinical studies using IV rt-PA for stroke had been published. In May, 1992, four separate scientific reports of the clinical use of rt-PA to treat stroke were published in widely circulated peer reviewed journals, Stroke and Neurology (24,25,26,28). In July, 1992, another report describing a pilot trial of rt-PA for stroke was published in Annals of Neurology (27). In December, 1992, a metaanalysis of reports of the use of rt-PA for strokes was reported by Warlow in the journal Stroke (29). This report from a group in Edinborough reported that the data from trials already performed suggest that thrombolytic therapy is effective for stroke. The trial reported by Mori was a randomized placebo controlled clinical trial that suggested efficacy (28).

From a broader perspective, other randomized, controlled multicenter trials have begun to investigate the use of heparinoids, calcium channel blockers, gangliosides, aminosteroids, and NMDA antagonists as treatment for ischemic stroke. Other large trials are planned. There is an air of anticipation, and, lacking an effective treatment, a growing demand that something be done for the multitude of stroke victims.

In this atmosphere, the clinical investigators of the NINDS t-PA Stroke Trial became concerned that thrombolytic therapy may become widely used before adequate

justification is available. The doubt and uncertainty that would arise from the use of an agent, with serious potential risks and unknown benefit would be unacceptable. It is likely that any publication of data from Part A, whether negative or positive, would have a significant impact on the practice of stroke care and make continuation of the trial unfeasible. This impact is expected even though the primary outcome for Part A was activity measured by the 24-hour stroke scale. Additional prospective clinical trial data as is required to justify acceptance of a therapy with possible serious risks or rejection of a therapy that offers great potential. In an ideal world, the current trial would have stopped when Part A was completed. The data would have been analyzed and another prospective randomized trial with efficacy as the primary outcome would be done. However, the inevitable delay of at least 12 to 18 months between these trials would destroy the remarkably successful, objective, and highly motivated trial organization responsible for the successful early completion of Part A. Publication of the Part A results from the current trial could be very confusing. Many cooperating physicians do not make a careful distinction between activity and efficacy. For a patient suffering from acute stroke, evidence of activity may be enough to justify use of even a risky therapy. Support from these cooperating physicians is critical to successful recruitment soon after the onset of symptoms.

For these reasons, the Project Officer and Clinical t-PA Trial Investigators decided to extend the current trial. Rather than be unblinded to any results, they have decided to continue the current trial without any change other than a redefinition of the primary hypothesis. No one in the trial except the statisticians for the trial will be unblinded regarding any treatment differences. The unblinded independent data monitoring committee has seen interim analyses and made recommendations concerning the sample size for the trial and the primary hypothesis that is incorporated in this protocol.

III. Objectives

The goals of the NINDS t-PA Stroke Trial are stated both as primary and secondary hypotheses. The clinical investigators, without knowledge of any treatment comparisons from Part A of this trial, chose the Barthel Index as the primary indicator of clinical efficacy. The independent Safety and Data Monitoring Committee at the request of the FDA and the clinical investigators have reviewed the outcome data from Part A. They have recommended that further supporting evidence be required, namely, that the primary endpoint be a consistent and persuasive difference between the t-PA treatment group and placebo group enrolled within 180 minutes of stroke onset as defined below under Primary Hypothesis 1. Also specified are sub-group hypotheses to be tested by comparison of outcomes in particular strata within the study population.

A. Primary Hypothesis

There is a consistent and persuasive difference between the rt-PA treatment group and placebo group enrolled in Part B within 180 minutes of stroke onset in the proportion with a 90 day:

Barthel Index $\geq$ 95
Rankin Score of "no significant disability or symptoms"
Glasgow Outcome Score of "good recovery"
and
NIH Stroke Scale of $\leq$ 1

B. Secondary Hypotheses

1. There is a consistent and persuasive difference between the rt-PA treatment group and placebo group enrolled within 90 minutes of stroke onset in the proportion with a 90 day:

   Barthel Index $\geq$ 95
   Rankin Score of "no significant disability or symptoms"
   Glasgow Outcome Score of "good recovery"
   and
   NIH Stroke Scale of $\leq$ 1

2. There is a consistent and persuasive difference between the rt-PA treatment group and placebo group enrolled between 91 and 180 minutes of stroke onset in the proportion with a 90 day:

   Barthel Index $\geq$ 95
   Rankin Score of "no significant disability or symptoms"
   Glasgow Outcome Score of "good recovery"
   and
   NIH Stroke Scale of $\leq$ 1

3. There is a difference between the rt-PA treated group and placebo group

enrolled within 180 minutes of stroke onset in the proportion showing significant, early improvement. Significant early improvement is defined as a decrease from baseline of 4 or more points in the NIH stroke scale or a complete resolution of neurological deficit within 24 hours from onset of stroke. The expected outcome is improvement in the rt-PA treatment group. It is also of interest to detect an improvement in the placebo as compared to the rt-PA group. Thus a two sided alternative was chosen. For the same reasons, the secondary hypotheses also have been formulated as two sided alternatives. Onset of stroke is defined as the beginning of symptoms related to the stroke.

4. There is a difference between the rt-PA treated group and placebo group enrolled within 90 minutes of stroke onset in the proportion showing significant early improvement.

5. There is a difference between the rt-PA treated group and placebo group enrolled between 91 and 180 minutes of stroke onset in the proportion showing significant early improvement.

6. There is a difference between the rt-PA and placebo groups in the proportion showing on the table improvement two hours from the start of treatment. On the table improvement is defined as a decrease from baseline of 2 or more points in the NIH stroke scale or complete resolution of the neurological deficit.

7. There is a difference between the rt-PA and placebo groups in the proportion showing improvement at 7-10 days post stroke onset. Improvement is defined as a decrease from baseline of 5 or more points in the NIH stroke scale or a complete resolution of the neurological deficit.

8. There is a difference between the rt-PA and placebo groups in proportion with a Barthel Index at 7-10 days of 95 or 100.

9. There is a difference between the rt-PA and placebo groups in the median modified Rankin score at 7-10 days and 3 months.

10. There is a difference between the rt-PA and placebo groups in median Glasgow outcome scale at 3 months.

11. There is a difference between the rt-PA and placebo groups in the proportion showing deterioration between 2 and 24 hours post stroke onset. A deterioration is defined as a four point increase in NIH stroke scale score or death.

12. There is a difference between the rt-PA and placebo groups in CT infarct volume as measured at 7-10 days and 3 months.

13. There are significant correlations between the NIH Stroke Scale at 24 hours post stroke onset and lesion volume, NIH Stroke Scale, Barthel Index, modified Rankin and Glasgow Outcome Scale at 3 months post stroke onset.

C. Additional Objectives

Additional objectives are to:

1. Evaluate the possible adverse effects of rt-PA use in stroke patients.

2. Describe the natural history of stroke in the placebo group.

3. Sensitize the medical community to the need for rapid action in managing patients with acute stroke and to describe various measures developed for speeding treatment to these patients.

1. Additional Sub-Group Hypotheses

Because of the reduced sample in the sub-groups the power of the tests for these sub-groups is reduced below the power of the study as a whole. The following sub-group hypotheses will be tested:

1. The proportion of patients showing improvement at 24 hours from onset of stroke will differ between patients receiving rt-PA within 90 minutes of onset of stroke and patients receiving rt-PA between 91 and 180 minutes from onset of symptoms. Improvement is defined as a four point decrease in the NIH stroke scale or a complete resolution of the neurological deficit.

2. Patients with and without "on the table improvement" will differ in median standardized (score ÷ number of items evaluable = standardized score) NIH stroke scale change score function at 3 months post stroke onset. On the table improvement is defined as a decrease of 2 or more points in the NIH stroke scale or a complete resolution of neurological deficit at 2 hours after onset of treatment.

3. Patients taking Acetylsalicylic Acid (ASA) prior to randomization
   a. and treated with rt-PA will be more likely to show improvement at 24 hours from onset of stroke than patients treated with rt-PA with no previous usage of ASA due to ASA's effect on platelets and reocclusion problems;
   b. will have a higher rate of symptomatic intracranial bleeding defined as either hemorrhagic conversion of cerebral infarction, or intracerebral hematoma in the area of the brain separate from the infarct, or subarachnoid hemorrhage, or subdural hematoma.

4. Patients who have a greater activation of the fibrinolytic system, as evidenced by a lower fibrinogen and higher FDP level will have a greater risk of symptomatic intracerebral hemorrhage as defined in 3b above.

IV. Design

A. Introduction

The target population for the rt-PA trial includes men and women aged 18 and older admitted to a hospital emergency room with a confirmed ischemic stroke and able to begin treatment within 180 minutes of onset of symptoms. Every effort will be made to include women and minorities in the trial. Also included in the target population are patients in the same age group who have a stroke while already hospitalized. Patients who have contraindications to the use of rt-PA or other conditions thought likely to impair follow-up to three months will not be eligible for the study.

After completion of baseline examination, required laboratory tests, CT scan, informed consent, and randomization, rt-PA treatment will be initiated in eligible patients. Patients will be followed for one year.

Specifications for the estimation of sample size for the study are given in Appendix A.

B. Enrollment

1. Recruitment

All centers will have a recruitment coordinator who will be responsible for enhancing efforts to recruit stroke patients soon after onset of symptoms. This person will be responsible for coordinating education of local communities and physicians. The goal of the educators program will be to encourage early presentation to the emergency department following the onset of stroke. There will also be education of prehospital care providers about the nature of the t-PA trial and their role in providing early assessment and transport.

2. Eligibility Assessment

a. Patient Log

For the purpose of documenting the emergency room and hospital population from which the patients in this trial are drawn, each clinical center will maintain a log of all patients with ischemic and hemorrhagic stroke presenting within 24 hours of the onset of their symptoms. This information will be helpful in planning the recruitment of patients if there is a future Phase III trial. Included in the log will be information regarding the

time of onset of stroke symptoms, circumstances at the time of onset, the means by which the patient was transported to the emergency department, date and time of admission to the emergency department, age, sex, race and zip code. For those patients who are not randomized, the primary reason should also be recorded. Also, if trial medication was mixed but not used this should be recorded along with the medication packaging number. Patients, for whom the drug was mixed but not administered, will be followed to determine hospital discharge status (dead, alive and discharged to home, nursing home, rehabilitate unit, etc.)

b. Eligibility Assessment

Prior to treatment blood must be drawn for:

Complete blood count (CBC) and platelet count
Prothrombin time
Partial thromboplastin time
Blood glucose
Serum electrolytes and creatinine

A 12 lead electrocardiogram must be done but can be started as soon as possible after treatment has been initiated.

Prior to treatment the investigator must know the results of the following:

CBC and platelet count
Prothrombin time if the patient has a history of oral anticoagulant therapy in the week prior to treatment initiation. (Those currently on oral anticoagulants are excluded, see exclusion criteria 5).
Partial Thromboplastin Time if patient has received heparin within 48 hours of treatment initiation.
Blood Glucose (serum or fingerstick)
CT Scan (non-contrast) of the brain

The beginning of treatment will not be delayed because test results other than those listed are not available. After randomization and beginning of treatment, if the results of tests exclude the patient, treatment will be stopped and the patient will continue to be followed in the group to which they are randomized.

To be eligible the following inclusion conditions must be met:

1. Age 18 or older.
2. Clinical diagnosis of ischemic stroke causing a measurable neurological deficit defined as impairment of language, motor function, cognition and/or gaze, vision, or neglect. Ischemic stroke is defined as an event characterized by the sudden onset of an acute focal neurologic deficit presumed to be due to cerebral ischemia after CT excludes hemorrhage.

3. Time of onset well-established to be less than 90 minutes before treatment would begin, or time of onset well-established to be less than 180 minutes before treatment would begin and the total number of patients at the Clinical Center that have been treated within 180 minutes from onset does not exceed by more than 2 the number of patients treated within 90 minutes.

c. Exclusion Criteria

Patients are not eligible when any of the following exclusion conditions apply:

1. Patient has (a) only minor stroke symptoms or (b) major symptoms which are rapidly improving by the time of randomization.
2. There is evidence of intracranial hemorrhage on CT scan.
3. Patient has a clinical presentation that suggests subarachnoid hemorrhage, even if the initial CT scan is normal.
4. Patient is a female and lactating or known or suspected to be pregnant.
5. Patient has platelet count less than 100,000, prothrombin time greater than 15, patient has received heparin within 48 hours and has an elevated partial thromboplastin time (> upper limit of normal for each laboratory) or patient is currently taking oral anticoagulants.
6. Patient has had major surgery or serious trauma excluding head trauma in the previous 14 days or serious head trauma in the previous 3 months.
7. Patient has history of gastrointestinal or urinary tract hemorrhage in previous 21 days.
8. Patient has had arterial puncture at a non-compressible site or a lumbar puncture in the previous 7 days.
9. On repeated measurement, patient has a systolic blood pressure > 185 or diastolic blood pressure > 110 at the time treatment is to begin or patient requires aggressive treatment to reduce blood pressure to within these limits.
10. Patient has a history of stroke in the previous 3 months or has ever had a intracranial hemorrhage considered to put the individual at an increased risk for intracranial hemorrhage.
11. Patient has a serious medical illness that is likely to interfere with this trial.
12. Patient has abnormal blood glucose (< 50 or > 400 mg/dl).
13. Patient has clinical presentation consistent with acute myocardial infarction or patient has a clinical presentation suggesting post-myocardial infarction pericarditis.
14. Patient who in the judgement of the investigator cannot be followed for three months.
15. Patient had a seizure at onset of stroke.

d. Informed Consent

Informed consent will be obtained prior to randomization.

3. Baseline

Prior to randomization blood must be drawn for later analysis of fibrinogen and fibrinogen degradation products. Also a baseline NIH stroke scale should be obtained. As soon as possible after randomization, additional data should be obtained as described in the manual of procedures.

4. Randomization

A stratified blocked randomization will be used to assign patients to rt-PA or placebo. The strata will include clinical center and time from onset of symptoms ($\leq$ 90, >90 minutes to $\leq$ 180 minutes). Block size will be varied. Prior to randomization the treating physician must confirm that the patient meets eligibility criteria.

Because of the limited time available for randomization, randomization will take place at the hospital where treatment will be given. The medication, rt-PA must be obtainable 24 hours a day in order to guarantee drug availability. A patient is considered randomized and in the trial at the time infusion of trial medication begins.

Within the first two hours of administration of the trial medication, the Coordinating Center must be called and given the time, date, location, study ID number, date of birth, sex and race of the patient. The Clinical Center must also keep a careful inventory of drugs for the trial and complete the drug accountability form.

5. Unblinding

The trial medication, rt-PA, will be provided by Genentech along with an identically packaged placebo. The Coordinating Center will know the medication assignment for a patient. Each medication package will have a removable label to be opened at the Clinical Center if unblinding is necessary. Conditions requiring unblinding are generally those occurring within a few days of treatment. To unblind, the patient's physician must require knowledge of the drug in order to provide appropriate patient care. Additionally, because the clinical centers will be carrying out laboratory analyses which may provide information which will unblind, procedures for storing this information as described in the manual of procedures must be followed.

For some conditions such as myocardial infarction or surgery, unblinding the specialist rather than informing the trial investigator should be considered. The trial investigator would remain blinded.

The Executive Committee consists of the Coordinating Center Principal Investigator and Co-principal Investigator and the Project Officer. The Executive Committee of the Steering Committee will review all unblindings. Where the decision to unblind is not related to an emergency situation, the clinical investigator should discuss the need to unmask with the Executive Committee prior to unmasking. Two months after the drug is administered, the unopened drug label should be mailed to the Coordinating Center.

If the label has been opened, an unblinding form must be completed and mailed to the Coordinating Center. The Coordinating Center should be notified by telephone on the first working day after any unblinding.

All except the Principal Investigator at the Coordinating Center and the biostatisticians at the Coordinating Center and the Data and Safety Monitoring Committee will be kept blinded to the analysis of Part A of the trial. Also, in order to maintain blinding with respect to outcome the investigators will not be given the specifics of the sample size calculations for Part B.

C. Treatment Protocol

1. Dosage Schedule

The dose was based primarily on data from the results of the Phase I portion of this trial. The chosen dose represents the dose that appears to offer the best potential for benefit while maintaining an acceptably low risk of hemorrhage. The dosage will be 0.90 mg/kg (90 mg maximal dose), 10% to be given as an initial bolus over one minute, followed by continuous IV infusion over sixty minutes. Pending Safety and Data Monitoring Committee review, dosage adjustments may be made.

2. Follow-up

Upon notification that a patient has been enrolled in the study, the Coordinating Center will transmit a patient follow-up schedule to the Clinical Center. The patient follow-up schedule is given in the manual of procedures. The NIH stroke scale will be completed at 2 hours after initiation of treatment, and at 24 hours (± 20 minutes), 7-10 days and 3 months (± 2 weeks) after onset of stroke. Also a Glasgow outcome score will be completed at 3 months. The Barthel Index should be completed at 7-10 days and 3 months. A modified Rankin will be completed to describe the patient's condition prior to stroke and at 7-10 days and 3 months. Follow up CT scans will be performed at 24 hours, 7-10 days and 3 months after onset of symptoms. In addition to baseline, blood will be drawn for analysis of fibrinogen and fibrinogen degradation products at 2 hours after treatment initiation and 24 hours post stroke onset. Other follow-up data to be collected are discussed in the manual of procedures.

After the 3 month visit, all patients will continue to be followed and a health history questionnaire will be completed at six months and one year.

3. General Patient Management Guidelines and Other Therapy

For all patients:

a. Admission to a Neurology Special Care area or Intensive Care Unit.
b. Therapy for acute stroke other than rt-PA (including heparin or antiplatelet

drugs) will not be initiated during the infusion or for 24 hours following the onset of symptoms (see C.4).

c. Supplemental oxygen as indicated.
d. Initially NPO; later, diet as tolerated and no nasogastric tube (NGT) for at least 24 hours except when clinically indicated and no other less invasive option is available.
e. IV fluids to maintain adequate urine output during the first 24 hours.
f. Cardiac Monitoring.
g. Blood pressure monitoring and appropriate measures to control BP within acceptable limits.
h. Central venous pressures, pulmonary artery pressure, and cardiac output monitored as indicated but not prior to, during the 60 minute rt-PA infusion, or for 30 minutes following the end of rt-PA infusion unless an emergency situation intervenes. Pre-existing peripheral lines may be maintained but will be closely watched and compressed if removed.
i. As already noted, uncontrolled hypertension would pose a potentially serious risk of bleeding to these patients; but, rather than continuously monitoring blood pressure with an arterial cannula, which would pose its own set of risks, we prefer to rely on frequent (every 15 minute) blood pressure monitoring during the first two hours and every 30 minute monitoring for 6 hours thereafter before relaxing the monitoring to every 60 minutes for the next 16 hours.
j. Neurological check by evaluation of level of consciousness and weakness in the arms and legs performed every 15 minutes for the first 2 hours, every 30 minutes for the next 6 hours, and hourly thereafter for the next 16 hours (using methods for motor arm and leg described in the NIH Stroke Scale).
k. Urinalysis (results to be reported when obtained for medical indications). Urinary catheterization shall be avoided if possible, especially until 30 minutes following completion of rt-PA treatment.
l. Stool guaiac and NGT guaiac (results to be reported when obtained for medical indications).
m. The patient will be under the immediate supervision of one of the named trial investigators until after the 2 hour post treatment exam is completed.
n. If the patient is admitted to the emergency department, the goal will be to begin treatment <u>within 55 minutes</u> from the time of admission. If the patient is hospitalized at the time of stroke onset, the goal is to begin treatment within 55 minutes of the time of notification of a trial investigator. In other words, the only acceptable delays are those which delay the arrival of the patient to the hospital.
o. Physical, occupational and speech therapy should be started as soon as possible when appropriate.

If, during or after infusion, sudden worsening occurs in any patient, (a) a neurological evaluation will be performed and reported, (b) a CT scan will be done as soon as possible, and, (c) if hemorrhage is discovered, the Coordinating Center will be notified within two hours. If worsening occurs after infusion, clinical management will

proceed at the discretion of the investigator.

4. Adjunctive Stroke Therapy

Although heparin and to a lesser extent aspirin are conventionally used in patients with coronary thrombosis to prevent re-occlusion and maintain arterial patency after rt-PA therapy, no adjunctive antithrombotic therapy will be given in this trial during the first 24 hours after the onset of symptoms, until after the patient has had their 24 hour exam and the CT scan has been evaluated. At that time, standard medical therapy can be given. Standard medical therapy may include aspirin (325-1300 mg daily), or heparin (for example mini-dose for prevention of deep vein thrombosis or full dose for prevention of recurrent cerebral embolism). If full dose heparin is used, the 24 hour CT scan should be reviewed and if clinically indicated a new CT scan obtained to exclude cerebral hemorrhage. No heparin bolus should be given, and the PTT should be maintained no more than 2 x control. If full dose of heparin is used, the PTT should be monitored daily and platelet count every other day, and these results recorded on the case report form. A form will be completed documenting any aspirin or other antithrombotic therapy used by any patients during the two weeks prior to entry in the trial. IV crystalloid therapy should be given to maintain euvolemia, but hypervolemic therapy with either colloid or blood substitutes should not be used. Standard antihypertensive therapy may be used as needed. If calcium channel blockers are used, their use should be carefully recorded. Osmotic agents such as mannitol or glycerol, intubation with hyperventilation, muscular paralysis, and sedation can be used in the setting of uncontrolled elevation of intracranial pressure. Corticosteroids should be avoided unless specifically indicated for a systemic condition. Central nervous system active drugs such as sedatives and hypnotics should be avoided. Antidepressant drugs can be given if clinically indicated.

These limitations on adjunctive therapy will minimize any risk of cerebral hemorrhage associated with combined rt-PA and antithrombotic therapy. They will also prevent the introduction of a second form of therapy which would confound the interpretation of data.

5. Management of Hemorrhage

If clinical worsening occurs during the infusion and intracerebral hemorrhage is suspected, the rt-PA infusion will be discontinued. Each center will be responsible for having personnel trained and available at all times to treat intracerebral hemorrhage in patients receiving rt-PA. A suggested management plan approved by the Steering Committee is given in the Manual of Procedures.

6. Mortality and Adverse Effects

Although mortality is not an outcome for this trial, mortality will be evaluated. The Clinical Center will call the Coordinating Center within two hours after a patient's death is ascertained. Calls will be monitored regularly by Coordinating Center staff to insure notification of the FDA within the required time frame. The Clinical Center will obtain the death certificate and other documentation (information from witnesses, relatives, autopsy information, hospital or physicians records, if possible), and complete the relevant study forms. This documentation should be forwarded to the Coordinating Center. All names should be removed and a study I.D. should be written on each document. Deaths will be coded by a nosologist retained by the Coordinating Center using the death certificates and other relevant medical information. Should there be a need for a more detailed review in the future, the information will be available in the patient's file. Data on mortality will be provided to the Safety and Data Monitoring Committee and to the FDA.

A similar procedure should be followed for any life threatening/serious adverse effects (see manual of procedures for definition). The Coordinating Center should be called within 2 hours of the Clinical Center's notification that a life threatening adverse effect occurred. For any other adverse effects, an adverse experience form should be completed and forwarded to the Coordinating Center as soon as possible. The Coordinating Center will notify Genentech within 72 hours of any adverse event or death reported to the FDA.

V. Conduct Of The Trial

A. Training and Orientation

Initial training and information meetings were held for patient coordinators, clinical coordinators, neurologists, laboratory staff and neuroradiologists from each clinical center prior to the start of data collection for the trial in Part A.

B. Patient Safety and Confidentiality

Patients participating in a clinical trial have the right to privacy. Thus, stringent policies relating to confidentiality will be adopted for this trial. Name, social security numbers or medical record numbers will not be included on forms sent to the Coordinating Center. A stringent password protection program is in effect for the data files. Additionally, any forms collected for the study will be stored in locked file cabinets. As further protection study staff will sign a confidentiality policy.

On monitoring visits Coordinating Center staff will need to review some patient charts. However, no names or medical record numbers will be recorded on any monitoring visit reports. Coordinating Center staff will be bound by the same

confidentiality policies as noted previously.

With regard to patient safety, interim analyses will be carried out prior to Safety and Data Monitoring Committee meetings. Tabulations of all side effects, by treatment group, will be prepared and presented to the Data and Safety Monitoring Committee as requested.

C. Data Monitoring and Quality Control

Once a year a team from the Coordinating Center will visit each clinical center to monitor clinical performance. At some centers, the Coordinating Center team may need to visit more frequently. Additionally a clinical research nurse (CRN) will make frequent unscheduled visits to the centers to review and compare case report forms and medical records.

The Study Coordinator at each clinical center has responsibility for all data collected in that center. Information gathering is standardized at the clinical centers through the manual of procedures and training mentioned previously.

At the Coordinating Center quality assurance procedures will be carried out for data entry, data coding, laboratory analysis and CT readings.

D. Data Analysis

The primary hypothesis will be tested with a global statistic (Wald test) computed using generalized estimating equations as described by Lefkopoulo, Moore, and Ryan (30). The stratifying variables, center and time ($\leq$ 90, < 91-180 minutes from stroke onset) will be included in the model as covariates. This global test statistic was chosen because it will test the consistency in direction of all four functional outcome measures specified in the primary hypothesis. The critical level for the test will be 0.05. All patients randomized to Part B will be included in the primary analysis. Treatment failure will be ascribed to any who have died before an outcome was determined and to any who could not have an outcome determined, regardless of the reason.

If after the global test statistic is computed, the differences between treatment groups are found to be statistically significant, then further testing will be done using logistic regression with center and time as covariates at the 0.05 level for each of the four functional scales used in the global test. The purpose of this testing is to describe the effect of treatment on each of the functional scales.

An interim analysis using the primary outcome will be carried once during the trial as requested by the Data Monitoring and Safety Committee. Critical levels will be adjusted using the methods of Lan and DeMets (21) with an O'Brien and Fleming spending function. More frequent interim analysis of adverse events, especially death and hemorrhage will be carried out in conjunction with the Monitoring Committee. The

results of any interim analysis of adverse event or any outcome will be seen only by the trial statisticians and the Monitoring Committee. Any decision to stop the trial for safety or efficacy will not be based solely on statistical considerations.

E. Authorship of Papers

As previously agreed any publications or presentations of data on trial patients must have the permission of the NINDS. Also centers are not to separately present or publish data relating to the objectives of the trial. The NINDS t-PA Stroke Trial Group will be listed as author on major trial publications. Investigators, the NINDS project officer, and committees will be listed at the end of the paper.

VI. Organizational Structure

A. Introduction

The participating units in this collaborative clinical trial include: eight individual clinical centers, a Coordinating Center which includes a laboratory and CT scan reading center, the NINDS Division of Stroke and Trauma.

B. Participating Sites

1. Clinical Centers

Eight clinical centers will be responsible for recruiting the required number of patients, administering the study drugs, coordinating patient care, and collecting the information required by the trial protocol. The Principal Investigator is responsible for the overall conduct and performance of the clinical center. The organization of each clinical center will differ, but each clinical center will have one person specifically identified as the clinical coordinator and one person identified as the patient recruitment coordinator. The clinical coordinator will be responsible for such critical matters as checking Emergency Room logs, appointment scheduling, checking the completeness of forms, and arranging the shipment of blood specimens and CT data to the Coordinating Center.

2. Coordinating Center

The Coordinating Center will provide overall guidance and coordination of the data management, quality control, data analysis for the study, centralized measurement of fibrin and fibrin-fibrinogen degradation products and monitoring for safety. This will include preparation of the procedure manual, data collection forms and the various

reports. Although the primary responsibility for early recruitment is at the clinical centers, the Coordinating Center will assist in this effort, not only by monitoring the degree of success at the various centers, but also by holding meetings of the patient recruitment coordinators to develop recruitment strategies for each of the clinical centers and then monitoring the success of the implementation of these plans.

a. Coagulation Laboratory

Frozen plasma and serum will be sent from the clinical centers to the Coordinating Center coagulation laboratory. The coagulation laboratory at Henry Ford Hospital will receive and analyze frozen plasma samples for fibrinogen and frozen serum for the fibrinogen degradation products.

b. CT Reading Center

The clinical centers will send CT scan data to the Coordinating Center for reading. Although centers will have read the baseline scan to determine eligibility, these scans should also be sent to the Coordinating Center to be read. All clinical center sites will be asked to send data tapes and hard copies of each CT of the brain.

The initial interpretation will include:

A description of the quality of the study
Presence or absence of an infarct
The arterial or venous distribution of the infarct.
The infarct and brain volume.
Any additional findings on the CT scan that can affect the management of the patient.
The presence or absence of hemorrhage

3. The NINDS Division of Stroke and Trauma

The NINDS provides research funding for the trial, monitors the trial with respect to safety, monitors performance, and gives scientific direction to the trial.

C. Study Administration

1. Steering Committee

The Steering Committee includes principal investigators from each clinical center, the NINDS Project Officer and the Coordinating Center principal investigator and co-principal investigator. The Steering Committee will prepare the final protocol and provide scientific direction for the study at the operational level. The Steering Committee will

advise and assist the Coordinating Center on operational matters, monitor the performance of the clinical centers and receive requests for any proposed ancillary studies. The Steering Committee will also report major problems and recommend changes in the protocol to the Project Officer and the Safety and Data Monitoring Committee. Beyond providing study guidance, the Steering Committee also provides the forum for Principal Investigators to serve as study collaborators.

2. Executive Committee

The Executive Committee consists of the Coordinating Center Principal Investigator and Co-principal Investigator and the Project Officer. The Executive Committee of the Steering Committee will review all unblindings. Where the decision to unblind is not related to an emergency situation, the clinical investigator should discuss the need to unblind with the Executive Committee prior to unblinding.

3. Sub-committees

The Steering Committee will appoint the following subcommittees:

- The **Publications Subcommittee** has established a procedure for reviewing every scientific paper or abstract using unpublished t-PA trial data (including ancillary study data) prior to forwarding the paper for approval by the NINDS. This subcommittee will also review every paper using published t-PA trial data which purports to represent official t-PA trial views or policy. This policy applies to papers prepared for publication or oral presentation. The subcommittee will also review ancillary studies to ensure patient safety, trial design and scientific integrity are not compromised prior to submitting the study to the Steering Committee for review and the NINDS for approval.

- The **Quality Assurance Subcommittee** will monitor the performance of the clinical centers, Coordinating Center, central laboratory and CT reading center. Outcome data is not reviewed by this subcommittee. The NINDS will appoint an independent board to monitor all aspects of the trial.

- The **Stroke Scale Subcommittee** will address issues relating to the reliability, validity, and redundancy in the NIH Stroke Scale and other measures of neurologic function. The subcommittee will also develop definitions for the manual of procedures and address procedural questions relating to the scales throughout the trial.

4. Safety and Data Monitoring Committee

The Safety and Data Monitoring Committee will act in an advisory capacity to the NINDS on policy matters throughout the trial. This group also periodically reviews trial results and reports of adverse effects. The Board is selected by the NINDS and is comprised of members who are not trial investigators. Board meetings are attended,

when appropriate, by senior investigators from the Coordinating Center including the principal investigator, co-investigator and other Coordinating Center investigators as necessary. The Board will review the final protocol, review any subsequent changes to the protocol including any necessary changes to the dose schedule, and make recommendations on early termination of the trial (if appropriate). The Board will also review clinical center performance, make recommendations and assist in resolution of problems reported by the Steering Committee.

5. Clinical Coordinators' Committee

The Clinical Coordinators' Committee will discuss issues relevant to patient management and data reporting. This committee is composed of all the clinical coordinators from the clinical centers and the clinical coordinator from the Coordinating Center.

6. Recruitment Coordinators' Committee

The Recruitment Coordinators' Committee will discuss issues relevant to community education and patient recruitment. This committee is composed of all the patient recruitment coordinators from the clinical centers and the coordinator and health educators from the Coordinating Center.

APPENDIX

A. Sample Size

Primary Hypothesis

Power curves are provided in Figure 1 for univariate analyses using various rates of success in the control group and various values for the absolute percent difference from control. The absolute percent difference represents the smallest difference detectable with the specified power. Calculations are based on a sample size of 160 per group, the sample size for Part A. The critical level for the test is 0.05 (two-sided). The power for a global test should be greater.

Subgroup Hypotheses

There will generally be lower power to address the subgroup hypotheses.

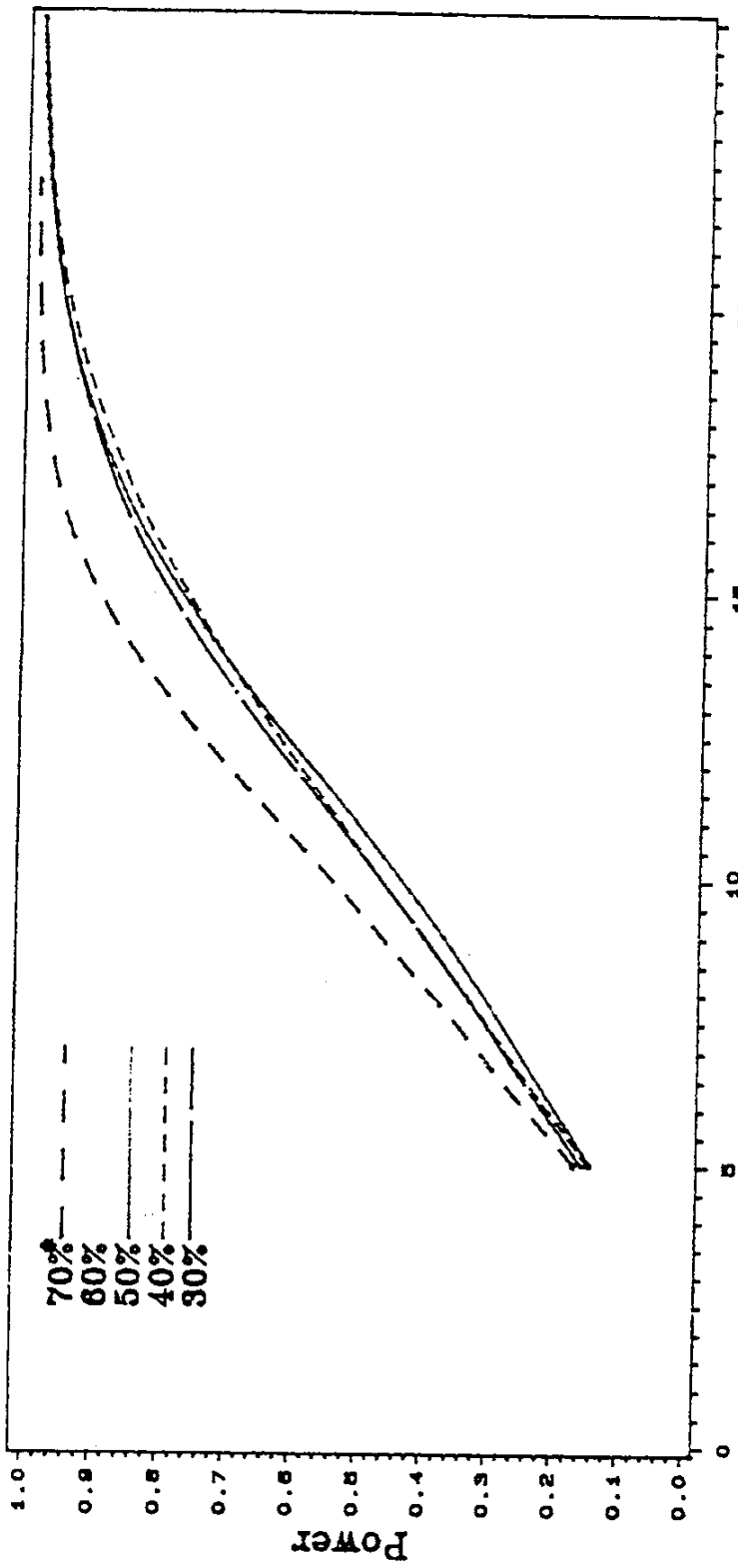

Figure 1. Minimum Absolute Percent Difference Detectable

*Success Rate in Placebo
N=160/group Alpha=0.05, Two-sided

APPENDIX

B. The NINDS t-PA STROKE TRIAL INVESTIGATORS

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APPENDIX

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