Ancrod in Acute Ischemic Stroke
Results of 500 Subjects Beginning Treatment Within 6 Hours of Stroke Onset in the Ancrod Stroke Program

David E. Levy, MD; Gregory J. del Zoppo, MD; Bart M. Demaerschalk, MD, MSc, FRCP(C); Andrew M. Demchuk, MD; Hans-Christoph Diener, MD; George Howard, DrPH; Markku Kaste, MD; Arthur M. Pancioli, MD; E. Bernd Ringelstein, MD, FAHA, FESO; Carmen Spatareanu, MD; Warren W. Wasiewski, MD

Background and Purpose—Previous studies of multiple-day dosing with the defibrinogenating agent, ancrod, in acute ischemic stroke yielded conflicting results but suggested that a brief dosing regimen might improve efficacy and safety. The Ancrod Stroke Program was designed to test this concept in subjects beginning ancrod or placebo within 6 hours of the onset of acute ischemic stroke.

Methods—Five hundred subjects with acute ischemic stroke who could begin receiving study material within 6 hours of symptom onset were infused intravenously with either ancrod (0.167 IU/kg per hour) or placebo over 2 or 3 hours. The primary efficacy outcome was a dichotomized, modified Rankin score at 90 days with less stringent cut-points for higher prestroke modified Rankin score and pretreatment NIHSS total score (“responder analysis”). Safety variables included mortality, major bleeding, and intracranial hemorrhage.

Results—Although the desired changes in fibrinogen level were seen in >90% of ancrod subjects, interim analysis for futility led to the study being halted for lack of efficacy. Positive responder status in the interim dataset was seen in 39.6% of ancrod subjects and 37.2% of placebo subjects (P=0.47). Ninety-day mortality did not differ between the 2 groups (ancrod, 15.6%; placebo, 14.1%; P=0.32), and the incidence of symptomatic intracranial hemorrhage within the first 72 hours, although not significantly different in ancrod compared to placebo subjects (P=0.19), was approximately twice as high (3.9% vs 2.0%; P=0.19).

Conclusion—These results demonstrate that intravenous ancrod starting within 6 hours after symptom onset in a broad selection of subjects with ischemic stroke did not improve their outcome and revealed a trend to increased bleeding despite successful efforts to achieve rapid initial defibrinogenation and avoid prolonged hypofibrinogenemia. (Stroke. 2009;40:3796-3803.)

Key Words: ancrod ◆ anticoagulant ◆ cerebral infarction ◆ defibrinogenation ◆ fibrinogen ◆ fibrinolytic agent ◆ recovery of function ◆ therapeutics ◆ treatment outcome

Elevated fibrinogen levels are a known risk factor for coronary artery disease, peripheral arterial disease, and stroke, as demonstrated in a meta-analysis of 31 studies by the Fibrinogen Studies Collaboration.1 Less well-documented is the impact of fibrinogen level on outcome from stroke. A report by Tanne et al2 of data from the NINDS study of recombinant tissue plasminogen activator in acute ischemic stroke suggested that acute stroke patients with elevated initial fibrinogen levels did less well than those with lower levels. The serine protease, ancrod, is a defibrinogenating agent derived from the venom of the Malayan pit viper, Calloselasma rhodostoma, that has been marketed in some countries for occlusive vascular disease. Ancrod-induced defibrinogenation results in anticoagulation, reduced blood viscosity, and indirect fibrinolysis,3,4 leading, in recent years, to several studies of its potential benefit for the management of acute ischemic stroke.

Sherman et al published a study5 showing a good functional outcome for ancrod based on the proportion of subjects at 90 days who were alive and had Barthel Index total scores of 95 to 100 or at least as high as the prestroke score (for subjects with prestroke disability), covariate-adjusted for age and pretreatment Scandinavian Stroke Scale score. When started within 3 hours of stroke onset and continued for 5

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From the Department of Neurology and Neuroscience (D.E.L.), Weill Cornell Medical College, New York, NY; Department of Hematology (G.J.d.Z.), University of Washington, Seattle, Wash; Department of Neurology (B.M.D.), Mayo Clinic Hospital, Phoenix, Ariz; Foothills Hospital/Calgary Stroke Program (A.M.D.), Calgary, Alberta, Canada; Department of Neurology (H.C.D.), University Hospital Essen, Essen, Germany; Department of Biostatistics (G.H.), UAB School of Public Health, Birmingham, Ala; Helsinki University Central Hospital (M.K.), Helsinki, Finland; Department of Emergency Medicine (A.M.P.), University of Cincinnati, Cincinnati, Ohio; Department of Neurology (E.B.R.), University of Münster, Münster, Germany; Memorial Sloan-Kettering Cancer Center (C.S.), Mineola, NY; Infracare (W.W.W.), Trevose, Pa.
Correspondence to David E. Levy, MD, 622 Greenwich St, Apt. 4-F, New York, NY 10014-3305. E-mail delmd@nyc.rr.com
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days, 42.2% of ancrod subjects achieved a good functional outcome vs 34.4% of placebo subjects (P=0.04; effect size, 7.8%). In that trial the incidence of symptomatic intracranial hemorrhage (sICH) in the active group was only 2.5-times that of placebo (5.2% vs 2%; P=0.06). This was followed by a trial of ancrod started within 6 hours of stroke onset that failed to show benefit (good functional outcome of 42% in both groups). Although failure was attributed to the longer time window, a larger per-patient dose of ancrod and liberal entry criteria for blood pressure probably contributed as well. Supporting the report by Tanne et al., our recently published observation from these ancrod trials suggests that higher fibrinogen levels measured in placebo patients within 6 hours of stroke onset were associated with poor functional outcome.

Post hoc analyses of these 2 ancrod trials as well as an earlier but smaller trial with a 6-hour window suggested that a modified dosing regimen designed to achieve rapid initial defibrinogenation should enhance efficacy and that avoidance of prolonged hypofibrinogenemia may reduce the incidence of hemorrhage. This new dosing regimen led to the re-evaluation of ancrod for the treatment of acute ischemic stroke: the Ancrod Stroke Program (ASP), which was designed to test the hypothesis that this modified ancrod administration would achieve both efficacy (functional improvement) and safety in the setting of acute ischemic stroke. ASP began as 2 parallel randomized, double-blind trials, each planned for 650 subjects to begin within 6 hours of stroke onset a 2- or 3-hour infusion of the study material, based on pretreatment fibrinogen levels.

The 2 phase 3 studies had identical inclusion and exclusion criteria. Therefore, an interim analysis of futility was planned when the first 500 treated subjects across the 2 studies completed the 90-day follow-up. On reviewing that analysis, the Data Safety Monitoring Board recommended that the studies be halted for futility. Data from this interim population are the basis for this report.

Subjects and Methods

ASP subjects were initially enrolled into 2 parallel double-blind, randomized, placebo-controlled studies: NTI-ASP-0502 (ASP-I) began in September 2004, and NTI-ASP-0503 (ASP-II) began in January 2005. Both studies were international and included sites in the US, Canada, Austria, the Czech Republic, Poland, Slovakia, Russia, Israel, South Africa, Taiwan, Australia, and New Zealand. Both studies were approved by all appropriate national authorities and ethics committees; written informed consent was obtained either from the subject or from representatives per local rules.

Entry criteria for ASP-I and ASP-II were identical (Table 1). Noncomatose subjects were eligible if they were at least 18 years of age (no upper limit) with a clinical diagnosis of ischemic stroke, were able to begin study treatment within 6 hours of symptom onset, had an initial NIHSS score of 5 to 25 (later changed to any score ≥5 with no upper limit), did not have hypertension even after antihypertensive medications (blood pressure ≤185/105 mm Hg), and did not have intracranial extravascular blood on pretreatment head neuroimaging (CT or MRI). Use of heparin, warfarin, and recombinant tissue plasminogen activator excluded subjects from enrollment, but aspirin and other antiplatelet agents were permitted.

Ancrod was purified from the venom of the Malayan pit viper (Calloselasma rhodostoma). Identical-appearing clear vials of the study material containing either ancrod (70 WHO IU/mL) or placebo in 10 mmol/L sodium phosphate and 0.9% sodium chloride, pH 6.8, were packaged by an independent manufacturer and provided with unique vial numbers. A randomization scheme was prepared by an independent statistician contracted by the sponsor. The randomization was designed to stratify equal numbers of ancrod and placebo subjects to the 2 treatment groups by geographical region and pretreatment stroke severity (NIHSS 5–15 and ≥16). An age-based adaptive randomization process using a biased coin approach was also integrated to promote balanced enrollment by age (65 years or younger, 66–75, 76 years or older). Vial assignment was performed in real time using an interactive voice response system.

Each vial was to be diluted into 250 mL saline and administered at the same infusion rate (0.6 mL/kg per hour) for 3 hours (infusing 0.5 IU/kg ancrod) for pretreatment fibrinogen levels ≥200 mg/dL or 2 hours (infusing 0.33 IU/kg ancrod) for pretreatment fibrinogen levels of 100 to 199 mg/dL; subjects with pretreatment fibrinogen levels <100 mg/dL were excluded. Blood samples were collected at prespecified time intervals for fibrinogen determination and sent to a central laboratory to assure constancy in the methodology for the fibrinogen assay.

Efficacy end points were based on the modified Rankin scale (mRS) and Barthel Index obtained at 10 and 90 days and the NIHSS obtained at prespecified time points over the first 72 hours and at 90 days. The primary efficacy outcome measure was a responder analysis of the mRS at 90 days, similar to that published by Adams et al. Responders were defined as follows: (1) subjects with a prestroke mRS of 0 to 1 and pretreatment NIHSS score of 5 to 15 who achieved a 90-day mRS of 0 to 1; (2) subjects with a prestroke mRS of 0 to 1 and pretreatment NIHSS scores ≥16 who achieved a 90-day mRS of 0 to 2; and (3) subjects with a prestroke mRS ≥2 and any pretreatment NIHSS score who at 90 days returned to their prestroke mRS or better. The utility of this responder definition was tested before the ASP trial was broken and found to be robust using data from the NINDS recombinant tissue plasminogen activator study. Secondary efficacy outcomes were assessed hierarchically, with neurological recovery (improvement of NIHSS at 90 days by ≥11 points or a reduction of the NIHSS to 0–1) followed by the Barthel Index total score. Safety end points included mortality, intracranial hemorrhage, major bleeding, and review of other physical and laboratory measurements.

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**Table 1. Major Entry Criteria**

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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<tr>
<td>Age ≥18 years</td>
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<tr>
<td>Signs and symptoms of acute ischemic deficit (any distribution)</td>
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<tr>
<td>Able to begin study material treatment within 6 hours of symptom onset</td>
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<tr>
<td>Pretreatment NIHSS ≥ 5</td>
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<tr>
<td>Prestroke mRS 0–1*</td>
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<tr>
<td>Written consent signed by the subject or his/her representative</td>
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</table>

<table>
<thead>
<tr>
<th>Exclusion criteria</th>
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</thead>
<tbody>
<tr>
<td>Neuroimaging evidence of intracranial, extravascular blood</td>
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<tr>
<td>Coma (pretreatment NIHSS 1a &gt;2)</td>
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<tr>
<td>Improvement prior to study material administration to NIHSS &lt;5</td>
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<tr>
<td>Use or intended use of thrombolytic agent</td>
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<tr>
<td>Previous stroke within prior 6 weeks</td>
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<tr>
<td>Last pretreatment blood pressure &gt;185/105</td>
</tr>
<tr>
<td>Pretreatment fibrinogen &lt;100 mg/dL</td>
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<tr>
<td>Intrinsic or extrinsic coagulation disorders</td>
</tr>
<tr>
<td>Medical condition likely to interfere with survival or evaluation through 90 days after stroke</td>
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<tr>
<td>Previous exposure to ancrod or pit viper snake bites</td>
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</tbody>
</table>

*ASP-I initially permitted enrollment of subjects with prestroke mRS ≥2.

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For efficacy, the full analysis data set (FADS) population was used. The full analysis data set was defined as randomized subjects infused with any study material who had at least one measurement of efficacy for a given end point. Subjects in the full analysis data set population were grouped according to the study material assignment. The safety population was defined as all randomized subjects infused with any study material grouped according to the study material actually received (Figure 1).

A serious adverse event (SAE) of sICH was defined in ASP as evidence of extravascular blood on head neuroimaging temporally related to neurological worsening that, in turn, was judged by the investigator to be causally related to the hemorrhage. Asymptomatic intracranial hemorrhage was defined as evidence of extravascular blood on neuroimaging that was not associated with neurological worsening as judged by the investigator. Additional analyses utilized ECASS II criteria (including adjudication) and neuroradiological criteria differentiating 4 grades of intracranial hemorrhage as defined for ECASS. The primary analysis of intracranial hemorrhage incidence was performed on intracranial hemorrhages occurring within the first 72 hours after beginning the infusion. Major hemorrhagic events were defined as bleeding adverse events that met criteria for an SAE, transfusions for excessive bleeding, and sICH (ASP criteria).

The sample size in each study of 650 was predicated on a 2-tail alpha level of 0.05, power of at least 90%, a placebo response rate of 32%, and an ancrod response rate of 45%, yielding a treatment effect of 13%. The primary end point was to have been analyzed using logistic regression, corrected for age, pretreatment stroke severity, geographic region, and pretreatment fibrinogen level. A 2-sided alpha level of 0.05 was set for the significance of differences in efficacy end points.

An interim analysis of safety and key efficacy data were planned after a combined total of 500 treated full analysis data set subjects across both studies had either completed the day 90 evaluation or died. East software was used to conduct the interim analysis of the primary efficacy end point. The stopping boundary for futility used the O'Brien-Fleming boundary shape. The test of futility compared the primary end point across treatment groups at a 0.2266 signific-
cance level; assuming the placebo group had a functional success rate of 32%, this would have corresponded to an approximate ancrod functional success rate of $\leq 36.9\%$, an absolute difference of $\leq 4.9\%$ between the rates for the 2 groups. Efficacy and safety end points were analyzed using a logistic regression model including terms for treatment group, study center, treatment-by-center interaction, and as continuous variables, age (years), pretreatment NIHSS score, and pretreatment fibrinogen level (mg/dL, centrally determined); individual adverse events and SAEs were analyzed using the Fisher exact test.

The studies were designed and funded by the sponsor, Neurobiological Technologies, Inc, and the analyses were conducted by a contractor to the sponsor with input from all of the authors. The study designs were provided before first subject enrollment at www.clinicaltrials.gov (NCT00141011 and NCT 00300196).

### Results

By August 17, 2008, 500 subjects had been randomized and treated with study material (Figure 1). These were drawn from 508 subjects, 277 in ASP-I and 231 in ASP-II, who were randomized; 8 patients were randomized but not treated. Among the most common reasons for excluding screened patients presenting within 6 hours of onset were inability to begin study material within the 6-hour time window, mild deficits, rapidly improving deficits, and extravascular blood on the pretreatment head CT scan.

Because there were no meaningful differences between ASP-I and ASP-II and the plan had been to combine the 2 studies for analysis, the following is based on the combined analysis of the interim 500-subject population. The ancrod and placebo groups were similar at baseline (Table 2) with respect to age, gender, pretreatment NIHSS total score, time-to-treat, blood pressure, and history of diabetes mellitus, and tobacco use. The mean dose of study material (IU/kg for ancrod) received in each group was identical and very close to the intended dose of 0.5 IU/kg over 3 hours or 0.33 IU/kg over 2 hours.

There was no difference between ancrod and placebo in the primary efficacy end point at 90 days; similar proportions of subjects achieved responder status in each group (ancrod 39.6% vs placebo 37.2%; $P=0.47$; Table 3). No trends suggested benefit in subgroups based on age or pretreatment NIHSS. The proportion of subjects who achieved the first secondary end point based on improvement in the NIHSS was also similar in each group (ancrod 37.3% vs placebo 38.9%). The NIHSS total score for ancrod subjects did not improve more than for placebo between baseline and 24 hours (difference in mean scores: ancrod 1.4 vs placebo 2.1).

Mortality at 90 days was not different between the groups (ancrod 15.6% vs placebo 14.1%; $P=0.32$; Table 3). Stroke was the most common cause of death, accounting for 38% of deaths in each treatment group. The time to death was similar between groups (data not shown; $P=0.71$).

Using the ASP criteria, sICH occurred within the first 72 hours in 10 ancrod subjects (3.9%) compared with 5 placebo subjects (2.0%; $P=0.19$; Table 3). sICH in the ancrod subjects developed early, with 6 of the 10 hemorrhages occurring within the first 24 hours, 7 within 48 hours, and the remaining 3 between 48 and 72 hours. In contrast, only 2 of the 5 sICH in placebo subjects occurred within the first 48 hours. Asymptomatic hemorrhages within the first 72 hours were similar in the 2 groups (ancrod 6.7% vs placebo 6.5%; $P=0.85$). Adjudication of sICH was performed by a subcommittee of the Steering Committee based on ECASS II criteria, resulting in fewer subjects in each group with an sICH, but the difference between the 2 groups was more marked and was statistically significant (ancrod 3.5% vs placebo 0.4%; $P=0.021$). Using neuroradiological criteria from ECASS, there was very little difference between the 2 treatment groups among the 4 grades of intracranial hemorrhage. Within the first 10 days, there was an increased incidence of major hemorrhagic events in the ancrod subjects (6.3% vs 2.9%; $P=0.054$); the difference for major hemorrhage within the first 72 hours was more marked and was statistically significant (5.5% vs 1.6%; $P=0.028$).

There was a trend for more frequent adverse events in the placebo group (93.5%) than in the ancrod group (89.0%; $P=0.028$).

### Table 2. Baseline and Dosing Characteristics: FADS Population

<table>
<thead>
<tr>
<th></th>
<th>Placebo n=247</th>
<th>Ancrod n=253</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr: mean/median</td>
<td>69.6/73</td>
<td>70.2/73</td>
</tr>
<tr>
<td>Age 65, n (%)</td>
<td>89 (36.0%)</td>
<td>82 (32.4%)</td>
</tr>
<tr>
<td>Age &gt;65–75, n (%)</td>
<td>50 (20.2%)</td>
<td>59 (23.3%)</td>
</tr>
<tr>
<td>Age &gt;75, n (%)</td>
<td>108 (43.7%)</td>
<td>112 (44.3%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>122 (49.4%)</td>
<td>124 (49.0%)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>125 (50.6%)</td>
<td>129 (51.0%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, n (%)</td>
<td>195 (78.9%)</td>
<td>210 (83.0%)</td>
</tr>
<tr>
<td>Black, n (%)</td>
<td>32 (13.0%)</td>
<td>19 (7.5%)</td>
</tr>
<tr>
<td>Asian, n (%)</td>
<td>8 (3.2%)</td>
<td>9 (3.6%)</td>
</tr>
<tr>
<td>Other, n (%)</td>
<td>12 (4.9%)</td>
<td>15 (5.9%)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latino, n (%)</td>
<td>6 (2.4%)</td>
<td>6 (2.4%)</td>
</tr>
<tr>
<td>Non-Latino, n (%)</td>
<td>240 (97.6%)</td>
<td>247 (97.6%)</td>
</tr>
<tr>
<td>Known diabetes, n (%)</td>
<td>73 (29.6%)</td>
<td>65 (25.7%)</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>68 (27.5%)</td>
<td>54 (21.3%)</td>
</tr>
<tr>
<td>Pretreatment NIHSS, mean/median</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment NIHSS 5–7, n (%)</td>
<td>86 (34.8%)</td>
<td>93 (36.8%)</td>
</tr>
<tr>
<td>Pretreatment NIHSS 8–15, n (%)</td>
<td>110 (44.5%)</td>
<td>108 (42.7%)</td>
</tr>
<tr>
<td>Pretreatment NIHSS ≥16, n (%)</td>
<td>51 (20.6%)</td>
<td>52 (20.6%)</td>
</tr>
<tr>
<td>Time-to-treat, hours, mean/median</td>
<td>5.2/5.5</td>
<td>5.1/5.3</td>
</tr>
<tr>
<td>Blood pressure, mm Hg, median</td>
<td>153/82</td>
<td>153/80</td>
</tr>
<tr>
<td>Dose (IU/kg) of study material received, 3-hour infusion, N: mean/median</td>
<td>239: 0.506/0.504</td>
<td>244: 0.517/0.504</td>
</tr>
<tr>
<td>Dose (IU/kg) of study material received, 2-hour infusion, N: mean/median</td>
<td>8: 0.285/0.336</td>
<td>9: 0.305/0.333</td>
</tr>
</tbody>
</table>

*None of these differences is statistically significant. †Calculated on the basis of volume received as if placebo actually contained ancrod.
Adverse events leading to subject withdrawal were uncommon, occurring in only 3 placebo-treated subjects and 1 ancrod-treated subject. Table 4 displays the 10 most common adverse events. Although not reflected in Table 4, there was a statistically significant increase in the incidence of infections in the ancrod subjects (38.8% ancrod vs 29.0% placebo; \(P = 0.023\)). This increase was the result primarily of an increased incidence of pneumonia (9.0% vs 6.5%; \(P = 0.069\)), upper respiratory tract infections (2.7% vs 0.4%; \(P = 0.069\)), and bronchitis (2.4% vs none; \(P = 0.031\)).

More ancrod subjects had adverse event terms reflecting renal failure (renal failure, acute renal failure, and renal impairment) than placebo (2.7% vs 0.4%; \(P = 0.034\)), and hematuria was more common in ancrod than placebo subjects (7.8% vs 4.9%; \(P = 0.20\)).

There was a trend for more frequent SAEs in the ancrod-treated subjects (38.0%) than in placebo-treated subjects (33.1%; \(P = 0.26\); Table 3). SAEs that occurred in at least 1% of the safety population are also shown in Table 4. Stroke-in-evolution as an SAE occurred with approximately equal frequency in the placebo and ancrod groups.

Table 3. Efficacy and Safety End Points

<table>
<thead>
<tr>
<th>FADS Population</th>
<th>Placebo n=247</th>
<th>Ancrod n=253</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary responder analysis (proportion achieving functional outcome), n (%)</td>
<td>92 (37.2%)</td>
<td>99 (39.6%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td>1.124 (0.763, 1.796)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIHSS of 0–1 or reduction by (\geq) 11 points, n (%)</td>
<td>96 (38.9%)</td>
<td>94 (37.3%)</td>
<td>0.94</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td>0.983 (0.660, 1.645)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement in mean NIHSS total score from baseline to 24 hours</td>
<td>2.1</td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Adverse Events in the Safety Population*

<table>
<thead>
<tr>
<th>Placebo n=245</th>
<th>Ancrod n=255</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ten most common adverse events</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>51 (20.8%)</td>
<td>45 (17.6%)</td>
</tr>
<tr>
<td>Constipation</td>
<td>45 (18.4%)</td>
<td>55 (21.6%)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>40 (16.3%)</td>
<td>46 (18.0%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>36 (14.7%)</td>
<td>40 (15.7%)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>35 (14.3%)</td>
<td>32 (12.5%)</td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>28 (11.4%)</td>
<td>34 (13.3%)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>26 (10.6%)</td>
<td>31 (12.2%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>25 (10.2%)</td>
<td>23 (9.0%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>22 (9.0%)</td>
<td>19 (7.5%)</td>
</tr>
</tbody>
</table>

SAs in at least 1% of the safety population

| Stroke-in-evolution | 9 (3.7%) | 12 (4.7%) | |
| Pneumonia | 5 (2.0%) | 13 (5.1%) | |
| Cerebrovascular accident | 5 (2.0%) | 5 (2.0%) | |
| Myocardial infarct | 5 (2.0%) | 3 (1.2%) | |
| Pulmonary embolus | 5 (2.0%) | 3 (1.2%) | |
| Atrial fibrillation | 4 (1.6%) | 2 (0.8%) | |
| Brain edema | 3 (1.2%) | 2 (0.8%) | |
| Transient ischemic attack | 3 (1.2%) | 2 (0.8%) | |
| Aspiration pneumonia | 2 (0.8%) | 3 (1.2%) | |
| Congestive heart failure | 2 (0.8%) | 4 (1.6%) | |

Central nervous system SAEs occurring in at least 3 subjects in 1 treatment group of the safety population

| All | 34 (13.9%) | 40 (15.7%) | |
| Stroke-in-evolution | 9 (3.7%) | 12 (4.7%) | |
| Cerebrovascular accident | 5 (2.0%) | 5 (2.0%) | |
| Brain edema | 3 (1.2%) | 2 (0.8%) | |
| Transient ischemic attack | 3 (1.2%) | 2 (0.8%) | |
| Brain herniation | 2 (0.8%) | 1 (0.4%) | |
| Cerebral hemorrhage | 1 (0.4%) | 3 (1.2%) | |
| Hemorrhagic transformation | 1 (0.4%) | 4 (1.6%) | |

*None of these differences is statistically significant.
acute treatment of ischemic stroke suggested that, when

frequency in ancrod and placebo subjects (4.7% vs 3.7%; 
P = 0.66), similar to SAEs of hemorrhagic transformation of
the index stroke (1.6% vs 0.4%; P = 0.37). The increased
incidence in infections noted as adverse events is also
reflected as an SAE, with >2-fold but insignificant increase
in pneumonia for the ancrod subjects (5.1% vs 2.0%; 
P = 0.092).

There was a trend for central nervous system SAEs being
slightly increased in the ancrod-treated subjects (15.7% vs
13.9%; P = 0.62), resulting primarily from nonsignificant
increased incidences of cerebral hemorrhage, stroke-in-
evolution, and hemorrhagic transformation of the index
stroke. Table 4 lists the central nervous system-related SAEs
that occurred in ≥3 subjects in either treatment group.

The dosing regimen used in these studies was designed to
achieve rapid initial defibrinogenation (0 to 3-hour defibrino-
genation rate ≥30 mg/dL per hour) and avoid prolonged
hypofibrinogenemia (9 to 72-hour time-weighted fibrinogen
level >70 mg/dL); Figure 2 shows that this was generally
achieved. Subjects who received ancrod were categorized in
terms of success at meeting these criteria and whether success
was related to key efficacy and safety outcomes. Calculated
defibrinogenation rates were available in 194 ancrod subjects;
most (177; 91.2%) achieved the desired rate of initial defi-
brinogenation, >30 mg/dL per hour, and 71 of those achieved
the primary efficacy end point (40.1%). The response rate in
those with lower defibrinogenation rates was 10 of 17
(58.8%). Calculated mean 9 to 72-hour fibrinogen levels were
available in 240 ancrod subjects; nearly all (235, 97.9%) had
levels greater than the target floor of 70 mg/dL, and 9 of those
experienced sICH (3.8%). None of the 5 subjects with lower
mean fibrinogen levels had sICH.

Discussion
Ancrod is a serine protease that has been shown to rapidly
decrease serum fibrinogen levels in healthy volunteers and
stroke patients. A previous study of intravenous ancrod for
acute treatment of ischemic stroke suggested that, when

started within 3 hours of stroke onset, ancrod improved
outcome. In an early study of 132 subjects starting treatment
within 6 hours of stroke onset, there was evidence of efficacy
when the data were analyzed using a patient-weighted anal-
ysis. The larger ESTAT study using a 6-hour window,
however, failed to confirm this finding, and there was a
significant increase in mortality and sICH with ancrod in
ESTAT. The ASP studies were designed based on a retrospective
review of these studies. The highest initial infusion rate
used in the previous studies, 0.5 IU/kg administered over a
short duration (3 hours), was chosen to rapidly reduce fibrinogen and prevent long periods of low fibrinogen levels.
Initially rapid defibrinogenation was achieved in >91% of
ancrod subjects, and avoidance of prolonged hypofibrinogen-
emia was achieved in >97% of ancrod subjects for whom
data were available.

The interim study population was well-balanced for the
key prognostic factors for stroke outcome (eg, age, and pretreatment stroke severity). Ancrod did not improve out-
come and was associated with a nonsignificant increase in
sICH and major bleeding events and a significantly increased
incidence of infection. The lack of efficacy in this population
may be the result of the time to treat, which on average was
>5 hours. Ancrod may still possess efficacy when adminis-
tered within 3 hours as demonstrated in the STAT study.

The dosing period of 3 hours in this program differs from
that used in previous studies of ancrod in acute ischemic
stroke, when dosing continued over several days. An
early treatment effect in the present study therefore might
have been diluted by the lack of continued treatment. Incon-
sistent with this hypothesis, however, is the observation that
unlike STAT, when neurological scores improved in the first
24 hours, more in ancrod than placebo subjects, no such early
treatment effect was seen in the interim ASP database.

Although sICH was relatively uncommon in this study, the
incidence of sICH using ASP criteria in the ancrod subjects
was twice that seen with placebo (a difference that failed to
reach statistical significance). The overall incidence of sICH
was even lower when characterized with ECASS II criteria,15
but the difference between ancrod and placebo was more
marked and was statistically significant. There was virtually
no ancrod–placebo difference, however, using ECASS neu-
radiological criteria.16 These discrepancies highlight the
sensitivity of sICH reporting to the definitions used. None-
theless, the fact that sICH by ASP and ECASS II criteria
increased at all and that major bleeding increased signifi-
cantly was unexpected because the analysis of previous data
suggested that the risk of hemorrhage with ancrod was related
to prolonged low fibrinogen levels, and these did not occur in
this study. An association of efficacy to initial defibrino-
genation rates and sICH to maintenance fibrinogen levels in
the ancrod subjects could not be determined in this study because
so few ancrod subjects failed to achieve the desired targets.

There are 2 unexpected safety findings: the increased
incidence of infections and the increased incidence of renal
failure. There was a significant increase in the incidence of all
infections attributable in large measure to the increases in
pulmonary and upper respiratory tract infections. Ancrod is
not known to induce immunodeficiency or inhibit white blood cell production or function; thus, the mechanism leading to this finding is unclear. There was also a significant increase in adverse events related to renal failure in the ancrod subjects. Although ancrod is excreted in the kidneys, these observations have not been previously reported. Both ancrod subjects with SAEs of renal failure had progressive stroke and multisystem organ failure; both died, but renal failure was not the primary cause of death in either of the subjects. Whether the observations of increased incidence of renal failure and infections are chance occurrences or true safety signals is unclear. Nevertheless, based on the data from this cohort of patients, any future investigations with ancrod should include close surveillance for infections and renal impairment as well as bleeding.

Two of the previous stroke studies with ancrod showed a benefit from ancrod, whereas 1 did not. The briefier but more intense dosing used in ASP was expected to improve both efficacy and safety, but that was not the case. Reasons for failure to show efficacy in ASP are most easily attributed to the delay in treatment, the same rationale used to explain the negative ESTAT results. Other factors, however, may have contributed to the failure of ESTAT, including inclusion of subjects with blood pressures up to 220/120 mm Hg and a greater overall dose than that used in STAT, but these factors were controlled in ASP. Moreover, the time-to-treat in one of the earlier studies was virtually identical to that in ASP. Unlike most acute stroke trials, ASP enrolled subjects with mean treatment intervals of 5 hours as in ASP.

Appendix. ASP Investigators and Staff

Australiа: C. Bird, Box Hill, Victoria; S. Davis, Melbourne; G. Donnan, Heidelberg Heights, Victoria; T. Phan, Clayton, Victoria; J. Sturm, Gosford, NSW

Austria: F. Faezekas, Graz; F. Gruber, Linz

Canada: A. Shaih, Corner Brook, Newfoundland, and Labrador

Czech Republic: J. Rektor, Brno; P. Geier, Pardubice; H. Lachmann, Prague; J. Polivka, Pilsen

Israel: N. Bornstein, Tel Aviv; E. Dorodnicov, Ashkelon; B. Gross, Nahariyya; Y. Lampel, Holon; R. Leker, Jerusalem; D. Tanne, Tel Hashomer; G. Telman, Haifa; Y. Wirguin, Beer Sheva

New Zealand: A. Barber, Auckland; J. Fink, Christchurch

Poland: A. Czlonkowska, Warsaw; J. Wronka, Poznan

Russia: V. Gusev, Petrozavodsk; J. Kolomoets, Moscow; T. Lukshchhanoa, Samara; V. Shmyrev, Moscow; V. Skvortsova, Moscow

Slovakia: M. Dvorak, Nova Ves; L. Gurick, Levocha

South Africa: M. Basson, Cape Town; J.S. Roos, Somerset West; L.J. Van Zyl, Worcester

Taiwan: J.-T. Lee, Taipei; T.-H. Lee, Taoyuang; H.-J. Lin, Tainan; R.-T. Lin, Kaohsiung; C.-W. Liou, Kaohsiung; C.-H. Liu, Taichung; M.-H. Sun, Taichung

US: A. Ahuja, Milwaukee, WI; C. Benesch, Rochester, NY; D.M. Brown, Newport Beach, CA; D. Camp, Austin, TX; C. Chang, Honolulu, HI; D. Chiu, Houston, TX; S. Cruz-Flores, St. Louis, MO; T. Cutfman, Fort Wayne, IN; I. DaSilva, Tallahassee, FL; B. Dandapani, Melbourne, FL; B. Demaerschalk, Phoenix, AZ; T. Devlin, Chattanooga, TN; J. Erickson, Tacoma, WA; G. Ferenz, Toms River, NJ; M. Gizzi, Edison, NJ; D.G. Gorman, Bellevue, WA; J.O. Harris, Fort Lauderdale, FL; M.E. Hecker, Buffalo, NY; W.A. Holt, Port Charlotte, FL; W.S. Holt, Duluth, MN; J. Hollander, Rochester, NY; R. Jackel, Doylestown, PA; J. Kramer, Chicago, IL; J. Krauss, Detroit, MI; K. Levin, Ridgewood, NJ; S. Mallenbaum, Virginia Beach, VA; J. McDowell, Olympia, WA; A.W. McElveen, Bradenton, FL; M. Nash, Decatur, GA; K. Ng, Ogala, FL; H. Rabiee, Redding, CA; D. Rodriguez, Billings, MT; A. Runheim, Winston-Salem, NC; M.K. Sauter, Greensburg, PA; J.L. Schindler, New Haven, CT; T. Schoonover, Kettering, OH; G. Scott, Anderson, SC; S. Selco, Las Vegas, NV; P. Sethi, Greensboro, NC; S. Silliman, Jacksonville, FL; S. Starkman, Los Angeles, CA; D. Steiner, Lawrence, NY; J. Stevens, Fort Wayne, IN; G. Tietjen, Toledo, OH; M. Torbay, Milwaukee, WI; M. Tremwel, Fort Smith, AR; T.R. Vidic, Elkhart, IN; T. Warwick, Fresno, CA

Sponsor: Neurobiological Technologies, Inc, Emeryville, CA

Steering Committee: G.J. del Zoppo, Seattle, WA (chair); B. Demaerschalk, Phoenix, AZ; A.M. Demchuk, Calgary, Alberta, Canada; H.-C. Diener, Essen, Germany; G. Howard, Birmingham, AL; M. Kaste, Vantaa Finland; A.M. Pancioli, Cincinnati, OH; E.B. Ringelstein, Munster, Germany

Intracranial Hemorrhage Adjudication Committee: A.M. Demchuk, Calgary, Alberta, Canada (chair); G.J. del Zoppo, Seattle, WA; A.M. Pancioli, Cincinnati, OH

Data Safety Monitoring Board: M.D. Walker, Bradenton, FL; B. Coull, Tucson, AZ; R. Kromlal, Seattle, WA

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Drs Levy, Wasiewski, and Spatareau were employees of the sponsor, NTI. Drs Levy and Wasiewski own stock in NTI. Dr del Zoppo received honoraria from NTI. Professor Kaste received travel expenses and honoraria from Boehringer Ingelheim, Paion AG, Forest Laboratories, and Lundbeck for serving as a member of Steering Committees of ECASS, ECASS II, ECASS III, DIAS, DIAS 2, and DIAS 4, and as a speaker and as a consultant. Dr Demchuk received honoraria for participation in clinical trials, contribution to advisory boards, or oral presentations from: Abbott, AstraZeneca, Bayer Vital, BMS, Bőhringer Ingelheim, CoAxaix, D-Pharm, Fresenius, GlaxoSmithKline, Janssen Cilag, MSD, MindFrame, NTI, Novartis, Novo-Nordisk, Paion AG, Parke-Davis, Pfizer, Sanofi-Aventis, Sankyo, Servier, Solvay, Thrombogenic, Wyeth, and Yamanouchi. H.C. Diener has no ownership interest and does not own stock in any pharmaceutical company. Professor Ringelstein has received travel expenses and honoraria from Boehringer Ingelheim, Sygma, NTI, Novartis, Novo-Nordic, Sanofi-Aventis, Solvay, Bayer Vital, M’s Science, Servier,UCB, and Trommsdorff for serving
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References


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In the article “Ancrod in Acute Ischemic Stroke: Results of 500 Subjects Beginning Treatment Within 6 Hours of Stroke Onset in the Ancrod Stroke Program”, by Levy et al,1 an author is missing from the byline. E. Bernd Ringelstein, MD, FAHA, FESO should be listed before Carmen Spatareanu, MD. The publisher regrets this error.

The corrected version can be viewed online at http://stroke.ahajournals.org.