Early stroke treatment associated with better outcome: The NINDS rt-PA Stroke Study

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Neurology 2000;55;1649-1655

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The NINDS rt-PA Stroke Study

J.R. Marler, MD; B.C. Tilley, PhD; M. Lu, PhD; T.G. Brott, MD; P.C. Lyden, MD; J.C. Grotta, MD; J.P. Broderick, MD; S.R. Levine, MD; M.P. Frankel, MD; S.H. Horowitz, MD; E.C. Haley, Jr., MD; C.A. Lewandowski; and T.P. Kwiatkowski, MD, for the NINDS rt-PA Stroke Study Group*

Article abstract—*Background:* The National Institute of Neurological Disorders and Stroke (NINDS) rt-PA Stroke Study showed a similar percentage of intracranial hemorrhage and good outcome in patients 3 months after stroke treatment given 0 to 90 minutes and 91 to 180 minutes after stroke onset. At 24 hours after stroke onset more patients treated 0 to 90 compared to 91 to 180 minutes after stroke onset had improved by four or more points on the NIH Stroke Scale (NIHSS). The authors performed further analyses to characterize the relationship of onset-to-treatment time (OTT) to outcome at 3 months, early improvement at 24 hours, and intracranial hemorrhage within 36 hours. *Methods:* Univariate analyses identified potentially confounding variables associated with OTT that could mask an OTT–treatment interaction. Tests for OTT–treatment interactions adjusting for potential masking confounders were performed. An OTT–treatment interaction was considered significant if $p \leq 0.10$, implying that treatment effectiveness was related to OTT. *Results:* For 24-hour improvement, there were no masking confounders identified and there was an OTT–treatment interaction ($p = 0.08$). For 3-month favorable outcome, the NIHSS met criteria for a masking confounder. After adjusting for NIHSS as a covariate, an OTT–treatment interaction was detected ($p = 0.09$): the adjusted OR (95% CI) for a favorable 3-month outcome associated with recombinant tissue-type plasminogen activator (rt-PA) was 2.11 (1.33 to 3.35) in the 0 to 90 minute stratum and 1.69 (1.09 to 2.62) in the 91 to 180 minute stratum. In the group treated with rt-PA, after adjusting for baseline NIHSS, an effect of OTT on the occurrence of intracranial hemorrhage was not detected. *Conclusions:* If the NINDS rt-PA Stroke Trial treatment protocol is followed, this analysis suggests that patients treated 0 to 90 minutes from stroke onset with rt-PA have an increased odds of improvement at 24 hours and favorable 3-month outcome compared to patients treated later than 90 minutes. No effect of OTT on intracranial hemorrhage was detected within the group treated with rt-PA, possibly due to low power.

NEUROLOGY 2000;55:1649–1655

Both trials in the National Institute of Neurological Disorders and Stroke (NINDS) rt-PA Stroke Study[1] demonstrated the effectiveness of recombinant tissue-type plasminogen activator (rt-PA) for the treatment of acute ischemic stroke when started within 3 hours of stroke onset. Because time from stroke onset to treatment was considered an important possible influence on subsequent outcome, randomization of patients in these two trials included stratification on time from stroke onset (0 to 90 minutes, 91 to 180 minutes). In the initial analysis of the results, there was little apparent observed difference

*See the Appendix on page 1654 for a list of study participants.

From the National Institute of Neurological Disorders and Stroke, Rockville, MD.

Supported by the National Institute of Neurological Disorders and Stroke (NINDS) (N01-NS-02382, N01-NS-02374, N01-NS-02377, N01-NS-02381, N01-NS-02379, N01-NS-02373, N01-NS-02378, N01-NS-02376, N01-NS-02380). Genentech, Inc., South San Francisco, provided for supply and distribution of study drug (placebo and Activase, Alteplase) as well as clinical site monitoring.

Received October 21, 1998. Accepted in final form August 24, 2000.

Address correspondence and reprint requests to Dr. John R. Marler, Associate Director for Clinical Trials, National Institute of Neurological Disorders and Stroke, Neuroscience Center, Room 2216, 6001 Executive Boulevard, Rockville, MD 20892.

between the two time strata in the number of favorable outcomes at 3 months or in the number of intracranial hemorrhages. The apparent lack of additional benefit for earlier treatment was unexpected because pilot studies[2,3] and laboratory research[4] had shown reduced rates of hemorrhage and increased benefit with earlier treatment. A recent study has commented on the strength of the research data supporting the concept that earlier treatment would be expected to produce a better outcome.[5] Although there appeared to be a difference between the two time strata in the proportion of patients showing either complete improvement or a four-point improvement in NIH Stroke Scale (NIHSS) at 24 hours, formal testing of an interaction was not conducted.

Seeking to understand the unexpected lack of difference between patient outcomes in the two time strata of these two trials, we proposed several explanations for the apparent lack of effect of time on patient outcome at 3 months: 1) patients treated sooner after stroke onset could have come to medical attention earlier because their presenting symptoms were more severe or noticeable; 2) patients starting treatment sooner after stroke onset may have come at a different time of day and received different care in the emergency department or intensive care unit; 3) patients treated earlier may have ischemic stroke subtypes less responsive to thrombolytic treatment; 4) for patients treated earlier compared to those treated later, baseline characteristics predicting better outcomes may have been distributed differently between the rt-PA and placebo groups.

After adjusting for patient baseline characteristics, as necessary, we performed further analyses of the data from the two trials in the NINDS rt-PA Stroke Study to determine if we could detect an onset-to-treatment time (OTT)–treatment interaction association with any of the outcomes of interest (24 hour improvement, 3 month favorable outcome, or hemorrhage in the first 36 hours). If an interaction was detected this would imply that treatment effect varied depending on OTT.

Methods. The NINDS rt-PA Stroke Study was performed in two parts, each of which was a separate trial conducted at eight centers using over 40 hospitals. The two parts differed only in the prospectively defined primary outcome. Data from both parts of the study were combined for the analyses reported here to obtain more statistical power and a more complete picture of the effect of OTT on patient outcomes.

Hypotheses. The protocols in place before the start of both parts of the study specified testing the secondary hypotheses relating to treatment effectiveness within two OTT strata. The most powerful approach to testing these hypotheses is to test for an interaction between OTT by treatment interaction treating time as a continuum. Such an interaction, if present, implies that treatment effectiveness varies across OTT intervals and treatment effects within OTT strata should be assessed.

Definition of outcomes. As described in the original report, rt-PA was considered to be effective 3 months after stroke if there was a consistent and persuasive difference in the proportions of rt-PA compared to placebo-treated patients with favorable outcome. A favorable outcome was defined as recovery with minimal or no deficit 3 months after treatment using four outcome measures: the Barthel Index[6] $\geq$ 95, modified Rankin Scale[7] $\leq$ 1, Glasgow Outcome Scale[8] of 1, and NIHSS[9] score $\leq$ 1.

A patient was considered to show improvement 24 hours after stroke if the patient had at least a four-point improvement from baseline in NIHSS score or a complete resolution of symptoms. For the analysis of hemorrhage, both symptomatic and asymptomatic intracranial hemorrhages occurring within 36 hours were included because the total number of symptomatic hemorrhages was so small. The definition of intracranial hemorrhage within 36 hours of treatment and methods for assessing hemorrhage are described elsewhere.[10] More detail on the assessment of outcomes is given in the initial report on the NINDS trials.[1]

Determination of time of stroke onset. Time of stroke onset was determined by interviewing patients and any available observers present when the stroke was first noticed. Physicians sought corroborating evidence (such as ambulance reports) and carefully screened for the possibility of onset during sleep. If the patient awoke with stroke symptoms, the time of onset was taken as the last time the patient was known to be awake and without any symptoms of stroke. If the onset time could not be established with confidence, the patient was not randomized. After randomization, the time of onset was re-reviewed using the emergency medical system logs and notes in the medical record. If discrepancies were found, corrections were made. It was through this review that two patients were identified who were randomized outside the 180-minute window. Audits of source records for all patients were performed to confirm consistency of stroke onset time and treatment start time determinations. The difference between these two times is referred to as the OTT.

Randomization and treatment. In both the Part 1 and Part 2 trials, a permuted block randomization was used with varying block sizes stratified by clinical center and OTT (0 to 90 or 91 to 180 minutes). No other covariates were used as stratifying variables. To balance the number of patients treated in 0 to 90 minutes from stroke onset with those treated 91 to 180 minutes, no center was to randomize a patient at 91 to 180 minutes when they had already treated two more patients in the 91 to 180 minute group than in the 0 to 90 minute group. Patients received alteplase (Activase, an rt-PA manufactured by Genentech, South San Francisco) or placebo, 0.9 mg/kg (maximum 90 mg), with 10% given as a bolus followed by the remainder as a constant infusion over 60 minutes.

Patient selection. A total of 624 patients were randomized to both trials in the study. The two patients inadvertently randomized outside the protocol-specified limit of 180 minutes were included in the original analyses based on the intention-to-treat principle. For one of these patients study treatment was started 195 minutes from stroke onset; for the second patient, treatment was started 24 hours after stroke onset. The focus of this report was treatment within 180 minutes of stroke onset. Whereas inclusion of these two patients did not change our results, we did not believe we should be extrapolating our results beyond 180 minutes based on data from two patients. Thus

we excluded these two patients from the analysis. Data from the remaining 622 patients are included in the tables and analyses presented in this report.

Statistical analysis. To understand more about OTT, we assessed the association between OTT and two other time-related variables (time of day and time from emergency department admission to treatment) using analyses of variance on the ranked data.

The primary objective was to determine if confounding variables could be masking the association of the OTT–treatment interaction with treatment outcomes; i.e., 3-month favorable outcome, 24-hour improvement, or hemorrhage within 36 hours. (In other reports on 3-month favorable outcome and hemorrhage, no baseline variable by treatment interactions had been detected, including interactions with OTT.[10,11]) As a sufficient condition for a baseline variable to be a masking confounder for the OTT–treatment interaction, a variable must be associated with both the OTT–treatment interaction and the outcome of interest. As a necessary condition, leaving this variable out of the model testing the association of the interaction with treatment outcome would mask the association.[12]

Because the main effects for OTT and treatment would be in any regression model testing the baseline variable associations with the OTT–treatment interaction, we first tested for associations between the relevant baseline variables and OTT. OTT was uncategorized and we tested for associations by testing whether the Spearman's correlations were equal to zero. The baseline variables included in the analysis were those variables considered in previous reports to be associated with the outcomes of interest (3 months favorable outcome, 24-hour improvement, and hemorrhage)[11] because these variables would be the only variables that could meet the sufficient condition above. We had previously tested for associations with treatment and found only associations with weight and aspirin. Neither variable was associated with outcome (sufficient condition).[1]

If potential confounding variables were not identified, we tested directly for OTT–treatment interactions using a logistic model (24 hours improvement and hemorrhage) or global test (3-month favorable outcome). More detail on the global analytical approach is published elsewhere.[13] Where potential masking confounders were identified, we fit multivariable models to test for the OTT–treatment interactions, adjusting for the potential confounders again using the logistic regression or global tests depending on the outcome of interest. As noted above, in the models testing for OTT–treatment interactions, OTT and treatment main effects were included. Consistent with our previous work,[10,11] an interaction was considered significant if the p value was ≤ 0.10. We chose a significance level greater than 0.05, recognizing the limitations in power when testing for multiplicative interactions in multiplicative models. We also checked the underlying assumption of linearity in the log odds for the interaction terms and could not detect violations of this assumption. Where an interaction was detected, we present OTT stratified as 0 to 90 and 91 to 180 minutes from stroke onset, the strata used in randomization, along with odds ratios and 95% confidence limits. We present a graphic representation of the OTT–treatment interaction, with OTT uncategorized, to give a further illustration of the OTT–treatment interaction and its effect on treatment outcome. For the analysis considering NIHSS as a masking confounder where the outcome was hemorrhage, the baseline NIHSS was divided into five different groups (NIHSS <5, 6 to 10, 11 to 15, 16 to 20, and >20) to induce linearity in the associated log odds of hemorrhage. For all other analyses, the NIHSS was treated as a continuum. Because there were only 10 symptomatic and asymptomatic intracranial hemorrhages in the placebo group, analysis of the OTT hemorrhage association within the rt-PA treated group was planned regardless of the computed p value for the test of the interaction.

Analyses were repeated excluding 35 patients reported to have edema or mass effect on the initial CT scan.[1] These patients might represent patients whose time of stroke

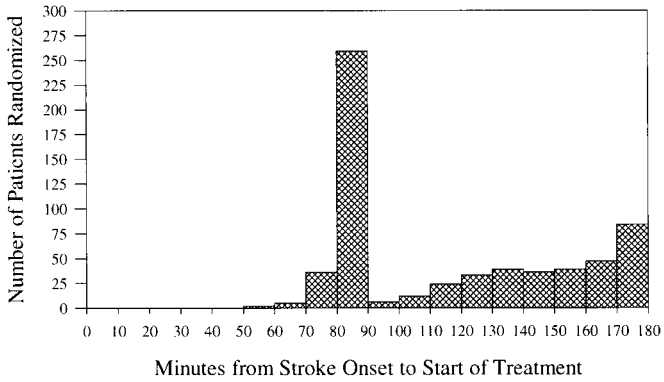

Figure 1. Histogram shows number of patients with time from stroke onset to start of treatment (onset-to-treatment time [OTT]) in intervals of 10 minutes. Total number of patients is 622 (excludes two patients randomized outside the 180-minute window).

Table 1 *Time from stroke onset (onset-to-treatment time [OTT]), delay after admission, and time of stroke onset*

	0–90 Minute OTT stratum, n = 302		91–180 Minute OTT stratum, n = 320	
Variable	rt-PA	Placebo	rt-PA	Placebo
No. of patients	157	145	153	167
OTT, min, mean (SD); median	86.2 (5.4); 89	86.4 (5.4); 88	153.1 (22.4); 156	149.0 (23.8); 151
Onset time of day (24 hour clock), mean time (SD)	14:03 (4:36)	13:33 (4:10)	13:19 (5:11)	13:51 (4:49)
Delay from admission to treatment, min, mean (SD); median	53.7 (14.9); 53	55.2 (17.8); 54	84.7 (26.9); 84	81.9 (27.0); 79

rt-PA = recombinant tissue-type plasminogen activator.

Table 2 *Similarity of baseline deficits: NIH Stroke Scale (NIHSS) components by stroke onset to treatment (onset-to-treatment time [OTT]) strata and treatment group*

	Patients having NIHSS component with abnormal score >1, %*			
	OTT 0–90 min		OTT 91–180 min	
Characteristics	rt-PA	Placebo	rt-PA	Placebo
No. of patients	157	145	153	167
Level of consciousness	31	23	35	36
Best gaze	56	58	46	51
Best visual	61	65	48	57
Ataxia	6	10	5	7
Sensory	67	72	61	71
Best language	57	49	48	54
Dysarthria	80	85	81	85
Extinction and inattention	57	63	53	59
Average motor (face, arm, leg)	73	75	63	77

* Patients may have more than one abnormal component.

rt-PA = recombinant tissue-type plasminogen activator.

onset was incorrectly classified and could have a longer time between stroke onset and treatment than reported.

Results. *Baseline characteristics.* From January 1991 through October 1994, 624 patients were randomized in the two trials, Part 1 and Part 2. Excluding the two patients described in Methods, 302 patients were randomized in the 0 to 90 minute stratum and 320 in the 91 to 180 minute stratum. The clinical investigators were able to maintain the balance between the two OTT strata throughout the two trials. Based on the final determination of OTT, on 25 occasions (4% of randomizations) patients were randomized to the 91 to 180 minute strata at centers where there were already two more patients in the 91 to 180 minute stratum than the 0 to 90 minute stratum.

The distribution of OTT is shown in figure 1 for the combined Part 1 and Part 2 trial cohorts. OTT for the patients in the 0 to 90 minute stratum clustered close to the 90-minute limit. OTT for the patients in the 91 to 180 stratum was more evenly distributed across the time interval. Table 1 gives the distribution of time-related variables within the OTT strata and treatment categories. We detected no OTT strata or treatment group associations for time of day of stroke onset ($p > 0.7$). For time from emergency department admission to treatment there was no detectable association with treatment group ($p > 0.63$), but there was an association with OTT strata ($p < 0.001$). Delay from emergency department admission to start of treatment was 30 minutes longer for patients in the 91 to 180 minute strata (see table 1).

In order to compare differences in presenting signs, the components of the stroke scale are presented by treatment group and OTT strata in table 2. There are no apparent differences in the type of neurologic symptoms between OTT strata.

There was an association between OTT and baseline NIHSS whether or not the stroke scale was categorized (Spearman correlation $p = 0.05$, NIHSS as continuous variable; $p = 0.04$, NIHSS as categorical variable). We also noted that the mean and median NIHSS values at baseline were higher for the rt-PA–treated group compared to placebo group treated 0 to 90 minutes after onset and lower for the rt-PA compared to placebo groups treated at 91 to 180 minutes, confirmed by a significant OTT × treatment interaction with NIHSS as the outcome (table 3). Because of the presence of the interaction we tested the association within treatment group. Within the rt-PA group, the association between OTT and baseline NIHSS remained significant (Spearman correlation $p = 0.04$, NIHSS as continuous variable; $p = 0.02$, NIHSS as categorical variable), but there was no significant association detected in the placebo group (Spearman correlation $p = 0.52$, NIHSS as continuous variable; $p = 0.60$, NIHSS as categorical variable).

Table 3 *Baseline NIH Stroke Scale (NIHSS) by time from stroke onset to treatment (onset-to-treatment time [OTT]) strata and treatment group*

	OTT strata				
	0–90 Minute, n = 302		91–180 Minute, n = 320		
NIHSS	rt-PA	Placebo	rt-PA	Placebo	*p* Value*
No. of patients	157	145	153	167	
NIHSS, mean (SD); median	15.2 (7.2); 15	15.0 (6.7); 14	13.5 (7.7); 12	15.4 (6.9); 15	0.06
NIHSS groups, percent					0.03
0–5	8.3	6.2	19.0	4.2	
6–10	19.1	25.5	24.2	27.5	
11–15	24.8	21.4	17.0	21.0	
16–20	25.5	25.5	21.6	19.8	
>20	22.3	21.4	18.3	27.5	

* *p* Value associated with test of the OTT strata by treatment interaction using analysis of variance on ranked data with NIHSS as the outcome. The presence of an interaction implies the NIHSS magnitude varies by OTT and treatment strata.

rt-PA = recombinant tissue-type plasminogen activator.

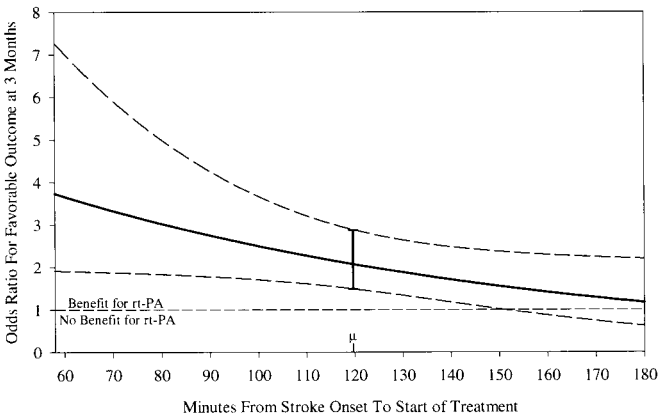

Figure 2. Graph of model estimating OR for favorable outcome at 3 months in recombinant tissue-type plasminogen activator (rt-PA) treated patients compared to placebo treated patients by time from stroke onset to treatment (onset-to-treatment time [OTT]) with 95% confidence intervals, adjusting for the baseline NIH Stroke Scale. OR > 1 indicates greater odds that rt-PA treated patients will have a favorable outcome at 3 months compared to the placebo treated patients. Range of OTT was 58 to 180 minutes with mean (μ) of 119.7 minutes.

Previous work[10,11] had also shown that there was a relationship between the NIHSS favorable outcome at 3 months ($p < 0.001$), and with hemorrhage within 36 hours ($p < 0.001$). Thus, NIHSS was a potential masking confounder. Because by chance the rt-PA treated patients in the 91 to 180 minute stratum had less severe strokes than the patients in the placebo group, the effect of rt-PA may have appeared to be greater than it actually was. In the 0 to 90 minute stratum the stroke severity was more equal between treatment groups. Comparison of the effects of rt-PA in the two strata may be misleading if the imbalance in stroke scales between the rt-PA and placebo groups is not taken into account.

There was also an association between OTT and presumptive stroke subtype determined at baseline. Patients with small vessel strokes were treated later than patients with cardioembolic or large vessel strokes ($p = 0.02$). Stroke subtype was not associated with the 24-hour outcome and was not associated with 3-month favorable outcome or hemorrhage (all p values > 0.05) after adjusting for the NIHSS. Thus stroke subtype was not a potential masking confounder. There was no association detected (all p values > 0.05) between OTT and the other baseline variables considered.

Improvement from baseline at 24 hours. For the 24-hour outcome (complete resolution of symptoms or a four or more point improvement in the NIHSS) an OTT–treatment interaction was detected (OR 0.992, 95% CI 0.98 to 1.00, $p = 0.08$) without adjustment for any baseline variables. Because we are considering a multiplicative interaction on a multiplicative scale, the interaction effect size appears small. To further represent the interaction, we categorized OTT into the two strata used for randomization.

The OR comparing rt-PA to placebo for patients treated within 0 to 90 minutes of stroke onset was 1.71 (95% CI 1.09 to 2.70, $p = 0.02$). An OR >1 indicates that the odds of a four or more point NIHSS improvement at 24 hours are greater in rt-PA treated patients compared to patients receiving placebo. The OR for patients treated between 91 and 180 minutes after stroke onset was 1.12 (95% CI 0.71 to 1.76, $p = 0.62$).

Favorable clinical outcome at 3 months. In the model without covariates an OTT–treatment interaction was not detected ($p = 0.31$, OR 0.996, 95% CI 0.988 to 1.004). In the model including the NIHSS, an OTT–treatment interaction was detected ($p = 0.09$, interaction OR 0.993, 95% CI 0.983 to 1.001). The magnitude of the interaction is again small, given the use of the multiplicative model. To characterize the relationship of OTT to favorable outcome, figure 2 shows a graphical representation of the OTT–treatment interaction (with OTT as a continuum) in terms of the OR for a favorable outcome, after adjusting for NIHSS. As we did for 24-hour outcome, we also categorized OTT by the randomization strata. We then computed the OR (95% confidence limits) for a favorable outcome at 3 months, adjusting for baseline NIHSS. The adjusted OR for the rt-PA treated group as compared to placebo group was 2.11 (1.33 to 3.35), $p = 0.002$ for the 0 to 90 minute time strata. The adjusted OR was 1.69 (1.09 to 2.62), $p = 0.02$ for the 91 to 180 minute time strata.

The OTT–treatment interaction remained significant, $p = 0.04$, OR 0.991 (0.98 to 1.00), in a multivariable model of 3-month favorable outcome including the NIHSS and all other covariates identified in a previous report as being associated with 3-month favorable outcome.[11] The adjusted OR (95% CI) for a favorable outcome at 3 months for the rt-PA treated group as compared to placebo group was 2.53 (1.53 to 4.19), $p = 0.0003$ for the 0 to 90 minute time strata. The adjusted OR was 1.61 (1.02 to 2.55), $p = 0.04$ for the 91 to 180 minute time strata.

Symptomatic and asymptomatic hemorrhage within 36 hours. Because baseline NIHSS was associated with both intracranial hemorrhage within 36 hours and OTT, we tested for an interaction between treatment group and OTT adjusting for categorized NIHSS. No interaction was detected for all hemorrhages, $p = 0.24$, OR 0.99 (0.97 to 1.01), or for symptomatic hemorrhages, $p = 0.74$, OR 0.99 (0.95 to 1.03). Within the rt-PA group alone, after adjusting for baseline NIHSS, no OTT association with hemorrhage was detected ($p > 0.76$).

Exclusion of patients with midline shift and edema. When the 35 patients with severe edema and midline shift at baseline were excluded, the results were similar to the analyses including these 35 patients presented above.

Discussion. OTT was shown to be associated with a differential response to treatment both at 24 hours and 3 months after stroke. Due to chance, there was an imbalance in the NIHSS severity of stroke randomized in the two treatment groups at different OTT. Because baseline NIHSS is a good predictor of outcome, this imbalance in the treatment groups increased the number of favorable outcomes in the rt-PA group treated 91 to 180 minutes from stroke onset and reduced them in the group treated with rt-PA between 0 and 90 minutes. The imbalance obscured or confounded the increased response to treatment in the rt-PA group treated early compared to the rt-PA group treated later.

Before this analysis, the apparent similarity of the

outcomes for the patients treated early and late could have encouraged the natural proclivity to delay treatment until the last possible minute. The clustering of patients in the 90 minute group (see figure 1) and the longer delay from emergency department admission to treatment in the 91 to 180 minute patient group demonstrates this tendency. The US Food and Drug Administration approval of rt-PA for use in all patients up to 3 hours from stroke onset could lead to clustering of treatment at 3 hours rather than 90 minutes. We observed a demonstration of this phenomenon in one of our clinical centers. In order to avoid turning away patients, investigators at this center did not open the 91 to 180 arm of the Part 1 trial at their center until there were 11 patients enrolled in the 0 to 90 minute stratum. Once they opened the 91 to 180 minute arm, time from admission to treatment increased from a median of 58 minutes to a median of 65 minutes and within 19 months the "surplus" of 0 to 90 minute patients was decreased to the two patient difference tolerated by the protocol.[14] Whereas it is good to have some benefit to offer patients who come late for treatment with rt-PA, the results of our modeling suggest that the effort should be to treat each patient as early as possible before 3 hours. The fact that the models are consistent with our understanding of the pathophysiology of stroke and the mechanism of action of rt-PA adds to the significance of the findings reported here.

Before the initiation of the NINDS rt-PA Stroke Study, data from laboratory and pilot clinical studies with tissue plasminogen activator for ischemic stroke had suggested time was an important factor in determining outcome and the number of symptomatic hemorrhages.[2,3] Improved functional outcome in a laboratory model of multifocal ischemic brain injury is dose dependent.[15] This is demonstrated by a graded response with reduced recovery rates as the delay between onset of ischemia and start of rt-PA infusion increases from 15 to 30, 45, and 60 minutes. In the 74-patient 0 to 90 minute open-label pilot study for the NINDS rt-PA Stroke Study, 46% of patients showed a four point improvement at 24 hours on the NIHSS and 4% had symptomatic intracranial hematoma. In a parallel 21-patient 91 to 180 minute pilot study, 15% of patients had a four-point improvement on the NIHSS at 24 hours and 10% had symptomatic intracranial hematoma rate. Comparison of these two open label studies suggested that there would be less benefit and more hemorrhages in patients treated after 90 minutes. An independent 93-patient open label study of angiographic patency[16] showed a statistically significant difference in the number of intracerebral hemorrhages related to the time from stroke onset to treatment with tissue plasminogen activator. In that study patients who developed hemorrhagic transformation had a mean OTT of 6.1 $\pm$ 1.5 hours compared to 5.3 $\pm$ 1.7 hours for those who did not show evidence of hemorrhagic infarction.

The failure to detect the treatment $\times$ OTT interaction in the initial analyses of stroke outcome[13] highlights a limitation of hierarchical modeling. In hierarchical modeling, the best set of three variables is chosen from the best set of two plus a new variable chosen from all variables not yet included. If we were truly looking for the best set of three variables, we would choose from among all sets of three variables, not just from the best set of two variables plus one. This limitation was acknowledged in the previous report. Best subset selection, used in traditional regression analyses,[17] would have identified this OTT $\times$ treatment interaction, but this approach was not computationally feasible given the large number of variables and potential interactions, and the lengthy calculation required by the Generalized Estimating Equations (GEE) approach to developing a global test statistic. In the future, we expect that researchers will develop a computationally efficient approach to best subset selection that might be used in selecting subsets using GEE.

It is possible that, using a best subsets approach to data analysis, other confounding variables could be identified that would influence the OTT–treatment relationship. It is also possible that other unmeasured variables could influence the relationship. As another limitation, we initially analyzed OTT as a continuous variable (to increase our power) rather than as a categorized variable as specified in the protocol, and thus the analyses could be considered as post hoc. We also cannot draw conclusions regarding the OTT–treatment relationship beyond 180 minutes.

The investigators in the NINDS rt-PA Stroke Study urge continued efforts to treat patients eligible for rt-PA as quickly as possible, preferably within 60 minutes of emergency department arrival, but always within the currently required 3-hour time window using the NINDS protocol and the methods of the NINDS TPA Stroke Study Group.[14,18,19] Even though the importance of early treatment was a prospectively defined hypothesis, the results reported here are the result of an exploratory analysis. It would be important to test whether the results of other trials of thrombolytic therapy confirm this result.

Appendix

The following persons and institutions participated in the National Institute of Neurological Disorders and Stroke rt-PA Stroke Study. Participants are listed by their affiliation *at the time* the trial was conducted. Some of the investigators are now at other institutions.

Clinical centers: **University of Cincinnati (150 patients)—**Principal Investigator: T. Brott; Co-investigators: J. Broderick, R. Kothari, M. O'Donoghue, W. Barsan, T. Tomsick; Study Coordinators: J. Spilker, R. Miller, L. Sauerbeck; Affiliated sites: *St. Elizabeth (South)*, J. Farrell, J. Kelly, T. Perkins, R. Miller, *University Hospital*, T. McDonald, *Bethesda North Hospital*, M. Rorick, C. Hickey, *St. Luke (East)*, J. Armitage, C. Perry, *Providence*, K. Thalinger, R. Rhude, *The Christ Hospital*, J. Armitage, J. Schill, *St. Luke (West)*, P.S. Becker, R.S. Heath, D. Adams, *Good Samaritan Hospital*, R. Reed, M. Klei, *St. Francis/St. George*, A. Hughes, R. Rhude, *Bethesda Oak*, J. Anthony, D. Baudendistel, *St. Elizabeth (North)*, C. Zadicoff, R. Miller, *St. Luke-*

Kansas City, M. Rymer, I. Bettinger, P. Laubinger, *Jewish Hospital*, M. Schmerler, G. Meirose.

University of California, San Diego (146)—Principal Investigator: P. Lyden; Co-investigators: J. Dunford, J. Zivin; Study Coordinators: K. Rapp, T. Babcock, P. Daum, D. Persona; Affiliated sites: *UCSD*, M. Brody, C. Jackson, S. Lewis, J. Liss, Z. Mahdavi, J. Rothrock, T. Tom, R. Zweifler, *Sharp Memorial*, R. Kobayashi, J. Kunin, J. Licht, R. Rowen, D. Stein, *Mercy Hospital*, J. Grisolia, F. Martin, *Scripps Memorial*, E. Chaplin, N. Kaplitz, J. Nelson, A. Neuren, D. Silver, *Tri-City Medical Center*, T. Chippendale, E. Diamond, M. Lobatz, D. Murphy, D. Rosenberg, T. Ruel, M. Sadoff, J. Schim, J. Schleimer, *Mercy General, Sacramento*, R. Atkinson, D. Wentworth, R. Cummings, R. Frink, P. Heublein.

University of Texas Medical School, Houston (104)—Principal Investigator: J.C. Grotta; Co-investigators: T. DeGraba, M. Fisher, A. Ramirez, S. Hanson, L. Morgenstern, C. Sills, W. Pasteur, F. Yatsu, K. Andrews, C. Villar-Cordova, P. Pepe; Study Coordinators: P. Bratina, L. Greenberg, S. Rozek, K. Simmons; Affiliated sites: *Hermann Hospital*, *St. Lukes Episcopal Hospital*, *Lyndon Baines Johnson General Hospital*, *Memorial Northwest Hospital*, *Memorial Southwest Hospital*, *Heights Hospital, Park Plaza Hospital*, *Twelve Oaks Hospital.*

Long Island Jewish Medical Center (72)—Principal Investigators: T.G. Kwiatkowski (6/92–), S.H. Horowitz (12/90–5/92); Co-investigators: R. Libman, R. Kanner, R. Silverman, J. LaMantia, C. Mealie, R. Duarte; Study Coordinators: R. Donnarumma, M. Okola, V. Cullin, E. Mitchell.

Henry Ford Hospital (62)—Principal Investigator: S.R. Levine; Co-investigators: C.A. Lewandowski, G. Tokarski, N.M. Ramadan, P. Mitsias, M. Gorman, B. Zarowitz, J. Kokkinos, J. Dayno, P. Verro, C. Gymnopoulos, R. Dafer, L. D'Olhaberriague; Study Coordinators: K. Sawaya, S. Daley, M. Mitchell.

Emory University School of Medicine (39)—Principal Investigator: M. Frankel (7/92–10/95), B. Mackay (11/90–6/92); Co-investigators: J. Weissman, J. Washington, B. Nguyen, A. Cook, H. Karp, M. Williams, T. Williamson; Study Coordinators: C. Barch, J. Braimah, B. Faherty, J. MacDonald, S. Sailor; Affiliated sites: *Grady Memorial Hospital*, *Crawford Long Hospital*, *Emory University Hospital*, *South Fulton Hospital*; M. Kozinn, L. Hellwick.

University of Virginia Health Sciences Center (37)—Principal Investigator: E.C. Haley, Jr.; Co-investigators: T.P. Bleck, W.S. Cail, G.H. Lindbeck, M.A. Granner, S.S. Wolf, M.W. Gwynn, R.W. Mettetal, Jr., C.W.J. Chang, N.J. Solenski, D.G. Brock, G.F. Ford; Study Coordinators: G.L. Kongable, K.N. Parks, S.S. Wilkinson, M.K. Davis; Affiliated sites: *Winchester Medical Center*, G.L. Sheppard, D.W. Zontine, K.H. Gustin, N.M. Crowe, S.L. Massey.

University of Tennessee (14)—Principal Investigator: M. Meyer (2/93–), K. Gaines (11/90–1/93); Study Coordinators: A. Payne, C. Bales, J. Malcolm, R. Barlow, M. Wilson; Affiliated sites: *Baptist Memorial Hospital*, C. Cape, *Methodist Hospital Central*, T. Bertorini, *Jackson Madison County General Hospital*, K. Misulis, *University of Tennessee Medical Center*, W. Paulsen, D. Shepard.

Coordinating center: **Henry Ford Health Sciences Center—**Principal Investigator: B.C. Tilley; Co-investigators: K.M.A. Welch, S.C. Fagan, M. Lu, S. Patel, E. Masha, J. Verter; Study Coordinators: J. Boura, J. Main, L. Gordon; Programmers: N. Maddy, T. Chociemski; *CT Reading Centers:* Part A–*Henry Ford Health Sciences Center,* J. Windham, H. Soltanian Zadeh; Part B–*University of Virginia Medical Center,* W. Alves, M.F. Keller, J.R. Wenzel; *Central Laboratory*: *Henry Ford Hospital*, N. Raman, L. Cantwell; *Drug Distribution Center:* A. Warren, K. Smith, E. Bailey.

Committees: Executive: K.M.A. Welch, B.C. Tilley, J.R. Marler; *Steering:* K.M.A. Welch (Chair), T. Brott, P. Lyden, J.C. Grotta, T.G. Kwiatkowski, S.R. Levine, M. Frankel, E.C. Haley, Jr., M. Meyer, B.C. Tilley, J.R. Marler; *Genentech, Inc. Participants:* J. Froehlich, J. Breed; *Data And Safety Monitoring Committee:* J.D. Easton (Chair), J.F. Hallenbeck, G. Lan, J.D. Marsh, M.D. Walker.

Project office: National Institute of Neurologic Disorders and Stroke, Project Officer: J.R. Marler.

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