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Division of Clinical Trial Design and Analysis
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Memorandum

Date: June 12, 1996

From: Marc Walton, Clinical Reviewer & Chair, PLA Committee

Subject: Clinical Review for PLA 96-0350

Through: Karen Weiss, Director, DCTDA 6/19/96

To: PLA 96-0350 File

OVERVIEW

Genentech, Inc. submitted PLA Supplement 96-0350 on March 19, 1996 for tissue plasminogen activator (tPA; Activase®) with the proposed additional indication of:

> "Activase is indicated for the management of acute ischemic stroke in adults for improving functional and neurological recovery and reducing the incidence of disability associated with stroke."

Scope of the review

The focus of this review is upon the information derived from the NINDS Stroke Study, Genentech Protocol # A0228s. This was a double-blind study comparing Activase to placebo administered to patients within 3 hours of the onset of stroke. The study was conducted from January 1991 to December 1994. Additional information submitted in the PLA are from two smaller pilot studies and a study report of the European Cooperative Acute Stroke Study (ECASS). These trials are briefly reviewed and information relevant to the issues under consideration highlighted. However, these studies are not extensively reviewed.

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INTRODUCTION

Stroke

Ischemic stroke is the most common neurological disorder causing death and disability among adults with incidence in the U.S. of approximately 500,00 per year. It is the third-ranked cause of death in adults in the U.S. (following heart disease and cancer). Incidence increases with age. Within the U.S. population, incidence rates are higher among persons with African ancestry than among those with European ancestry.

Acute ischemic events are often classified as transient ischemic attack (TIA), reversible ischemic neurological deficit (RIND) and stroke. These are all events of the same general etiology, differentiated by the time course and degree of recovery for a specific event. A TIA is an ischemic deficit that completely resolves with 24 hours (although most resolve within an hour of onset), a RIND completely resolves within 3 weeks. Ischemic events that are more long lasting than RINDs may still appear to clinically completely resolve, but most leave at least a mild amount of residual abnormality on examination.

On the order of 10% of strokes are hemorrhagic, with a hematoma in the brain parenchyma and/or blood in the CSF spaces. This type of stroke is associated with poor outcome. Between 1/4 and 1/2 of hemorrhagic strokes are fatal, and many more show marked degrees of residual disability.

Treatment of Stroke

There are presently no licensed products for the treatment of acute ischemic stroke. If approved, this application will be the first product indicated for the treatment of acute stroke.

Outcome of stroke varies considerably with the size as well as brain location of the brain injury. Mortality in studies ranges from a few percent to over 1/4 of patients. Early in the course deaths tend to be related to the acute injury, with resultant edema and risk of brain herniation. Later deaths are more related to medical complications which may occur in a severely disabled patient. Most of the survivors will have mild to moderate deficits, while approximately 1/3 of surviving patients have severe disability long term following stroke.

A CT scan is often obtained in early the evaluation of a patient with stroke. An early CT scan may show no acute abnormalities. The typical abnormalities resulting from brain edema may not be visible on CT scan for 1-2 days, and depend on the size and extent of the ischemic region. The initial CT scan is often utilized to rule out the presence of intracranial hemorrhage.

Treatment of acute stroke at present focuses upon medical and supportive care. These include adequate pulmonary care and maintenance of respiratory function, prevention of aspiration, cardiovascular monitoring and management, and fluid, electrolyte, and metabolic monitoring and management.

Because there are complex and multifaceted processes that contribute to the ultimate neurological deficit, there are a wide variety of hypothesized approaches to improve neurological

function, many of which are under investigation. Rheological treatments have been evaluated, without definitive demonstration of efficacy at present. Anticoagulant agents have also been of longstanding interest. No studies have demonstrated a definitive benefit, but these investigations continue. Other approaches in development and evaluation include neutrophil inhibitors and excitotoxicity protective agents.

The rationale for the use of thrombolytic agents in acute thrombotic stroke is readily apparent. The results of many studies of thrombolytics in patients with stroke are reported in the medical literature. Some reports are encouraging with respect to demonstrating improvement in outcome, but the reports also highlight the risk of intracranial hemorrhage associated with this class of agents.

Activase

Activase is recombinant human tissue-type plasminogen activator (t-PA). It is produced by expression of the human gene for tPA in CHO cells. Activase is glycosylated, with 527 amino acids. The mechanism of action is believed to be the enzymatic cleaving of plasminogen into plasmin, which will then increase fibrinolysis. Activase exhibits fibrin specificity. It is considerably more active when bound to the surface of fibrin, thus promoting release of plasmin in the direct vicinity of fibrin. Activase is distributed largely to the vascular space and is rapidly cleared from the plasma; the half life is a few minutes.

Activase is currently indicated for acute myocardial infarction (AMI) and massive pulmonary embolism. There are two recommended dosage regimens for AMI. The older regimen (termed 3-hour infusion) is 100mg total, given 6-10% as a bolus, 50-54% as infusion in the first hour, then 20% over each succeeding hour. For patients weighing less than 65kg, the dose is 1.25 mg/kg, distributed in the same proportions. The second, newer AMI regimen is termed the accelerated infusion. It is, for patients weighing more than 67kg, a total of 100 mg, given as a 15 mg iv bolus, followed 50mg in a 1/2 hour infusion, followed by 35 mg over the next hour. Total infusion duration is 90 minutes. For patients weighing less than 67kg, the dose is 15mg bolus, 0.75mg/kg in a 1/2 hour infusion, followed by 0.5mg/kg in a 1 hour infusion.

The pulmonary embolus regimen is 100mg administered by infusion over 2 hours.

For comparison purposes, the proposed regimen used in the phase 3 trial in stroke was 0.9mg/kg total, given as 0.09mg/kg in a bolus, followed by 0.81mg/kg as a 1 hour infusion. For a 65kg person, this becomes 58.5mg total dose, 5.8mg as a bolus, and 52.6mg in a 1 hour infusion. Thus, the accelerated infusion for AMI includes higher total dose, with a considerably larger bolus and a greater infusion rate during the first half-hour, but a lower infusion rate during the second half hour.

PROTOCOL A0228s

Title: The NINDS t-PA Stroke Trial

Study dates: January 1991 to December 1994
Study Funding: Supported by NINDS contract to [redacted] & co-investigators
Additional support from Genentech, Inc.

Overview

This protocol includes two studies that were conducted with identical procedures at the same set of study sites, distinguished by the different primary endpoints and the prospective designation that patients enrolled on November 3, 1992 and thereafter will be considered part of the second study. For purposes of reference, these two studies will be called the Part 1 study and the Part 2 study. The written protocol refers to these as Part A and Part B studies. The paper published in NEJM refers to the Part 1 study and the Part 2 study, and the materials submitted in the PLA refer to the studies in both manners. This review will utilize the Part 1 & Part 2 nomenclature solely for consistency with the NEJM paper.

The Part 1 study was designed as a study of early activity effects of Activase in stroke, with planned size of 280 patients, and no plans for an immediate continuation into an additional study. By late in the conduct of this study the investigator group had determined that the outcome at 90 days was more informative of clinical benefit, and for a variety of reasons wished to proceed directly into a phase 3 efficacy study. Thus it was decided to add a second study, of approximately 300 patients, which would commence immediately upon ending the enrollment into the Part 1 study. Interim analyses of the Part 1 study results were used in the selection of the Part 2 study's primary endpoint. CBER was involved with the discussions leading to this plan of immediate initiation of the Part 2 study, and the analytic plan differences between the studies.

All procedures were the same for the Part 1 study and the Part 2 study. The analytic plans and endpoints were not identical. The following review of the study design applies to both Part 1 and Part 2 studies, except as noted. Furthermore, the analytic plan of the protocol was that selected by the investigator group which had obtained the contract from NINDS to conduct the study. Additional discussions were conducted between the manufacturer, Genentech, and CBER regarding an analytic plan to be used by Genentech for consideration of the study in support of a license application for this indication. These analysis plans evolved with time and further discussions, and are detailed further below.

OBJECTIVES

The objectives as stated in the written protocol were:

Part 1 Study

This study was designed to assess the early activity of tPA as a treatment.

Primary Objective:

Evaluation of "Significant Early Improvement" between the treatment groups in :

a) Patients in the two arms in the 0-90 minute treatment stratum
b) Patients in the two arms in the 91-180min stratum
c) Patients in the 0-180 minutes combined strata

Secondary Objectives:

1) Evaluation of percentage of patients with "on table improvement"
2) Evaluation of percentage of patients with improvement by the day 7-10 evaluation
3) Comparison of change in NIH Stroke Scale at 90d
4) Comparison of infarct volume as determined from CT scans
5) Comparison of outcome at 1 week and 90d by the Barthel Index
6) Comparison of the outcome at 90 days with the Glasgow Scale
7) Comparison of the outcome at 1 week and 90 days by the Modified Rankin Scale
8) Comparison of percentage of patients showing neurologic deterioration
9) Evaluations of correlations of 1day NIH Stroke Scale score with infarct volume, and the 90 day outcomes.

Subgroup Objectives:

1) Comparison of percentages with early improvement between the time strata
2) Determination if "on table improvement" will be predictive of 90d outcome
3) Evaluation of patients with aspirin use prior to the stroke for improvement and risk of intracranial hemorrhage.
4) Evaluation of patients with lower fibrinogen or higher FDP will have greater risk of intracranial hemorrhage.

Part 2 Study

This study was designed to assess the efficacy of tPA as a treatment.

Primary Objective:

To assess the hypothesis that: There is a consistent and persuasive difference between the t-PA treatment group and the placebo group enrolled within 180 minutes of stroke onset in the proportion with a 90 day outcome of :

a) Barthel Index ≥ 95
b) Rankin Score of 0 or 1 (No significant disability or symptoms)
c) Glasgow Outcome Score of 1 (good recovery)
d) NIH Stroke Scale of 0 or 1

Secondary Objectives:

1) To assess the primary hypothesis of "consistent and persuasive" benefit in the 0-90min stratum
2) To assess the primary hypothesis of "consistent and persuasive" benefit in the 90-180 min stratum
3) To evaluate percentage of patients with "Significant early improvement" overall.
4) To evaluate the percentage of patients with "significant early improvement" in the 0-90 min stratum
5) To evaluate the percentage of patients with "significant early improvement" in the 90-180min stratum.
6) To evaluate the percentage of patients with "on table improvement"
7) To evaluate the percentage of patients with improvement at 1 week
8) To evaluate the percentage with a Barthel Index of 95-100 at 1 week
9) To evaluate the median Rankin score difference between arms at 1 week, 90 days.
10) To evaluate the median Glasgow Outcome score at 90 days
11) To evaluate the percentage with deterioration within 1 day.
12) To evaluate the infarct volume effects of treatment at 1 week, 90 days
13) To evaluate correlations between the 24 hr NIH Stroke Scale score and infarct volume, and outcome scales at 90 days.

Sub-group Objectives:

The objectives were the same as stated for the Part 1 Study.

Definitions:

Significant early improvement is defined as improvement in NIH Stroke Scale (NIHSS) from baseline to the 24 hr exam by 4 points or complete resolution to a score of 0.

On table improvement is defined as improvement in NIHSS from baseline by 2 points at the 2 hr evaluation, or complete resolution to a score of 0.

Improvement for the 7-10day exam is defined as improvement in NIHSS from baseline by 5 points or more, or complete resolution to a score of 0.

Deterioration is defined as a 4 point increase in the NIHSS from the prior score.

"Consistent and persuasive" benefit was not defined further in the protocol with regard as to how to perform the calculation. This was extensively discussed during the course of the Part 2 Study, with the result being the analytic plan eventually agreed upon.

DESIGN

Overview

These were two consecutive, analytically separate, double-blind, randomized, placebo-controlled studies. The studies were conducted at 39 treatment sites. Each group of treatment sites was administered by 1 of 9 local Clinical Centers. The single central Coordinating Center was at Henry Ford Hospital.

Patients with acute ischemic stroke were randomized to one of 2 treatment arms, iv tPA or iv placebo. Treatment had to be initiated within 180 minutes of stroke onset. Patient randomization was stratified by the time from stroke onset to initiation of treatment into two cohorts, those treated within 90 minutes of onset (0-90min) and those treated after (91-180min). It was expected that the later cohort would be easier to accrue patients into, so the protocol required that each regional group of treatment sites maintain accrual in a close to balanced manner. The regional group of sites could not enroll any additional patients into the 91-180 time stratum whenever the total number of patients enrolled by the regional group into that stratum exceeded the number in the 0-90 stratum by three patients. Thus, three patients per Clinical Center was the greatest imbalance that could occur in accrual. This exerted pressure on the treatment sites to use the utmost of speed to treat any patient who arrived earlier than 90 post-onset minutes within the 90 minute cutoff.

The primary clinical outcome evaluations consisted of 4 outcome scales: the Barthel Index (Barthel), the Modified Rankin Scale (Rankin), the Glasgow Outcome Scale (Glasgow) and the NIH Stroke Scale (NIHSS).

Randomization

The study required the individual treatment sites to be able to rapidly move through the screening process to enrollment and initiation of treatment. To facilitate this the randomization process was decentralized.

The coordinating center received blind-labeled vials prepared by Genentech, along with a code list for the vial contents. The coordinating center established a study patient ID number that was then attached to the labels, and became the number used for tracking the patient throughout the study. The patient ID numbers were randomly ordered, and randomly assigned to tPA or placebo, with blocking on a by-clinical-center level rather than a treatment-site level. The two time strata were separately randomized. All treatment sites within each clinical center's administration received an identically labeled supply of blinded vials, along with a list indicating the order in which the patient numbers were to be utilized. The ID number order was random, not numerically sequential. As each treatment site had a supply of the same patient ID numbered vials, a system was in place to notify all treatment sites when a patient was enrolled at one of the sites, so that all sites would mark-off the utilized patient ID number, and go to the next in the list for the next enrolled patient. Unused vials were returned to the HFH Coordinating Center where they were relabeled with new patient ID numbers and sent back out to sites to have on hand.

Because of the short time frame from patient arrival at the treatment site to the 90 or 180 min time limit for beginning the infusion, certain preparations were conducted prior to completion of all screening procedures and confirmation of eligibility. In particular, when a likely-eligible patient was in the midst of the screening process, the investigator would notify the pharmacy of a possible enrollment. The pharmacy would at that time begin to prepare the blinded study treatment, by looking at the randomization list, selecting the box of vials with the appropriate ID#, and reconstituting the material. The pharmacy might need to rush the solutions to the location of the patient, where the investigator would complete the screening (usually this was completion of the CT scan). If the patient was determined to be not eligible, the prepared solutions were disposed of, and the patient ID# remained available for the next patient. This

patient was not deemed randomized or entered into the study. If the patient completed screening and was eligible, then the infusion of study material was started. It was only at that moment that the patient was deemed to have been enrolled and randomized. Thus were no patients who were entered into the study who received no study material at all. There were many cases of patients becoming excluded from the study after the pharmacy had prepared the study material.

Although the analytic plans regard the two studies as completely independent, they were not entirely so with regards to randomization. The two studies in effect used a single randomization list. The only apparent interaction between the studies with regard to this is that the randomization lists were blocked (within each clinical center), and there will exist a transition block at each clinical center where the earlier patients were enrolled into the Part 1 Study, and the remainder of the block were enrolled into the Part 2 Study.

Comment:

This is unlikely to have had a significant effect upon the studies.

Blinding

Blinding was incorporated into the studies by using blind labeled vials and identical administration regimens for the two treatment arms. The potentially unblinding laboratory results of fibrinogen and FDP were sent as blood samples to the HFH coordinating center where the assays were performed; results were not returned to the treatment sites. Additionally, the outcome assessments of 1day, 1 week and 3 months were to be performed by personnel not present during the study treatment infusion.

PATIENT POPULATION

Inclusion Criteria

1) Age 18 or older
2) Clinical diagnosis of acute ischemic stroke with measurable neurologic deficit
3) Time of onset is <180 min of when treatment can begin,
4) The Clinical Center administering the specific treatment site has adequate balance of patients between strata (enrollment into 90-180 min stratum was permitted only if the number of patients in 90-180 min. stratum is not more than 2 greater than in the 0-90 stratum).

Exclusion Criteria

1) Only minor stroke or symptoms rapidly improving at time of infusion start
2) Evidence of hemorrhage on CT scan
   No other formal CT scan exclusion criteria.
3) Clinical presentation suggests subarachnoid hemorrhage
4) Female & lactating or pregnant
5) Platelet count < 100,000, PT > 15, Heparin within 48 hrs & PTT > normal, or
   Patient on oral anticoagulants.
6) Major surgery or body trauma within 14 d prior; serious head trauma within 3 mo

7) Hx of GI or UT hemorrhage in prior 21 d.
8) Noncompressible arterial puncture within 7d; LP within 7 d
9) Systolic BP > 185 or diastolic > 110, or aggressive management (IV medication) necessary to reduce BP to these limits
10) Hx of stroke in prior 3 mo, prior ICH suggesting risk factor.
11) Serious medical illness that would interfere with trial
12) Blood glucose <50 or > 400
13) Clinical presentation consistent with MI or postMI pericarditis
14) Seizure at onset of stroke

Comment:

There were no formal CT scan based exclusion criteria other than the finding of hemorrhage. However, Dr. John Marler (NINDS Project Officer for the study) stated at the prePLA meeting in December 1995 that there were practices that may have resulted in *de facto* exclusion criteria. When investigators observed what was subjectively felt to be significant early infarct signs on the screening CT scan, they would frequently proceed to re-question the patient and/or family as to the time of onset of the stroke. In the course of doing this, they would often determine that the stroke onset was earlier than previously reported, and the patient was excluded on the basis of too long an elapsed time from onset. Thus, in practice, there was exclusion from the study on the basis of infarct related CT scan findings.

TREATMENTS

Study Treatment

Study treatment consisted of either tPA or placebo, given IV, with time from stroke onset to start of the study treatment determining the time stratum of the patient. Patients were deemed to be enrolled and randomized only at the moment the study treatment began. The dosage of tPA was 0.9 mg/kg up to maximum of 100kg of body weight, and 90 mg for all patients of weight > 100 kg. The treatment was given as 10% of the material as a 1-2 minute bolus, followed by the remainder as an infusion to be completed at 60min after the start of the bolus. Placebo was prepared in equal volumes and given identically.

Concomitant Treatments

Anticoagulant and anti-platelet agents were prohibited during the first 24 hours. After that time, use of these agents was at the discretion of the investigators after confirming absence of hemorrhage on the 24 hr CT scan, and was recorded in the concomitant medication list.

EVALUATIONS

In addition to clinical laboratory evaluations and general medical care evaluations in keeping with standard care, study specific assessments included the following:

Baseline and Screening:
- NIH Stroke Scale
- CT Scan
- Estimated pre-stroke Modified Rankin Scale

2 Hours after initiation of treatment
- NIH Stroke Scale (also at several additional timepoints during the succeeding day)

24 Hours
- NIH Stroke Scale
- CT Scan

7-10 Days
- NIH Stroke Scale
- Barthel Index, Modified Rankin Scale
- CT Scan

1 Month
- Barthel Index, Modified Rankin Scale, Glasgow Outcome Score

3 Months
- NIH Stroke Scale
- Barthel Index, Modified Rankin Scale, Glasgow Outcome Score
- CT Scan.

ENDPOINTS AND PLANNED ANALYSES

These studies were designed and conducted by the NINDS Investigator group. Although Genentech had ongoing discussions with the investigators during the design and did provide support for the study, selection of objectives, endpoints, and analytic plan did not require concurrence from Genentech. Selection of the Global Statistic as the primary endpoint, and the uncertainty of interpreting it for clinical meaningfulness caused some concern for its suitability for regulatory purposes. Consequently, Genentech began in 1994 to discuss alternative analytic plans with CBER for the NINDS Stroke Studies to use in potential support of a licensure application. All discussions were conducted without either Genentech personnel or CBER reviewers aware of any results from the interim analyses (see pg. 16). Discussions initially involved different primary endpoint definitions, but by late 1994 Genentech was proposing that the Genentech analytic plan for licensure would utilize the Global Statistic and be the same analytic plan as devised by the investigator group. The final Genentech analytic plan for these studies retained the concept of the Part 1 study designed with the objective of a 24 hour assessment activity endpoint, and the Part 2 Study objective of the 3 month efficacy outcome.

Part 1 Study

Primary Endpoints (now regarded as activity endpoints)

Percentage of patients with "Significant Early Improvement"

a) in patients in the 0-90min stratum
b) in patients in the 91-180min stratum
c) in patients in the combined strata

Secondary Efficacy Endpoints

1) Percentage of patients with "On Table Improvement"
   a) in patients in the 0-90min stratum
   b) in patients in the 91-180min stratum
   c) in patients in the combined strata
2) Percentage of patients with Improvement at 1 week
   a) in patients in the 0-90min stratum
   b) in patients in the 91-180min stratum
   c) in patients in the combined strata
3) Global Statistic utilizing the dichotomized outcome scales as described under Objectives
4) Percentage of patients achieving "Recovery" as defined by each of the univariate dichotomized scales at 90 days
   a) NIH-SS
   b) Barthel Index
   c) Modified Rankin Scale
   d) Glasgow Outcome Score
5) Median outcome score on each of the univariate scales at 90 days
   a) NIH-SS
   b) Barthel Index
   c) Modified Rankin Scale
   d) Glasgow Outcome Score
6) Percentage with Clinical Improvement at 90 days, defined as improvement by at least 11 points or complete recovery on the NIH-SS at 90 days from baseline.

Safety Endpoints

Mortality rates at 30days, 90 days

Part 2 Study

Primary Efficacy Endpoint

Global Statistic utilizing the dichotomized outcome scales (with dichotomization as described under Objectives):

Barthel Index = 95 or 100
Modified Rankin Scale = 0 or 1
Glasgow Outcome Score = 1
NIH-SS = 0 or 1

Secondary Efficacy Endpoints

1) Percentage of patients achieving "Recovery" as defined by each of the univariate dichotomized scales at 90 days
   a) NIH-SS
   b) Barthel Index
   c) Modified Rankin Scale
   d) Glasgow Outcome Score

2) Median outcome score on each of the univariate scales at 90 days
   a) NIH-SS
   b) Barthel Index
   c) Modified Rankin Scale
   d) Glasgow Outcome Score
3) Percentage with Clinical Improvement at 90 days, defined as improvement by at least 11 points or complete recovery on the NIH-SS at 90 days from baseline.
4) Global Statistic of the dichotomized scales with adjustment for covariates (specific covariates not named)

Safety Endpoints

Mortality rates at 30days, 90 days

Activity Endpoints

Dichotomized Barthel Index at 1 week

Median Rankin Scale at 1 week

Percentages with Significant Early Improvement; On Table Improvement; Improvement at 1 week.

An Intent to Treat analysis was to be performed, and the data imputation plan for missing values as devised by the NINDS Investigator group would be utilized.

Note: Significant Early Improvement (NIH-SS improvement at 24 hours), On Table Improvement (NIH-SS improvement at 2 hours), and Improvement at 1 week (based on NIH-SS) used the same definitions as listed in the Objectives section above.

INTERIM ANALYSES

The NINDS Stroke Study conducted periodic safety interim analyses that were reviewed unblinded by the NINDS appointed Data and Safety Monitoring Committee.

The DSMC also conducted an interim analysis that contained the majority of the efficacy outcomes from the Part 1 Study at the time that the Part 2 Study was being designed. The DSMC was the source of the "Consistent and Persuasive" definition of the primary endpoint, and was fully aware of the results of the Part 1 study at the time they selected this as the primary efficacy endpoint for the Part 2 study. Thus, the Part 1 Study results can be considered as supportive results for this endpoint, but it is the Part 2 Study that serves to test the prospective hypothesis.

PLANNED FINAL ANALYSES

Subsequent to the completion of analyses by the NINDS Investigator group, and publication of the results, Genentech and CBER held discussions regarding the PLA submission. It was determined that in order to expedite the submission and review of the results, Genentech would limit the analyses they perform to those selected by CBER as critical to the review. These included the Global Statistic analysis, the tests of the univariate scales in dichotomized form, and

the univariate scales in their full ordinal form. Additionally, Genentech would perform and submit certain exploratory analyses specifically requested by CBER for purposes of sensitivity and safety. All analyses would be conducted using two-tailed tests.

STUDY PERFORMANCE & PATIENT DISPOSITION

Patient Disposition

Patients were enrolled into the two studies between January 1991 and October 1994. The distinction between the Part 1 study and the Part 2 study was prospectively designated such that November 3, 1992 began enrollment into the Part 2 study. There were 291 patients enrolled into the Part 1 study (144 Activase, 147 placebo) and 333 patients enrolled into the Part B study (168 Activase, 165 placebo), for a total of 624 patients in the combined studies (312 patients in each treatment arm).

There were 9 clinical centers, of which 8 contributed significant numbers to the studies. One clinical center, U. of Maryland, with only it's own hospital as a treatment site, contributed only a single patient to the studies, and then ceased to participate due to inability to establish an adequate patient screening rate. Each of the other 8 clinical centers administered from 1 to 12 treatment sites. The distribution of patient enrollment among the clinical centers was not uniform. The U. of California and U. Cincinnati were the two largest centers, each with approximately 150 recruited among their administered sites. Total recruitment at the other 6 Clinical Centers ranged from 14 to 104.

	Enrollment and Assignment as Analyzed							
Clinical Center	Part 1 Study				Part 2 Study			
		Number of Patients				Number of Patients		
	# Sites	Total	Placebo	Activase	# Sites	Total	Placebo	Activase
Emory U.	2	22	11	11	3	17	8	9
Henry Ford	1	28	15	13	1	34	18	16
Long Island Jewish	1	38	20	18	1	34	16	18
U. California	8	73	37	36	7	73	35	38
U. Cincinnati	11	50	24	26	12	100	52	48
U. Tennessee	3	10	5	5	4	4	1	3
U. Texas	7	47	23	24	7	57	29	28
U. Virgina	2	22	12	10	2	14	6	8
U. Maryland	1	1	0	1	0	0	0	0
Totals	36	291	147	144	37	333	165	168

Eligibility Violations

There are 54 patients of the 624 total patients reported with at least 1 eligibility violation. Of these, 29 are Activase patients, 25 placebo patients, with 63 total violations. The most common type of violation involved the blood pressure criteria; 29 of the 54 patients had such a violation. The other violations were broadly distributed in type, and included cases of not obtained clinical laboratories prior to initiating treatment. There was nor recognizable pattern nor imbalance in the violations between the treatment arms.

Randomization

The Randomization process was quite complex, involving the HFH Coordinating Center creating randomization lists, labeling vials, verifying contents by vial number, tracking the shipment to sites and back to the coordinating center, removing labels and re-storing vials for future re-labeling, monitoring treatment site usage of vials, etc. The PLA submission reports that very few errors occurred in the randomization process.

Out of Order Patients

The submission reports that there were 13 patients for whom the treatment site used the wrong box of vials, assigning an unintended patient ID# to that patient. In each case, the assigned ID# was for an ID# intended to be used for a patient 1-2 patients later in the study. These were termed "out of order" patients. This occurred 8 times in the Part 1 study, 5 times in the Part 2 study. All are reported accidental occurrences, and all are believed to have been without any unblinding at the time of enrollment and randomization. The practice was for the coordinating center to notify the clinical center when this was discovered, and to have the next patient use the skipped patient ID#.

Of these 13 patients, 11 patients who should have received Activase instead received placebo. The remaining two patients received a placebo and would have received placebo had they been assigned the correct patient ID# as well. There were no cases of patients intended to receive placebo receiving Activase instead. Eight patients skipped over only 1 ID# (7 of which contained Activase, 1 placebo) to receive the box of vials actually utilized. Five patients skipped over 2 ID#s (3 Activase-Activase, 1 Activase-placebo, 1 placebo-placebo).

Comment:

The baseline characteristics of these patients were examined. There does not appear to be any distribution of these patients with regard to baseline disease characteristics or demographics that distinguishes them from the patients in the study overall.

Of the 11 patients for whom this resulted in a change from Activase to placebo, the day 90 Barthel Index was 95-100 in 6. This 55% good outcome rate is higher than the 38% good outcome rate seen overall in the placebo group in the dataset of the combined studies. Thus, this error in the randomization process appears not to have contributed any bias to overestimating the treatment effect.

Incorrect Vial Shipment

Only one instance of a patient treated with vials containing the opposite-from-intended treatment occurred (placebo vials packed under an ID# intended for Activase). The Coordinating Center record keeping system appears to be reliable in the assurance that this occurred for no other patients. This one instance was for patient ——— who received vials of placebo when Activase was assigned to that ID#.

Stratum Code Discrepancies

There are 18 patients for whom the ID# used came from the wrong time stratum. The PLA initially reported that this was due to a (single) period during the study when there was a shortage of vials, and the Coordinating Center was unable to keep the treatment sites adequately stocked. Treatment sites were instructed that if they had an eligible patient, but no vials for the appropriate stratum, then they should use the next patient ID# package from the list for the other time stratum. This maintained full blinding, but did cause some intermixing of the randomization process between the two strata. This occurred for 15 patients in the 90-180min stratum, and 3 patients in the 0-90min stratum.

Comment:

The database was examined with regard to these patients. The 18 patients do contain an apparent discrepancy between the time to treatment and the ID# indicated stratum. However, these patients were enrolled between early 1991 and mid 1994, which is at odds with the explanation of a vial shortage at a single period of time. An explanation was requested from the applicant. Genentech has responded that [illegible]

Additionally, the database shows no other discrepancies between codes and times. The randomization process required investigators in the process of screening a patient for eligibility call the pharmacy to request study drug reconstitution and indicate to the pharmacist which time stratum the patient will be treated within. The lack of further discrepancies implies that the investigators predicted with complete accuracy how long it would take to complete the screening, and there were no patients treated at 91 or 92 minutes who had been expected to complete screening and start treatment within the 90 minute window, and similarly for the 180 minute limit.

Of the 18 patients who do appear to have received vials from the wrong randomization list, 7 received the same treatment as the next available ID# in the appropriate stratum's list would have provided (5 Activase, 2 placebo). There were 11 patients where use of the wrong-stratum vials resulted in changing the treatment actually received. There were 10 who should have received Activase, but instead received placebo, and 1 patient who should have received placebo and instead received Activase. The one patient who was both a wrong-stratum and ID#-used-out-of-order was changed from Activase to placebo if the stratum-shift if considered, and was to have received placebo under both ID#s when the out-of-order effect is considered.

Of these 11 wrong-stratum treatment-change patients, 4 of the 10 changed to placebo had 90 day Barthel Index values of 95-100, as did the 1 patient changed from placebo to

Activase. The 40% good outcome rate in the erroneous-placebo patients is not different from the 38% rate in the combined studies dataset for the placebo patients as a whole. Thus, these errors do not seem to have altered the overall outcome of the studies.

Effects upon analysis

Because it was believed that blinding was maintained throughout, the NINDS Stroke Study group elected to analyze these patients in the group as treatment received, not as a true intent to treat analysis.

Comment

] However. since upon the discovery of the error, subsequent patients were then assigned to the skipped ID#s, there were generally clusters of 2-4 patients who received different ID#s than they would have had the out-of-order error not occurred. Furthermore, most of these additional patients were enrolled in treatment sites other than the one where the out-of-order error occurred. For the wrong-stratum patients, it becomes even more complex, as every subsequent patient, in both time strata, received an other-than-originally-intended ID#, of which many would be different in the actual treatment received. Thus, the group of patients affected in performing a "true" intent-to-treat analysis is not well defined. It remains notable however, that of the 22 patients who had treatment changed due to the randomization difficulties that occurred at the treatment sites, 21 of these involved a patient who should have received Activase being changed to receive placebo. Only one of the 22 was changed from placebo to Activase.

Unblinding

Unblinding envelopes were to be returned to the Coordinating Center at the 10 day time point after enrollment. Envelopes were logged in and examined for opening or other damage. There are 16 patients of the total 624 reported as having been unblinded during the study. This includes 12 patients in the Activase group, and 4 in the placebo group. Unblindings with listed reasons include 6 for intracranial bleed or subarachnoid hemorrhage, 1 for groin hematoma, and 1 for "reperfusion vs. reocclusion".

CT Analysis Procedure and ICH Review

The central review procedure for the CT scans was conducted at Henry Ford Hospital by Dr. —, a neuroradiologist, who was not participating in the patient treatment aspects of the protocol (HFH was both the site of the coordinating center as well as a treatment site). CT scans were submitted to the coordinating center with labels that contained the date of the scan, the patient ID#, and no other identifying information. Dr. — reviewed the scans for evidence of hemorrhage and for other specific CT abnormalities (edema, mass effect, etc.). Treatment site investigators notified the coordinating center when ever an ICH was found, both symptomatic and asymptomatic. Dr. — was aware of these reports when reviewing the submitted scans,

and would either confirm or reject the finding of ICH. There were 19 patients in whom the treatment site reported ICH and a determination of hyperdense infarct (called a Type II scan, not ICH) was made by Dr. —, and 9 patients in whom a determination of neither ICH nor hyperdense infarct was made.

The determination of symptomatic or asymptomatic ICH was made by Dr. — at HFH, with full knowledge of the CT scan results as well as clinical events. In cases of uncertainty, Dr. — would consult the site investigator directly prior to making the determination.

The protocol had planned for the 90 day CT scan to be used for lesion volume measurements. However, difficulties arose in performing these measurements. The measurements were made in a blinded manner, but many occurred after the studies clinical results were known. At the time of the PLA submission, the CT volume measurements were not completed, nor the results analyzed. No CT lesion volume measurements were submitted as part of the PLA Supplement.

Treatment Characteristics

Incomplete Treatment

There were 16 patients of the 624 who had an interruption of the treatment infusion. These included 7 in the placebo group, and 9 in the Activase group. In the Activase group, one was for occurrence of an ICH, 2 for baseline laboratory values that subsequently were reported as out of the eligibility range, 3 for adverse events (1 each vaginal bleeding, oral bleeding, seizure) and 4 for events unrelated to the Activase (consent withdrawal, iv line failure, etc.). When the interruption cause could be corrected with only brief delay, the investigators were to resume treatment to the full dose.

Inexact Dosing

There were few patients who received a dosage different than planned (based on the weight used to calculate dosage at the time of enrollment). In the Part 1 study, 5 placebo and 10 Activase patients had a >5% difference from the planned dosage. Most were for the above noted incomplete treatment patients. In the Part 2 study, there were 4 placebo, and 5 Activase patients with > 5% difference from the planned dose, 5 of which are in the treatment interruptions noted above. The other patients, in both Studies, were errors in dosage, and were divided between over- and under-dosing.

Non-Ideal Dosing

Overall, most patients were close to appropriately dosed, but many patients had dosage that was >5% different from the ideal, protocol intended dose, when based on the actual patient weight. These were divided between both over- and under-dosing. Of the 611 patients (those without missing information, 304 placebo, 307 Activase), 67% were within ±5% of the intended dose. 14% received a dose more than 5% low (6% a more than 10% low dose) and 19% received a dose more than 5% higher then intended (11% a more than 10% higher dose). The distributions were not different between the two treatment groups.

Most patients were treated on the basis of estimated weights (93%), and most differences between the intended dose and the delivered dose were entirely due to differences between the estimated weight and the actual body weight measured within 48 hours of admission to the hospital. However, as these differences tended to be small, the resulting dosing differences were similarly small.

Study Participation Withdrawal

There were few patients who withdrew from the study. Since after the completion of the one hour infusion, study participation consisted solely of evaluations without further therapy, these withdrawals are all included in the missing data accounting.

Missing Evaluations

At the time of the 90 day endpoint, The Part 1 Study has 8 of 236 living patients (each with 4 outcome scale evaluations expected) missing 18 individual scale values. Three patients with 6 missing scales are in the placebo arm, and 5 patients with 12 missing scales in the Activase arm.

The Part 2 Study has 4 of 271 living patients (each with 4 outcome scale evaluations expected) missing 7 individual scale values, with all in the placebo group.

All missing values were imputed according to the prospective plan for such. Most patients merely had the last value carried forward from the day 7-10 evaluation.

Comment:

> Overall there are 7 placebo patients (of 312 total) missing 13 scale values (of 1248 total, including imputed scores on the basis of death), and 5 Activase patients missing 12 scale values (also of 1248 total expected). Thus the total missing number is approximately 1% in each treatment group. Since most were imputed by last observation carried forward (LOCF) from the 1 week score, they will tend to be close to the actual score to some degree. This low a percentage of missing values does not significantly alter the overall results.

There are larger numbers of patients with missing evaluations at the day 7-10 evaluation; however most of these patients apparently complied with the requests to return for the 90 day evaluation, and thus are not missing from the primary efficacy analyses.

Bioresearch Monitoring Inspections

Inspections by the field offices were requested by Bioresearch Monitoring for the following sites:

Bioresearch Monitoring Inspections			
Inspection Site	Clinical Center administering site	Number of Patients	
		Part 1 Study	Part 2 Study
Henry Ford Hospital	Henry Ford Hosp.	28	34
Long Island Jewish	Long Island Jewish	38	34
U. Cincinnati Hosp	U. Cincinnati	7	14
Bethesda-North Hosp	U. Cincinnati	4	16
Mercy Hosp	U. Cal.	7	19
Sharp Mem. Hosp.	U. Cal.	10	18
Herman Hosp.	U. Texas	17	17
St. Luke's Hosp.	U. Texas	13	10
U. Va. Med. Ctr.	U. Virgina	15	10
Henry Ford Hosp	Coordinating Center	(0)	(0)
Patient Totals (% of Study total)		122 (42%)	172 (52%)

No discrepancies of significant enough to call into question the studies' validity or integrity were discerned in any of these inspections.

STUDY POPULATION CHARACTERISTICS

Screened Patient Characteristics

A screening log was maintained in the Emergency Department of each treatment site. It was designed to capture a record of all patients evaluated for stroke in the ER within 24 hours of event onset, and the reason for exclusion from the study if not enrolled. Over the course of almost 4 years, 17367 patients were recorded as screened but not enrolled. Patients may have had more than one reason for exclusion, with the primary reason for exclusion distributed as follows:

Exclusion reason	Number	%
Time from onset too long	8708	51.6
Symptoms rapidly improving	1749	10.4
Intracranial Hemorrhage	1306	7.8
Symptoms too minor	1106	6.6
Outside age range	1021	6.1
Other serious illness	490	2.9
Seizure at stroke onset	391	2.3
Stroke not present	373	2.2
Time from onset 90-180 min[1]	267	1.6
Recent prior stroke	219	1.3
Oral anticoagulants	210	1.2
Subarachnoid Hemorrhage	169	1.0

[1]Site enrollment unbalanced at the time the specific patient was screened. Individual sites were required to maintain the number enrolled in the two time strata equal within 3 patients

Thus the patients enrolled in the study represent a small minority (approximately 3½%) of the patients who have had a potential stroke and evaluated in the emergency room setting. The majority of patients were not eligible based simply on time from onset to potential treatment. Whether or not the patients enrolled in the study are adequately representative of the stroke population as a whole when the time from onset element is eliminated is not known.

Comment:

Of interest for consideration with later exploratory analyses of intracranial hemorrhage during the studies is that intracranial hemorrhage at the time of screening was a more common exclusion reason for Hispanic patients than for either white or black screened patients (18% of 701 Hispanic exclusions, 7% of 11,166 white exclusions and 9% of 4337 black exclusions).

Study population

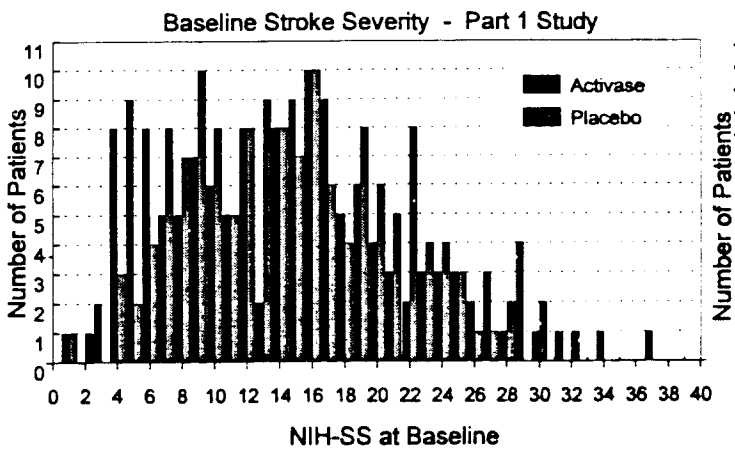
The characteristics of the study population with regard to demographics, prior medical history, and baseline status are presented in the following tables:

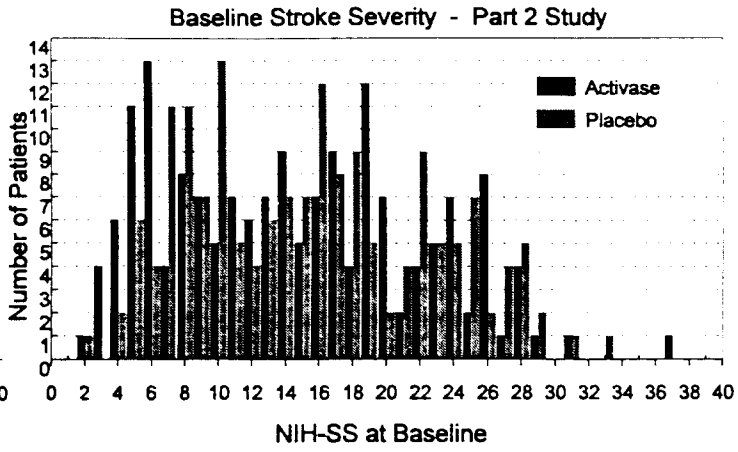
Demographics and Baseline Status							
Characteristic	criterion	Part 1 Study			Part 2 Study		
		Placebo	Activase	p-value	Placebo	Activase	p-value
Number	enrolled	147	144		165	168	
Age (years)	Median	67	68.7	0.62	67.9	71.4	0.01
	Range	34 - 92	40 - 88.5		26 - 100.6	31 - 97.9	
Gender (%)	male	59.9	57.6	0.70	58.2	56.5	0.76
	female	40.1	42.4		41.8	43.5	
Race (%)	Caucasian	60.5	61.8	0.09	66.1	69.0	0.46
	Black	31.3	29.2		26.1	22.6	
	all other	8.2	9.0		7.8	8.3	
Weight (kg)	median	81.6	75	0.02	78.2	76.8	0.25
	range	42-136.1	46-125.1		44 - 179.6	41-137.4	
NIHSS - baseline	median	14	14	0.26	15	14	0.20
	range	1 - 32	1 - 37		2 - 33	2 - 37	
Systolic BP	median	152	155	0.49	152	154	0.99
	range	90 - 220	102- 227		100 - 210	106- 215	
Diastolic BP	median	85	84	0.73	87	83	0.08
	range	49 - 120	54 - 124		10 - 120	47 - 134	

Evident are the small, but statistically notable imbalances in age (Part 2, Activase patients are older) and weight (Part 1; Activase patients are lighter), and lesser imbalances in Race (Part 2), weight (Part 2; Activase patients are again lighter), and baseline NIH Stroke Scale score (Activase patients have slightly less severe strokes in both Part 1 & 2).

Comment:

To examine the characteristics of the study population as well as balance of the two treatment arms, the baseline NIH Stroke Scale was examined in detail.





Comparisons of these groups showed a trend towards imbalance in the baseline severity. This imbalance was more pronounced in the patients enrolled with less severe strokes, and occurred to similar degrees in both studies. In both studies, within the patients with the most mild of strokes (NIH-SS at baseline of 2-6) there is a larger number of patients in the Activase group than in the placebo group. Particularly for the dichotomized endpoints, this has potential to bias the study as there are more patients who need to improve only mildly to meet the criteria for success on outcome.

Baseline NIH-SS in Individual Studies				
	Part 1 Study		Part 2 Study	
	Placebo	Activase	Placebo	Activase
Mean	15	14.3	15.4	14.5
Std. Dev	6.55	7.47	7.06	7.47
Median	14	14	15	14

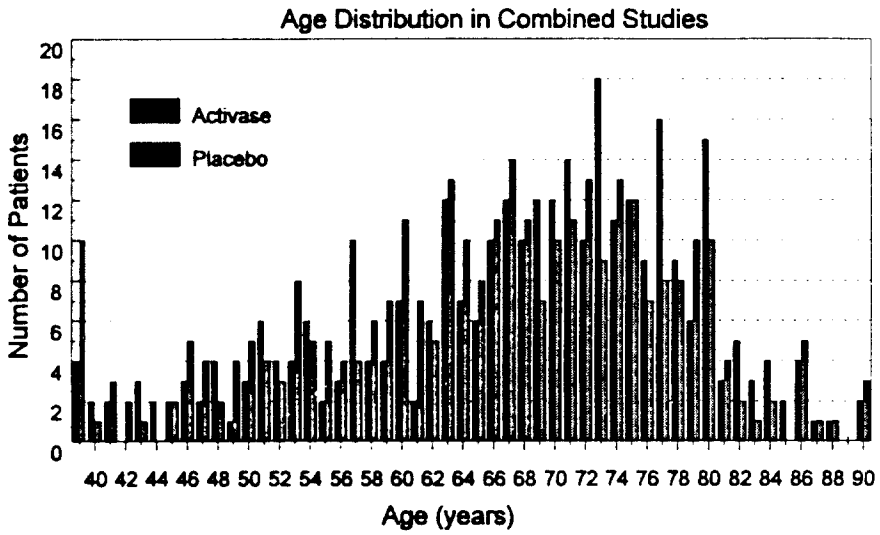
The mean baseline NIH-SS was different by almost a point in both studies, in both cases in direction of lesser stroke severity in the Activase group. The following table shows the degree of disparity in the numbers of patients with the least severe strokes in the combined studies dataset. A Rank-sum comparison of the entire set of patients in each treatment group shows the small difference in mean and median baseline NIH-SS is persists in the larger comparison as a strong trend of imbalance.

Baseline NIH-SS Distribution in Combined Studies Dataset			
	Placebo	Activase	p-value
Median	15	14	0.092[1]
Patients with Baseline NIH-SS within stated range: [n (%)]			
0 - 1	1 (0.3)	1 (0.3)	
0 - 2	3 (1.0)	2 (0.6)	
0 - 3	3 (1.0)	8 (2.6)	
0 - 4	8 (2.6)	22 (7.1)	
0 - 5	16 (5.1)	42 (13.5)	
0 - 6	24 (7.7)	63 (21.2)	

[1] Rank-sum test

Comment:

The distribution of age was also examined in detail. This distribution shows a peak frequency in the range of the low-70s years-old, with the Activase patients slightly older in both studies.



Mean Age			
Part 1 Study		Part 2 Study	
Placebo	Activase	Placebo	Activase
65.7	66.6	66.2	69.2

Genentech submitted analyses of the distribution of patients with specific medical history items collected in this study:

Percentages of patients with Prior Medical History Elements						
Characteristic	Part 1 Study			Part 2 Study		
Number enrolled	147	144		165	168	
History of:	Placebo	Activase	p-value	Placebo	Activase	p-value
Prior stroke	16.6	17.4	0.85	8.6	12.0	0.32
TIA	14.4	21.6	0.12	19.5	13.5	0.16
Diabetes	20.5	23.6	0.53	20.1	20.5	0.94
Hypertension	64.1	66.4	0.68	67.1	66.7	0.94
CHF	17.5	13.7	0.38	19.2	15.8	0.43
Disability	8.8	10.4	0.65	6.7	5.4	0.62
Smoking	36.6	42.9	0.28	35.2	26.7	0.10
ASA usage	22.4	34.0	0.03	24.2	31.5	0.14

Also recorded were history of prior MI, atrial fibrillation, angina pectoris, valvular heart disease, renal disease, and hepatic disease. None of these were significantly imbalanced.

Comment:

There are degrees of imbalance in several characteristics, often with direction of imbalance opposite between the Part 1 and Part 2 studies (prior TIAs, hypertension, disability, smoking). However, the approximately 3½% difference in history of CHF occurs in both studies in the direction of less CHF in the Activase patients. Of some note is the patients with pre-existing disability. While small numbers, these patients are of especial concern with the selected primary endpoint, as pre-existing disability may be sufficient to prevent a patient reaching criteria for "excellent recovery" on one or more scales even if the stroke leaves no residual. There is no further information on these patients with regard to amount or kind of pre-existing disability. The imbalance in percentages is in opposite directions between the two studies, however, so that balance occurs in the overall dataset of the combined studies.

Also notable is the difference in pre-stroke aspirin usage (within the two weeks prior to the stroke). More patients in the Activase arms had pre-stroke usage of aspirin than in the placebo arm, and this occurred in both studies. This information was collected as part of the admission survey of medical history, when inquiring about all medications the patient has taken in the two weeks prior to admission. Non-aspirin NSAID medications were not counted in this category as submitted in the PLA by the manufacturer. However, in the investigator analysis, a broader definition was used that included NSAID medications.

Baseline Laboratory Findings						
Characteristic	Part 1 Study			Part 2 Study		
	Placebo	Activase	p-value	Placebo	Activase	p-value
Number	147	144		165	168	
Platelet count (mean)	$275x10^3$	$259x10^3$		$263x10^3$	$244x10^3$	
PT, median (sec)	12	11.9	0.86	11.9	11.7	0.01
PT > 12 sec (%)	11.7	15.4	0.36	22	9.1	0.001
PTT, median (sec)	25.8	25.6	0.97	25.5	26.0	0.29
Fibrinogen, median	330.0	326.0	0.38	311.5	298.0	0.46
Glucose, median	125	123.5	0.66	121	126	0.64

These baseline findings were notable for the difference in percentage of patients with elevated PT. While the percentages of patients with PT > 12 sec. was imbalanced in each study, it was in opposite directions between the two studies.

CT Scan Findings at Baseline

The baseline CT scans were read in a blinded manner at the coordinating center to confirm the absence of hemorrhage as well as to identify a number of potential CT characteristic findings.

Baseline CT Scan Readings									
	Part 1 Study			Part 2 Study			Combined Studies		
	placebo n (%)	Activase n (%)	p-value	placebo n (%)	Activase n (%)	p-value	placebo n (%)	Activase n (%)	p-value
N enrolled	147	144		165	168		312	312	
Edema	4 (2.7)	6 (4.2)	0.491	9 (5.6)	7 (4.2)	0.582	13 (4.2)	13 (4.2)	0.99
Mass Effect	3 (2.1)	4 (2.8)	0.675	6 (3.7)	5 (3.0)	0.736	9 (2.9)	9 (2.9)	0.99
Midline Shift	0	1 (0.7)	0.310	0	0		0	1 (0.3)	0.316
Hyperdense Infarct	1 (0.7)	0	0.323	0	0		1 (0.3)	0	0.318
Hematoma	0	1 (0.7)	0.310	0 (0)	1 (0.6)	0.321	0	2 (0.7)	0.156
Ventricular Hemorrhage	0	1 (0.7)	0.310	0	0		0	1 (0.3)	0.316
SAH	0	1 (0.7)	0.310	0	0		0	1 (0.3)	0.316
Intraarterial Thrombus	14 (9.6)	10 (7.0)	0.434	29 (17.9)	23 (13.9)	0.327	43 (14.0)	33 (10.7)	0.226

Note: Percentages are of available CT scans with answers to each specific item
p-values calculated with Chi-Square test

There were three patients (two Part 1 Study, one Part 2 study) who were judged to have actually had a hemorrhage at the time of the baseline CT scan. All three were in the Activase groups. Overall, only approximately 4% of patients were identified as showing edema, and 3% showing mass effect on the baseline CT scans. The degree to which this may have been due to investigators re-inquiring as to stroke onset time in patients with these CT findings is uncertain. A localized hypodense infarct sign was not a recorded CT finding during the coordinating center CT reading. Overall, there were only a minority of patients who had abnormal CT scans as relates to the acute stroke.

Time to Treatment

Slightly more patients are enrolled in the 91-180 min stratum than in the 0-90min stratum:

Distribution of Patients into Time to Treatment Strata

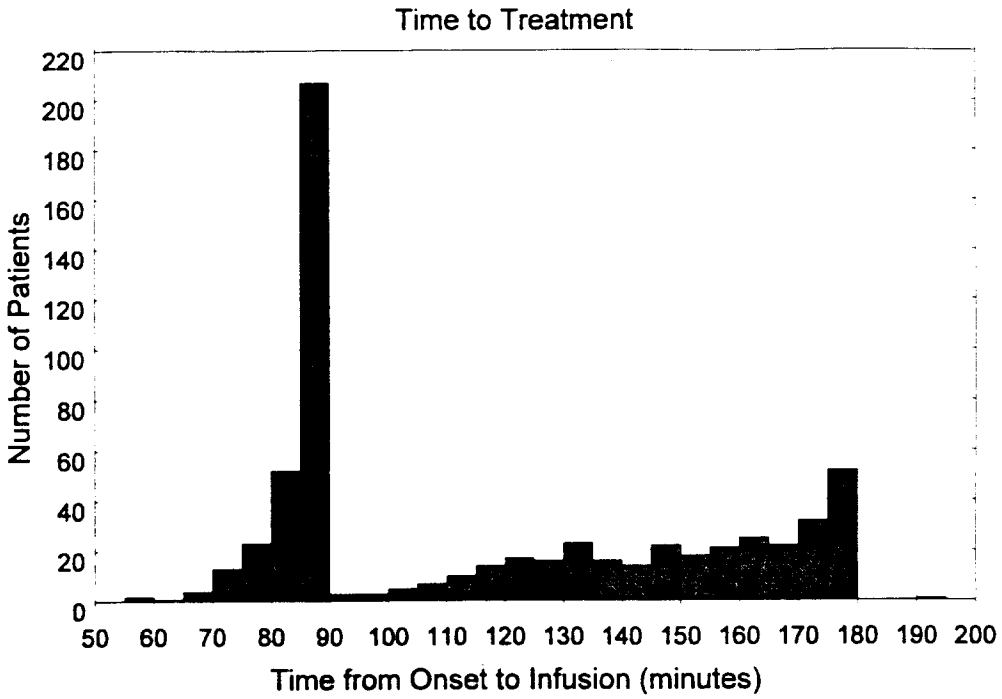
Stratum	Part 1 Study			Part 2 Study		
	Placebo	Activase	p-value	Placebo	Activase	p-value
Combined	147	144		165	168	
0 - 90 min	68 (46.3%)	71 (49.3%)	0.60	77 (46.7%)	86 (51.2%)	0.41
91 - 180 min	79 (53.7%)	73 (50.7%)		88 (53.3%)	82 (48.8%)	

p-values from Chi-Square test

This difference (13 for the Part 1 Study, 7 for the Part 2 Study) is fully consistent with the protocol which would allow an imbalance of up to 24 patients to occur (3 patients imbalance at each of 8 active clinical centers).

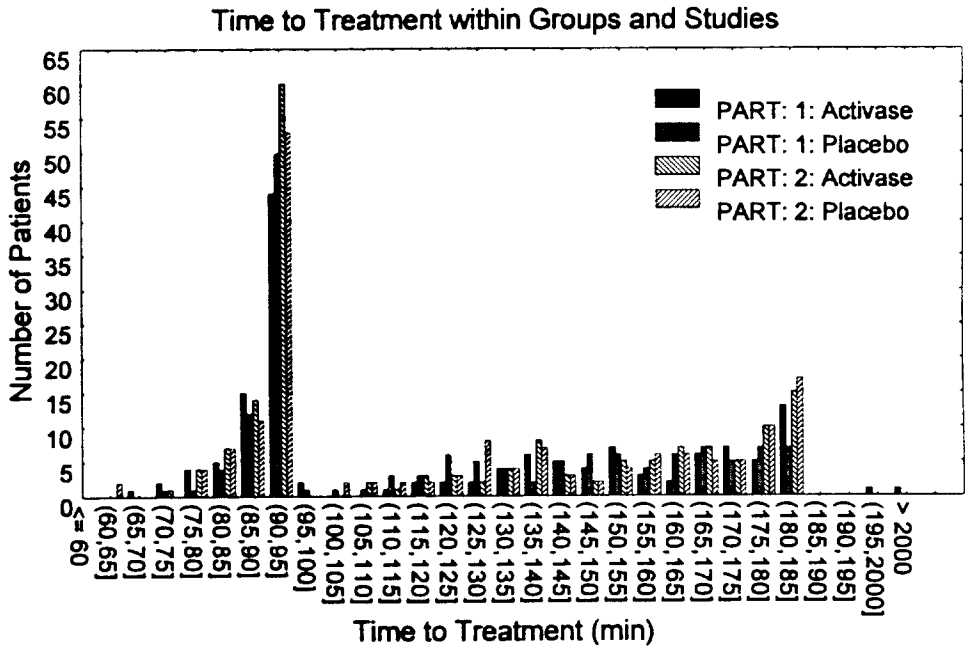
Comment:

The actual time to treatment was examined in the database supplied. The distribution of time to treatment for all patients combined was not uniform throughout the 3 hour time window, as shown in the following figure:



Comment:

However, the distribution in each of the treatment arms of each of the studies was similar to that of the overall dataset. Thus, there does not appear to have been any bias in the time to treatment of patients, and procedures appear to have been consistently applied between the two sequential studies.



Comment:

The supplied dataset was examined with regards to the time from onset to when first contact with the medical system was made, and to when the patient arrived in the hospital emergency room for evaluation. This was limited to those patients that were listed in the screening log datafile (%). There was a much broader distribution of time from onset to arrival than for time to treatment. No significant amounts or patterns of discrepancies were seen in these comparisons. Most patients required more than 60 minutes between arrival at the hospital when the study treatment was initiated. The duration of screening was shorter in the 0-90min stratum than in the 91-180min stratum. As arrival time approached the time limits for the stratum (especially for the 0-90 min stratum) the screening duration became shorter, and was under 20 minutes for some of the patients in the 0-90 min stratum. It is notable that there are reportedly no patients assigned to the 0-90 min stratum who actually had the treatment infusion begin at minute 91 or 92.

RESULTS: PRIMARY EFFICACY ENDPOINTS

Efficacy within the Part 2 Study was prospectively defined as the 90 day outcomes. However, the Part 1 Study protocol did not have a primary efficacy endpoint identified. After subsequent discussions with CBER, the prospectively written analytic plan for primary and secondary analyses to be performed by Genentech was not fully executed,. While not formally written, an overview conception of the efficacy analyses, consistent with all prior discussions, is that the global endpoint analysis of the dichotomized outcome scales will be the primary efficacy endpoint for both studies. Of only somewhat less importance are the analyses of the individual outcome scales, both in the dichotomized and native forms. The Part 2 study is regarded as the primary efficacy study, with the Part 1 study providing a second trial, supportive evidence role. Analysis of the combined results of the studies was to be reserved for exploratory analyses of risk-benefit in subgroups.

The results are presented here for all of the focused-upon efficacy endpoints. These include the global analysis for the dichotomized outcomes, the individual scale dichotomized outcomes, and the individual scales in their native form.

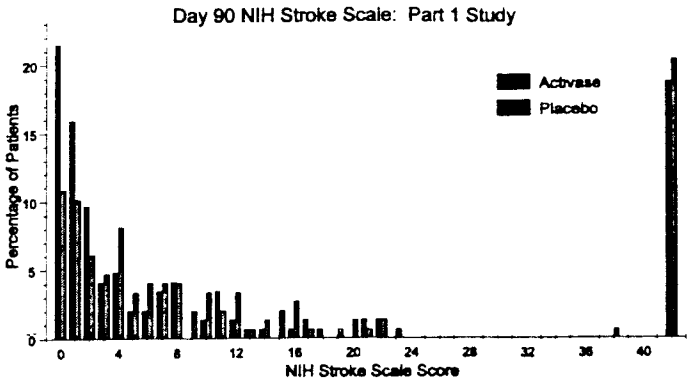
90 Day Outcome Assessment								
	Part 1 Study							
Outcome	Dichotomized Outcome ("Excellent Recovery")					Native Scale		
N	147	144				147	144	
	Placebo n %	Activase n %	%Recovery Difference	Relative Recovery[3]	p-value[1]	Placebo median	Activase median	p-value[2]
Global				1.44	0.005			
Barthel	57 (38.8)	78 (54.2)	15.4	1.40	0.010	75	95	0.016
Rankin	40 (27.2)	68 (47.2)	20.0	1.74	0.001	3	2	0.010
Glasgow	45 (30.6)	67 (46.5)	15.9	1.52	0.006	2	2	0.016
NIHSS	31 (21.1)	54 (37.5)	16.4	1.78	0.003	7	3	0.006
	Part 2 Study							
Outcome	Dichotomized Outcome ("Excellent Recovery")					Native Scale		
N	165	168				165	168	
	Placebo n %	Activase n %	%Recovery Difference	Relative Recovery[3]	p-value[1]	Placebo median	Activase median	p-value[2]
Global				1.34	0.018			
Barthel	62 (37.6)	84 (50)	12.4	1.33	0.024	65	92.5	0.064
Rankin	43 (26.1)	65 (38.7)	12.6	1.48	0.015	3	3	0.035
Glasgow	52 (31.5)	74 (44)	12.5	1.40	0.020	2	2	0.050
NIHSS	33 (20)	52 (31)	11.0	1.55	0.024	7	4.5	0.033

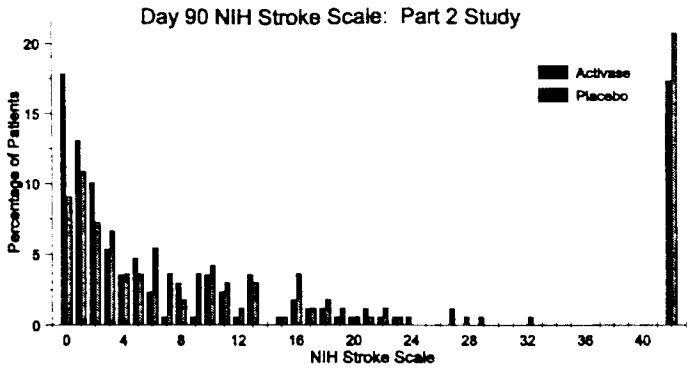
[1] p-value from GEE with log link; for univariate scales equivalent to CMH test

[2] p-value from Wilcoxon rank-sum test; all results in direction of benefit with Activase, including instances when median scores are identical (Rankin and Glasgow)

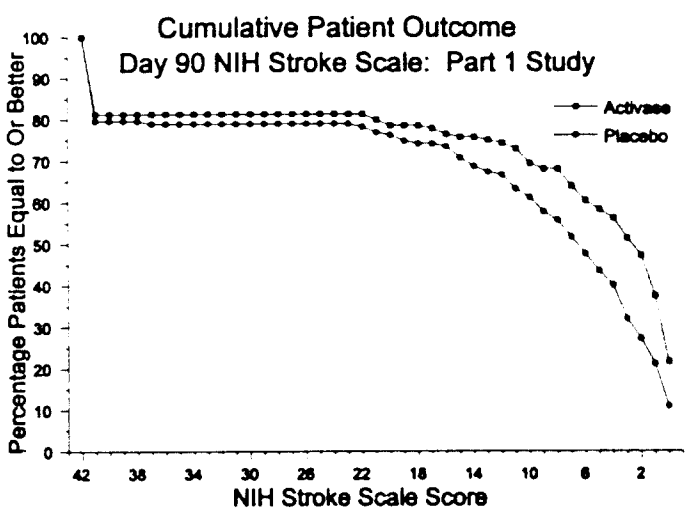
[3] Relative Recovery is the relative frequency of patients with outcomes meeting the "Recovery" criterion

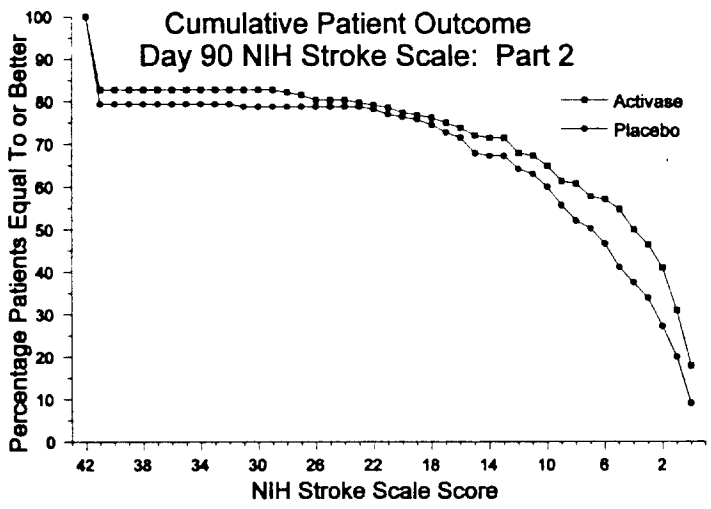
The distribution of the 90 day NIH-SS is represented in the following figures:





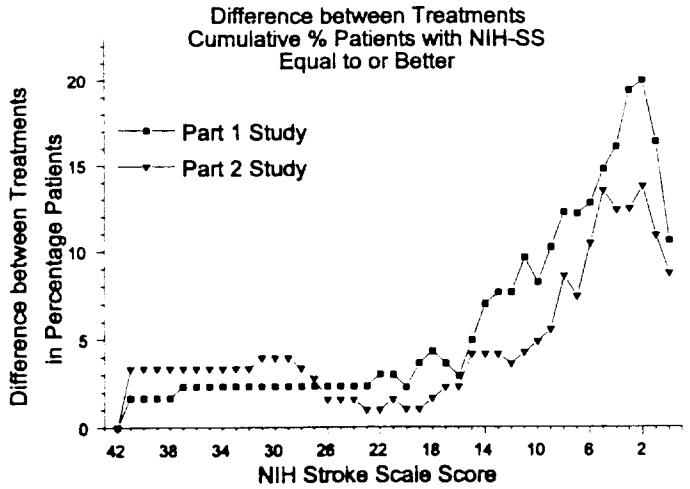
These figures illustrate the higher percentage of patients in the Activase groups with the best outcomes (scores of 0-2), and lower percentage with the worst score (42). The middle range of outcomes are unclear as to relative distributions. To better understand the treatment effects upon the groups, the cumulative NIH score was also examined. These figures display the percentage of patients with an outcome equal to or better than the plotted score. Thus, a higher curve describes a group with more patients distributed at better outcome scores. In comparing two curves, the higher curve identifies the group with better outcome than the other, and the area between the two curves a sense of the amount of benefit. In particular, these figures indicate that for the Part 1 study, 10% of the placebo patients had a score of 0, while 18% of the Activase patients did. Alternatively, 63% of the placebo patients had a score of 10 or better, while 73% of the Activase patients did. Note that the horizontal scale is drawn reversed from the prior figures, with better outcome scores to the right.



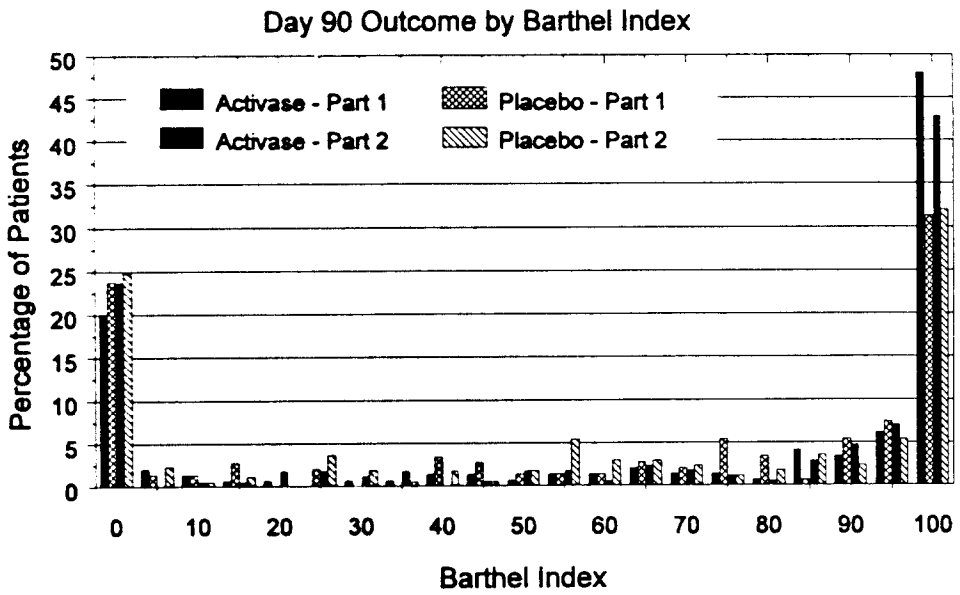


The following figure illustrates the differences between the cumulative score curves. This figure illustrates the difference in percentage of patients that would be defined as "recovering" associated with the Activase treatment if the NIH-SS was dichotomized at any specific score. When dichotomized at a score of 1 or better, the Part 1 study showed a treatment effect of approximately 16% of the patients benefiting. The maximum apparent benefit occurs when the

NIH-SS is dichotomized at 2 or 3, when the Part 1 study indicated approximately 20% of the Activase patients benefiting.

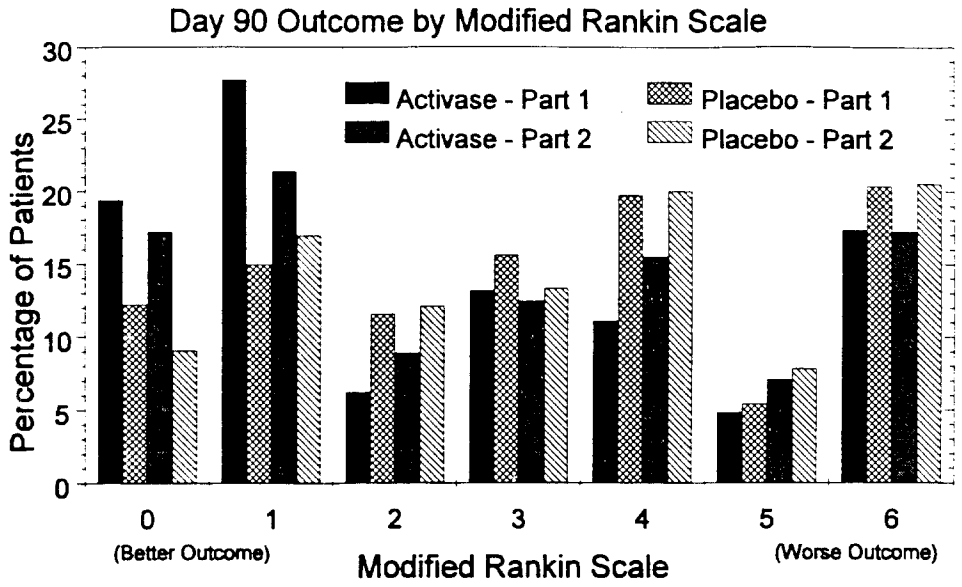


The following figure illustrates the 90 day Barthel Index outcome. The figure shows the greater number of patients in the Activase groups with outcome scores of 100, and fewer with outcomes of 0. This latter difference is quite modest for the Part 2 study. Also notable is that the Barthel Index is largely distributed between the single best and single worst scores. A minority of patients receive scores that distribute between these. This indicates that the Barthel Index will be relatively insensitive to smaller clinical changes.

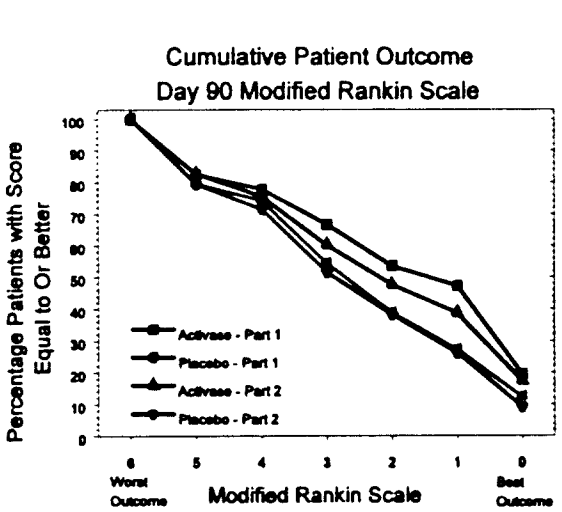


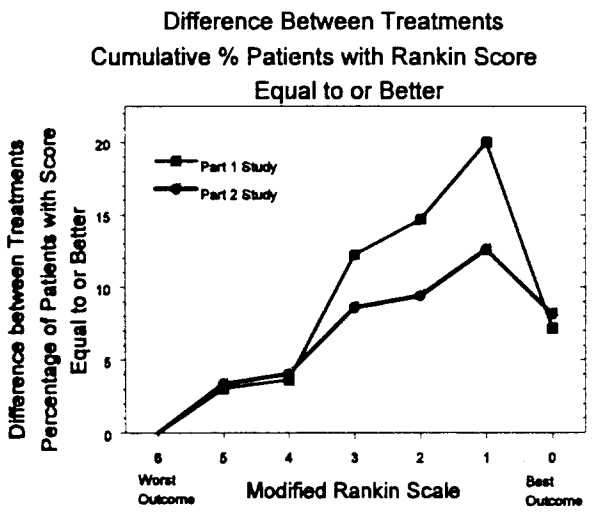
The day 90 Modified Rankin Scale outcome is shown in the following figure. Although there are relatively few categories in the Rankin Scale, the patients are broadly distributed across the available categories. This figure again illustrates the increased number of patients with the best of outcomes (scores of 0 or 1) and decreased number of patients with the worst outcome (score of

6; death). The consistency of the results between the two studies is also apparent.



The cumulative Rankin score was also examined to better understand the treatment effects. These figures display the percentage of patients with an outcome equal to or better than the plotted score. As before, a higher curve describes a group with more patients distributed at better outcome scores, and the area between two curves provides a sense of the amount of additional good outcomes. In particular, these figures indicate that for the Part 1 study, 12% of the placebo patients had a score of 0, while 20% of the Activase patients did. Alternatively, 39% of the placebo patients had a score of 2 or better, while 53% of the Activase patients did. Note that the horizontal scale is again drawn with better outcome scores to the right.

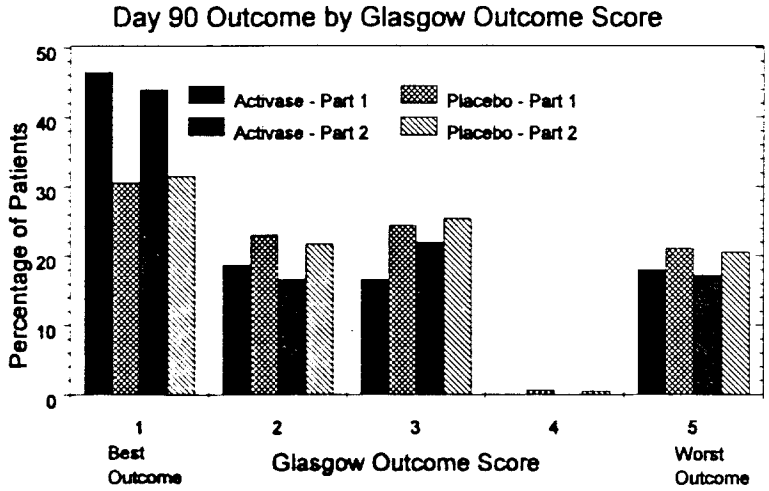




The figure showing the differences between the cumulative score curves indicates the change in percentage of patients that would be defined as "recovering" with the Activase treatment if the Rankin Scale were dichotomized at any specific score. When dichotomized at a score of 1 or

better, the Part 1 study showed a treatment effect of approximately 20% more of the patients with recovery. The maximum apparent treatment effect occurs when the Rankin Scale is dichotomized at 1. However, dichotomizing the Rankin Scale at any value consistently describes more "recovery" associated with Activase treatment, only the percentage of patients affected changes.

The Glasgow Outcome Score at 90 days shows a similar consistency of results between the two studies. Of note, the Glasgow Outcome Score is largely a 4 category scale for stroke patients. Very few patients enter a persistent vegetative state following a stroke.



RESULTS: OTHER ACTIVITY AND EFFICACY ENDPOINTS

Efficacy in the separate time strata

A prospectively stated subset analysis was defined by the time from stroke onset to initiation of treatment. This was considered to potentially be an important enough factor that the patient randomization was stratified on this basis. In both studies both time-to-treatment subsets (0-90 min, 91-180 min) showed better outcome with Activase. In the Part 1 study the later patients had a larger increase in the frequency of favorable outcome associated with Activase treatment than occurred in the earlier treated group, while this was reversed for the Part 2 study. This resulted in a similar amount of apparent treatment effect in the two subgroups when the combined studies dataset was examined (e.g. for the dichotomized Rankin scale, recovery percentages in the earlier patients of 28.3% placebo, 40.1% Activase, in the later patients 25.1% placebo, 45.2% Activase).

Comment:

Because of the highly nonuniform distribution of time to treatment among the patients, the combined studies dataset was used to divide the patients into four groups that might better reveal any disparity based on time to treatment. Patients were divided into groups by times of 0-85 min, 85-94 min, 95-170 min, and ≥171 min. Results were similar across the outcome assessments. The results with the Rankin Scale are shown in the following table.

90 Day Rankin Outcome Assessment - Time to Treatment Subsets							
Time to Treatment		Combined Studies Dataset					
	N	Dichotomized Outcome ("Recovery")			Native Scale		
	placebo : Activase	Placebo n %	Activase n %	%Recovery Difference	Placebo median	Activase median	p-value[2]
0-84 min	28 : 37	5 (17.9)	16 (43.2)	25.3	4	2	0.15
85-94 min	118 : 121	36 (30.5)	48 (39.7)	9.2	3	3	0.13
95-170min	118 : 99	26 (22.0)	48 (48.5)	26.5	"3.5"	2	0.001
> 170 min	48 : 55	16 (33.3)	21 (38.2)	4.9	3	3	0.98

This indicated increased numbers of patients with the recovery outcome in the Activase treated group in all of these post hoc subgroups. While the amount of treatment effect was least in the > 170 min subgroup of patients, there remained a trend of benefit in the dichotomized form of the outcome. This subgroup happened to have the best performing subset of the placebo patients, although there is no reason to expect any of the placebo subgroups to have different outcomes. Also importantly, there was no consistent trend in treatment effect across the time-based subsets. Furthermore, on division of the > 170min patients into two small groups (171-175 vs. > 175 min) the patients in the first 5 min of this subset had less treatment effect, while the patients treated within the last 5 min of the study's time window had larger treatment effects, again illustrating the absence of a consistent trend with time-to-treatment. Thus, there does not seem to be a major differential of efficacy with time to treatment within the 3 hour time window used in these studies.

Significant early improvement

The percentages of patients showing "early improvement", defined as an improvement of 4 points on the NIH-SS or complete resolution at 24 hours, was the primary endpoint for the Part 1 study, and was examined by Genentech. There was a trend to larger numbers of patients showing early improvement with Activase in both Part 1 and Part 2 studies (8.1% Part 1, 8.2% Part 2) which did not reach statistical significance. This remains solely an indicator of early activity, and is not an important definition of efficacy.

Improvement at 1 week

Improvement at 1 week (the day 7-10 evaluation) was also examined by Genentech. The Part 1 study indicated a larger percentage of patients with 5 points or more improvement on NIH-SS from baseline (48% placebo, 62% Activase, p = 0.02), while this was not seen in the Part 2 study (47% placebo, 45% Activase, p =0.80).

Deterioration

Deterioration was defined by the protocol as a worsening in NIH-SS by at least 4 points between hours 2 and 24. There were more patients in the Activase group who exhibited deterioration than in the placebo group in the Part 1 study (12% placebo, 18% Activase, p=0.21), while the reverse occurred for the Part 2 study (24% placebo, 17% Activase, p=0.15). Thus, overall, there was no

difference in percentage of patients with deterioration between the two treatment groups.

CT scan abnormalities in followup assessments

CT scans were scheduled to be performed at 1 day, 1 week, and 3 months after the stroke onset. CT abnormalities typical for acute changes of stroke occurred in many patients during this period.

CT Scan Readings									
	Part 1 Study			Part 2 Study			Combined Studies		
	placebo n (%)	Activase n (%)	p-value	placebo n (%)	Activase n (%)	p-value	placebo n (%)	Activase n (%)	p-value
N enrolled	147	144		165	168		312	312	
Any ICH	9 (6.1)	19 (13.2)		11 (6.7)	29 (17.3)		20 (6.4)	48 (15.4)	
24 Hour CT Scan									
Edema	102 (70.8)	75 (55.1)	0.007	103 (65.6)	101 (60.1)	0.307	205 (68.1)	176 (57.9)	0.009
Mass Effect	86 (59.7)	58 (42.6)	0.004	101 (64.3)	96 (57.1)	0.185	187 (62.1)	154 (50.7)	0.004
Midline Shift	10 (6.9)	12 (8.8)	0.559	12 (7.6)	20 (11.9)	0.198	22 (7.3)	32 (10.5)	0.165
Hyperdense Infarct	14 (9.7)	14 (10.3)	0.873	8 (5.1)	9 (5.4)	0.916	22 (7.3)	23 (7.6)	0.904
Hematoma	4 (2.8)	13 (9.6)	0.018	7 (4.5)	19 (11.3)	0.023	11 (3.7)	32 (10.5)	0.001
Ventricular Hemorrhage	1 (0.7)	5 (3.7)	0.085	1 (0.6)	8 (4.8)	0.024	2 (0.7)	13 (4.3)	0.004
SAH	0	3 (2.2)	0.073	1 (0.6)	2 (1.2)	0.602	1 (0.3)	5 (1.6)	0.103
Intraarterial Thrombus	38 (26.4)	15 (11.0)	0.001	38 (24.2)	30 (17.9)	0.160	76 (25.2)	45 (14.8)	0.001

Note: Percentages are of available CT scans with answers to each specific item
p-values calculated with Chi-Square test

CT scan abnormalities at the 1 week evaluation were similar to the 24 hour scan results in the relative distribution of abnormalities. These abnormalities, which are typical for the more acute stage of stroke, had largely resolved at the 3 month CT examination. The two studies are entirely in agreement in these findings as to treatment effects.

Comment:

Most notable in this information is the higher incidence of ICH in the Activase treated patients. Also evident is the lower incidence of edema and mass effect in the Activase treated patients. This is in spite of the larger numbers of patients with hematoma, who are likely to have mass effects based just on the volume of hematoma. This effect of treatment was most evident in the Part 1 study, with only a trend in the same direction evident in Part 2. Thus there is objective evidence in the CT scan changes for the presence of a treatment effect.

RESULTS: SAFETY

Mortality

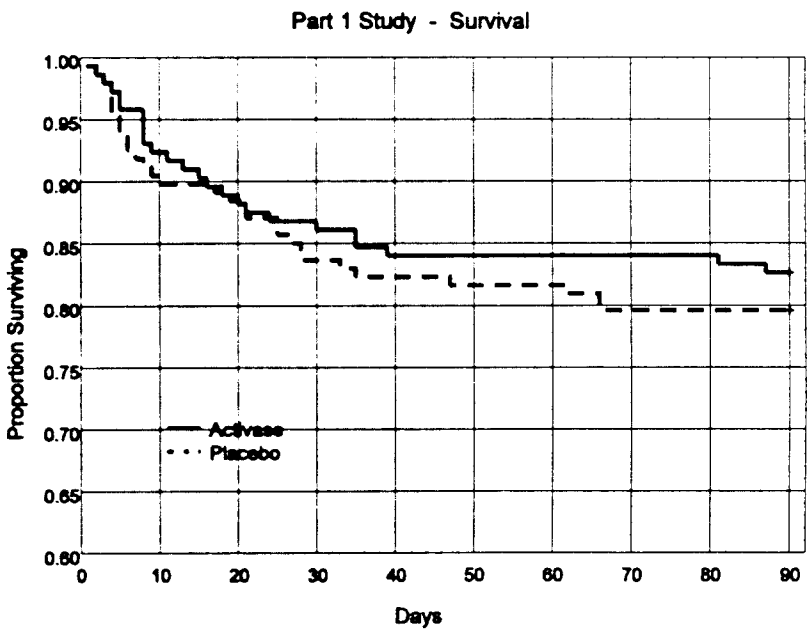
Mortality is an important safety parameter for this patient population. Mortality rates were examined in the two studies.

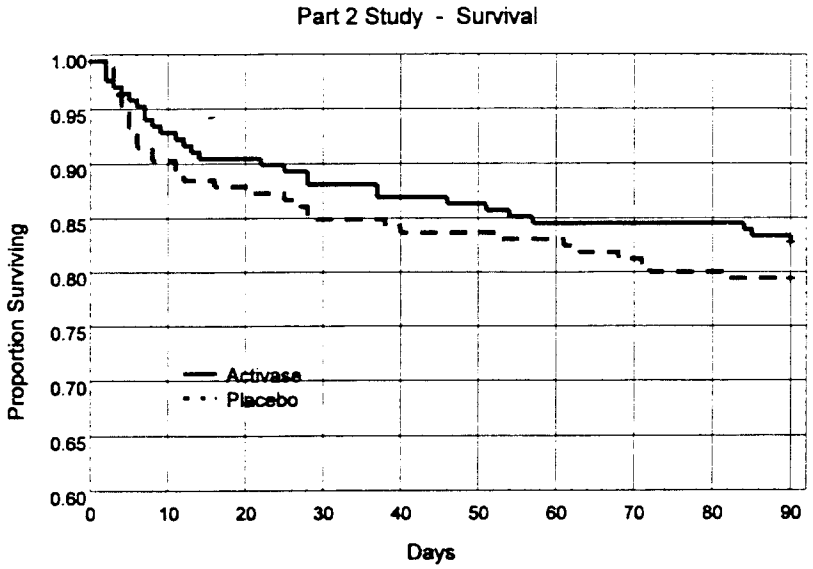
	Mortality Rates									
Timepoint	Part 1 Study					Part 2 Study				
	Placebo (n=147)		Activase (n=144)		p-value	Placebo (n=165)		Activase (n=168)		p-value
	N	%	N	%		N	%	N	%	
30 days	24	16.3	20	13.9	0.62	25	15.2	20	11.9	0.42
90 days	30	20.4	25	17.4	0.55	34	20.6	29	17.3	0.48
All avail. f/u	47	32.0	34	23.6	0.12	43	26.1	44	26.2	1.0

p-values by Fisher's Exact test

While there were no statistically significant differences between the treatment groups, there was a trend of lower mortality at 90 days in the Activase group that was seen in both studies.

Within the 90 day time frame the Kaplan-Meier curves also indicated weak trends towards increased survival with Activase treatment, without any excessive early mortality that might have been associated with the increased ICH incidence. The differences in the survival curves were consistent between the studies, but did not reach statistical significance (Part 1 study p = 0.505; Part 2 Study p = 0.424; Combined studies p = 0.296).





Intracerebral Hemorrhage (ICH)

Intracranial hemorrhage was determined according the previously described process. Occurrence of ICH was greater in the Activase group than placebo, as shown in the following table:

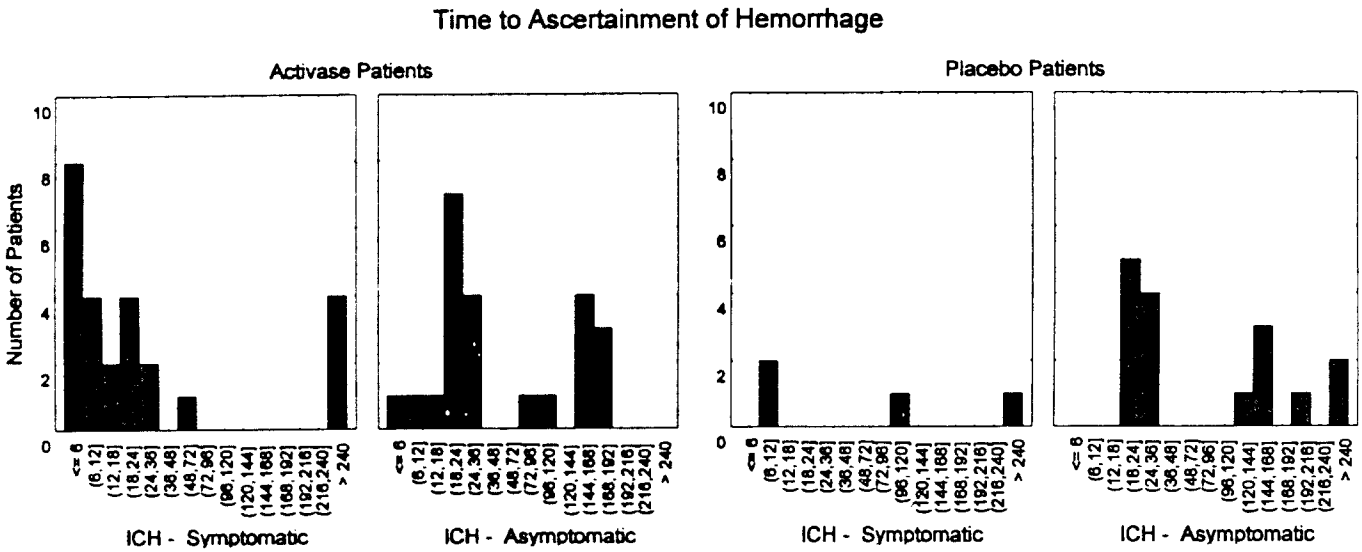
Intracranial Hemorrhage									
	Part 1 Study			Part 2 Study			Combined Studies		
	placebo n (%)	Activase n (%)	p-value	placebo n (%)	Activase n (%)	p-value	placebo n (%)	Activase n (%)	p-value
N enrolled	147	144		165	168		312	312	
All ICH	9 (6.1)	19(13.2)	0.04	11(6.7)	29(17.3)	0.003	20 (6.4)	48 (15.4)	<0.001
Symptomatic ICH	2 (1.4)	11 (7.6)	0.01	2 (1.2)	14 (8.3)	0.003	4 (1.3)	25 (8.0)	<0.001
Asymptomatic	7 (4.8)	8 (5.6)	0.80	9 (5.4)	15 (8.9)	0.29	16 (5.1)	23 (7.4)	0.32
ICH Within 36 hours of Treatment	3 (2.0)	13 (9.0)	0.009	8 (4.8)	21 (12.5)	0.01	11 (3.5)	34 (10.9)	<0.001
Symptomatic ICH	0	8 (5.6)	0.003	2 (1.2)	12 (7.1)	0.011	2 (0.6)	20 (6.4)	<0.001
Fatal Symp. ICH	0	5		1	4		1	9	
Asymptomatic ICH	3 (2.0)	5 (3.5)	0.46	6 (3.6)	9 (5.4)	0.45	9 (2.9)	14 (4.5)	0.29

NOTE : Fatal Symptomatic ICH does not include patient# ——— who is reported as a death from a recurrent ICH which occurred 3 mo after first event. The table also does not include 3 ICH events occurring prior to treatment.
p-values from Chi-Square test

As previously noted, there were three patients who were enrolled into the studies and

subsequently noted to have pre-existing hemorrhage on the baseline CT scan. All three of these patients received Activase, and one of these patients subsequently had enlargement of the hemorrhage, further neurological decline, and death related to brain edema and herniation. The other two patients had lesser hemorrhages at enrollment, and did not appear to have any clinically significant deterioration subsequent to Activase treatment.

The time course of occurrence of the ICH is of interest in understanding the risks of this therapy. The time of ascertainment of hemorrhage is likely to be near the time of the event occurrence for symptomatic hemorrhages, but for asymptomatic hemorrhages will largely be dependent upon the scheduled CT scans. ICH did not occur with uniform risk per time within the Activase arm, as may be seen in the following figures of when hemorrhages were ascertained. Note that the time duration encompased by each bar in the figures are not all equal (ranging from 6 hour increments early to 24 hour increments later).



Asymptomatic hemorrhages were largely ascertained at the time of the 24 hour CT scan. A small additional number were ascertained at the succeeding scheduled CT scan obtained sometime between days 7 to 10. Symptomatic hemorrhages occurred with a decreasing frequency with time in the Activase arm. There is an apparent change in the differential incidence of hemorrhage between the two groups at approximately 36 hours after the stroke onset. While the Activase group has markedly more hemorrhages than the placebo group before 36 hours (20 symptomatic, 14 asymptomatic in Activase patients, 2 symptomatic, 9 asymptomatic in placebo patients) after 36 hours the incidences are more similar (5 symptomatic, 9 asymptomatic in Activase, 2 symptomatic, 7 asymptomatic in placebo patients). Therefore, the increased hemorrhages related to Activase treatment appear to manifest themselves largely within 36 hours of treatment.

Systemic Hemorrhage and Transfusion

Systemic bleeding events were also higher in the Activase group. However, serious adverse events of hemorrhage, while increased in frequency, did not reach statistical significance.

Systemic Bleeding									
	Part 1 Study			Part 2 Study			Combined Studies		
	placebo n (%)	Activase n (%)	p-value	placebo n (%)	Activase n (%)	p-value	placebo n (%)	Activase n (%)	p-value
Any Bleeding	33 (22.4)	71 (49.3)	<0.001	71 (43.0)	97 (57.7)	0.007	104 (33.3)	168 (53.8)	<0.001
Serious Bleeding	1 (0.7)	4 (2.8)	0.169	4 (2.4)	3 (1.8)	0.685	5 (1.6)	7 (2.2)	0.560

p-values calculated with Chi-square test

The numbers of patients receiving transfusions during the study was also examined.

Patients Receiving Transfusions During Studies									
	Part 1 Study			Part 2 Study			Combined Studies		
	placebo n (%)	Activase n (%)	p-value	placebo n (%)	Activase n (%)	p-value	placebo n (%)	Activase n (%)	p-value
Any type transfusn.	5 (3.4)	13 (9.0)	0.047	8 (4.8)	20 (11.9)	0.021	13 (4.2)	33 (10.6)	0.002
RBC transfusions	5 (3.4)	7 (6.9)	0.371	7 (4.2)	13 (7.8)	0.334	12 (3.8)	20 (6.4)	0.187
1-2 U RBC	3 (2.0)	3 (2.1)		4 (2.4)	9 (5.4)		7 (2.2)	12 (3.8)	
>2 U RBC	2 (1.4)	4 (2.8)		3 (1.8)	4 (2.4)		5 (1.6)	8 (2.6)	
Plasma transfusion	1 (0.7)	5 (3.5)	0.077	0 (0)	5 (3.0)	0.030	1 (0.3)	10 (3.2)	0.006
1-2 U Plasma	1 (0.7)	3 (2.1)		0 (0)	1 (0.6)		1 (0.3)	4 (1.3)	
>2 U Plasma	0 (0)	2 (1.4)		0 (0)	4 (2.4)		0 (0)	6 (0.9)	
Cyropecipitate transfusn.	0 (0)	6 (4.2)	0.025	0 (0)	4 (2.4)	0.046	0 (0)	10 (3.1)	0.005
Platelet transfusions	0 (0)	3 (2.1)	0.104	0 (0)	5 (3.0)	0.033	0 (0)	8 (2.6)	0.008

p-values using Mantel-Haenszel Chi-Square

There were significantly more patients in the Activase group than placebo that required transfusions with the various kinds of transfusion products. However, the actual numbers of patients requiring transfusions were relatively few.

Other Adverse Events

Genentech has supplied a datafile of all adverse events reported by the investigators. The classification system used for organizing this information was not COSTART or any other widely utilized system, so that the summary incidence counts are not meaningful to compare either between treatment arms or to medically expected rates of specific types of events. The specific descriptions of the events were surveyed, and do not reveal an obvious incidence of unexpected types of events given the clinical setting of acute stroke, a generally elderly population, and the administration of a thrombolytic agent to half the patients.

The risks of thrombolytic agents are understood in general based upon the extensive use of these agents in clinical practice, particularly for the treatment of acute myocardial infarction. This setting treats a patient group that can be expected to be similar in general prior medical characteristics, has experience in a far larger number of patients than the 312 patients treated in these studies. Additionally, the dosage of Activase is larger than that studied in acute stroke (see pg 7). Thus, general adverse events can be expected to be well understood in acute ischemic stroke based on prior experience with Activase in acute MI patients.

RESULTS: EXPLORATORY ANALYSES OF EFFICACY AND SAFETY

Analysis of Efficacy Endpoints with Covariates

No covariates for the analysis of the efficacy endpoints were specified prospectively. Exploratory analyses were conducted by both Genentech and CBER to examine the effects that any imbalances in the randomized assignments on the dichotomized efficacy outcome results.

Genentech approach to selection of Covariates

Genentech submitted analyses of the dichotomized efficacy outcomes with covariate adjustment. The selection of *post hoc* covariates was based on the size of the imbalance between the groups in the demographics and baseline parameters. The criterion for imbalance was a test of homogeneity with a p-value threshold of <0.05; however, some variables with p-values of 0.10 - 0.05 were also included. For many continuous or multi-value parameters Genentech created groups of patients based upon a categorizing of these parameters in 2-3 ordinal values.

For the Part 1 Study, prior aspirin use, and weight-category were examined for importance. The weight-category variable had a greater influencing effect in the model of the global outcome test, with a p-value of 0.144 as a predictor. Incorporating both variables into the efficacy analysis as covariates did not effect the results. The odds ratio for the global outcome test remained at 2.06, but the p-value changed from 0.001 without covariates to 0.002 with the covariates. The odds ratios of the univariate scales were also only minimally affected.

For the Part 2 Study, prior aspirin use, smoking history, age group category, diastolic BP > 85, and baseline PT > 12 were examined. Only the age group category variable had a significant effect upon the global test in the model (p=0.001). Incorporating all 5 variables as covariates into the efficacy analysis altered the odds ratio slightly. The odds ratio for the global outcome

test changed from 1.71 (p=0.009) without covariates to 2.23 (p<0.001) with covariates. The univariate scale odds ratios changed similarly.

This analysis was also conducted for the combined study data. Parameters of prior aspirin use, weight category, age category, NIHSS baseline category, PT baseline category, and stroke subtype were used as covariates. The results of the global statistic test with covariates was changed moderately from 1.87 without covariates, to 2.07 with covariates (p<0.001 both cases). The univariate scales were similarly affected. Only age category and baseline NIHSS category had important effects on outcome (p<=0.001) for the global statistic.

CBER approach to covariate analysis

The CBER Biostatistian also performed covariate adjusted analyses of the two studies. Based on the medical literature, age and baseline stroke severity (represented as the baseline NIH-SS) were utilized as covariates, as well as prior aspirin usage, due its prospective selection as a potentially important factor in the protocol, and its relatively large imbalance between the treatment groups. A logistic model was utilized for each of the univariate dichotomized outcome scales. Age and baseline NIH-SS were incorporated as continuous variables, rather than the categorized forms utilized in the Genentech analysis. Odds ratios for recovery and p-values for the odds ratios were calculated for each of the univariate scales for both of the studies. In all cases treatment remained a statistically significant predictor of outcome. In general, the odds ratios were slightly larger with the covariate adjustment than without, and the p-values were slightly larger. In almost all cases age and baseline NIH-SS were also statistically significant predictors of outcome. In all cases, prior aspirin usage was not statistically significant as a predictor of outcome.

Outcomes by centers

Genentech performed analyses of the effect of clinical center on the results of the dichotomized scales. Judged by the global test statistic, treatment by center interaction was seen to occur in the Part 1 study (p=0.073). The Barthel Index outcome was as sensitive, while the strength of the interaction was less for the Rankin and Glasgow scales (p=0.126-0.128), and the NIHSS (p=0.313). Two centers (Henry Ford Hospital and U. Tennessee) favored placebo, while 5 centers favored Activase and one favored neither. As the two centers favoring placebo contributed relatively few patients to the Part 1 study (38 of 291, and U. Tennessee was the smallest clinical center, enrolling only 10 patients) the Part 1 study continued to show significant treatment effect with Activase even when the center effects are included.

The treatment by center interaction analysis did not indicate any significant interaction for the Part 2 study (p=0.53), nor for the combined dataset of the studies (p=0.23). As the centers that participated in the studies were largely the same between the Part 1 and Part 2 studies, an important interaction between treatment effect and investigative center was not likely.

Intent-to-Treat Sensitivity Analysis

As described above the investigator group and manufacturer have performed an "as treated" analysis of the data. CBER requested an intent to treat analysis also be performed, as a sensitivity analysis. The definition of what should constitute an actual "intent to treat" group was not a simple issue (see Randomization section above, pg 18). The randomization list was prepared in advance, and the errors that occurred of skipped-over ID codes affected the order in which study kits were assigned for a cluster of several patients each time an error occurred for the out-of-sequence patients, and for all succeeding patients for the wrong-stratum patients. The "intent to treat" group was defined considering only the patient in whom the error occurred, not the subsequent few who received different ID numbers than they would otherwise have. This analysis was performed by changing the treatment group for the 7 patients in the Part 1 study who received placebo due to use of a patient ID# out-of-order, and the 4 similar patients in the Part 2 study. The patients who may have received the wrong treatment due to the wrong-stratum ID# were not included. This produced a small inequality in the overall number of patients assigned to each treatment group.

Patients with Recovery Outcomes in Intent to Treat Analysis

	Part 1 Study				Part 2 Study			
	placebo n (%) (n= 140)	Activase n (%) (n=151)	Odds Ratio	p-value	placebo n (%) (n=161)	Activase n (%) (n=172)	Odds Ratio	p-value
Global statistic			2.30	<0.001			1.68	0.012
Barthel Index	52 (37.1)	83 (55.0)	2.07	0.003	61 (37.9)	85 (49.4)	1.60	0.035
Rankin	35 (25.0)	73 (48.3)	2.81	<0.001	42 (38.4)	66 (38.4)	1.76	0.017
Glasgow	40 (28.6)	72 (47.7)	2.28	0.001	51 (31.7)	75 (43.6)	1.67	0.025
NIH-SS	28 (20.0)	57 (37.7)	2.43	0.001	32 (19.9)	53 (30.8)	1.80	0.023

p-values using GEE with logit link (equivalent to CMH for univariate outcomes)

This altered the results only slightly quantitatively, by slightly increasing the apparent effect of Activase treatment compared to the results of the prior analysis. Thus, the use of the "As Treated" dataset did not appear to introduce any bias in favor of Activase.

Consequences of Intracranial hemorrhage

Comment:

Intracranial hemorrhage has long been regarded as the single most important risk of thrombolytic therapy of acute ischemic stroke. A first step to evaluating the safety aspects of this proposed therapy is to evaluate the consequences of ICH. In particular, is occurrence of ICH in these studies associated with poor outcome (or death)? If so, is this true for both the ICH ascertained due to the acute decline in neurologic status (symptomatic ICH) and for the ICH ascertained only incidentally on the scheduled CT scans (asymptomatic ICH)?

The outcome distribution of patients with ICH was compared to patients without ICH using the Rankin Scale. Due to the relatively small number of ICH events, this was examined only for the combined studies dataset.

Outcome of Patients with ICH						
	Activase			Placebo		
	ICH	No ICH	p-value	ICH	No ICH	p-value
N	48	264		20	292	
Recovery by Rankin	5 (10.4%)	128 (48.5%)	<0.001[1]	1 (5%)	82 (28.1%)	0.02[1]
Median Rankin	5	2	<0.001[2]	4	3	<0.01[2]
Mortality - all ICH	23 (47.9%)	31 (11.7%)	<0.001[1]	5 (25%)	59 (20.2%)	0.61[1]
Mortality-Symptomatic ICH	15 (60%)	n.a.		2 (50%)	n.a.	
Mortality Asymptomatic ICH	8 (34.8%)	n.a.		3 (18.8%)	n.a.	
Mortality Symptomatic ICH within 36 hours	15 (75%)	n.a.		1 (50%)	n.a.	

1 p-values from Chi-Square test
2 p-values from Rank-sum test

Thus, patients who had an ICH generally had a worse outcome than patients who do not experience ICH. The baseline characteristics of the ICH vs. nonICH patients were not identical, but were not extensively compared due to the small sample size of the ICH patients. In general, ICH patients in this study (and the literature) tended to be older and have more severe strokes at baseline. Thus, from this analysis it was not possible to identify ICH as a completely independent predictor of poor outcome.

The type of ICH (symptomatic vs. asymptomatic) was also evaluated. This analysis was limited to the Activase patients, as there were too few symptomatic hemorrhages in the placebo group. There was no difference between the two types of hemorrhage in percentages who achieved the "good recovery" outcome (2 of 25 symptomatic ICH, 3 of 23 asymptomatic ICH by Rankin Score, $p = 0.46$, Fisher's Exact test). The outcomes were further examined by the full ordinal range of Rankin Scale, which indicated a worse outcome with symptomatic hemorrhages ($n = 25$) than asymptomatic ICH ($n = 23$) in the Activase group ($p = 0.08$, Rank-sum test). This difference in outcome was also true for ICH occurring within 36 hours of treatment ($p = 0.02$). Rank-sum statistics utilizing the NIH-SS or Barthel Index outcomes produced similar results. Asymptomatic ICH patients had worse Rankin Score outcomes than non-ICH patients ($p < 0.001$, rank-sum test, $n = 23$ asymptomatic ICH, 264 non-ICH). Thus, although the asymptomatic patients had better outcomes than the symptomatic ICH, they still had lesser amounts of recovery than the patients without ICH. Therefore, both symptomatic and asymptomatic ICH are an important

safety concern to include in further exploratory analyses regarding the safety of Activase treatment as concerns the incidence of ICH.

Genentech exploratory analyses of patient subgroups for treatment interaction effects

Genentech performed exploratory analyses for patient subgroups that may not derive benefit from Activase. Statistical models of baseline and demographic parameters were examined to identify factors that could predict response to treatment in the combined studies dataset, due to its larger pool of patients. Diabetes was found to have a strongly interactive term with treatment, CHF and race had moderately interactive terms, while age had interaction effects with treatment only for the Rankin and Glasgow outcomes.

History of Diabetes Mellitus

Overall 21% of the patients were diabetic. The global statistic odds ratio (OR) for recovery with Activase treatment in patients with diabetes was 1.02, with similar ORs for the univariate scales. The p-value associated with the diabetes-by-treatment interaction was 0.04, and the interaction p-values were 0.02-0.07 for the Barthel, Rankin and Glasgow outcomes. Genentech examined the diabetes patients for balance in other characteristics. The diabetes subset showed more imbalance in age then the overall population. The Activase patients were older than the placebo diabetes patients. Baseline NIH-SS was slightly imbalanced, but in a reverse manner to the overall trend; the Activase diabetes patients have slightly more severe strokes than the placebo diabetes patients. Other factors were balanced between the two patient subgroups. When patient age category and baseline NIH-SS were added as covariates to the model, the interaction coefficient declined from -0.78 without the covariates, to -0.41 with the covariates, and the p-values for the interaction with treatment based on the global statistic became 0.32 (0.09-0.23 for the Barthel, Rankin and Glasgow univariate scale outcomes). Genentech suggested that this eliminates concern for diabetes as a subgroup with significantly different efficacy with Activase treatment.

History of Congestive Heart Failure

Overall 16% of the patients have a history of CHF, and the OR for recovery in the CHF patients is 1.03 for the global statistic, and similar in 3 of the univariate scales (recovery by NIH-SS has an OR of 1.5, but with a very wide confidence interval). The p-values for the CHF-x-treatment interaction effect is 0.13 using the global statistic, and 0.03-0.15 for the Barthel, Rankin and Glasgow outcomes. When age and baseline NIH-SS were added to the model as covariates, the coefficient of interaction using the global statistic changed slightly from -0.664 without covariates to -0.549 with covariates. However, the p-value associated with the interaction changed to 0.22 for the global statistic, and 0.04-0.24 for the univariate Barthel, Rankin, Glasgow outcomes. Thus, Genentech suggests that CHF was not an important treatment effect modifier.

Differential efficacy based on racial demographics

Overall 65% of the patients were white, and the OR for non-white patients (as a whole) is 1.28, less than for the white patients, with the p-value of the interaction 0.09 for the global statistic. There were no important imbalances between these two subgroups in other factors that might account for the differential efficacy. When race was re-categorized into the finer distinctions of

white (n= 205 Activase, 198 placebo), black (n= 89 Activase, 80 placebo), Hispanic (n= 18 Activase, 19 placebo) (with elimination of 13 patients that were either Asian (8) or other), the OR using the global statistic changed slightly to 1.36, while the Hispanic subgroup had an OR of 0.38 for the global statistic. The p-value for the treatment interaction effect for black patients increased to 0.28 for the global statistic. Genentech suggests this eliminates concern for an important interaction leading to loss of efficacy in the black patients.

The Hispanic patients were imbalanced in both age (Activase patients older by a mean 7 years) and baseline NIH-SS (Activase patients more severe strokes by a mean of 3 points). Genentech suggested that this may account for the apparent loss of efficacy in this small subset.

Genentech concludes that there are no important treatment interaction effects based on patient demographics or baseline characteristics.

CBER Exploratory Analyses for Differential Efficacy

Note: All analyses of this section, pg 49-58, were performed by CBER.

CBER conducted a screening review of the results obtained in the dataset of the combined studies to identify subgroups of patients with results different from the overall results for further in depth review. Outcome on the dichotomized Modified Rankin Scale was utilized for this screening process (see Appendix B). Reliance on p-values was not used in the screening review for selection of subgroups of interest. Rather, the size of the treatment effect was examined, and any subgroup that had a point estimate of only 1/3 of the overall efficacy, either in absolute or relative terms, was selected for further examination. For purposes of this process, the parameters of age, weight and stroke severity (baseline NIH-SS) were categorized into approximate quintiles of patients.

The screening review involved a large number of subgroups. Concern for the meaningfulness of differences identified through multiple subset comparisons should be borne in mind. When large numbers of subsets are examined, it is statistically expected that some will exhibit results different from the study as a whole.

The purpose of the screening review was to identify subgroups with fewer good outcomes that warranted further examination, comparing demographic and baseline balance as well as outcome on the other scales, in both dichotomized form and rank comparisons of the full ordinal scale, to either strengthen or alleviate the concern of differential effect. From this screening the following parameter-based subgroups were identified for further consideration:

Efficacy in Selected Patient Subgroups: Recovery based on Rankin Scale								
Grouping Factor	Criterion	Placebo		Activase		Treatment Effect		
		Number: recover total	% recovery	Number: recover total	% recovery	% patients with benefit	Relative Benefit	p-value
All Patients		83 312	26.6	133 312	42.6	16	1.60	<0.001
Prior Stroke	History of	13 38	34.2	17 45	37.8	3.6	1.10	0.737
Prior TIA	History of	18 49	36.7	21 50	42.0	5.3	1.14	0.593
Diabetes	Pre-existing	18 63	28.6	21 68	30.9	2.3	1.08	0.773
CHF	Pre-existing	16 55	29.1	11 44	25.0	-4.1	0.86	0.651
Valvular Heart Disease	Pre-existing	7 19	36.8	11 25	44.0	7.2	1.19	0.636
CT: Intravasc. Thrombus	visualized in baseline CT	7 43	16.3	5 33	15.2	-1.1	0.93	0.894
Age by group	77-100 yrs (oldest)	14 55	25.5	16 71	22.5	-2.9	0.89	.702
Race	Hispanic	6 18	33.3	5 19	26.3	-7.0	0.79	0.642

14 patients with ICH after 36 hours are excluded from this table
p-values based on test of Relative Risk by z-score

Two additional subgroups chosen for further examination. Patients with a history of aspirin usage within the two weeks prior to the stroke was included due to the prospective identification of this subgroup for analysis. Stroke severity prior to treatment was also included due to this parameter's previously identified importance as a predictor of outcome. These subgroups were examined for a broader range of the evaluated outcomes; dichotomized recovery on the NIH-SS and Barthel Index (along with the Rankin outcome as above), and rank comparisons of the full ordinal scales. Mortality and ICH were also examined. The more detailed results are listed in Appendix D.

Of note, when outcomes were examined by quintile groups based on patient weight, all quintiles of patients appeared to have improved outcome associated with Activase treatment. There was no consistent differential trend across the groups in percentage of patients with "recovery" outcome nor in mortality risk. There were also no significant gender differences in either recovery outcome or ICH incidence.

The more detailed examination of subgroup results discussed in the following sections are contained in Appendix D.

Subgroups of Prior Stroke, TIA History, Valvular Heart Disease, Prior Aspirin Usage.
The patient subgroups determined by patients with a prior stroke, a history of TIA, valvular heart disease, or use of aspirin within the two weeks prior to the acute stroke, all show improved outcome with Activase treatment. Genentech and the investigator group identified slightly different sets of patients for the subgroup of prior aspirin usage (175 patients by Genentech, 216 by the investigator group). Review of the relevant medication histories indicated that NSAID medications were included by the investigators within this subgroup but not by Genentech. The results of all analyses, for both efficacy and safety, were qualitatively the same for both subgroupings. Because the NSAIDs may have similar mechanisms and effects as aspirin itself, the CBER analyses were conducted with the investigator-identified patient subgrouping that includes NSAID usage.

The trend of better outcome in these subgroups is consistent across all 3 outcome scales in the dichotomized form as well as on the full ordinal scale when examined by rank sum score. Although the associated p-values are generally well above 0.05, these analyses of small groups have little statistical power. Also, while the increased risk of ICH is apparent in all of these subgroups, this has limited impact upon outcome as a whole for each of the subgroups. The efficacy trend was maintained and mortality in these patients trended in most of the subgroups towards lower mortality with Activase. Only the aspirin-use subgroup failed to show this trend, where death rates were approximately the same in the Activase and placebo subgroups. Thus, while the data were not capable of demonstrating efficacy in these subgroups independently, there appeared to be no evidence of an unfavorable risk-benefit comparison in these patient subgroups.

Severity of Stroke
Distinguishing patients based on the severity of the stroke (defined as the NIH-SS at baseline) also did not raise concerns for an absence of benefit in these subgroups (see Appendix D). Of note, on the dichotomized recovery outcomes, patients with the least severe strokes had reduced amounts of treatment effect. This may be a limitation of the recovery dichotomization procedure in these patients where many are expected to nearly fully recover. However, all quintile groups showed favorable treatment effects on all three of the dichotomized recovery outcomes, as well as favorable trends on the rank sum comparisons of the native ordinal scales.

The quintile of patients with the most severe strokes showed a reduced amount of treatment effect; the additional percentage of patients reaching the recovery criteria with Activase is relatively small (4-6%). This group showed markedly increased rates of ICH with Activase administration, and increased mortality, in contrast to the trend toward lower mortality in the study overall. Thus, this most severe stroke subgroup appeared to have a less favorable risk-benefit comparison than the overall study population.

Diabetes Mellitus
Patients with diabetes constituted 21% of the study overall (68 Activase, 63 placebo), and raised concerns about an unfavorable risk-benefit comparison with Activase. In the recovery-dichotomized outcomes, the Activase patients did better than placebo only on the NIH-SS, and have fewer patients with recovery on the Barthel Index Recovery outcome (32.4% Activase, 36.5% with placebo). Reviewing the full ordinal scale showed that the Activase-treated patients

had small trends to worse outcome than placebo on both the Rankin Scale and Barthel Index, while the NIH-SS was not different.

ICH was markedly increased in these patients with Activase administration, while for diabetic placebo patients ICH was only slightly increased over the rate for the non-diabetic patients. However, mortality with Activase was not increased above the rate with placebo. In spite of the larger number of ICH events in this subgroup, it did not appear that ICH was the driving mechanism for the difference in outcome. When the patients who had ICH within 36 hours of treatment (9 Activase, 4 placebo) were removed from consideration, the same differentials in percentage recovery remained. Neither were there any favorable trends in comparing the full ordinal scales.

Examination of the baseline characteristics of these patients revealed that the patients with diabetes who received Activase were older, had slightly more severe strokes, and may have slightly more pre-existing disability than the placebo diabetes patients (mean baseline NIH-SS 15.38 Activase, 14.1 placebo; mean age 69.2 Activase, 65.5 placebo, history of pre-existing disability 14.7% Activase, 11.1% placebo; and mean estimated pre-stroke Rankin Score 0.51 Activase, 0.37 placebo). To examine if these may influence the comparison between the two subgroups, a comparison was made of all patients with adjustment for covariates of age and baseline NIH-SS (both treated as continuous variables, not categorized) and history of diabetes using a General Linear Model (GLM) using the full ordinal Rankin Scale outcome. While history of diabetes was a significant predictor factor ($p<0.01$) for the model, an interaction between diabetes and treatment was not significant ($p=0.22$). Thus, even in spite of the noticeably increased risk of ICH, there was no evidence that this subgroup had a markedly unfavorable risk-benefit comparison.

History of Congestive Heart Failure

Patients with CHF comprised 15.9% of the study population (44 Activase, 55 placebo). The CHF patients treated with Activase had lesser percentages of recovered patients than the CHF patients in the placebo group for both the Barthel Index (-0.5%) and the Rankin (-4.1%), while the NIH-SS recovery-dichotomized outcome did indicate more recovered patients (+6.8%) with Activase. All three of the full ordinal scales showed slight trends in the direction of worse outcome with Activase. In addition, the mortality rate was higher with Activase in this subgroup of patients (36% Activase, 29% placebo). None of these trends were statistically significant, but the power to demonstrate effects was low.

Few of these patients who died had ICH prior to 36 hours (3 of 16 Activase, 2 of 16 placebo), so that even after exclusion of patients with ICH within 36 hours, the trend of higher mortality remained. Comparison of the baseline characteristics showed that the Activase patients with CHF had similar severity of stroke (mean baseline NIH-SS 17.2 Activase, 16.5 placebo), but were older (mean age 71.2 Activase, 67.7 placebo, and had more pre-stroke disability; mean estimated pre-stroke Rankin Score was 0.73 Activase, 0.42 placebo).

When the GLM method with the full ordinal Rankin Score and covariates of baseline NIH-SS, age, and history of CHF, was applied, it indicated that history of CHF was not a significant predictor of outcome ($p=0.22$). There was a non-significant, but possibly suggestive interaction between history of CHF and treatment ($p=0.18$). The estimated adjusted mean outcome Rankin

Score for these patients was shifted to indicate no difference (with adjustment 3.1 Activase, 3.1 placebo; without covariate adjustment: 3.6 Activase, 3.4 placebo). Thus, the amount of improved outcomes associated with Activase treatment in these patients was uncertain, but there was no strong evidence that they were qualitatively different than the patients without a history of CHF, nor that the overall outcome was toward worse degrees of disability. The increased mortality rate remains disturbing, however.

Visualization of Intravascular Thrombus on CT Scan

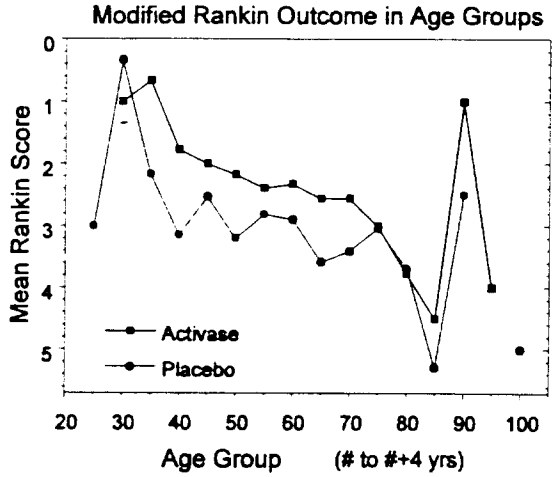
These comprise 12.2% of the patients overall, and were more prevalent in the placebo group (33 Activase, 43 placebo). While these patients showed an increase in the percentage recovered using the NIH-SS, the treatment effect was modest for the Barthel Index, and trended slightly towards worse outcome for the dichotomized Rankin Scale. The comparison based on the full ordinal scales was consistent between the scales; it trended slightly to worse outcome for all three scales. The trend in incidence of ICH was only 5% higher in the Activase patients, less than that seen overall. Mortality trended to lower with Activase. This subgroup also showed a trend for imbalance in baseline NIH-SS and age (more severe strokes in the Activase subgroup and older). When the GLM comparison was performed with covariates, the presence of thrombus on baseline CT scan showed a trend towards significance in predicting outcome ($p=0.11$), but no interaction between treatment and CT visualized thrombus was supported ($p=0.66$). Thus, while uncertainty remains as to the amount of improved outcome associated with Activase in these patients, there was no evidence of an unfavorable risk-benefit comparison.

Patient Age

Previous analyses showed patient age to be a significant predictor of outcome, both for the dichotomized scales and the full ordinal scales. Older patients tended to have worse outcomes in both treatment groups. This effect is not unexpected, having been previously described in the medical literature. An increase in incidence of ICH with increasing age, both with and without thrombolytic treatment, has also been noted in the literature. Evaluation for a possible interaction between treatment and age such that older patients have risks that outweigh benefits is thus appropriate.

This issue was approached by dividing the patients into quintiles based on age. The quintile of oldest patients (77-100yo) showed little benefit in the dichotomized recovery outcomes. The comparison using the full ordinal scales in this quintile showed neither a trend to better nor worse outcome with Activase. Mortality in this quintile showed a slight trend toward lower mortality. Overall there were no differences in outcomes between treatment arms in this subgroup. This is in spite of the noticeably higher incidence of ICH in the Activase treated patients (almost half of all ICH). Most of these ICHs were within 36 hours of treatment (14 of 22).

To determine any general trends of outcome with age in each of the two treatment groups, we examined mean Rankin Scale score in cohorts of patients divided by 5 year windows of age (standard errors are not drawn, but are sizable and overlap at all points):



These mean values were not different for the cohorts at ages 75-79 and 80-84, but again showed better mean outcome for the subsequent two cohorts. Thus, there was a trend to less-good outcome at older ages, but no indication of a likelihood of harm from Activase treatment at older ages.

Patient Race

In these studies 64.6% of the patients overall were white, 27.1 were black, 5.9% Hispanic, with 2.4% of other demographics. White patients as whole derived the majority of the improvement in outcome.

Black patients (80 Activase, 89 placebo) had improved outcome associated with Activase treatment on the dichotomized outcome scales, but this was less clear when the full ordinal scales were compared. There were no differences between treatments for the any of the three ordinal scales. Furthermore, while the increase in ICH with Activase was in concert with the studies' overall results, there was an increase in the mortality (21% Activase, 17% placebo) that was opposite to the overall result. Much of this was attributable to the excess ICH. When ICH within 36 hours of treatment was eliminated (6 patients Activase, 4 placebo) the mortality rates were identical in the two treatment subgroups due to the elimination of 4 deaths in the black Activase treated patients. Under those circumstances the percentage of patients with recovery approached the overall rates, and the full ordinal scales showed trends toward better outcomes.

When adjusted for age and baseline NIH-SS by GLM comparison of the full ordinal Rankin Scale (retaining all patients), the estimated mean Rankin scores for black patients trended toward better outcome with Activase treatment. Thus, some black patients did appear to have improved outcomes associated with Activase treatment, but there was a predominance of poor outcomes associated with the increased ICH in the Activase subset.

Hispanic patients comprised a small part of this study (19 Activase, 18 placebo) but show some evidence of have worse outcomes with Activase. All of the three scales showed slight trends to worse outcome with Activase, both when examined in the dichotomized form and in the full ordinal scales. Furthermore, ICH was markedly increased in this subgroup of Activase patients (7 of 19 Activase, 37%; 1 of 18 placebo, 6%) and mortality trended to worse in the Activase subgroup as well. All of the ICH were prior to 36 hours. However, the small sizes of these two

treatment subgroups leaves the meaningfulness of these trends in considerable doubt.

When examined for baseline characteristics, the Activase hispanic subgroup had slightly less severe strokes, but were slightly older, and did have greater estimated pre-existing disability (worse mean estimated pre-stroke Rankin Score). When adjustment for age and baseline NIH-SS was performed with the GLM comparison of the full ordinal Rankin Scale (retaining all patients), the estimated mean Rankin scores were different, in the direction of further increase in worse outcome for the Activase treated patients. For this patient subgroup, there appeared to be a trend of increased risk of ICH associated with Activase treatment, and this increased ICH was associated with higher mortality and worse disability. Adjustment for covariates did not eliminate this difference. The small size of this group precludes forming conclusions with any degree of confidence, however this subgroup remains an unresolved concern for an unfavorable risk-benefit comparison.

Adverse Outcome of Death OR Severe Disability

The potential for significant adverse consequences of the increase in ICH were further examined by CBER by examining the incidence of the combined unfavorable outcome of death or severe disability. This is equivalent to dichotomizing the outcome endpoints at the separation between severe and moderate disability. As this is a *post hoc* analysis, no prospective criteria for locating this distinction were specified. However, when this dichotomization was performed with post hoc selections of the separation score for each of the outcome assessments, there was a consistent trend in all of the outcome scales to have lower percentages of patients with death or severe disability in the Activase group than the placebo group. Examples of this can be seen in the accumulated patient figures previous shown (pg 34-36). Thus, in spite of the increased ICH, there was no trend to increased frequency of patients with unfavorable outcomes in these studies.

Exploratory analyses of ICH and mortality in patient subgroups

Because of the central importance of ICH as a safety issue, patient subgroups were screened for those that may have differentially higher rates of ICH, and thus an altered risk to benefit comparison. The subgroups of Activase treated patients were reviewed for substantially elevated rates of ICH, determined by a 50% differential in the rate of ICH occurring within 36 hours of treatment (Appendix C). Not included in these selections were those subgroups previously examined above. The following subgroups were selected for further analysis:

Safety in Selected Patient Subgroups: Incidence of ICH within 36 Hours in Activase Patients								
Grouping Factor	Criterion	Without Factor		With Factor		Factor Effect on ICH		
		Number: with ICH total	% ICH	Number: with ICH total	% ICH	% of patients	Relative Risk	p-value
All Patients	Activase Tx	n= 34 ICH n= 298 total		11.4 %				
Aspirin	Use within prior 2 wks	17 180	9.4	17 118	14.4	5.0	1.53	0.190
Atrial Fibrillation	History of	25 243	10.3	8 51	15.7	5.4	1.52	0.262
Fibrinogen	baseline <200	25 249	10.0	5 22	22.7	12.7	2.26	0.061
Hypertension	within 24 hrs after Tx	23 250	9.2	11 48	22.9	13.7	2.49	0.006
CT: Intravasc. Thrombus	visualized in baseline CT	28 261	10.7	6 32	18.8	8.1	1.75	0.172
CT: Edema	visualized in baseline CT	31 280	11.1	3 13	23.1	12.0	2.08	0.169
CT: Mass Effect	visualized in baseline CT	31 284	10.9	3 9	33.3	22.4	3.05	0.026
Age by quintile group	77-100 yrs (oldest)	(n.a.)		14 63	22.2			

p-values based on test of Relative Risk by z-score

Prior Aspirin Usage

Use of aspirin within the two weeks prior to the acute stroke was prospectively identified as a subgroup of interest by the study protocol. Genentech and the investigator group have identified slightly different sets of patients for this subgroup (175 patients by Genentech, 216 by the investigator group). Review of the relevant medication histories indicate that NSAID medications were included by the investigators within this subgroup but not by Genentech. The results of all analyses, for both efficacy and safety, are qualitatively the same for both subgroupings. Because the NSAIDs may have similar mechanisms and effects as aspirin itself, the CBER analyses have been conducted with the investigator identified patient subgrouping that includes NSAID usage. The results of these studies indicate a greater incidence of ICH in Activase patients with a history of aspirin usage than without. This is true for both all ICH (Appendix B) and ICH within 36 hours (table above). However, this does not appear to have effects upon outcome in these patients large enough to eliminate the efficacy of Activase. The differences between the two With-prior-aspirin treatment subgroups are somewhat less than the differences between the treatment subgroups of without-prior-aspirin. However, this appears to be due as much to better outcome of the with-prior-aspirin placebo patients compared to the without-prior-aspirin placebo patients as it is to lesser good outcomes with-aspirin than without

patients compared to patients without these acute CT scan changes at baseline, but was not different between treatments for the subgroup. Thus, these patients retained a favorable risk-benefit comparison in spite of the increased ICH incidence.

The medical literature raises concern of worse outcome for patients with baseline CT scan abnormalities of acute stroke, and particularly for patients with focal hypodensities seen in the baseline CT scan. CT scan readings in this study did not specifically include a report of this finding. Based on the relative incidence of other types of acute CT scan changes, it can be expected that there were relatively few patients in these studies with focal hypodensity abnormalities on the baseline CT exam. Thus, even if it had been specifically reported, there would appear be little basis for reaching a conclusion regarding the safety of Activase treatment in this type of patient.

INFORMATION FROM OTHER STUDIES: ECASS

The ECASS protocol was conducted in Europe by a different manufacturer of alteplase, Actilyse. It is important as it provides an equally large group of patients in a similar setting treated with a similar regimen. ECASS did not show improvement of patient outcome with alteplase treatment. However, there are many differences that must be borne in mind when extrapolating the ECASS results to the proposed therapy of Activase.

Protocol: ECASS: European Cooperative Acute Stroke Study

Sponsor: Boehringer Ingelheim Deutschland GmbH
Protocol#: 135.63
Title: European Cooperative Acute Stroke Study (ECASS)
Dates: September 1992 to June 1994
Publication: JAMA (1995) 274:1017-25

Objective

To evaluate the efficacy and safety of alteplase in acute ischemic stroke patients who are treated within 6 hours from the onset of symptoms.

Design

This was a randomized, double blind, placebo controlled, multicenter clinical trial conducted in 14 European countries in 75 hospitals. It enrolled 620 acute ischemic stroke patients randomized to either Actilyse (Alteplase) or placebo. Patients were enrolled only if treatment could be initiated within 6 hours from the time of stroke onset.

The study was planned to enroll 300 patients per arm. An interim analysis was planned to occur after 160 patients were enrolled.

in the Activase patients. In spite of the higher percentage of ICH, the with-prior-aspirin Activase patients do not have a higher mortality rate than the without-prior-aspirin. Thus prior usage of aspirin in a patient does not appear to significantly increase the risk of poor outcome when treated with Activase.

History of Atrial Fibrillation

In these studies 18.4% of the patients overall had a history of atrial fibrillation (58 Activase, 57 placebo). The incidence of ICH was higher in Activase patients with a history of atrial fibrillation than those without. This was also true of the placebo patients, although ICH incidence in atrial fibrillation patients remained higher with Activase than without (26% Activase, 12% placebo). In spite of this increase in ICH, the outcome scales, whether examined in the dichotomized percent-recovery form or the full ordinal scales, trended to improved outcome in all cases. Furthermore, while mortality was higher in atrial fibrillation patients than in those without this history, there was no difference in mortality rate between the treatment subgroups in patients with a history of atrial fibrillation. The patients with atrial fibrillation, as a whole, were older and had more severe strokes than those without this history. This may have contributed to some of the differences between outcomes when comparing subgroups on the basis of this criterion. There did not seem to be evidence of an unfavorable risk-benefit comparison.

Baseline Fibrinogen

The percentage of patients with abnormal baseline fibrinogen of < 200 was small in these studies, 5.9%, and not evenly distributed between the treatment arms (23 patients Activase, 14 placebo). While these patients showed increased percentage with recovery associated with Activase on the dichotomized outcome scales, there was no benefit or slight trends of poorer outcome when comparing the full ordinal scales. This was in spite of the baseline stroke severity being slightly less severe in the Activase portion of this subgroup, although Activase patients were also slightly older. The mortality rate was not different between the two treatments in this subgroup, although higher in both treatment arms of the subgroup than in the patients without low baseline fibrinogen. Because this analysis included only a small subset of the patients, firm conclusions cannot be reached.

Hypertension within 24 hrs of Treatment

This group of patients was defined by a systolic BP > 185 or diastolic BP > 110 during first 24 hours. In this subgroup, 15.7% of the patients overall, the ICH rate was higher than in the non-hypertensive patients. However, all of the outcome scales indicated better outcome associated with Activase on both the dichotomized and full ordinal scales. Additionally, mortality was lower with Activase treatment. There appears to be no remaining concern for this hypertensive-defined subset of patients.

Baseline CT Scan Abnormalities

Baseline CT scan abnormalities typical for acute ischemic stroke included edema (4.2% of patients), and mass effect (2.3%). It is unknown to what extent the acute changes may have acted as a *de facto* eligibility exclusion criterion (see above, Eligibility Criteria).

While in these patients the ICH rate was higher, these patients improved on both the dichotomized and full ordinal scales. Mortality was generally somewhat higher overall for these

There were also 81 patients enrolled in a preceding study, an unrandomized, open label, pilot study to assess safety of the selected regimen.

Treatments

Study Treatment and Regimen

Actilyse was given IV at 1.1mg/kg, to a maximum of 100mg, with a patient weight limit of 100 kg . Identically reconstituted placebo was utilized. The study treatment was given 10% as a bolus, the remainder as an infusion over 60 min. Note that this dose is 22% higher than that used in the NINDS Stroke Trial.

Concomitant Therapy

SC heparin could be given immediately after hospital admission, upon investigator discretion. IV heparin (systemic anticoagulation) was prohibited for 24 hrs. All oral anticoagulants, antiplatelet, and hemorrheologic agents were prohibited for 24 hrs.

Antiplatelet agents were recommended, beginning at 24 hrs after the infusion.

Eligibility Criteria

Inclusion:

1) 18-80 yo
2) Maximum weight 100kg
3) "moderate to severe" stroke identified by neurologist
4) Onset of symptoms within 6 hours of when study Tx will begin
5) Symptoms are present for at least 1 hour, and still present at time of Tx start
6) Normal cerebral CT scan
7) CT scan determined hypodensity in < 1/3 of MCA territory
8) Sulcal effacement in < 1/3 of MCA territory
9) Additional items of CT scan interpretation in amendment of 11/20/92 were considered; these are not detailed in the study report.

Note- item 6 may be meant to imply no hemorrhage seen on CT scan

Exclusion:

1) Onset time not known
2) Severe deficit of coma, severe stupor, hemiplegia with fixed eye deviation
3) Complete global aphasia
4) Only minor stroke (SSS score > 50 of 58)
5) Rapid improvement of symptoms
6) Presumed vertebrobasilar stroke
7) CT finding of acute hypodense parenchymal lesions in > 1/3 MCA territory
8) Seizure disorder or within 6 hours of stroke
9) Prior stroke within 3 months
10) Prior ICH, tumor, SAH, AVM, aneurysm

11) presumed SAH
12) Hypertension (>200 systolic, 110 diastolic, or iv medications to control BP
13) Septic embolus or infective endocarditis
14) List of other characteristics concerning for a potential increase in hemorrhage

Endpoints

Primary Endpoint
- Barthel scale
- Rankin scale

Dual primary endpoints were specified. Both were to be evaluated at 90 days, with deaths included as lowest score.

Secondary Endpoints
- Combined Barthel-Rankin Scale
- Scandinavian Stroke Scale at 2, 8, 24 hrs; 7, 30, 90 days
- NIH stroke Scale at 'near' baseline, 24 hrs, 90 days; modified for European setting
- Mortality rates at day 30

Subgroup analyses
- Comparison of time stratum - 1-3 hrs vs 3-6 hrs
- Outcome by severity of stroke

Statistical Plan

The primary analysis was to be an intent-to-treat analysis. An Evaluable Patient population was also defined for a secondary analysis. This was a treated per protocol, eligible per protocol, and completed trial (or known withdrawal) population.

The alpha level of the dual primary endpoints were planned to be adjusted for correlation of the two endpoints. At the end of the trial this correlation was determined to be -0.95. This resulted in the final alpha for each of the dual primary endpoints being set at 0.039 for each test.

The primary endpoint analysis was to include deaths, scored as the lowest possible score. Last value carried forward was to be used for withdrawals or for deaths for analysis of SSS and NIH-SS. Patients not actually treated were differentially incorporated into the ITT analysis. Actilyse patients received the worst scores, placebo patients the best possible scores.

Study Subjects

There were 620 subjects enrolled and randomized.

ECASS Enrollment		
	Placebo	Actilyse
Enrolled (ITT pop.)	307	313
Treated	305	310
Eligibility & other viol.	41	63
Per Protocol (PP pop.)	264	247

There were a total of 109 patients retrospectively excluded from the evaluable population (43 placebo, 66 Actilyse). The reasons for exclusion were as follows:

Evaluation-Exclusion Reasons		
	Placebo	Actilyse
CT Scan basis	26	40
major early infarct signs	21	31
hemorrhage	0	2
not available/adequate	5	7
Not treated (cause not stated)	2	3
Hypertension	1	0
Heparin IV within 24 hrs	4	6
Other prohibited medication	3	5
Dose below 1.1 mg/kg	0	1
Information obtained by phone	1	3
Lost to followup	2	7
day 90 evaluation outside ± 14 d	8	7
Patients with more than 1 reason	5	5
Total Patients Excluded	43	66

Note: numbers do not tally correctly; 1 off in each column, reason unknown

Note that almost half of the excluded patients in each arm were excluded on the basis of CT scan findings of major early infarct signs that were not noted by the investigator at the treatment site at the time of screening. The number of patients with early infarct signs less than major is not known, but can be presumed to have occurred in at least some of these patients.

The baseline characteristics and demographics resemble, in part, the characteristics seen in the NINDS Stroke Study. The ECASS patients were almost entirely white, slightly lighter in

weight, and with somewhat lower percentages of selected pre-existing medical disorders. ECASS patients may also have had slightly less severe strokes at baseline, if the NIH-SS can be compared between the two studies. The time to treatment was very different in ECASS. Only a minority of patients in ECASS were treated within 3 hours of stroke onset.

Demographic and Baseline Characteristics					
Characteristic	Criterion	ITT Population		PP Population	
		Placebo	Actilyse	Placebo	Actilyse
N	enrolled	307	313	264	247
Age (yrs),	median : mean	67 : 65	69 : 65	68 : 65	69 : 66
Gender	male	201 (65.5%)	188 (60.1%)	173 (65.5%)	149 (60.3%)
Race	white	303 (98.7%)	312 (99.7%)	260 (98.5%)	246 (99.6%)
Weight, male (kg)	median : mean	78 : 78	78 : 77	78	78
Weight, female (kg)	median : mean	65 : 66	68 : 69	64	68
Medical Hx:	% with Hx				
Hypertension		134 (43.6%)	124 (39.6%)	118 (44.6%)	97 (39.2%)
Coronary Ht dis.		82 (26.5%)	72 (22.8%)	73 (29.4%)	59 (23.7%)
Atrial Fibr.		58 (18.8%)	55 (17.5%)	48 (18.1%)	38 (15.3%)
Diabetes		45 (14.6%)	38 (12.1%)	42 (15.7%)	29 (11.7%)
Time to Tx (hrs)	median : mean	4.5 : 4.4	4.5 : 4.3	4.52	4.42
Time to Tx < 3hrs	% of patients	12	16		
SSS	baseline median	26	28	28	29
NIH SS	baseline, median	13	12	12	11

Efficacy Results

The efficacy results of ECASS are shown in the following table. This includes both primary and secondary endpoints for the protocol.

ECASS Efficacy Results						
90 day outcomes	ITT Analysis			PP Analysis		
	Placebo	Actilyse	p-value	Placebo	Actilyse	p-value
N	307	313		264	247	
Barthel Index, median - mean	75 61.7	85 62.9	0.99	80 63.2	90 66.4	0.18
Rankin Scale, median : mean	3 3.1	3 2.9	0.41	3	2	0.035
Rankin Scale, score 0-1 - (%)	29	35				
90 d Deaths	48 (15.6%)	69 (22%)	0.04	39 (14.8%)	48 (19.4%)	0.17
SSS, median	36	39	0.54[6]	37	43	0.033[6]
30d Mortality	39 (12.7%)	56 (17.9%)	0.076	31 (11.7%)	36 (14.6%)	0.36
Combined Barthel-Rankin	90	"97.5"	0.003	90	100	0.0001
NIHSS, median	5	4	0.096[6]	5	2	0.011[6]

Notes: Barthel includes deaths, as lowest score
Rankin include deaths as lowest score
SSS analysis includes deaths by LOCF
Combined Barthel-Rankin excludes deaths
NIHSS includes deaths by LOCF
6: These p-values were not substantiated in the detailed statistical tables

Subgroup Analyses

A division of patients into time-to-treatment strata of 1-3 hrs, and 3-6 hrs showed neither significant differences between Actilyse and placebo in either stratum, nor statistically significant differences between strata. The difference in apparent treatment effect between the two strata appears largely due to a difference in the outcome of the placebo subgroups in each of the strata, where patients who presented earlier had worse outcomes. Such a difference related to time of presentation at the hospital is not clinically expected. As there was no assessment of baseline balance in these subsets, no further conclusions can be made.

Barthel Index Outcome				
	Time to Tx <= 3 hrs		Time to Tx > 3hrs	
	Placebo	Actilyse	Placebo	Actilyse
n	37	48	266	256
mean	52.4	63.9	63	62.7
median	50	87.5	80	85
p-value	0.19		0.80 (trend in favor of placebo)	

Rankin Scale Outcome				
	Time to Tx <= 3 hrs		Time to Tx > 3hrs	
	Placebo	Actilyse	Placebo	Actilyse
n	40	52	267	261
mean	3.2	3.0	3.0	2.9
median	4	3	3	3
p-value	0.27		0.33	
% with score 0-1	30	38		

Division of the patients into strata of baseline stroke severity, based on the baseline Scandinavian Stroke Scale (SSS), with subgroups of 2-14 points (severe stroke patients) vs ≥ 15 points showed worse outcome overall with more severe stroke (both placebo and Actilyse), but there was no clear difference in treatment effects between the two subgroups.

Separation of the patients into age strata utilizing a division at age 70 years also did not clearly indicate any difference in treatment effects between the older and younger patients.

It should be noted that the Boehringer-Ingelheim study report states these results are indications of differential effectiveness in each of these analyses. However, this is not apparent by examination of the results.

Mortality

Mortality was also reported as a specific outcome of the study.

ECASS Mortality Results								
Mortality Outcomes	ITT Analysis			PP Analysis			Excluded Patients	
	Placebo	Actilyse	p-value	Placebo	Actilyse	p-value	Placebo	Actilyse
N	307	313		264	247		43	66
Deaths by day 90	48 (15.6%)	69 (22%)	0.04	39 (14.8%)	48 (19.4%)	0.20	9 (20.9%)	21 (31.8%)
Deaths by day 30	39 (12.7%)	56 (17.9%)	0.08	31 (11.7%)	36 (14.6%)	0.36	8 (18.6%)	20 (30.3%)
ICH related deaths (30d)	7 (2.3%)	19 (6.1%)		7 (2.7%)	10 (4.0%)		0 (0%)	9 (13.6%)
neurologic death, but non-ICH-related	19 (6.2%)	26 (8.3%)		12 (4.5%)	16 (6.5%)		7 (16.3%)	10 (15.2%)
Time to treatment < 3hrs	21% of 40	26% of 52	0.62	21% of 29	26% of 39	0.78		
Time to treatment > 3hrs	15% of 267	22% of 261	0.06	14% of 235	18% of 208	0.24		
90d mortality; only patients with early major infarct signs							6 / 21 (28.6%)	15 / 31 (48.4%)

p-values with Fisher's's Exact test
mortality of patients with early major infarct signs inferred from supplied results, some uncertainty exists in this result

Thus, mortality was higher in the Actilyse treated group. As this was also true in the small subset of patients who were treated within 3 hours of stroke onset, the difference in mortality between the ECASS and NINDS Stroke Study cannot be attributed to just the difference in time to treatment between the two studies.

Hemorrhage Events

ICH in the ECASS study occurred at higher rates than was seen in the NINDS Stroke Trial, as seen the following table.

Mortality at day 90, while higher in patients with early major CT infarct signs overall, was doubled with Actilyse treatment as compared with placebo. Thus, these patients may be a definable subset that are at particular risk for mortality related to ICH. They are particularly important to consider because the NINDS Stroke Trial did not contain many patients with any early CT changes. Thus the ECASS patients may contribute strongly to considerations regarding this subset of patients.

INFORMATION FROM OTHER STUDIES: NINDS PILOT STUDY

Preliminary development of this therapy included a phase 2 pilot study that examined a range of doses and a different dosage regimen. The information submitted by Genentech is largely limited to that of the published reports.

Design: Conducted in sequential dose tiers, this is an open label study except for the small last group which was placebo controlled and blinded. Safety was the primary objective, assessed as bleeding events, both intracranial and systemic serious bleeding. Clinical status at 3 months and CT scans were also evaluated to provide a potential indicator of therapeutic activity. There were not angiograms performed as part of this study.

Patient population: Initially limited to patients with acute ischemic stroke presenting at the three participating centers soon after onset of the stroke such that treatment could be initiated within 90 minutes of stroke onset. Modifications to the original protocol subsequently included a group of patients in the 90 to 180 minute period from onset to treatment, to assess safety in these patients. The eligibility criteria were essentially the same as for the pivotal trial conducted by the NINDS investigator group. Small modifications occurred in eligibility criteria during the trial, such as in laboratory results and blood pressure limits, that eventually led to those adopted for the pivotal trial.

Dose Tiers, Regimens and Patient Enrollment

Tier	Dose amount	Reported as (mg/kg)	Regimen	Total Patients
I:	0.35 mg/mg (max 25mg)	0.35	no bolus, 1 hr infusion;	6
II:	0.6 mg/kg (max. 40mg)	0.60	no bolus, 1 hr infusion;	20
III:	0.85 mg/kg (max 60mg)	0.85	no bolus, 1 hr infusion;	10
IV:	32mg/m2 (max 90mg)	0.85	10% bolus, 1hr infusion	26
V:	37.6 mg/m2 (max 90mg)	0.95	10% bolus, 1 hr infusion	3
IVExt	32mg/m2 (max 90mg) + 5.5mg/m2	0.95	no bolus, 1 hr infusion + 30 min infusion for following part	28
VI	45mg/m2 (max 100mg)	1.08	10% bolus, 45% in 30 min infusion then 45% in 1 hr infusion	1
Controlled	same dose as IVExt.	0.85	placebo controlled, blinded; IVExt regimen	14 + 13

ECASS Hemorrhage Events								
	ITT Analysis			PP Analysis			Excluded Patients	
	Placebo (n=302)	Actilyse (n=305)	p-value	Placebo (n=264)	Actilyse (n=245)	p-value	Placebo (n=38)	Actilyse (n=60)
Total ICH	113 (36.8%)	134 (42.8%)	0.10	97 (36.7%)	107 (43.7%)	0.148	16 (42.1%)	27 (45%)
Hemorrhagic Infarction	93 (30.8%)	72 (23.6%)		79 (29.9%)	59 (23.9%)		14 (36.8%)	13 (21.7%)
Parenchymal Hematoma	20 (6.6%)	62 (20.3%)		18 (6.8%)	48 (19.4%)		2 (5.3%)	14 (23.3%)

p-values using Fisher's Exact test
n's exclude patients with missing CT scans: ITT - 5 placebo, 8 Actilyse; PP - 0 placebo, 2 Actilyse;

Comment:

The difference between the ECASS rates of ICH and the NINDS rates of ICH cannot be explained solely on the basis of different criteria of reading the CT scans, particularly different judgements on the parenchymal hyperdense regions that the NINDS study designated as not indicating hemorrhage. These were a small number of patients in the NINDS study, and also unlikely to be confused with a parenchymal hematoma where the largest difference in this study is seen. There were, overall, higher rates of ICH in ECASS than seen in the NINDS studies. This is true for both the alteplase groups as well as for the placebo groups. The higher rates of hemorrhagic infarction in the placebo arm than the Actilyse group may imply that some of the hemorrhagic infarction patients were converted into parenchymal hematomas in the Actilyse group.

Conclusions

ECASS was a large placebo controlled, double blind, randomized trial of alteplase in acute ischemic stroke, and thus relevant to considerations of benefit and risk of tPA in stroke. ECASS results showed an unfavorable risk-benefit comparison of the thrombolytic regimen. Outcome scales were not significantly different between the two treatment groups, but there was a higher mortality rate with Actilyse. This was likely related to the higher rate of ICH with Actilyse, particularly parenchymal hematomas. When 18% of the patients were excluded, there were some trends to efficacy; the excess ICH and mortality still remained, however. There were no statistical differences between the patients treated within 3 hours of stroke onset and those within the 3-6 hour period.

There were also considerable differences from the NINDS Stroke Trial. The tPA product itself is from a different manufacturer, so that there may be differences in the activities of the two products. ECASS used a dose of tPA approximately 22% higher than the NINDS study. This might indicate a narrow therapeutic window for tPA.

The "per protocol" excluded patients are of particular interest. Almost half of these patients had marked changes on the screening CT scans. Although these patients did not have ICH rates different from the other patients, their mortality rates were somewhat higher, and there was a three-fold increase in ICH-related deaths in the Actilyse vs. placebo subgroups.

Notes: 1) Results were reported as approximate average mg/kg received for the patients actually enrolled in groups IV to VI, so that all groups were regarded as dosed on mg/kg basis.
2) The protocol designates the controlled patient group to receive the Tier IVExt regimen, the published account describes the Tier IV regimen as utilized.

Safety results in the open label portion indicated that 5 ICH occurred, 1 in Tier IV, 3 in Tier IVExt, 1 in Tier V, with a scattering of hemorrhagic transformation without hematoma or other asymptomatic hemorrhage in dose tiers I to IVExt. There were 18 deaths within the 90 day period, distributed through dose tiers I to IVExt.

In the controlled and blinded portion, there were no ICH in the Activase treated patients. There were 3 deaths in the placebo group and 1 in the Activase group by 3 months. These results were deemed encouraging as to the likelihood of relative safety of the regimen, and worthy of further evaluation in a controlled trial.

INFORMATION FROM OTHER STUDIES: TTATTS

This was a multicenter, dose escalation, angiographic study of Activase in acute stroke.

Design

This study was an open label, non-randomized dose escalation study of intravenous Activase in acute ischemic stroke patients. In addition to an eligibility screening process similar to that used in the NINDS pilot and pivotal trials, these patients were required to demonstrate obstruction to flow (TIMI Grade 0) in an arterial vessel appropriate for the clinical signs in an angiogram performed as part of the screening. Patients with a complete occlusion were treated with Activase at dose levels of 0.8 and 1.0 mg/kg, given as 10% bolus, the remainder by a 1 hr infusion. Dose levels of 1.2 and 1.4 mg/kg had been planned, but were not initiated due to the early termination of the study. Eligibility was restricted to patients who could begin treatment within 6 hours of stroke onset. In addition to clinical assessments of outcome, a repeat angiogram was performed at 2 hrs after initiation of the Activase. Patient followup was only through 10 days.

Results

There were 24 patients enrolled at 0.8mg/kg, and 14 patients at 1.0mg/kg. There were 10 ICH in the 24 patients in the 0.8mg/kg group (4 symptomatic, 6 asymptomatic) , and 7 ICH in the 14 patients in the 1.0mg/kg group (4 symptomatic, 3 asymptomatic). The occurrence of 4 of 14 patients with symptomatic ICH in the 1.0mg/kg group let to early termination of the study for safety reasons. Of the 8 patients with symptomatic ICH, 4 died (2 each in each dose level).

In the 0.8 mg/kg dose group, 2 of 18 patients had a complete response of flow restoration, 4 of 18 had a partial response, and 12 had no response. In the 1.0 mg/kg group, 1 of 13 had complete response, 2 of 13 had a partial response, and 10 had no response. Thus, recanalization rates were low in this study.

The review of the adverse events in these few patients and their characteristics indicated that

more of the patients with medical histories of CHF or atrial fibrillation experience serious adverse events than without these characteristics. Patients with more severe strokes had a tendency toward a greater incidence of ICH. There was also a tendency of patients with ICH having had higher systolic blood pressures. None of these associations were statistically significant in this small group of patients.

Conclusion

This study utilized a protocol that involved selection of patients with angiographically proven occlusions and a repeat angiogram to assess recanalization. The patient population also differed from the NINDS studies in allowing up to 6 hours from onset to initiation of treatment. The results of this study indicated higher rates of ICH than was observed in either of the NINDS studies. The rates of ICH were not dissimilar between the two dose levels, but this study does not have sufficient power to demonstrate even moderate differences. Whether the angiogram, the time from onset to treatment, or other factors influenced the ICH rate cannot be determined.

SUMMARY

- The NINDS Stroke Trial consisted of two randomized, double-blind, placebo controlled, multicenter studies designed to assess the efficacy and safety of 0.9mg/kg IV Activase in acute ischemic stroke patients who could have treatment initiated within 3 hours of stroke onset.

- The primary efficacy endpoint was a global statistic comprised of the dichotomized forms of four stroke outcome scales, assessed at 90 days after the stroke onset. Supportive analyses included individual consideration of each of the four univariate scales, in both the dichotomized and full ordinal forms.

- The two trials were conducted immediately sequentially. All procedures, investigators and treatment sites were identical in the two studies. Each trial was of a size large enough to be well powered to detect treatment effect.

- The Primary Efficacy Endpoint of the Part 2 Study was selected by the unblinded Data and Safety Monitoring Committee with complete knowledge of a late trial interim analysis of the Part 1 Study. Thus the Part 2 Study is the hypothesis testing study, the Part 1 Study providing confirmatory evidence.

- Approximately 3½% of patients screened were enrolled into the study.

- The 90 day outcome assessments, in the tests of the global statistic, the univariate scales in dichotomized form, and the univariate scales in full ordinal form all demonstrated significantly better outcomes in the Activase group. This was true for the Part 1 Study and the Part 2 Study.

- The two treatment groups exhibited modest imbalances in some characteristics that may be significant predictors of outcome. Covariate-adjusted analyses that accounted for the baseline imbalances did not qualitatively alter the comparison between treatment groups.

- There was a trend to lower mortality with Activase that did not reach statistical significance in either study alone, nor in the combined dataset.

- Intracranial hemorrhage (ICH) was significantly higher with Activase treatment vs placebo. This was effect was most pronounced within 36 hours of treatment. Although ICH is associated with poor outcome, the Activase group did have significantly better 90 day outcomes in spite of the increase in ICH.

- Severity of the acute stroke and patient age are important predictors of outcome, independent of treatment. Patients with more severe strokes or patients who are older may have decreased amounts of benefit from treatment with Activase.

- Multiple exploratory analyses to identify patient subgroups that might have unfavorable risk -to-benefit comparisons were examined.

  Patients with diabetes constituted 21% of the study. There was no significant difference between the treatment groups within this subset. Adjustment for covariates did not eliminate the uncertainty of efficacy in diabetic patients.

Patients with a history of CHF comprised 16% of the total studied. These patients exhibited a trend to higher mortality with Activase treatment compared to placebo. Outcome scale comparisons did not show any difference between the treatment groups for this subset.

Visualization of an intravascular thrombus on the baseline CT scan was associated with a trend to worse outcomes, independent of treatment. While there was no significant interaction with treatment, the amount of benefit, if any, associated with Activase treatment for these patients is uncertain.

Patient race was another factor leading to difficulty in interpretation of the results. In black patients Activase treatment was associated with higher mortality than placebo. However, covariate adjusted analyses indicated that Activase treatment showed trends towards improvement on the full ordinal outcome scales.

In Hispanic patients Activase treatment was associated with trends of a higher ICH rate, a higher mortality rate , and worse outcome on the full ordinal outcome scales, even after adjustment for baseline characteristics.

Baseline CT scan changes typical for acute stroke were associated with a higher rate of ICH. These CT scan findings were not associated with worse outcome on the stroke scales.

- The European Cooperative Acute Stroke Study (ECASS) was a large, placebo controlled, European study with a dose regimen of Actilyse -tPA that was 22% higher than in the NINDS Stroke Trial. Most patients in ECASS were treated more than 3 hours after onset of stroke.

  ECASS did not demonstrate a beneficial treatment effect utilizing the outcome scales. There was no significant difference in outcome between the ECASS patients treated prior to 3 hours after onset and those treated more than 3 hours after stroke onset. Mortality was significantly higher in Actilyse patients, as was ICH. Patients with early major infarct signs on the baseline CT scan appeared to have a particularly increased rate of parenchymal hematoma and mortality associated with Actilyse treatment.

- Adverse events other than ICH seen in these studies were not unusual for this patient population. The adverse events were not different from the known risks of treatment with Activase based on the experience in the setting of acute myocardial infarction.

Recommendation

These data demonstrate a favorable risk-benefit comparison for Activase treatment in acute ischemic stroke patients (as a group) at the proposed dose and regimen. Activase should be approved for the additional indication of treatment of acute ischemic stroke. The labeling should take particular care to highlight the factors for consideration in selecting patients for this therapy and to fully describe the risks accompanying treatment with Activase.

Appendix A: The Stroke Outcome Scales

Barthel Index

This is an Activities of Daily Living (ADL) scale. Eleven areas of functioning are assessed and given point values, which are then summed for a single, final score. The scale ranges from 100 for a fully functional patient to 0 for the worst possible score, with steps of 5 only possible. Death is not explicitly included in the scale. These studies did incorporate death by assigning a score of 0.

Item#	Functional Area	Point Range
1	Self Feeding Ability	0-10
2	Transfer (bed <-> chair)	0-15
3	Personal Toilet (self washing)	0-5
4	Commode usage ability	0-10
5	Bathing ability	0-5
6,7	Locomotion - Walking/Wheelchair ability	0-15
8	Locomotion - stairs	0-10
9	Dressing ability	0-10
10	Bowel continence	0-10
11	Bladder continence	0-10

Modified Rankin Scale

This is a single choice, global disability assessment scale. The scale ranges from 0 for No Symptoms, to 5 for Severe Disability. Only a brief phrase of description of the criteria for each level are provided, so that a potentially significant element of rater judgement is incorporated. A further modification of the scale often used is to incorporate death explicitly into the scale as a score of 6.

0 - No Symptoms
1 - No significant disability
2 - Slight disability
3 - Moderate disability
4 - Moderate-severe disability
5 - Severe disability
6 - Death (not part of original scale, often added for clinical trials)

Glasgow Outcome Scale

This is a single choice, global assessment of outcome from a disabling event scale. The scale ranges from a score of 1 for good outcome to 5 for death. This is the only commonly used scale that included a special, specific category for death. However, a score of 4, for vegetative survival is a relatively uncommon outcome in stroke, so that this becomes a 4 level scale in stroke studies.

1 - Good recovery (but not necessarily normal neurologic exam)
2 - Moderately disabled (impairment present, patient independent)
3 - Severe disability (patient highly dependent on others for daily activities)
4 - Vegetative survival
5 - Death

NIH Stroke Scale

This is a neurologic exam scale in which each of the specific exam findings are assigned point values. The individual items are summed to give a single score for the patient. The scale ranges from a score of 0 for normal in all items to 42 for the worst possible score. Death is not an explicitly incorporated status in the scale, but was included in this study by assigning a score of 42.

Item	Exam Area	Point range
1a,b,c	Level of Consciousness (3,2,2)	0-7
2	Eye movement ability	0-2
3	Visual Fields	0-3
4	Facial motor ability	0-3
5a,b	Arm strength vs gravity (r&l)	0-4 x 2
6a,b	Leg strength vs gravity (r&l)	0-4 x 2
7	Cerebellar ataxia of limbs	0-2
8	Sensory loss	0-2
9	Aphasia	0-3
10	Dysarthria	0-2
11	Sensory input extinction/inattention	0-2

Scandinavian Stroke Scale

This also is a neurologic exam scale in which each of the specific exam findings are assigned point values. The individual items are summed to give a single score for the patient. The scale ranges from a score of 58 for normal in all items to 0 for the worst possible score. Death is not an explicitly incorporated status in the scale. Some studies have utilized a baseline exam that does not test gait, in which case 46 is the maximum (and normal exam) score. ECASS utilized a subset for the 90 day outcome that excluded the Consciousness and Eye movements areas, for a maximum score of 48.

Area of Examination		Max. points	Distribution of point choices
1)	Consciousness	6	(2,4,6)
2)	Orientation	6	(0,2,4,6)
3)	Facial palsy	2	(0,2)
4)	Speech (aphasia)	10	(0,3,6,10)
5)	Eye movements	4	(0,2,4)
6)	Arm strength (affected side)	6	(0,2,4,5,6)
7)	Hand strength (affected side)	6	(0,2,4,6)
8)	Leg strength (affected side)	6	(0,2,4,5,6)
9)	Gait (strength)	12	(0,3,6,9,12)

Appendix B: Subgroup Analyses focused on Efficacy

Pages 75-78 follow in this section.

Treatment Effect and Relative Benefit on Rankin Outcome

Grouping Factor		Number of Patients with Recovery By Rankin Scale				Percentage of Patients Recovering			Relative Recovery (% recovery Activase / % recovery placebo)	
		Placebo		Activase						
	category	Recover	total	Recover	total	Placebo	Activase	Tx Effect	R.R.	p-value
Combined Studies										
All Patients	Barthel	119	312	162	312	38.1	51.9	13.8	1.36	0.001
(all outcomes)	Rankin	83	312	133	312	26.6	42.6	16.0	1.60	0.000
	Glasgow	97	312	141	312	31.1	45.2	14.1	1.45	0.000
	NIH-SS	64	312	106	312	20.5	34.0	13.5	1.66	0.000
Gender	Female	34	128	55	134	26.6	41.0	14.5	1.55	0.015
	Male	49	184	78	178	26.6	43.8	17.2	1.65	0.001
Time stratum	0-90 min	41	145	63	157	28.3	40.1	11.9	1.42	0.033
	91-180 min	42	167	70	155	25.1	45.2	20.0	1.80	0.000
Prior Disability	Yes	2	24	3	24	8.3	12.5	4.2	1.50	0.640
	No	81	288	130	288	28.1	45.1	17.0	1.60	0.000
Prior Stroke	Yes	13	38	17	45	34.2	37.8	3.6	1.10	0.737
	No	67	269	116	266	24.9	43.6	18.7	1.75	0.000
Hypertension Hx	Yes	51	203	76	205	25.1	37.1	12.0	1.48	0.010
	No	32	106	57	103	30.2	55.3	25.2	1.83	0.000
Atrial Fibril. Hx	Yes	11	57	18	58	19.3	31.0	11.7	1.61	0.155
	No	72	254	113	250	28.3	45.2	16.9	1.59	0.000
Prior TIA Hx	Yes	18	49	21	50	36.7	42.0	5.3	1.14	0.593
	No	58	239	108	240	24.3	45.0	20.7	1.85	0.000
Diabetes	Yes	18	63	21	68	28.6	30.9	2.3	1.08	0.773
	No	65	247	112	242	26.3	46.3	20.0	1.76	0.000
Prior MI	Yes	17	61	31	70	27.9	44.3	16.4	1.59	0.059
	No	63	237	100	227	26.6	44.1	17.5	1.66	0.000
Angina Hx	Yes	21	67	28	64	31.3	43.8	12.4	1.40	0.147
	No	61	229	103	237	26.6	43.5	16.8	1.63	0.000
CHF Hx	Yes	16	55	11	44	29.1	25.0	-4.1	0.86	0.651
	No	65	244	120	253	26.6	47.4	20.8	1.78	0.000
Valvular Ht Dis	Yes	7	19	11	25	36.8	44.0	7.2	1.19	0.636
	No	71	279	120	274	25.4	43.8	18.3	1.72	0.000

Grouping Factor		Number of Patients with Recovery By Rankin Scale				Percentage of Patients Recovering			Relative Recovery (% recovery Activase / % recovery placebo)	
		Placebo		Activase						
	category	Recover	total	Recover	total	Placebo	Activase	Tx Effect	R.R.	p-value
Race	Black	24	89	28	80	27.0	35.0	8.0	1.30	0.260
	White	52	198	95	205	26.3	46.3	20.1	1.76	0.000
	Hispanic	6	18	5	19	33.3	26.3	-7.0	0.79	0.642
NIH-SS	1-7	30	43	56	72	69.8	77.8	8.0	1.11	0.359
(Baseline cat.)	8-12	29	78	34	64	37.2	53.1	15.9	1.43	0.058
	13-16	13	66	21	57	19.7	36.8	17.1	1.87	0.039
	17-21	8	57	16	61	14.0	26.2	12.2	1.87	0.111
	22-37	3	68	6	58	4.4	10.3	5.9	2.34	0.213
Age Category	25-56	17	68	34	53	25.0	64.2	39.2	2.57	0.000
	57-65	22	71	26	58	31.0	44.8	13.8	1.45	0.107
	66-71	15	64	28	70	23.4	40.0	16.6	1.71	0.047
	72-76	15	54	29	60	27.8	48.3	20.6	1.74	0.031
	77-100	14	55	16	71	25.5	22.5	-2.9	0.89	0.702
Weight Category	40-62 kg	12	52	24	61	23.1	39.3	16.3	1.70	0.074
	62-73 kg	20	61	30	70	32.8	42.9	10.1	1.31	0.243
	73-82 kg	13	58	41	80	22.4	51.3	28.8	2.29	0.002
	82-93 kg	27	70	22	52	38.6	42.3	3.7	1.10	0.676
	93-200kg	11	71	16	49	15.5	32.7	17.2	2.11	0.031
Prior Aspirin	Yes	27	89	53	127	30.3	41.7	11.4	1.38	0.096
	No	56	223	80	185	25.1	43.2	18.1	1.72	0.000
Hypertension	Yes	6	50	15	48	12.0	31.3	19.3	2.60	0.029
(within 24 hr)	No	77	262	118	264	29.4	44.7	15.3	1.52	0.000
Edema on CT	Yes	2	13	4	13	15.4	30.8	15.4	2.00	0.369
	No	81	295	126	294	27.5	42.9	15.4	1.56	0.000
Mass effect on CT	Yes	2	9	3	9	22.2	33.3	11.1	1.50	0.604
	No	81	299	127	298	27.1	42.6	15.5	1.57	0.000
Thrombus visual.	Yes	7	43	5	33	16.3	15.2	-1.1	0.93	0.894
(on CT)	No	76	265	125	274	28.7	45.6	16.9	1.59	0.000
Smoking Hx	Yes	37	111	52	104	33.3	50.0	16.7	1.50	0.015
	No	46	199	78	201	23.1	38.8	15.7	1.68	0.001

Grouping Factor		Number of Patients with Recovery By Rankin Scale				Percentage of Patients Recovering			Relative Recovery (% recovery Activase / % recovery placebo)	
		Placebo		Activase						
	category	Recover	total	Recover	total	Placebo	Activase	Tx Effect	R.R.	p-value
Heparin (before	Yes	15	48	31	54	31.3	57.4	26.2	1.84	0.013
24 hr)	No	68	264	102	258	25.8	39.5	13.8	1.53	0.001
Heparin use	Yes	39	156	66	154	25.0	42.9	17.9	1.71	0.001
(after 24 hr)	No	44	156	67	158	28.2	42.4	14.2	1.50	0.010
Aspirin use	Yes	24	78	45	80	30.8	56.3	25.5	1.83	0.002
(after 24 hrs)	No	59	234	88	232	25.2	37.9	12.7	1.50	0.004
Aspirin during	Yes	27	110	44	94	24.5	46.8	22.3	1.91	0.001
1st week	No	56	202	89	218	27.7	40.8	13.1	1.47	0.006
Heparin use	Yes	41	159	65	152	25.8	42.8	17.0	1.66	0.002
during 1st wk.	No	42	153	68	160	27.5	42.5	15.0	1.55	0.006
Stroke Subtype	Small vessel	12	30	32	51	40.0	62.7	22.7	1.57	0.070
	Cardioembolic	38	137	51	136	27.7	37.5	9.8	1.35	0.088
	Large Vessel	30	135	47	117	22.2	40.2	17.9	1.81	0.003
PT baseline	<13	67	256	120	271	26.2	44.3	18.1	1.69	0.000
	>= 13	15	53	13	37	28.3	35.1	6.8	1.24	0.489
PTT baseline	< 30	68	270	114	271	25.2	42.1	16.9	1.67	0.000
	>= 30	14	40	18	37	35.0	48.6	13.6	1.39	0.229
Platelet count	<= 180K	10	38	16	47	26.3	34.0	7.7	1.29	0.448
	> 180K	72	273	117	263	26.4	44.5	18.1	1.69	0.000
Fibrinogen baseln.	<= 225	11	29	18	39	37.9	46.2	8.2	1.22	0.504
	> 225	67	258	105	244	26.0	43.0	17.1	1.66	0.000

Appendix C: Subgroup analyses focused on ICH

ICH within 36 hours in patient subsets.

Pages 80 - 81 follow in this section.

ICH within 36 hours: R[illegible] and Relative Risk

Grouping Factor	category	Activase Patients ICH	Activase Patients total	Percentage with ICH	Relative Risk (% with factor / % with factor in next line) Risk	p-value
Overall		34	298	11.4		
Gender	Female	17	130	13.1	1.29	0.427
	Male	17	168	10.1		
Prior Aspirin use	Yes	17	118	14.4	1.53	0.190
	No	17	180	9.4		
Hypertension Hx	Yes	22	193	11.4	1.05	0.896
	No	11	101	10.9		
Atrial Fibril. Hx	Yes	8	51	15.7	1.52	0.262
	No	25	243	10.3		
Diabetes	Yes	9	63	14.3	1.39	0.369
	No	24	233	10.3		
CHF Hx	Yes	7	42	16.7	1.61	0.224
	No	25	242	10.3		
Hypertension	Yes	11	48	22.9	2.49	0.006
(within 24 hr)	No	23	250	9.2		
Race	Black	6	76	7.9	0.74	0.490
	White	21	196	10.7	0.29	0.001
	Hispanic	7	19	36.8		
NIH-SS Category	1-7	5	72	6.9	1.46	0.595
(baseline)	8-12	3	63	4.8	0.37	0.139
	13-16	7	55	12.7	1.15	0.795
	17-21	6	54	11.1	0.46	0.089
	22-37	13	54	24.1		
Age Category	25-56	3	53	5.7	0.62	0.501
	57-65	5	55	9.1	1.22	0.745
	66-71	5	67	7.5	0.64	0.423
	72-76	7	60	11.7	0.53	0.131
	77-100	14	63	22.2		
Weight Category	40-62 kg	6	60	10.0	1.12	0.841
	62-73 kg	6	67	9.0	0.61	0.303
	73-82 kg	11	75	14.7	1.05	0.917
	82-93 kg	7	50	14.0	1.61	0.422
	93-200kg	4	46	8.7		

ICH within 36 hours: R.. .e and Relative Risk

Grouping Factor		Activase Patients			Percentage		Relative Risk (% with factor / % with factor in next line)	
	category	ICH	total		with ICH		Risk	p-value
Edema on CT	Yes	3	13		23.1		2.08	0.169
(baseline)	No	31	280		11.1			
Mass effect on CT	Yes	3	9		33.3		3.05	0.026
(baseline)	No	31	284		10.9			
Thrombus visual.	Yes	6	32		18.8		1.75	0.172
(on CT)	No	28	261		10.7			
Prior MI	Yes	8	66		12.1		1.05	0.894
	No	25	217		11.5			
Angina Hx	Yes	7	58		12.1		1.15	0.727
	No	24	229		10.5			
Valvular Ht Dis	Yes	1	23		4.3		0.41	0.368
	No	28	263		10.6			
PT baseline	<13	27	258		10.5		0.65	0.292
	>= 13	6	37		16.2			
PTT baseline	< 30	28	229		12.2		0.90	0.825
	>= 30	5	37		13.5			
Platelet count	<= 180K	6	42		14.3		1.34	0.481
	> 180K	27	254		10.6			
Fibrinogen baseln.	<200	5	22		22.7		2.26	0.061
	>=200	25	249		10.0			
Heparin (before	Yes	2	52		3.8		0.30	0.087
24 hr)	No	32	246		13.0			
Heparin after	Yes	3	144		2.1		0.10	0.000
24 hr.	No	31	154		20.1			
Heparin after	Yes	4	143		2.8		0.14	0.000
1 week	No	30	155		19.4			
Aspirin before	Yes	8	80		10.0		0.84	0.645
24 hrs	No	26	218		11.9			
Aspirin use	Yes	4	90		4.4		0.31	0.023
after 24 hrs.	No	30	208		14.4			

Appendix D: Detailed Information of Subgroups

More extensive efficacy and safety outcome analyses for patient subsets are contained in this section, on pages 83 - 92.

Subgroup Basis	Subgroup Category	Treatment	N	Number with Recovery by:			Percentage with Recovery by:			Treatment Effect (Change in % recovery) (Activase - Placebo)		
				NIH-SS	Barthel	Rankin	NIH-SS	Barthel	Rankin	NIH-SS	Barthel	Rankin
Overall		Activase	312	106	162	133	34.0	51.9	42.6	13.5	13.8	16.0
		Placebo	312	64	119	83	20.5	38.1	26.6			
Diabetes	Yes	Activase	68	15	22	21	22.1	32.4	30.9	7.8	-4.2	2.3
		Placebo	63	9	23	18	14.3	36.5	28.6			
	No	Activase	242	91	140	112	37.6	57.9	46.3	15.3	19.0	20.0
		Placebo	247	55	96	65	22.3	38.9	26.3			
CHF Hx	Yes	Activase	44	11	15	11	25.0	34.1	25.0	6.8	-0.5	-4.1
		Placebo	55	10	19	16	18.2	34.5	29.1			
	No	Activase	253	92	141	120	36.4	55.7	47.4	15.1	16.0	20.8
		Placebo	244	52	97	65	21.3	39.8	26.6			
Valvular Ht Dis	Yes	Activase	25	11	15	11	44.0	60.0	44.0	17.7	7.4	7.2
		Placebo	19	5	10	7	26.3	52.6	36.8			
	No	Activase	274	94	144	120	34.3	52.6	43.8	14.6	15.6	18.3
		Placebo	279	55	103	71	19.7	36.9	25.4			
Race	Black	Activase	80	21	36	28	26.3	45.0	35.0	3.8	7.9	8.0
		Placebo	89	20	33	24	22.5	37.1	27.0			
	White	Activase	205	77	116	95	37.6	56.6	46.3	18.4	18.2	20.1
		Placebo	198	38	76	52	19.2	38.4	26.3			
	Hispanic	Activase	19	5	5	5	26.3	26.3	26.3	-1.5	-23.7	-7.0
		Placebo	18	5	9	6	27.8	50.0	33.3			
Prior Stroke	Yes	Activase	45	11	22	17	24.4	48.9	37.8	11.3	1.5	3.6
		Placebo	38	5	18	13	13.2	47.4	34.2			
	No	Activase	266	95	140	116	35.7	52.6	43.6	14.5	15.8	18.7
		Placebo	269	57	99	67	21.2	36.8	24.9			
Prior TIA Hx	Yes	Activase	50	18	26	21	36.0	52.0	42.0	9.5	7.1	5.3
		Placebo	49	13	22	18	26.5	44.9	36.7			
	No	Activase	240	86	130	108	35.8	54.2	45.0	16.2	16.9	20.7
		Placebo	239	47	89	58	19.7	37.2	24.3			
Prior Aspirin use	Yes	Activase	127	43	62	53	33.9	48.8	41.7	13.6	10.6	11.4
		Placebo	89	18	34	27	20.2	38.2	30.3			
	No	Activase	185	63	100	80	34.1	54.1	43.2	13.4	15.9	18.1
		Placebo	223	46	85	56	20.6	38.1	25.1			

Subgroup Detailed Analyses: Dichoton d and Full Ordinal Outcome Scales art 2

Subgroup Basis	Subgroup Category	Treatment	Rankin Scale Mean	Rankin Scale median	NIH-SS Mean	NIH-SS median	Barthel Index Mean	Barthel Index median	p-values - Rank-sum test (Activase to placebo) Rankin	NIH-SS	Barthel
Overall		Activase	2.7	2	11.7	4	66.7	95			
		Placebo	3.2	3	14.0	7	58.5	72.5			
Diabetes	Yes	Activase	3.4	4	15.3	6	52.1	65	0.702	0.623	0.702
		Placebo	3.2	3	15.4	6	56.8	70			
	No	Activase	2.5	2	10.6	3	71.2	95	0.000	0.000	0.000
		Placebo	3.2	3	13.5	7	59.2	75			
CHF Hx	Yes	Activase	3.6	4	18.9	7.5	50.2	52.5	0.629	0.937	0.681
		Placebo	3.4	4	16.6	9	53.4	55			
	No	Activase	2.5	2	10.2	3	69.7	95	0.000	0.000	0.001
		Placebo	3.1	3	13.3	7	60.1	75			
Valvular Ht Dis	Yes	Activase	2.4	2	8.1	2	77.8	100	0.341	0.197	0.286
		Placebo	3.0	3	11.5	7	62.9	95			
	No	Activase	2.6	2	11.4	4	67.1	95	0.001	0.000	0.002
		Placebo	3.2	3	14.2	7	58.2	70			
Race	Black	Activase	3.0	3	14.3	6	59.3	85	0.633	0.660	0.681
		Placebo	3.1	3	13.7	8	59.5	75			
	White	Activase	2.5	2	10.4	3	71.4	100	0.000	0.000	0.001
		Placebo	3.2	3	13.6	7	59.3	72.5			
	Hispanic	Activase	3.6	4	16.5	7	46.8	35	0.630	0.865	0.417
		Placebo	3.2	3	15.9	7.5	56.7	80			
Prior Stroke	Yes	Activase	2.7	3	10.8	5	67.4	90	0.667	0.240	0.795
		Placebo	2.9	3	12.8	6.5	62.8	90			
	No	Activase	2.7	2	11.8	3	66.7	95	0.001	0.001	0.002
		Placebo	3.3	3	14.4	8	57.5	70			
Prior TIA Hx	Yes	Activase	2.5	2	10.8	3.5	69.3	95	0.441	0.582	0.451
		Placebo	2.8	3	11.3	4	66.2	90			
	No	Activase	2.6	2	11.3	3	67.8	95	0.000	0.000	0.003
		Placebo	3.3	3	14.2	8	58.2	70			
Prior Aspirin use	Yes	Activase	2.6	3	11.4	3	68.5	90	0.141	0.043	0.106
		Placebo	3.0	3	12.6	7	62.1	75			
	No	Activase	2.7	2	11.9	4	65.4	95	0.004	0.008	0.016
		Placebo	3.3	3	14.5	7	57.1	70			

Subgroup Basis			ICH (all)		Mortality by 90 d		Disability	NIH-SS Baseline		Age		PreStroke Rankin (est)		p-values - Rank-sum test (Activase to placebo)	
Subgroup Category		Treatment	n	%	n	%	n	Mean	Median	Mean	Median	Mean	Median	NIH-SS (b	Age
Overall		Activase	48	15.4	54	17.3		14.4	14	68.0	70.0	0.3	0		
		Placebo	20	6.4	64	20.5		15.2	15	66.0	67.2	0.3	0		
Diabetes	Yes	Activase	14	20.6	18	26.5	10	15.4	16	69.2	70.0	0.5	0	0.397	0.058
		Placebo	6	9.5	16	25.4	7	14.1	14	65.5	65.4	0.4	0		
	No	Activase	33	13.6	36	14.9	14	14.1	14	67.5	69.9	0.2	0	0.024	0.126
		Placebo	14	5.7	47	19.0	17	15.4	15	66.1	67.4	0.3	0		
CHF Hx	Yes	Activase	9	20.5	16	36.4	9	17.3	18	71.8	71.7	0.7	0	0.602	0.149
		Placebo	5	9.1	16	29.1	8	16.5	17	67.7	69.6	0.4	0		
	No	Activase	36	14.2	34	13.4	15	13.9	14	67.2	69.4	0.2	0	0.078	0.107
		Placebo	14	5.7	45	18.4	15	14.9	14.5	65.7	67.1	0.2	0		
Valvular Ht Dis	Yes	Activase	3	12.0	3	12.0	7							0.740	0.028
		Placebo	2	10.5	3	15.8	0								
	No	Activase	39	14.2	45	16.4	16							0.103	0.125
		Placebo	18	6.5	58	20.8	24								
Race	Black	Activase	10	12.5	17	21.3	9	15.1	15	65.2	67.1	0.4	0	0.886	0.161
		Placebo	5	5.6	15	16.9	10	15.2	14	62.3	63.7	0.3	0		
	White	Activase	30	14.6	30	14.6	12	14.1	14	69.4	70.9	0.2	0	0.159	0.108
		Placebo	12	6.1	42	21.2	12	15.0	15	67.9	69.0	0.2	0		
	Hispanic	Activase	7	36.8	6	31.6	3	13.5	13	69.6	72.4	0.5	0	0.094	0.060
		Placebo	1	5.6	5	27.8	2	16.6	15.5	62.8	67.7	0.3	0		
Prior Stroke	Yes	Activase	8	17.8	5	11.1	9							0.442	0.010
		Placebo	2	5.3	6	15.8	7								
	No	Activase	39	14.7	49	18.4	15							0.097	0.108
		Placebo	18	6.7	58	21.6	17								
Prior TIA Hx	Yes	Activase	7	14.0	7	14.0	2							0.894	0.675
		Placebo	2	4.1	8	16.3	3								
	No	Activase	36	15.0	40	16.7	19							0.041	0.005
		Placebo	16	6.7	49	20.5	18								
Prior Aspirin use	Yes	Activase	26	20.5	22	17.3	12	14.2	14	69.9	71.9	0.3	0	0.504	0.202
		Placebo	6	6.7	14	15.7	10	14.6	14	68.3	68.2	0.4	0		
	No	Activase	22	11.9	32	17.3	12	14.4	15	66.7	68.9	0.2	0	0.133	0.171
		Placebo	14	6.3	50	22.4	14	15.4	15	65.1	67.1	0.2	0		

Subgroup Basis	Subgroup Category	Treatment	N	Number with Recovery by:			Percentage with Recovery by:			Treatment Effect (Change in % recovery) (Activase - Placebo)		
				NIH-SS	Barthel	Rankin	NIH-SS	Barthel	Rankin	NIH-SS	Barthel	Rankin
NIH-SS Category (baseline)	1-7	Activase	72	47	61	56	65.3	84.7	77.8	11.8	5.7	8.0
		Placebo	43	23	34	30	53.5	79.1	69.8			
	8-12	Activase	64	26	42	34	40.6	65.6	53.1	13.7	16.9	15.9
		Placebo	78	21	38	29	26.9	48.7	37.2			
	13-16	Activase	57	18	26	21	31.6	45.6	36.8	10.4	7.7	17.1
		Placebo	66	14	25	13	21.2	37.9	19.7			
	17-21	Activase	61	11	20	16	18.0	32.8	26.2	11.0	13.5	12.2
		Placebo	57	4	11	8	7.0	19.3	14.0			
	22-37	Activase	58	4	13	6	6.9	22.4	10.3	4.0	6.2	5.9
		Placebo	68	2	11	3	2.9	16.2	4.4			
Age Category	25-56	Activase	53	25	39	34	47.2	73.6	64.2	17.8	20.6	39.2
		Placebo	68	20	36	17	29.4	52.9	25.0			
	57-65	Activase	58	18	34	26	31.0	58.6	44.8	11.3	16.4	13.8
		Placebo	71	14	30	22	19.7	42.3	31.0			
	66-71	Activase	70	19	37	28	27.1	52.9	40.0	10.0	21.6	16.6
		Placebo	64	11	20	15	17.2	31.3	23.4			
	72-76	Activase	60	28	30	29	46.7	50.0	48.3	26.3	20.4	20.6
		Placebo	54	11	16	15	20.4	29.6	27.8			
	77-100	Activase	71	16	22	16	22.5	31.0	22.5	8.0	0.1	-2.9
		Placebo	55	8	17	14	14.5	30.9	25.5			
Weight Category	40-62 kg	Activase	61	19	30	24	31.1	49.2	39.3	19.6	18.4	16.3
		Placebo	52	6	16	12	11.5	30.8	23.1			
	62-73 kg	Activase	70	22	37	30	31.4	52.9	42.9	5.2	8.6	10.1
		Placebo	61	16	27	20	26.2	44.3	32.8			
	73-82 kg	Activase	80	35	46	41	43.8	57.5	51.3	24.8	17.8	28.8
		Placebo	58	11	23	13	19.0	39.7	22.4			
	82-93 kg	Activase	52	17	26	22	32.7	50.0	42.3	-0.2	5.7	3.7
		Placebo	70	23	31	27	32.9	44.3	38.6			
	93-200kg	Activase	49	13	23	16	26.5	46.9	32.7	15.3	16.0	17.2
		Placebo	71	8	22	11	11.3	31.0	15.5			

Subgroup Basis			Rankin Scale		NIH-SS		Barthel Index		p-values - Rank-sum test (Activase to placebo)		
Subgroup Category		Treatment	Mean	median	Mean	median	Mean	median	Rankin	NIH-SS	Barthel
NIH-SS Category (baseline)	1-7	Activase	1.1	1	2.6	1	91.9	100	0.370	0.082	0.593
		Placebo	1.3	1	4.0	1	89.0	100			
	8-12	Activase	1.9	1	5.2	2	84.6	100	0.038	0.034	0.029
		Placebo	2.5	2	9.1	3.5	73.2	90			
	13-16	Activase	2.8	3	11.7	4	66.3	90	0.079	0.180	0.275
		Placebo	3.4	3	13.8	7.5	60.4	72.5			
	17-21	Activase	3.5	4	16.3	11	50.3	55	0.577	0.573	0.494
		Placebo	3.8	4	16.9	11	44.8	50			
	22-37	Activase	4.4	5	25.5	25.5	33.1	0	0.708	0.721	0.747
		Placebo	4.5	5	23.6	18.5	32.1	7.5			
Age Category	25-56	Activase	1.8	1	7.3	2	85.4	100	0.001	0.014	0.009
		Placebo	2.9	2.5	12.1	4.5	70.5	95			
	57-65	Activase	2.6	2	11.0	3.5	69.7	95	0.310	0.148	0.238
		Placebo	2.9	3	11.3	7	66.5	80			
	66-71	Activase	2.5	2.5	10.0	5	69.1	95	0.007	0.013	0.009
		Placebo	3.5	4	15.4	9.5	51.0	55			
	72-76	Activase	2.6	2	12.3	2	64.0	92.5	0.129	0.033	0.118
		Placebo	3.2	4	14.3	7	54.4	57.5			
	77-100	Activase	3.6	4	16.8	11	50.0	55	0.830	0.665	0.827
		Placebo	3.6	4	17.7	12	46.3	40			
Weight Category	40-62 kg	Activase	2.6	2	10.6	4	66.6	90	0.014	0.009	0.036
		Placebo	3.6	4	16.2	10	48.8	57.5			
	62-73 kg	Activase	2.7	2.5	12.3	4.5	66.6	95	0.412	0.350	0.513
		Placebo	3.0	3	14.1	7	59.4	80			
	73-82 kg	Activase	2.4	1	10.7	2	69.7	100	0.010	0.014	0.034
		Placebo	3.3	3	14.7	7.5	58.8	80			
	82-93 kg	Activase	3.0	3	15.1	4	58.6	92.5	0.575	0.549	0.967
		Placebo	2.8	3	11.6	5	64.0	82.5			
	93-200kg	Activase	2.7	3	10.6	5	70.4	90	0.078	0.037	0.061
		Placebo	3.4	3	14.0	8	59.3	75			

Subgroup Basis			ICH (all)		Mortality by 90 d		Prior Disabilit	NIH-SS Baseline		Age		PreStroke Rankin (est)		p-values - Rank-sum test (Activase to placebo)	
Subgroup Category		Treatment	n	%	n	%	n	Mean	Median	Mean	Median	Mean	Median	NIH-SS (b	Age
NIH-SS Category (baseline)	1-7	Activase	5	6.9	1	1.4	5							0.004	0.278
		Placebo	1	2.3	2	4.7	1								
	8-12	Activase	4	6.3	2	3.1	7							0.330	0.935
		Placebo	4	5.1	9	11.5	5								
	13-16	Activase	9	15.8	9	15.8	1							0.908	0.014
		Placebo	1	1.5	13	19.7	1								
	17-21	Activase	13	21.3	14	23.0	6							0.664	0.763
		Placebo	8	14.0	14	24.6	7								
	22-37	Activase	17	29.3	28	48.3	5							0.091'	0.050
		Placebo	6	8.8	26	38.2	10								
Age Category	25-56	Activase	3	5.7	5	9.4	4	13.9	14	48.7	50.1	0.2	0	0.487	0.880
		Placebo	2	2.9	13	19.1	4	14.9	14.5	48.2	50.1	0.2	0		
	57-65	Activase	8	13.8	10	17.2	6	14.1	13.5	61.6	62.5	0.3	0	0.414	0.453
		Placebo	6	8.5	10	14.1	5	14.6	14	62.0	62.5	0.3	0		
	66-71	Activase	8	11.4	8	11.4	3	14.2	14	69.2	69.3	0.2	0	0.153	0.412
		Placebo	4	6.3	14	21.9	1	15.9	16	68.9	68.6	0.2	0		
	72-76	Activase	7	11.7	13	21.7	5	12.5	11	74.4	74.2	0.3	0	0.089	0.829
		Placebo	3	5.6	12	22.2	6	14.4	13	74.3	74.3	0.3	0		
	77-100	Activase	22	31.0	18	25.4	6	16.6	18	81.1	80.3	0.4	0	0.809	0.718
		Placebo	5	9.1	15	27.3	8	16.3	16	81.5	80.3	0.5	0		
Weight Category	40-62 kg	Activase	7	11.5	8	13.1	3							0.033	0.523
		Placebo	7	13.5	13	25.0	7								
	62-73 kg	Activase	9	12.9	14	20.0	8							0.496	0.498
		Placebo	2	3.3	13	21.3	2								
	73-82 kg	Activase	16	20.0	12	15.0	6							0.016	0.037
		Placebo	2	3.4	13	22.4	1								
	82-93 kg	Activase	9	17.3	14	26.9	1							0.473	0.147
		Placebo	2	2.9	11	15.7	7								
	93-200kg	Activase	7	14.3	6	12.2	6							0.442	0.122
		Placebo	7	9.9	14	19.7	7								

Subgroup Basis	Subgroup Category	Treatment	N	Number with Recovery by:			Percentage with Recovery by:			Treatment Effect (Change in % recovery) (Activase - Placebo)		
				NIH-SS	Barthel	Rankin	NIH-SS	Barthel	Rankin	NIH-SS	Barthel	Rankin
Atrial Fibril. Hx	Yes	Activase	58	16	23	18	27.6	39.7	31.0	15.3	11.6	11.7
		Placebo	57	7	16	11	12.3	28.1	19.3			
	No	Activase	250	88	136	113	35.2	54.4	45.2	12.8	13.8	16.9
		Placebo	254	57	103	72	22.4	40.6	28.3			
Hypertension (within 24 hr)	Yes	Activase	48	12	18	15	25.0	37.5	31.3	13.0	21.5	19.3
		Placebo	50	6	8	6	12.0	16.0	12.0			
	No	Activase	264	94	144	118	35.6	54.5	44.7	13.5	12.2	15.3
		Placebo	262	58	111	77	22.1	42.4	29.4			
Fibrinogen (baseline)	< 200	Activase	23	8	8	8	34.8	34.8	34.8	27.6	-8.1	13.4
		Placebo	14	1	6	3	7.1	42.9	21.4			
	>= 200	Activase	260	92	142	115	35.4	54.6	44.2	13.8	15.8	16.8
		Placebo	273	59	106	75	21.6	38.8	27.5			
Thrombus visual. (on CT)	Yes	Activase	33	5	11	5	15.2	33.3	15.2	8.2	3.1	-1.1
		Placebo	43	3	13	7	7.0	30.2	16.3			
	No	Activase	274	98	148	125	35.8	54.0	45.6	12.7	14.0	16.9
		Placebo	265	61	106	76	23.0	40.0	28.7			
Edema on CT	Yes	Activase	13	4	5	4	30.8	38.5	30.8	23.1	0.0	15.4
		Placebo	13	1	5	2	7.7	38.5	15.4			
	No	Activase	294	99	154	126	33.7	52.4	42.9	12.3	13.7	15.4
		Placebo	295	63	114	81	21.4	38.6	27.5			
Mass effect on CT	Yes	Activase	9	2	3	3	22.2	33.3	33.3	11.1	11.1	11.1
		Placebo	9	1	2	2	11.1	22.2	22.2			
	No	Activase	298	101	156	127	33.9	52.3	42.6	12.8	13.2	15.5
		Placebo	299	63	117	81	21.1	39.1	27.1			

Subgroup Basis			Rankin Scale		NIH-SS		Barthel Index		p-values - Rank-sum test (Activase to placebo)		
Subgroup	Category	Treatment	Mean	median	Mean	median	Mean	median	Rankin	NIH-SS	Barthel
Atrial Fibril. Hx	Yes	Activase	3.3	3	16.3	8	54.3	65	0.226	0.170	0.323
		Placebo	3.8	4	18.1	13	46.8	50			
	No	Activase	2.5	2	10.6	3	69.3	95	0.003	0.002	0.006
		Placebo	3.0	3	12.9	6	61.4	77.5			
Hypertension (within 24 hr)	Yes	Activase	3.2	3	15.7	8	55.9	85	0.054	0.119	0.056
		Placebo	4.0	4	19.0	13	42.8	45			
	No	Activase	2.6	2	11.0	3	68.6	95	0.005	0.002	0.016
		Placebo	3.0	3	13.0	6	61.5	80			
Fibrinogen (baseline)	< 200	Activase	3.4	4	18.1	11	48.5	50	1.000	0.715	0.670
		Placebo	3.4	3.5	16.8	9	56.1	65			
	>= 200	Activase	2.6	2	10.8	3	69.0	95	0.001	0.000	0.001
		Placebo	3.1	3	13.5	7	59.7	75			
Thrombus visual. (on CT)	Yes	Activase	3.8	4	16.2	13	49.2	40	0.713	0.632	0.704
		Placebo	3.6	4	16.0	9	54.0	60			
	No	Activase	2.5	2	11.1	3	68.9	95	0.038	0.034	0.029
		Placebo	3.1	3	13.7	7	59.6	75			
Edema on CT	Yes	Activase	3.1	3	12.6	3	66.2	90	0.344	0.117	0.413
		Placebo	3.9	4	20.6	14	45.0	25			
	No	Activase	2.7	2	11.6	4	66.8	95	0.002	0.002	0.005
		Placebo	3.2	3	13.7	7	59.5	75			
Mass effect on CT	Yes	Activase	3.8	4	20.0	7	47.8	45	0.853	0.554	0.818
		Placebo	4.1	4	21.2	16	35.0	25			
	No	Activase	2.6	2	11.4	4	67.3	95	0.001	0.001	0.004
		Placebo	3.2	3	13.8	7	59.5	75			

Subgroup Basis			ICH (all)		Mortality by 90 d		Prior Disabilit	NIH-SS Baseline		Age		PreStroke Rankin (est)		p-values - Rank-sum test (Activase to placebo)	
Subgroup Category		Treatment	n	%	n	%	n	Mean	Median	Mean	Median	Mean	Median	NIH-SS (b	Age
Atrial Fibril. Hx	Yes	Activase	15	25.9	16	27.6	2	15.1	16	74.9	75.8	0.2	0	0.268	0.467
		Placebo	7	12.3	16	28.1	3	16.8	16	74.1	73.3	0.2	0		
	No	Activase	32	12.8	38	15.2	22	14.3	14	66.5	69.1	0.3	0	0.258	0.018
		Placebo	13	5.1	47	18.5	20	14.8	14	64.2	65.4	0.3	0		
Hypertension	Yes	Activase	11	22.9	11	22.9	5	14.7	13	67.7	67.8	0.4	0	0.231	0.486
(within 24 hr)		Placebo	4	8.0	15	30.0	3	16.2	16.5	66.0	68.0	0.3	0		
	No	Activase	37	14.0	43	16.3	19	14.3	14	68.1	70.2	0.2	0	0.217	0.029
		Placebo	16	6.1	49	18.7	21	15.0	14.5	66.0	67.1	0.3	0		
Fibrinogen	< 200	Activase	6	26.1	7	30.4	0	16.4	14	69.0	70.3	0.1	0	0.347	0.222
(baseline)		Placebo	1	7.1	4	28.6	0	18.8	20	63.6	66.8	0.1	0		
	>= 200	Activase	36	13.8	39	15.0	22	14.0	14	68.1	70.1	0.3	0	0.062	0.034
		Placebo	17	6.2	52	19.0	23	15.0	15	66.0	67.2	0.3	0		
Thrombus visual.	Yes	Activase	7	21.2	6	18.2	2	18.7	19	69.9	71.3	0.2	0	0.191	0.014
(on CT)		Placebo	7	16.3	11	25.6	2	17.0	17	64.2	65.7	0.2	0		
	No	Activase	41	15.0	46	16.8	22	13.8	13	67.9	69.7	0.3	0	0.030	0.125
		Placebo	13	4.9	53	20.0	22	14.9	14	66.3	68.0	0.3	0		
Edema on CT	Yes	Activase	3	23.1	3	23.1	0	14.3	13	67.0	72.4	0.1	0	0.837	0.522
		Placebo	1	7.7	4	30.8	3	14.6	14	64.8	66.5	0.8			
	No	Activase	45	15.3	49	16.7	24	14.3	14	68.1	69.9	0.3	0	0.077	0.028
		Placebo	19	6.4	60	20.3	21	15.3	15	66.0	67.4	0.3	0		
Mass effect on CT	Yes	Activase	3	33.3	4	44.4	0	19.1	20	72.3	73.1	0.1	0	0.330	0.691
		Placebo	2	22.2	3	33.3	0	15.2	17	72.7	70.4	0.0	0		
	No	Activase	45	15.1	48	16.1	24	14.2	14	67.9	69.8	0.3	0	0.054	0.021
		Placebo	18	6.0	61	20.4	24	15.2	15	65.8	67.2	0.3	0		