Introduction:

The NINDS tPA stroke study was published in 1995. This medication remains the only FDA approved medication for the treatment of acute stroke. The use of this drug has remained controversial despite proven benefit; as outcomes (with respect to level of disability) were improved at 90 days. Benefit (in terms of improvement based on neurological exam) was not established in the short term as the primary outcome of part I of the study was a 4 point or more improvement in the National Institutes of Health Stroke Scale (NIHSS) at 24 hours. As there is significant biological plausibility that improvement at 24 hours is predictive of ultimate outcome it would be useful to develop a model that could predict with confidence the final degree of improvement based on changes within the first 24 hours; as this might allow for future acute stroke trials to be expedited.

The Dataset:
A completely de-identified data set is available from the federal government with the data from all 624 patients enrolled in the trial. Of interest for this evaluation is the serial measurements of the NIHSS score (measured at baseline or prior to treatment, 2 hours, 24 hours and 90 days.) Data on level of disability at 90 days is also described using the modified Rankin Scale (mRS), which ranges from 0 (normal), 1 (no significant disability), 2 (some disability) to 6 (death).

The proposed data analysis:

I proposed the construction of 2 separate models:

Outcome: NIHSS at 90 days
Predictors: NIHSS at baseline, 2hr, 24 hours (slope), treatment (yes/no), age, systolic blood pressure, glucose, slope*treatment

Outcome: mRS at 90 days
Predictors: NIHSS at baseline, 2hr, 24 hours (slope), treatment (yes/no), age, systolic blood pressure, glucose, slope*treatment

Appropriate exploratory analysis including profile plots and transformation of variables (i.e. log(NIHSS)) as appropriate will occur.

Raghu's comments
This looks good. You might also want to study whether the longitudinal pattern of NIHSS differ by mRS by using NIHSS as outcome and mRS as between subject factor as it is measured only once at the 90 days.
Results:

Please see the appendix for SAS code. Based on the above objectives, I generated exploratory profile plots.

First in figure 1, I plotted all subjects by treatment group (tPA is blue dots connected by blue lines and placebo is open red boxes connected by red lines). Overall trend lines for each group (tPA versus placebo) were plotted. Given the time points were 0, 2, 24, 168 and 2160 hours I felt that plotting the time on the log base 10 scale would represent the data well. (Each of the time points was shifted ahead 1 hour since log(1) = 0.)

Visually comparing the trend lines suggests that the majority of the separation between the tPA and control groups is occurring when comparing baseline to two hours. This makes biological sense as well; since re-canalization of an occluded artery would be likely to lead to such observed rapid improvement.

In figure 2, the trend lines were plotted to examine trends in the change in NIHSS based on treatment group assignment and ultimate outcome (which was defined as good if the mRS was 0 or 1.) From this, one observes that in subjects who ultimately do not have a good outcome (mRS 2-6) there is very little difference in the trajectory of NIHSS over time. There is separation between the groups who ultimately have a good outcome. The tPA treated group has more rapid improvement and again this separation appears to occur in the first two hours.

In figure 3 we examined the overall trends in NIHSS over time depending on the ultimate outcome, when considered over the entire mRS. (SAS would not add a legend. The fitted line closest to the bottom of the graph represents a 3 month mRS of 0 and the top line represents a 3 month mRS of 6.) The best ultimate outcomes appear to be associated with the most sharp improvements.

Based on these findings, I assessed the ability of slope of NIHSS change (representing decrease in NIHSS points per hour) using the slope at 2 hours and at 24 hours. Interestingly, the majority of the slope was contributed by the change in the first 2 hours.

I examined whether the mean slope (2 hr) varied between treatment and placebo groups. The mean slope for tPA treated patients was 1.24 (95% CI 0.91-1.56) and for placebo was 0.74 (0.51-0.96). There was a significant difference (t=2.47 p = 0.014).

Since 2hr slope appeared to represent a potential variable of interest – I built a logistic regression model with the outcome of dichotomous mRS (0-1 = good outcome , 2-6 = bad outcome).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Point Estimate</th>
<th>95% Wald Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>2hr Slope</td>
<td>1.252</td>
<td>1.151 - 1.361</td>
</tr>
<tr>
<td>AGE</td>
<td>0.978</td>
<td>0.963 - 0.993</td>
</tr>
<tr>
<td>Serum Glucose</td>
<td>0.999</td>
<td>0.996 - 1.001</td>
</tr>
<tr>
<td>SBP</td>
<td>0.997</td>
<td>0.988 - 1.005</td>
</tr>
<tr>
<td>tpa treated</td>
<td>1.988</td>
<td>1.387 - 2.848</td>
</tr>
</tbody>
</table>

This demonstrated that an increase in the 2 hr slope (and thus a larger decrease in NIHSS at 2 hours from pre-treatment) was highly predictive of 3 month outcome. The results were quite similar when ordinal regression was used (and the full 0 to 6 range of mRS was employed as the outcome.)
The distribution of 2 hr slope per each treatment group was approximately normal and is depicted in the included histogram. To further describe the distribution of NIHSS at each time point by treatment group, boxplots were constructed (Figure 4.) The distributions in the tPA group appear to be shifted downward (meaning less neurological deficit) for all time points. Figure 5 demonstrates that the overall mRS outcome is better in the tPA group compared to placebo.

I examined whether quartile of 2hr slope was predictive of 3 month outcome. (The median of 2 hr slope was 0.5 with an IQR of 0 – 2. Therefore the 25th percentile was negative values for 2 hr slope and thus represented neurological deterioration from baseline.) tPA treatment was considered as a covariate in this logistic regression model of the dichotomous mRS outcome. (4th quartile represents most improvement.)

<table>
<thead>
<tr>
<th>Effect</th>
<th>Point Estimate</th>
<th>95% Wald Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>2hr slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>quartile 2 vs 1</td>
<td>1.149</td>
<td>0.625, 2.110</td>
</tr>
<tr>
<td>quartile 3 vs 1</td>
<td>1.647</td>
<td>0.996, 2.724</td>
</tr>
<tr>
<td>quartile 4 vs 1</td>
<td>3.829</td>
<td>2.326, 6.301</td>
</tr>
<tr>
<td>tpa treated 1 vs 0</td>
<td>1.856</td>
<td>1.309, 2.632</td>
</tr>
</tbody>
</table>

Not surprisingly, the subjects in the 4th quartile had a significantly higher odds of a good outcome than those in the lowest quartile. The raw number distributions of 3 month outcome on mRS by 2hr slope quartile are given in Figure 6.

I built a random effects model to predict NIHSS using PROC mixed, including potentially important co-variates.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>hour</td>
<td>1 2487</td>
<td>2487</td>
<td>0.70</td>
<td>0.4045</td>
</tr>
<tr>
<td>hr2</td>
<td>1 617</td>
<td>617</td>
<td>52.26</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>tpa</td>
<td>1 617</td>
<td>617</td>
<td>6.56</td>
<td>0.0106</td>
</tr>
<tr>
<td>AGE</td>
<td>1 617</td>
<td>617</td>
<td>4.57</td>
<td>0.0329</td>
</tr>
<tr>
<td>LGLU</td>
<td>1 617</td>
<td>617</td>
<td>5.97</td>
<td>0.0148</td>
</tr>
</tbody>
</table>

This appears to indicate that the effect of time on NIHSS was not significant. (Although the 2 hour slope was a significant predictor). As per your suggestion I added 3 month mRS into the model in an attempt to account for differences in trajectory of NIHSS between ultimate mRS.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>hour</td>
<td>1 2487</td>
<td>2487</td>
<td>0.70</td>
<td>0.4045</td>
</tr>
<tr>
<td>hr2</td>
<td>1 617</td>
<td>617</td>
<td>21.60</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>tpa</td>
<td>1 616</td>
<td>616</td>
<td>0.25</td>
<td>0.6153</td>
</tr>
<tr>
<td>AGE</td>
<td>1 616</td>
<td>616</td>
<td>0.98</td>
<td>0.3229</td>
</tr>
<tr>
<td>LGLU</td>
<td>1 616</td>
<td>616</td>
<td>1.90</td>
<td>0.1682</td>
</tr>
<tr>
<td>rank3m</td>
<td>1 616</td>
<td>1544.79</td>
<td>&lt;.0001</td>
<td></td>
</tr>
</tbody>
</table>

It is well known (and would be suspected) that the NIHSS would be closely related to the mRS at 90 days and this covariate has a stronger association with that than the others. However, the appeal of using the 2 hour slope to predict 3 month outcome is in the design of future stroke trials; one would not know what the mRS 3 months from now would be with good certainty.
I constructed a similar model in PROC mixed, but I restricted to the first two timepoints (0 and 2 hours). I did this based on what I observed in the profile plots.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num</th>
<th>Den</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>hour</td>
<td>1</td>
<td>604</td>
<td>25.47</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>hr2</td>
<td>1</td>
<td>600</td>
<td>27.03</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>tpa</td>
<td>1</td>
<td>600</td>
<td>0.90</td>
<td>0.3429</td>
</tr>
<tr>
<td>AGE</td>
<td>1</td>
<td>600</td>
<td>6.79</td>
<td>0.0094</td>
</tr>
<tr>
<td>LGLU</td>
<td>1</td>
<td>600</td>
<td>3.11</td>
<td>0.0783</td>
</tr>
<tr>
<td>CSYS</td>
<td>1</td>
<td>600</td>
<td>4.27</td>
<td>0.0391</td>
</tr>
<tr>
<td>hour*tpa</td>
<td>1</td>
<td>604</td>
<td>5.21</td>
<td>0.0228</td>
</tr>
</tbody>
</table>

Substantiating what was observed in the plots, there is a significant impact of change over time. The treatment time interaction is significant; but the tpa treatment main effect is not (likely owing to a lack of difference between the tpa and placebo groups at baseline – implying that randomization worked adequately.

Appendix – SAS Code

```sas
libname nind '.';
Options FMTSEARCH=(nind.formats);

data nind; set nind.ninds_updated_all; run;

proc format;
   value newrank_f
      0='0-No symptoms'
      1='1-No significant disability'
      2='2-Slight disability'
      3='3-Moderate disability'
      4='4-Moderately severe disability'
      5='5-Severe disability'
      6='6-Dead';
run;

data nind; set nind;
   format rank3m newrank_f.;
run;

proc contents; run;

data nind_shorter;
   set nind;
   keep record baseline nihhr2 nihhr24 nihday710 nihm3;
run;

data nind; *calculate slope of treatment response;
   set nind;
   hr2 = (baseline - nihhr2) / 2; *slope at 2 hours;
   hr22 = (nihhr2 - nihhr24) / 22; *slope from 2 - 24 hours;
   slope = hr2 + hr22; *total slope for first 24 hours - units NIHSS point per hour;
run;

ods pdf file = '.\histogram.pdf';
proc univariate data=nind noprint;
   class treatcd;
```
histogram hr2 / midpoints =-6 to 12 by 1 cfill=red normal;
title "Distribution of Delta NIHSS per hour based on 2 hr";
run; title;
ods pdf close;

proc ttest data=nind;
   class treatcd;
   var hr2 hr22 slope;
run;

data nind; set nind;
   if treatcd = 1 then tpa = 1;
   else if treatcd = 2 then tpa = 0;
   slope_treat = slope*tpa;
   hr2_treat = hr2*tpa;
run;

proc reg data=nind;
   model nihm3 = hr2 age lglu /*csys*/ tpa /*hr2_treat*/;
run;

proc logistic data=nind;
   class tpa(param=ref ref=first);
   model rank3m = hr2 age lglu /*csys*/ tpa / link=clogit;
run;

proc logistic data=nind;
   class tpa(param=ref ref=first);
   model rankin1(ref=first) = hr2 age lglu csys tpa;
run;

proc means data=nind median p25 p75;
   var slope hr2 hr22;
run;

* make a category for quartile of slope;
data nind;
   set nind;
   if slope < -0.1818182 then qslope = 1;
   if -0.1818182 <= slope < 0.7727273 then qslope = 2;
   if 0.7727273 <= slope < 2.1363636 then qslope = 3;
   if slope >= 2.1363636 then qslope = 4;
run;

data nind;
   set nind;
   if hr2 < 0 then qhr2 = 1;
   if 0 <= hr2 < 0.5 then qhr2 = 2;
   if 0.5 <= hr2 < 2 then qhr2 = 3;
   if hr2 >= 2 then qhr2 = 4;
run;

proc sort data=nind; by tpa; run;

/* ods rtf file='.ars_new.rtf';
proc freq data=nind;
   by tpa;
   tables qhr2*rank3m qslope*rank3m qhr2*rankin1 qslope*rankin1 / nocol norow nopct;
run;
ods rtf close;*/

proc logistic data=nind; * gives estimates of odds ratios for quartile;
   class qhr2(param=ref ref=first) tpa(param=ref ref=first);
model rankin1 (event='Yes') = qhr2 tpa;
run;

proc sort data=nind_shorter; by record; run;
proc sort data=nind; by record; run;

proc transpose data=nind_shorter out=wide;
  by record;
run;

proc print data=wide; run;
proc contents data=wide; run;

data nind_wide;
  merge wide nind;
  by record;
  rename COL1 = nih;
run;

data nind_wide;
  set nind_wide;
  if _name_ = 'BASELINE' then hour = 0;
  else if _name_ = 'nihhr2' then hour = 2;
  else if _name_ = 'nihhr24' then hour = 24;
  else if _name_ = 'nihday710' then hour = 168;
  else if _name_ = 'nihm3' then hour = 2160;
run;

proc sort data=nind_wide; by treatcd hour; run;
ods pdf file = '.\boxplots.pdf';
proc boxplot data=nind_wide;
  by treatcd;
  plot nih*hour / boxstyle=schematic boxwidth=5 ;
  title 'Figure 4: Distribution of NIHSS at each time point by treatment';
run;

proc sort data=nind; by treatcd; run;
proc boxplot data=nind;
  plot rank3m*treatcd / boxstyle=schematic boxwidth=5 ;
  title 'Figure 5: Distribution of mRS at 3 months by treatment';
run;
ods pdf close;

data nind_wide;
  set nind_wide;
  if hour ne 0 then log_hour = log(hour);
  else if hour = 0 then log_hour = 0;
run;

proc format;
  value outgroup_f
    1='tPA treated, mRS 0-1 at 3 months'
    2='tPA treated, mRS 2-6 at 3 months'
    3='placebo treated, mRS 0-1 at 3 months'
    4='placebo treated, mRS 2-6 at 3 months';
run;

data nind_wide;
  set nind_wide;
  hour1 = hour+1; *This sets the baseline time to 1 from zero so that the plots of hours on log scale look right;
if treatcd = 1 and rankin1 = 1 then outgroup = 1;
if treatcd = 1 and rankin1 = 0 then outgroup = 2;
if treatcd = 2 and rankin1 = 1 then outgroup = 3;
if treatcd = 2 and rankin1 = 0 then outgroup = 4;
format outgroup outgroup_f.;
run;

/* set graphic options for spaghetti plots */
goptions reset = all;
goptions colors=() ftext=simplex htext=10;
    axis1 width=3
        major=(h=1 w=3)
        minor=none
        label=( angle=90 'NIH Stroke Scale')
        offset=(2);
    axis2 width=3
        major=(h=1 w=3)
        minor=none
        label=( angle=0 'Hour of study (log base 10 scale) Baseline = Hour 1')
        offset=(2)
        logbase=10
        logstyle=expand;
ods pdf file ='.\profile.pdf' dpi=1200;
symbol1 interpol=join color=blue value=dot repeat=312 line=1; *treatment;
symbol2 interpol=join color=red value=square repeat=312 line=1; *placebo;
proc gplot data=nind_wide; *response based on treatment group;
*where hour in (0 2 24 168);
plot nih * hour1=record/ nolegend vaxis=axis1 haxis=axis2;
plot2 nih * hour1=treatcd /;
goptions htext=1;
symbol3 v=none i=sm50sm color=black width=6 line=1;
symbol4 v=none i=sm50sm color=black width=6 line=3;
title "Figure 1: Change in NIHSS based on treatment group";
run; quit;
proc gplot data=nind_wide; *response based on ultimate outcome;
*where hour in (0 2 24 168);
plot nih * hour1=record/ nolegend vaxis=axis1 haxis=axis2;
plot2 nih * hour1=outgroup /;
goptions htext=1;
symbol3 v=none i=sm50sm color=black width=6 line=1;
symbol4 v=none i=sm50sm color=black width=6 line=2;
symbol5 v=none i=sm50sm color=black width=6 line=3;
symbol6 v=none i=sm50sm color=black width=6 line=4;
symbol7 v=none i=sm50sm color=black width=6 line=5;
symbol8 v=none i=sm50sm color=black width=6 line=6;
symbol9 v=none i=sm50sm color=black width=6 line=7;
title "Figure 2: Trajectory of NIHSS over time based on treatment group and 3 month outcome";
run; quit;
proc gplot data=nind_wide; *response based on mRS at 3 months;
plot nih * hour1=record/ nolegend vaxis=axis1 haxis=axis2;
plot2 nih * hour1=rank3m /
legend position=bottom mode=protect;
goptions htext=1;
symbol3 v=none i=sm50sm color=black width=6 line=1;
symbol4 v=none i=sm50sm color=black width=6 line=2;
symbol5 v=none i=sm50sm color=black width=6 line=3;
symbol6 v=none i=sm50sm color=black width=6 line=4;
run; quit;
proc sort data=nind_wide;
   by tpa record;
run;

* model at 2 hours;
proc mixed method=reml noitprint dfbw maxiter=400 data=nind_wide;
   where hour < 3;
   model nih=hour tpa age lglu csys tpa*hour / solution;
   random intercept hour/subject=record type=un;
run;

* model including all times;
proc mixed method=reml noitprint dfbw maxiter=400 data=nind_wide;
   model nih=hour hr2 tpa age lglu / solution;
   random intercept hour/subject=record type=un;
run;

*above models repeated to account for differences in trajectory based on ultimate outcome;

* model at 2 hours;
proc mixed method=reml noitprint dfbw maxiter=400 data=nind_wide;
   where hour < 3;
   model nih=hour hr2 tpa age lglu csys tpa*hour / solution;
   random intercept hour/subject=record type=un;
run;

* model including all times - model fit improves if mRS at 3 months included;
proc mixed method=reml noitprint dfbw maxiter=400 data=nind_wide;
   model nih=hour hr2 tpa age lglu rank3m / solution;
   random intercept hour/subject=record type=un;
run;

proc freq data=nind_wide;
   tables rank3m;
run;
Figure 1: Change in NIHSS based on treatment group
Figure 2: Trajectory of NIHSS over time based on treatment group and 3 month outcome.
Figure 3: Trajectory of NIHSS over time based on mRS at 3 months

Hour of study (log base 10 scale) Baseline = Hour 1
Figure 4: Distribution of NIHSS at each time point by treatment
Treatment Code=t-PA
Figure 4: Distribution of NIHSS at each time point by treatment
Treatment Code=Placebo
Figure 5: Distribution of mRS at 3 months by treatment

Modified Rankin Scale at 90 Days (Intent to Treat Imputation)

- 6 - Dead
- 4 - Moderately severe disability
- 2 - Slight disability
- 0 - No symptoms

Treatment Code:
- t-PA
- Placebo
Distribution of Delta NIHSS per hour based on 2 hr
Figure 6: Distribution of outcomes based on 2 hr delta NIHSS

**tPA treated patients**

<table>
<thead>
<tr>
<th>Quartiles of 2 hr decrease in NIHSS</th>
<th>No symptoms</th>
<th>No significant disability</th>
<th>Slight disability</th>
<th>Moderate disability</th>
<th>Moderate severe disability</th>
<th>Severe disability</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>32</td>
<td>36</td>
<td>9</td>
<td>12</td>
<td>6</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>16</td>
<td>9</td>
<td>14</td>
<td>12</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>14</td>
<td>2</td>
<td>7</td>
<td>15</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>10</td>
<td>4</td>
<td>7</td>
<td>9</td>
<td>8</td>
<td>19</td>
</tr>
</tbody>
</table>

Proportion (Number in box) of patients based on 90 day mRS

**Placebo treated patients**

<table>
<thead>
<tr>
<th>Quartiles of 2 hr decrease in NIHSS</th>
<th>No symptoms</th>
<th>No significant disability</th>
<th>Slight disability</th>
<th>Moderate disability</th>
<th>Moderate severe disability</th>
<th>Severe disability</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>15</td>
<td>15</td>
<td>12</td>
<td>6</td>
<td>14</td>
<td>3</td>
<td>6</td>
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<tr>
<td>3</td>
<td>13</td>
<td>15</td>
<td>19</td>
<td>23</td>
<td>8</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>9</td>
<td>2</td>
<td>7</td>
<td>11</td>
<td>8</td>
<td>11</td>
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<tr>
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<td>4</td>
<td>11</td>
<td>8</td>
<td>13</td>
<td>14</td>
<td>2</td>
<td>27</td>
</tr>
</tbody>
</table>

Proportion (Number in box) of patients based on 90 day mRS