Proof-of-Concept Randomized Trial of the Monoclonal Antibody GSK249320 Versus Placebo in Stroke Patients

Steven C. Cramer, MD; Lori A. Enney, BS; Colleen K. Russell, MS; Monica Simeoni, PhD; Thomas R. Thompson, MD

Background and Purpose—One class of poststroke restorative therapy focuses on promoting axon outgrowth by blocking myelin-based inhibitory proteins such as myelin-associated glycoprotein. The purpose of the current study was to extend preclinical and clinical findings of GSK249320, a humanized monoclonal antibody to myelin-associated glycoprotein with disabled Fc region, to explore effects on motor outcomes poststroke.

Methods—In this phase IIb double-blind, randomized, placebo-controlled study, patients at 30 centers with ischemic stroke 24 to 72 hours prior and gait deficits were randomized to 2 IV infusions of GSK249320 or placebo. Primary outcome measure was change in gait velocity from baseline to day 90.

Results—A total of 134 subjects were randomized between May 2013 and July 2014. The 2 groups were overall well matched at baseline. The study was stopped at the prespecified interim analysis because the treatment difference met the predefined futility criteria cutoff; change in gait velocity to day 90 was 0.55±0.46 (mean±SD) in the GSK249320 group and 0.56±0.50 for placebo. Secondary end points including upper extremity function were concordant. The 2 IV infusions of GSK249320 were well tolerated. No neutralizing antibodies to GSK249320 were detected.

Conclusions—GSK249320, within 72 hours of stroke, demonstrated no improvement on gait velocity compared with placebo. Possible reasons include challenges translating findings into humans and no direct evidence that the therapy reached the biological target. The antibody was well tolerated and showed low immunogenicity, findings potentially useful to future studies aiming to use a monoclonal antibody to modify activity in specific biological pathways to improve recovery from stroke.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01808261.

Key Words: axon ▪ brain ▪ clinical trial ▪ gait ▪ stroke

After an injury from an acute stroke, numerous restorative events evolve within the brain. Targeting these events therapeutically may augment poststroke neural repair and favorably impact long-term outcome.1 Numerous biological targets are under study to develop restorative therapies. One class of therapy focuses on promoting recovery after stroke by blocking myelin-based inhibitory proteins that inhibit axon outgrowth. Three major inhibitors of such growth have been identified, 1 being myelin-associated glycoprotein (MAG). After stroke, MAG levels spontaneously increase in penumbra,2 suggesting that MAG may be a useful target to promote neural repair, an idea bolstered by previous observations that MAG blockade promotes axonal growth.3,5

The main objective of the current study was to determine whether a monoclonal antibody targeting MAG improves stroke recovery in patients with ischemic stroke. The specific therapy under study was GSK249320, an IgG1-type humanized monoclonal antibody to MAG with disabled Fc region. Anti-MAG antibodies have been shown to neutralize MAG-mediated inhibition in preclinical studies and to promote regeneration after peripheral nerve injury.7,8 Blocking the action of a related protein, Nogo, 7 days after ischemic stroke in rats improved behavioral recovery by promoting axonal growth.9 The preclinical program for GSK249320 included rodent studies that found that the antibody penetrated the infarct site and had small but significant effects on behavioral outcomes when initiated 24 hours poststroke without affecting infarct volume,10 and primate studies in which IV infusion of GSK249320 beginning 24 hours after experimental ischemic infarct facilitated behavioral recovery.11 GSK249320 was found to be safe in healthy...
human subjects, and a recent randomized, placebo-controlled phase II trial in patients 24 to 72 hours after ischemic stroke also found the antibody to be safe and suggested potential efficacy for improving recovery of gait.

The current study built on these findings as a phase IIb double-blind, randomized, placebo-controlled, multicenter study. Patients with ischemic stroke 24 to 72 hours prior and deficits in gait were randomized to receive 2 IV infusions of GSK249320 or placebo. The primary outcome measure was change from baseline to day 90 in gait velocity, which is valid, reliable, and sensitive after stroke. The study was stopped at the interim analysis because there was insufficient evidence to justify continuing the study given that the observed difference between treatment groups met the predefined futility cutoff.

**Methods**

**Study Overview**

Thirty centers across 4 countries enrolled subjects in the study, between May 2013 and July 2014. The study was approved by each site’s institutional review board. All subjects, or surrogates, gave written informed consent. Participation spanned 6 visits from baseline to day 180. Key entry/exclusion criteria appear in Table 1. See also online-only Data Supplement.

**Randomization**

Subjects were centrally randomized to GSK249320 15 mg/kg or placebo in a 1:1 allocation ratio, using permuted blocks, with treatment stratified according to baseline gait velocity (0, >0–<0.4, or 0.4–0.8 m/s). See also online-only Data Supplement.

**Study Assessments**

At baseline, prior to first infusion and thus <72 hours poststroke, assessments included National Institutes of Health Stroke Scale (NIHSS), modified Rankin Scale, gait velocity, and Box and Blocks (no. blocks transferred during 1 minute). All study assessors were formally trained and certified in each of these outcome measures (see online-only Data Supplement). Patients and assessors were blinded at all times. These were serially evaluated during the remaining 5 visits, as was the amount of rehabilitation (physical and occupational) therapy that patients received. Safety assessments included vital signs, clinical laboratories, ECGs, suicidality, adverse events (AE), serious adverse events, and falls and were monitored by the internal Safety Review Committee. Blood samples were collected at baseline, pre- and post-dosing of IP at visit 2 (day 6), as well as at visits 3 and 6 (day 30 and 180, respectively), or at the time of study withdrawal if applicable, from which free serum MAG levels and GSK249320 levels were measured. See also online-only Data Supplement.

**Data Analysis**

The primary efficacy end point was the mean change in gait velocity from baseline to day 90. To test the hypothesis that treatment with GSK249320 leads to an improvement of change in gait velocity compared with placebo at day 90, a repeated-measures mixed-effects model was used in a Bayesian framework, including fixed effects for treatment, visit, age, sex, treatment by visit interaction, baseline mean gait velocity by visit interaction, and baseline NIHSS by visit interaction. For additional information, see online-only Data Supplement. At the end of study, a positive signal of efficacy was to be declared if the posterior probability that the true improvement over placebo (GSK249320-placebo) was greater than zero is >95%, and a negative signal of efficacy was to be declared if the posterior probability that the true improvement over placebo is greater than zero is <85%; otherwise the result was to be interpreted as indeterminate. If the true mean gait velocity improvement with GSK249320 is 0.25 m/s over placebo, assuming variance as in the earlier placebo-controlled phase II study of GSK249320, enrolling 136 subjects with day 90 data would provide an 85% chance of observing a positive signal of efficacy. Assuming a 16% dropout rate to day 90, enrollment of 162 subjects was planned. Note that a change in gait velocity of 0.1 m/s has been suggested as clinically meaningful in populations with impaired walking speed, and an increase of 0.16 m/s is linked to a meaningful improvement in disability.

One interim and one headline data analysis were planned during the study. The interim analysis was planned for when >70 subjects completed the day 90 visit. At that time, the internal Safety Review Committee was to determine whether the estimated treatment effect of GSK249320 was likely to be futile based on a prespecified clinically meaningful treatment effect, that is, if the posterior probability that the true improvement over placebo is greater than zero is >70%. If the data hit the futility threshold, the internal Safety Review Committee would recommend discontinuation of the study.

The safety population was defined as subjects who received at least 1 infusion of IP. The intent-to-treat (ITT) population was defined as subjects in the safety population who underwent at least 1 post-baseline efficacy assessment, with subjects analyzed according to the treatment to which they were randomized. Intent to treat was the population used for the primary efficacy analysis. The per-protocol (PP) population was defined as all subjects in the intent-to-treat population, who were not protocol violators with regard to inclusion/exclusion criteria, blinding, IP administration, or gait velocity assessments. Subjects who did not receive both infusions of IP were also excluded from the PP population.

### Table 1. Key Entry and Exclusion Criteria

| Entry criterion                                                                                     | Exclusion criterion                                                                                   |
|----------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| Radiology confirmed supratentorial ischemic stroke; nonlacunar (either >15 mm diameter in 1 direction or >4 cc volume) | Ability to walk >0.8 m/s per gait velocity assessment                                               |
| Stroke onset within 24–72 h of IP infusion                                                          | Symptomatic stroke <3 mo before study entry                                                            |
| NIHSS score 3–21                                                                                   | Significant prestroke disability: Rankin score >2 before index stroke                                |
| Leg motor deficit: NIHSS Q6 score 1–4                                                              | Poorly responsive: NIHSS Q1a score 2 or 3                                                            |
| Impaired walking ability: gait velocity ≤0.8 m/s                                                    | Significant aphasia                                                                                    |
| Aged 18–90 y                                                                                       | Preexisting significant gait deficit, chronic liver disease, or prolonged QTc interval               |
| Expectation subject will receive standard physical, occupational, and speech rehabilitation therapies as indicated for poststroke deficits | Preexisting active poorly controlled neurological or psychiatric disease                               |
|                                                                                                   | Expected death because of index stroke or other preexisting condition                               |
|                                                                                                   | Participation in another investigational study targeting stroke recovery during study               |
|                                                                                                   | MRI contraindication                                                                                  |
|                                                                                                   | Pregnant/lactating                                                                                    |

MRI indicates magnetic resonance imaging; and NIHSS, National Institutes of Health Stroke Scale.
Results

Study Conduct
Across all 4 participating countries, 134 subjects were randomized, including 64 who were enrolled during the 3 months it took for the 70th subject to reach day 90, the futility criteria interim analysis to be completed, and the internal Safety Review Committee to make and communicate the decision to stop the study. Of the 133 who received investigational product, 64 subjects (48%) completed the study, and 69 subjects (52%) withdrew from the study or were lost to follow-up (Figure 1). The primary reason for withdrawal was that the study was terminated at the interim analysis. A total of 100 subjects (75%) were in the study for >90 days. A total of 116 subjects (87%) received both infusions of IP; 1 subject received no IP infusions, 10 subjects received only 1 IP infusion, 2 subjects received an incorrect dose for 1 infusion because of incorrect preparation of the dose, and 3 subjects received less than the full 100 mL volume of IP for at least 1 infusion. Overall, protocol deviations were reported for 109 subjects (81%), most of which were minor and did not require exclusion from the PP population (Table I in the online-only Data Supplement); all protocol deviations were collected, for transparency, regardless as to whether or not they had an impact on outcome. Of the 134 subjects randomized into the study, 133 were included in the safety population (placebo: n=68; GSK249320: n=65), 120 were included in the intent-to-treat population (placebo: n=60; GSK249320: n=60), and 104 were included in the PP population.

Subjects
Baseline data (Table 2) were generally balanced across treatment groups. The majority of enrollees (91%) had stroke involving the middle cerebral artery territory. During study participation, the amount of rehabilitation therapy, in minutes, provided to enrollees was substantial and variable, with subjects randomized to GSK249320 receiving a greater amount of therapy (Table 3).

Analysis of Treatment Efficacy
The study was stopped at the interim analysis because the posterior mean treatment difference was 0.027 at day 90 (95% credible interval, −0.146 to 0.199) and the posterior probability that true treatment difference was greater than zero was 0.621, which was lower than the predefined futility cutoff of 0.70 (Figure 2). Analysis of the PP population and using the final database including subject data for those subjects with an early withdrawal visit because of study termination were concordant (online-only Data Supplement).

Gait velocity data described the proportion of subjects in each gait impairment category (0, >0–< 0.4, 0.4–0.8, and >0.8 m/s) over time. Most subjects were nonambulatory at baseline and progressed to some level of ambulation by day 180, but a review of summary statistics for the secondary end points

---

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) diagram. ITT indicates intent to treat.
(change in gait impairment category, change in box and blocks score, distribution of modified Rankin Scale scores, and total NIHSS score) suggests no obvious differences between treatment groups (Table 4).

Analysis of Safety
The 2 IV infusions of GSK249320 were well tolerated as evidenced by an AE rate comparable to placebo, the majority of AEs having been reported as mild or moderate in severity, and the low withdrawal rate because of AEs (Table II in the online-only Data Supplement). No clinically important safety trends were observed post-dosing with GSK249320. There was no difference in the proportion of subjects having a fall, or in the number of falls, between treatment groups. The overall incidence of events common to stroke was comparable across the treatment groups (Table III in the online-only Data Supplement). AEs were reported in 57 subjects (84%) in the placebo group and in 49 subjects (75%) in the GSK249320 group. The most common AEs were constipation, nausea, and headache. No AE reports suggested peripheral neuropathy, infusion site reaction, or hypersensitivity reaction with GSK249320. Withdrawal from the study because of an AE occurred in 2 subjects in the placebo group and in no subjects in the GSK249320 group.

Sixteen subjects (24%) in the placebo group experienced serious adverse events, compared with 9 subjects (14%) in the GSK249320 group. Five subjects (7%) died in the placebo group. Two subjects (3%) died in the GSK249320 group: respiratory failure in a 90-year-old subject 4 days after first infusion and cardiorespiratory arrest in a 76-year-old subject 22 days after first infusion, both considered unrelated to IP infusions.

Immunogenicity
Five subjects had preexisting antibodies at low titers that were not related to treatment. Six of the 64 subjects who received GSK249320 developed antidrug antibodies. Eight of the 68 subjects in the placebo treatment group had antidrug antibodies against GSK249320 that were also not related to treatment. No neutralizing antibodies were detected.

GSK249320 Reduced Free Serum MAG Levels
Before administration of IP, soluble, free MAG plasma levels were similar between placebo and GSK249320 groups (33.0±42.0 versus 30.0±30.7 pg/mL, mean±SD). A progressive slow decline in free MAG level was seen after day 6 for placebo subjects, whereas subjects receiving GSK249320 exhibited an abrupt decline in free MAG level between day

### Table 2. Baseline Clinical Measures and Demographics

| Age, y* | Placebo (n=68) | GSK249320 (n=65) |
|---------|----------------|------------------|
| 67.1±11.2 | 68.2±11.9 |
| Sex (F/M) | 29/39 | 31/34 |
| Hypertension | 51 | 47 |
| Diabetes mellitus | 22 | 18 |
| Hyperlipidemia | 36 | 28 |
| Atrial fibrillation | 18 | 17 |
| History of angina pectoris/MI | 1 | 1 |
| History of stroke | 0 | 0 |
| Hispanic/Latino | 0 | 1 |
| Not Hispanic/Latino | 68 | 64 |
| Race | | |
| White | 62 | 62 |
| Black/African Heritage | 4 | 2 |
| American Indian/Alaskan Native | 1 | 0 |
| Asian | 1 | 1 |
| Received IV tPA | 29 | 25 |
| Received IA reperfusion therapy | 3 | 9 |
| Stroke subtype | | |
| Large-artery atherosclerosis | 24 | 20 |
| Cardioembolism | 19 | 25 |
| Small-vessel occlusion | 10 | 9 |
| Ischemic stroke other determined pathogenesis | 2 | 2 |
| Ischemic stroke undetermined pathogenesis | 13 | 9 |
| Gait impairment stratification | | |
| 0 | 55 | 53 |
| >0–<0.4 | 5 | 5 |
| 0.4–0.8 | 8 | 6 |
| >0.8 | 0 | 1 |
| NIHSS total score at day 1, median (range) | 9.5 (3–20) | 10.0 (3–19) |
| NIHSS Q6 leg deficit at day 1* | 2.4±1.20 | 2.1±1.09 |
| NIHSS Q5 arm deficit at day 1* | 2.7±1.29 | 2.4±1.34 |
| Box and blocks score at day 1* | | |
| Stroke-affected arm | 3.2±7.7 | 4.2±8.4 |
| Nonstroke arm | 25.1±13.3 | 23.3±12.4 |
| No. of hours between stroke onset and first IP infusion* | 52.7±14.4 | 52.4±13.3 |

Values are for safety population, except for box and blocks score at day 1, which is for per-protocol population. MI indicates myocardial infarction; NIHSS, National Institutes of Health Stroke Scale; and tPA, tissue-type plasminogen activator. *Values are represented in mean±SD.

## Table 3. Therapy Provided to Enrollees for the Duration of Study Participation

| | Placebo (n=52) | GSK249320 (n=52) |
|-------------------|----------------|------------------|
| Physical therapy | 1422 (0–10003) | 1610 (92–11285) |
| Occupational therapy | 771 (0–10003) | 1312 (0–11415) |
| Total therapy | 2241 (0–20006) | 3264 (184–22700) |

Results are for per-protocol population given in median (range) and represent minutes of therapy.
1 and day 6 that was maintained until at least day 30: median inhibition of free MAG in plasma was 97.5% after the first infusion of GSK249320 on day 1 and was maintained after the second infusion on day 6 at 97% until at least day 30, with free MAG levels in GSK249320-treated subjects resuming to levels similar to placebo group subjects at day 180 (Figure I in the online-only Data Supplement). The median GSK249320 concentration at the end of the second IP infusion, which can be considered the maximum concentration, was 494.5 μg/mL, and the mean half-life of GSK249320 was 23.7±5.2 days (Figure II in the online-only Data Supplement).

Discussion

The current study hypothesized that GSK249320, administered as 2 IV infusions beginning 24 to 72 hours poststroke and spaced 5±2 days apart, would improve gait recovery over 90 days in subjects with ischemic stroke and leg weakness with impaired walking ability. The data do not support this, and the study was stopped at interim analysis because observed difference between treatment groups met the pre-defined futility threshold.

The primary outcome measure was gait velocity, a choice that in retrospect had both advantages and disadvantages. Gait velocity has an established record as a valid, reliable assessment sensitive to treatment effects. Another advantage is that it measures function (ie, disability and activities limitations), rather than impairment, and can be directly linked with participation level (ie, handicap). As a modality-specific outcome measure, gait velocity has potential advantages compared with global outcomes for understanding recovery such as granularity of assessment. Furthermore, reduced gait velocity is common after stroke, gait improvements after stroke are linked to better quality of life, and in some studies gait recovery is ranked as the top priority by patients with hemiplegia after stroke. The value of gait velocity as primary end point was also based in part on its direct link with entry criteria (Table 1), which required slow gait for study entry. However, at baseline, >80% of subjects were entirely unable to ambulate at all (gait velocity=0 m/s), masking accurate understanding of within-subject gait recovery. This produced a floor effect such that several different degrees of neural abnormality were scored identically, although the study did make the key distinction between patients with gait velocity=0 m/s and patients in whom gait velocity could not be assessed. Another potential disadvantage of gait as the primary end point is that it is a complex behavior influenced by activity at multiple nervous system levels. Many patients with severe hemiparesis learn to walk on their spasticity, further complicating interpretation of changes in gait velocity after stroke. Putting it in perspective, the current placebo group mean gait velocity change from baseline to day 90 (0.56 m/s) was >3-fold greater compared with placebo group of the
previous phase II GSK249320 trial (0.18 m/s), a difference possibly because of play of chance but that reduced ability of the current study to detect a treatment group difference. Level of impairment also differed between studies, with median placebo group baseline total NIHSS score of 7 in the previous trial compared with 9.5 herein.

Other study design features may also be important for understanding results. Choice of patient population influences how hypotheses are tested. Patients with small-vessel infarcts, operationally <15 mm maximum diameter or 4 cc volume, were excluded given their comparatively favorable prognosis.24,25 Study entry required total NIHSS score of 3 to 21 and leg motor score of 1 to 4. This enrolled subjects with milder strokes, who might be expected to have a favorable prognosis regardless of treatment arm. The amount of IP infused could also be important. Median GSK249320 concentration at the end of the second infusion (maximum concentration) was lower herein as compared with subjects receiving the same dose in the previous study26 in which the second infusion was administered 9±1 days apart (median 494.5 versus 723.0 μg/mL); conceivably infusing a higher amount of antibody might have increased its effect size.

It is useful to revisit assumptions that supported current study design. The antibody showed a favorable preclinical and clinical profile. It was well characterized and the progression of therapy development confirmed to published recommendations. Preclinical studies in rodents48 and primates11 suggested efficacy. The antibody was found to be safe in 37 healthy subjects, who received a single IV infusion ≤25 mg/kg, and in a phase II study of 42 patients 24 to 72 hours after ischemic stroke, among whom 25 subjects received 2 IV infusions ≤15 mg/kg; significant benefit compared with placebo was found over time for gait velocity, an end point well aligned with preclinical behavioral end points.

Other issues relevant to current results pertain to translation from animals to humans. Behavioral recovery26,27 and neural plasticity28–30 after stroke are accelerated in rodents compared with humans. On the basis of this, time of first infusion in animals (24 hours poststroke) was extended to 72 hours in humans, but this may not have been an appropriate extrapolation. The same concern might extend to presence of MAG, the biological target: in rats with experimental stroke, MAG levels start to increase by 3 days poststroke and peak at 2 weeks poststroke, but it is uncertain whether this is true in humans. White matter constitutes 14% of rodent versus 50% of human brain volume31,32; axons might be more difficult for a large antibody to access in humans. Other limitations of animal models may also pertain, including that animal models incompletely capture the complex psychosocial issues that patients face after stroke, such as depression, caregiver support, and financial stressors.

Direct evidence that substantial quantities of the therapy reached the biological target was not available. Indirect evidence of target binding in the current study was suggested by the substantial reduction in free MAG plasma levels with GSK249320 treatment. The half-life of GSK249320 in the current study was 23.7±5.2 days, similar to the value of 21 days found in healthy control subjects and typical of a monoclonal antibody. Neutralizing antibodies were not detected and so did not contribute to current findings.

The experience of translating therapies targeting acute ischemic stroke has provided several lessons, and in many cases, these inform translation of restorative stroke therapies to clinical trials. Examples include stepwise translation from preclinical to clinical studies, the need to standardize performance of assessments, careful selection of study sample size to insure adequate study power, and centralized data management. However, neuroprotection differs in many ways from restoration—restorative trials are not simply delayed neuroprotection trials. On the contrary, trials targeting brain restoration must address unique aspects of study design issues within the context of topics such as end point selection, target population identification, and intervention timing because the optimal approach in these and other areas often does not directly extend from neuroprotection trials to restorative trials.

This proof-of-concept study for GSK249320, a monoclonal antibody GSK249320 administered IV and initiated within 72 hours of stroke onset, demonstrated no improvement on gait outcomes compared with placebo. As mentioned above, many possible reasons might have contributed to these findings, including using an end point with too large a floor effect at baseline, enrolling patients with too severe a level deficit, using too low an antibody dose, interspecies differences in pharmacokinetics, lack of direct evidence that the therapy reached the biological target, or simply that GSK249320 does not work in human stroke. In the current study, the antibody was well tolerated and showed low immunogenicity, findings that may prove useful to future studies aiming to use a monoclonal antibody to modify activity in specific biological targets to promote improved stroke recovery.

Sources of Funding
S.C. Cramer is supported by NIH K24 HD074722. This study was sponsored by GlaxoSmithKline.

Disclosures
S.C. Cramer has served as a consultant for GlaxoSmithKline, Roche, Dart Neuroscience, MicroTransponder, and RAND Corporation. L.A. Enney and M. Simeoni are employees of GlaxoSmithKline and own shares in the company. C.K. Russell and T.R. Thompson are former employees of GlaxoSmithKline and own shares in the company.

References
1. Cramer SC. Repairing the human brain after stroke. II. Restorative therapies. Ann Neurol. 2008;63:549–560. doi: 10.1002/ana.21412.
2. Li S, Carmichael ST. Growth-associated gene and protein expression in the region of axonal sprouting in the aged brain after stroke. Neurobiol Dis. 2006;23:362–373. doi: 10.1016/j.nbd.2006.03.011.
3. Mukhopadhyay G, Doherty P, Walsh FS, Crocker PR, Filbin MT. A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. Neuron. 1994;13:757–767.
4. Domeniconi M, Filbin MT. Overcoming inhibitors in myelin to promote axonal regeneration. J Neurosci. 2005;23:43–47. doi: 10.1016/j.jns.2005.03.023.
5. Walsmsley AR, Mir AK. Targeting the Nogo-A signalling pathway to promote recovery following acute CNS injury. Curr Pharm Des. 2007;13:2470–2484.
6. Irving EA, Vinson M, Rosin C, Roberts JC, Chapman DM, Facci L, et al. Identification of neuroprotective properties of anti-MAG antibody: a
novel approach for the treatment of stroke? J Cereb Blood Flow Metab. 2005;25:98–107. doi: 10.1038/sj.jbfm.9600011.

7. Torigoe K, Lundborg G. Selective inhibition of early axonal regeneration by myelin-associated glycoprotein. Exp Neurol. 1998;150:254–262. doi: 10.1006/exnr.1997.6775.

8. Mears S, Schachner M, Brushart TM. Antibodies to myelin-associated glycoprotein accelerate preferential motor reinnervation. J Peripher Nerv Syst. 2003;8:91–99.

9. Lee JK, Kim JE, Sivula M, Srtittmatter SM. Nogo receptor antagonist promotes stroke recovery by enhancing axonal plasticity. J Neurosci. 2004;24:6209–6217. doi: 10.1523/JNEUROSCI.1643-04.2004.

10. Cash D, Easton AC, Mesquita M, Beech J, Williams S, Lloyd A, et al. GSK249320, a monoclonal antibody against the axon outgrowth inhibitory molecule myelin-associated glycoprotein, improves outcome of rodents with experimental stroke. J Neurol Exp Neurosoc. 2016;2:28–33.

11. Barbay S, Plautz EJ, Zoubina E, Frost SB, Cramer SC, Nudo RJ. Effects of postinfarct myelin-associated glycoprotein antibody treatment on motor recovery and motor map plasticity in squirrel monkeys. Stroke. 2015;46:1620–1625. doi: 10.1161/STROKEAHA.114.008088.

12. Abila B, Cunningham E, Simeoni M. First-time-in-human study with GSK249320, a myelin-associated glycoprotein inhibitor, in healthy volunteers. Clin Pharmacol Ther. 2013;93:163–169. doi: 10.1038/cptl.2012.227.

13. Cramer SC, Abila B, Scott NE, Simeoni M, Enney LA; MAG111539 Study Investigators. Safety, pharmacokinetics, and pharmacodynamics of escalating repeat doses of GSK249320 in patients with stroke. Stroke. 2013;44:1337–1342. doi: 10.1161/STROKEAHA.111.674366.

14. Richards C, Maloun F, Dumas F, Tardif D. Gait velocity as an outcome measure of locomotor recovery after stroke. In: Craik R, Oates C, eds. Gait Analysis: Theory and Application. St. Louis: Mosby; 1995:355–364.

15. Duncan PW, Sullivan KJ, Behrmann AL, Azem SN, Wu SS, Nadeau SE, et al; LEAPS Investigative Team. Body-weight-supported treadmill rehabilitation after stroke. N Engl J Med. 2011;364:2026–2036. doi: 10.1056/NEJMoa1010790.

16. Fritz S, Lusardi M. White paper: “walking speed: the sixth vital sign.” J Geriatr Phys Ther. 2009;32:46–49.

17. Tilson JK, Sullivan KJ, Cen SY, Rose DK, Koradia CH, Azen SP, et al; Locomotor Experience Applied Post Stroke (LEAPS) Investigative Team. Meaningful gait speed improvement during the first 60 days poststroke: minimal clinically important difference. Phys Ther. 2010;90:196–208. doi: 10.2522/ptj.20090079.

18. Winnie CR, Stein J, Arena R, Bates B, Cherry LR, Cramer SC, et al; American Heart Association Stroke Council, Council on Cardiovascular and Stroke Nursing, Council on Clinical Cardiology, and Council on Quality of Care and Outcomes Research. Guidelines for Adult Stroke Rehabilitation and Recovery: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke. 2016;47:e98–e169. doi: 10.1161/STRK.0000000000000098.

19. Perry J, Garrett M, Cronley JK, Mulroy SJ. Classification of walking handicap in the stroke population. Stroke. 1995;26:982–989.

20. Cramer SC, Koroshetz WJ, Finklestein SP. The case for modality-specific outcome measures in clinical trials of recovery-promoting agents. Stroke. 2007;38:1393–1395. doi: 10.1161/STRK.0000000000000098.

21. Salbach NM, Mayo NE, Higgins J, Ahmed S, Finch LE, Richards CL. Responsiveness and predictability of gait speed and other disability measures in acute stroke. Arch Phys Med Rehabil. 2001;82:1204–1212. doi: 10.1016/sj.apmr.2001.24907.

22. Bohannon R, Andrews A, Smith M. Rehabilitation goals of patients with hemiplegia. Int J Rehab Res. 1988;11:181–183.

23. Fisher CM. Lacunar strokes and infarcts: a review. Neurology. 1982;32:871–876.

24. Pullicino P, Nelson RF, Kendall BE, Marshall J. Small deep infarcts diagnosed on computed tomography. Neurology. 1980;30:1090–1096.

25. Sprigg N, Gray LJ, Bath PM, Lindenstrøm E, Boysen G, De Deyn PP, et al; TAIST Investigators. Early recovery and functional outcome are related with causal stroke subtype: data from the tinzaparin in acute ischemic stroke trial. J Stroke Cerebrovasc Dis. 2007;16:180–184. doi: 10.1016/j.jstrokecerebrovasdis.2007.02.003.

26. Kawamata T, Dietrich WD, Schallert T, Gotts JE, Cocke RR, Benowitz LI, et al. Intracerebral basic fibroblast growth factor enhances functional recovery and up-regulates the expression of a molecular marker of neuronal sprouting following focal cerebral infarction. Proc Natl Acad Sci USA. 1997:94:8179–8184.

27. Duncan PW, Goldstein LB, Matchar D, Divine GW, Feussner J. Measurement of motor recovery after stroke. Outcome assessment and sample size requirements. Stroke. 1992;23:1084–1089.

28. Dijkstraen RM, Ren J, Mandeville JB, Wu O, Ozdag FM, Moskowitz MA, et al. Functional magnetic resonance imaging of reorganization in rat brain after stroke. Proc Natl Acad Sci USA. 2001;98:12766–12771. doi: 10.1073/pnas.231235598.

29. Calabretti C, Leroy F, Guinestre JY, Baron JC. Dynamics of motor network overactivation after striatocapsular stroke: a longitudinal PET study using a fixed-performance paradigm. Stroke. 2001;32:2534–2542.

30. Tombari D, Loubinoux I, Pariente J, Gerdelat A, Albucher JF, Tardy J, et al. A longitudinal fMRI study: in recovering and then in clinically stable sub-cortical stroke patients. Neuroimage. 2003;25:827–839. doi: 10.1016/j.neuroimage.2004.07.058.

31. Goldberg MP, Ransom BR. New light on white matter. Stroke. 2003;34:330–332.

32. Cramer SC. Clinical issues in animal models of stroke and rehabilitation. ILAR J. 2003;44:83–84.

33. Cramer SC. Drugs to Enhance Motor Recovery After Stroke. Stroke. 2015;46:2998–3005. doi: 10.1161/STROKEAHA.115.007433.

34. Fisher M, Feuerstein G, Ho FB, McArthur J, Hogg-Johnson S, Saposnik G, et al; STAIR Group. Update of the stroke therapy academic industry roundtable preclinical recommendations. Stroke. 2009;40:2244–2250. doi: 10.1161/STROKEAHA.108.541128.

35. Drinni U, Thomas Willis lecture: is translational stroke research broken, and if so, how can we fix it? Stroke. 2016;47:2148–2153. doi: 10.1161/STROKEAHA.116.013244.
Proof-of-Concept Randomized Trial of the Monoclonal Antibody GSK249320 Versus Placebo in Stroke Patients

Steven C. Cramer, Lori A. Enney, Colleen K. Russell, Monica Simeoni and Thomas R. Thompson

*Stroke*. 2017;48:692-698; originally published online February 22, 2017;
doi: 10.1161/STROKEAHA.116.014517

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2017 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/48/3/692

Free via Open Access

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2017/02/21/STROKEAHA.116.014517.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL

“A Proof of Concept Randomized Trial of the Monoclonal Antibody GSK249320 Versus Placebo in Stroke Patients”

Additional information on study overview

The 30 centers were located in the following countries: 5 in the US, 5 in Canada, 8 in the UK and 12 in Germany. Once a subject was randomized and had received at least one infusion of Investigational Product (IP), collection of survival status data was performed out to Day 180/Month 6 regardless of whether the subject was prematurely withdrawn from the study for any reason. The first infusion was on Study Day 1 and the second on Study Day 6±2 days.

Additional information on randomization

The choice of 15 mg/kg was based on: (1) level of demonstrated safety coverage in preclinical safety studies, (2) ability to provide sufficient exposure to achieve effective pharmacological levels as seen in preclinical studies, (3) safety in a phase I study that used doses up to 25 mg/kg\(^1\), and 4) safety plus potential efficacy in a phase II stroke trial that used doses up to 15 mg/kg\(^2\).

The randomization schedule was computer-generated using GlaxoSmithKline’s RandALL system. As each study site, open-label vials of GSK249320 and placebo were shipped directly to an unblinded pharmacist. When a patient was randomized into the study, the central study system provided a randomization number that was given to the unblinded pharmacist, who then prepared the infusion accordingly.

Additional information on study assessments

The iSRC was independent from the study team, consisted of senior GlaxoSmithKline staff plus independent external medical consultants, and served as the Data Monitoring Committee. Note that events common to stroke were a priori identified and were exempt from the standard AE/SAE reporting unless they were more severe than expected or attributed to IP. Actigraphy measurement of subject activity was also obtained and will be reported separately.

Plasma samples were analyzed for GSK249320 using a validated analytical method based on sample dilution, followed by immunoassay analysis. Free soluble MAG levels were measured by immunoassay. Immunogenicity was evaluated by measuring anti-GSK249320 antibodies in serum using an electrochemiluminescent assay.

Additional information on statistical analysis.
A non-informative prior distribution was assumed for each of the fixed effect terms (N(0,1e6)). Subject was fitted as a random effect; visit was fitted as a repeated effect within subject. The variance-covariance matrix was unstructured with a non-informative prior distribution (inverse Wishart).

The PP population was used for a sensitivity analysis of the primary endpoint and for secondary efficacy endpoints. The headline analysis was planned for when the last enrolled subject completed the Day 90 visit, at which time cumulative gait velocity data to Day 90 would be reviewed for all enrolled subjects to determine if GSK249320 had met the primary objective of the study and achieved proof of concept. Regardless of the outcome of the headline data analysis, the study was going to be taken to completion (i.e., completion of the Day 180 visit).

Additional information on behavioral assessments and assessor training:

*Gait velocity:* Gait velocity is an objective, quantitative measure of lower extremity motor recovery that has been shown to be reliable, valid and sensitive in the stroke population\(^3\). Normal gait velocity ranges between 1.2-1.4m/s and a change of 0.1m/s has been suggested as clinically meaningful in populations with impaired walking speed\(^4\). An increase of 0.16m/s has been shown to link to a meaningful improvement in disability\(^5\). Gait velocity was assessed over a level, indoor 10-meter distance, in a manner guided by the APTA StrokEDGE Taskforce. The time (in seconds) required for the subject to travel the 10-meter distance was recorded. The distinction was made between subjects who were assessed and found to be too incapacitated to walk (i.e., gait velocity = 0m/s) and subjects for whom gait velocity could not be assessed (i.e., truly missing data). Subjects were asked to walk at their usual or normal pace. Use of a subject’s normal assistive devices was permitted. Two trials of gait velocity were conducted at each time point. Only study personnel who completed and passed this study's Gait Velocity Training Module were permitted to perform the gait velocity assessment. Gait velocity assessors had to pass the Training Module prior to administering the assessment in the study and had to complete/pass additional in-stream training in order to ensure consistency in the conduct and measurement of this endpoint.

*Box & Blocks test:* The Box & Blocks test is an objective, gross manual dexterity test that has been shown to be reliable and valid in individuals with upper limb impairments\(^6\). Box & Blocks is an examiner-assessed, subject-completed test that requires the subject to move small wooden blocks from one side of a partitioned box to the other. The score is determined by the number of blocks transferred within a 60 second time period. Both the stroke-affected and the non-affected limbs were tested, starting with the non-affected limb. Only study personnel who completed and passed the Box & Blocks Training Module were permitted to perform this assessment during the study.

*Modified Rankin Scale:* The modified Rankin Scale (mRS) is a 6-level scale that measures activity limitations by evaluating limitations in activity and changes in lifestyle\(^8\). Only study personnel who completed and passed the mRS Training Module were permitted to perform the mRS.
NIH Stroke Scale: The NIH Stroke Scale (NIHSS) is a examiner-assessed, 15 item, standardized scale that measures neurological impairment and is used to quantify subject status by measuring the stroke severity. Only personnel who were formally certified on NIHSS scoring were permitted to perform the NIHSS.

Supplementary Results on Analysis of treatment efficacy

Regarding stopping the study at the interim analysis due to the posterior mean treatment difference, the main report provides analysis of treatment efficacy for the ITT population. Additional analysis using the PP population was concordant with this, with posterior mean treatment difference 0.041 at Day 90 (95% Credible Interval -0.131, 0.212) and posterior probability that true treatment difference was greater than 0 of 0.682. A final ITT group analysis was also performed using the final database including subject data for those subjects with an early withdrawal visit due to study termination. Findings were similar in this primary analysis using the final study database: the posterior mean treatment difference was 0.044 at Day 90 (95% Credible Interval -0.119, 0.200) and the posterior probability that true treatment difference was greater than 0 was 0.713; PP population analysis was again concordant, with posterior mean treatment difference 0.052 at Day 90 (95% Credible Interval -0.126, 0.228) and posterior probability that true treatment difference was greater than 0 of 0.722.
**Supplementary Table I. Summary of Protocol Deviations**

| Deviations                                                        | Placebo Group | GSK249320 Group |
|------------------------------------------------------------------|---------------|-----------------|
| n                                                                | 68            | 66              |
| Any protocol deviations                                          | 58 (85%)      | 51 (77%)        |
| Deviations that required exclusion from PP population             | 6 (9%)        | 4 (6%)          |
| Eligibility criteria not met                                      | 1 (1%)        | 1 (2%)          |
| Received wrong treatment or incorrect dose                        | 1 (1%)        | 2 (3%)          |
| Missed assessment or procedure                                    | 2 (3%)        | 1 (2%)          |
| Other assessment or procedure issue                               | 2 (3%)        | 0               |
| Deviations that did not require exclusion from PP population      | 57 (84%)      | 50 (76%)        |
| Eligibility criteria not met                                      | 1 (1%)        | 0               |
| Visit, assessment or time point window                            | 2 (3%)        | 2 (3%)          |
| Received wrong treatment or incorrect dose                        | 0             | 1 (2%)          |
| Assessments and/or procedures                                     | 57 (84%)      | 50 (76%)        |
| Informed consent process                                          | 5 (7%)        | 5 (8%)          |
| Failure to report SAE, pregnancy, or liver function abnormalities per-protocol | 1 (1%)        | 0               |
| Study blind/unblind procedures                                    | 1 (1%)        | 0               |
| Biological specimen sample procedures                             | 16 (24%)      | 14 (21%)        |
| Randomization procedures                                          | 2 (3%)        | 1 (2%)          |
| Missed assessment or procedure                                    | 29 (43%)      | 28 (42%)        |
| Administration of primary endpoint assessment not performed correctly | 0             | 1 (2%)          |
| Other                                                             | 44 (65%)      | 34 (52%)        |

Note that subjects may have more than one protocol deviation.
Supplementary Table II. Summary of Main Adverse Events (Safety Population)

| Adverse Event                  | Placebo Group | GSK249320 Group |
|-------------------------------|---------------|-----------------|
| n                             | 68            | 65              |
| Any Adverse Event             | 57 (84%)      | 49 (75%)        |
| Constipation                  | 6 (9%)        | 13 (20%)        |
| Nausea                        | 3 (4%)        | 5 (8%)          |
| Headache                      | 7 (10%)       | 12 (18%)        |
| Diarrhea                      | 2 (3%)        | 2 (3%)          |
| Dizziness                     | 2 (3%)        | 2 (3%)          |
| Urinary tract infection       | 4 (6%)        | 0               |
| Urinary retention             | 1 (1%)        | 3 (5%)          |
| Insomnia                      | 4 (6%)        | 5 (8%)          |
| Atrial fibrillation           | 0             | 4 (6%)          |
| Hypotension                   | 4 (6%)        | 2 (3%)          |
| Hypokalemia                   | 3 (4%)        | 2 (3%)          |
| Arthralgia                    | 2 (3%)        | 2 (3%)          |
| Contusion                     | 2 (3%)        | 3 (5%)          |
| Rash                          | 3 (4%)        | 4 (6%)          |

The number of subjects reporting a fall between baseline and end of the study was 18 (26%) in the placebo group and 16 (25%) in the GSK249320 group.

The severity of AE, when reported, was similar between the two treatment groups across all AE: mild in 24% of patients in the Placebo Group vs. 14% of the GSK 249320 Group; moderate in 37% vs. 49%; and severe in 24% vs. 11%.
### Supplementary Table III. Summary of Events Common to Stroke (Safety Population)

| Event                                                                 | Placebo Group | GSK249320 Group |
|----------------------------------------------------------------------|---------------|-----------------|
| n                                                                    | 68            | 65              |
| Any event                                                            | 59 (87%)      | 64 (98%)        |
| Joint or soft tissue pain                                            | 19 (28%)      | 22 (34%)        |
| Bladder incontinence                                                 | 11 (16%)      | 23 (35%)        |
| Depression/mood disorder                                             | 17 (25%)      | 16 (25%)        |
| Urinary tract infection                                               | 17 (25%)      | 13 (20%)        |
| Dysphagia                                                            | 12 (18%)      | 12 (18%)        |
| Bowel incontinence                                                   | 11 (16%)      | 12 (18%)        |
| Dysarthria                                                           | 11 (16%)      | 11 (17%)        |
| Confusion                                                            | 8 (12%)       | 13 (20%)        |
| Spasticity                                                           | 9 (13%)       | 9 (14%)         |
| Limb edema                                                           | 5 (7%)        | 12 (18%)        |
| Aspiration pneumonia                                                 | 5 (7%)        | 3 (5%)          |
| Hemorrhagic transformation (asymptomatic or symptomatic)             | 4 (6%)        | 4 (6%)          |
| Pressure ulcers                                                      | 3 (4%)        | 2 (3%)          |
| Deep vein thrombosis                                                 | 2 (3%)        | 0               |
| Seizures                                                             | 0             | 1 (2%)          |

The overall incidence of Events Common to Stroke was comparable across the two treatment Groups, with no apparent trends in reporting of the Events Common to Stroke to suggest an overall clinical worsening of subjects who received GSK249320 compared with those receiving placebo.
Supplementary Figure I. GSK249320 reduced free MAG levels in plasma but placebo did not, demonstrated by this graph of log free MAG in plasma vs. time for each of the two treatment groups. A progressive slow decline in free MAG level was seen after Day 6 for subjects in the Placebo Group. On the other hand, subjects in the GSK249320 Group exhibited an abrupt decline in free MAG level between Day 1 and 6 that was maintained until at least Day 30; median inhibition of free MAG in plasma among subjects in this Group was 97.5% after the first infusion of GSK249320 on Day 1 and was maintained after the second infusion on Day 6 at 97% until at least Day 30, with free MAG levels in GSK249320-treated subjects resuming to levels similar to placebo group subjects at Day 180.
**Supplementary Figure II.** Plot of median plasma GSK249320 Concentration over time for subjects in the GSK249320 Group. The median GSK249320 concentration at the end of the second IP infusion, which can be considered the maximum concentration, was 494.5 mcg/ml, and the mean half-life of GSK249320 was 23.7±5.2 days.
Supplement References

1. Abila B, Cunningham E, Simeoni M. First-time-in-human study with gsk249320, a myelin-associated glycoprotein inhibitor, in healthy volunteers. Clin Pharmacol Ther. 2013;93:163-169
2. Cramer SC, Abila B, Scott NE, Simeoni M, Enney LA. Safety, pharmacokinetics, and pharmacodynamics of escalating repeat doses of gsk249320 in patients with stroke. Stroke. 2013;44:1337-1342
3. Richards C, Malouin F, Dumas F, Tardif D. Gait velocity as an outcome measure of locomotor recovery after stroke. In: Craik R, Oates C, eds. Gait analysis: Theory and application. St. Louis: Mosby; 1995:355-364.
4. Fritz S, Lusardi M. White paper: "Walking speed: The sixth vital sign". J Geriatr Phys Ther. 2009;32:46-49
5. Tilson JK, Sullivan KJ, Cen SY, Rose DK, Koradia CH, Azen SP, et al. Meaningful gait speed improvement during the first 60 days poststroke: Minimal clinically important difference. Physical therapy. 2010;90:196-208
6. Desrosiers J, Bravo G, Hebert R, Dutil E, Mercier L. Validation of the box and block test as a measure of dexterity of elderly people: Reliability, validity, and norms studies. Archives PMR. 1994;75:751-755
7. Mathiowetz V, Volland G, Kashman N, Weber K. Adult norms for the box and block test of manual dexterity. The American journal of occupational therapy. 1985;39:386-391
8. Banks JL, Marotta CA. Outcomes validity and reliability of the modified rankin scale: Implications for stroke clinical trials: A literature review and synthesis. Stroke. 2007;38:1091-1096
9. Brott T, Adams H, Olinger C, Marler J, Barsan W, Biller J, et al. Measurements of acute cerebral infarction: A clinical examination scale. Stroke. 1989;20:864-870
We gratefully thank the patients and investigators at the study sites:

| Study Site Name                                      | Country     | Principal Investigator          |
|------------------------------------------------------|-------------|---------------------------------|
| London Health Sciences Centre                        | Canada      | Richard Chan                    |
| Sunnybrook Health Sciences Centre                    | Canada      | Richard Swartz                  |
| Hôtel-Dieu de St-Jérôme                              | Canada      | Yves Pesant                     |
| University of Alberta                                | Canada      | Ashfaq Shuaib                   |
| Grey Nuns Community Hospital                         | Canada      | Brian Buck                      |
| CSSS Champlain - Charles-LeMoyne                     | Canada      | Leo Berger                      |
| Universitaetsklinikum Freiburg                       | Germany     | Matthias Reinhard               |
| Allgemeines Krankenhaus Celle                        | Germany     | Wolfgang Heide                  |
| Universitaetsklinikum Ulm                            | Germany     | Eric Juettler                   |
| Universitaetsklinikum Erlangen                       | Germany     | Martin Koehrmann                |
| Universitaetsklinikum Leipzig                         | Germany     | Carsten Hobohm                  |
| Universitaetsklinikum Essen                          | Germany     | Christian Weimar                |
| Medizinische Hochschule Hannover                     | Germany     | Karin Weissenborn               |
| Klinikum Bremen Mitte gGmbH                           | Germany     | Andreas Kastrup                 |
| Asklepios Klinik Altona                              | Germany     | Joachim Roether                 |
| Asklepios Klinik Nord Helberg                        | Germany     | Guenter Seidel                  |
| Universitaetsklinikum Muenster                        | Germany     | Rainer Dziewas                  |
| Klinikum Friedrichshafen GmbH                         | Germany     | Roman Huber                     |
| Northwick Park Hospital                              | United Kingdom | David Cohen               |
| St George’s Hospital                                 | United Kingdom | Barry Moynihan            |
| Southern General Hospital                            | United Kingdom | Keith Muir              |
| King’s College Hospital                               | United Kingdom | Lalit Kalra              |
| Royal Victoria Infirmary                              | United Kingdom | Stephen Louw            |
| Barking, Havering & Redbridge University Hospitals NHS Trust | United Kingdom | Sreeman Andole          |
| Royal Devon and Exeter NHS Foundation Trust          | United Kingdom | Martin James           |
| Salford Royal Hospitals' Foundation Trust            | United Kingdom | Pippa Tyrrell              |
| Addenbrookes Hospital                                | United Kingdom | Elizabeth Warburton        |
| University of California Irvine Medical Center        | United States | Steve Cramer              |
| Vanderbilt University                                | United States | Howard Kirshner          |
| Oregon Health & Sciences University                  | United States | Helmi Lutsep           |
| University of Kentucky                                | United States | Luther Pettigrew          |
| University of Florida Health Sciences Center          | United States | Nader Antonios          |