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

Steven C. Cramer, MD[1], **Lori A. Enney, BS**[2], **Colleen K. Russell, MS**[2,3], **Monica Simeoni, PhD**[4], and **Thomas R. Thompson, MD**[2,5]

[1]Depts. of Neurology, University of California, Irvine

[2]GlaxoSmithKline Research & Development, Research Triangle Park, North Carolina

[3]PAREXEL International, Durham, North Carolina

[4]GlaxoSmithKline Research & Development, Stockley Park, United Kingdom

[5]Medpace Inc., Cincinnati, Ohio

Abstract

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

Methods—In this phase IIb double-blind, randomized, placebo-controlled study, patients at 30 centers with ischemic stroke 24–72 hours prior and gait deficits were randomized to two 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–July 2014. The two groups were overall well matched at baseline. The study was stopped at the pre-specified 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 endpoints including upper extremity function were concordant. The two 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 vs. 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

CORRESPONDING AUTHOR: Steven C. Cramer, MD, UCIrvine Medical Center, 200 S. Manchester Ave, Suite 206, Orange, CA 92868, Phone: (714) 456-6876, scramer@uci.edu.

Conflict of interests

SCC has served as a consultant for GlaxoSmithKline, Roche, Dart Neuroscience, MicroTransponder, and RAND Corporation. LAE and MS are employees of GlaxoSmithKline and own shares in the company. CKR and TRT are former employees of GlaxoSmithKline and own shares in the company.

monoclonal antibody to modify activity in specific biological pathways to improve recovery from stroke.

Clinical Trial Registration Information—https://clinicaltrials.gov/show/NCT01808261

Keywords

stroke recovery; axon; gait velocity; clinical trial

Subject terms

clinical studies; ischemic stroke; rehabilitation

After the injury from an acute stroke, numerous restorative events evolve within the brain. Targeting these events therapeutically may augment post-stroke 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, one being myelin-associated glycoprotein (MAG). After stroke, MAG levels spontaneously increase in penumbra[2], suggesting MAG may be a useful target to promote neural repair, an idea bolstered by prior 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 pre-clinical studies[6] and to promote regeneration after peripheral nerve injury[7, 8]. Blocking the action of a related protein, Nogo, seven 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 post-stroke without affecting infarct volume[10], and primate studies in which intravenous (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[12], and a recent randomized placebo-controlled Phase II trial in patients 24–72 hours after ischemic stroke also found the antibody to be safe, and suggested potential efficacy for improving recovery of gait[13].

The current study built on these findings as a Phase IIb double-blind, randomized, placebo controlled, multicenter study. Patients with ischemic stroke 24–72 hours prior and deficits in gait were randomized to receive two 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[14, 15]. 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 pre-defined futility cutoff.

Methods

Study overview

Thirty centers across 4 countries enrolled subjects in the study, between May 2013–July 2014. The study was approved by each site's Institutional Review Board. All subjects, or surrogates, gave written informed consent. This study was registered (clinicaltrials.gov NCT01808261). Participation spanned six visits from baseline-Day 180. Key entry/exclusion criteria appear in Table 1. See also Supplemental Material.

Randomization

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

Study assessments

At baseline, prior to first infusion and thus <72 hours post-stroke, assessments included NIHSS, modified Rankin Scale, gait velocity, and Box & Blocks (# blocks transferred during 1 minute). All study assessors were formally trained and certified in each of these outcome measures (see Supplemental Material). Patients and assessors were blinded at all times. These were serially evaluated over the remaining five visits, as was the amount of rehabilitation (physical and occupational) therapy that patients received. Safety assessments included vital signs, clinical labs, ECGs, suicidality, adverse events (AE), serious adverse events (SAE), and falls, and were monitored by the internal Safety Review Committee (iSRC). 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 Supplemental Material.

Data analysis

The primary efficacy endpoint 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 to 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 Supplemental Material. 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[13], 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 0.1m/s has

been suggested as clinically meaningful in populations with impaired walking speed[16] and an increase of 0.16m/s is linked to a meaningful improvement in disability[17].

One interim and one headline data analysis were planned during the study. The interim analysis was planned for when approximately 70 subjects completed the Day 90 visit. At that time, the iSRC was to determine if the estimated treatment effect of GSK249320 was likely to be futile based on a pre-specified clinically meaningful treatment effect, i.e., if the posterior probability that the true improvement over placebo is greater than zero is <70%. If the data hit the futility threshold, the iSRC would recommend discontinuation of the study.

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

Results

Study conduct

A total of 134 subjects were randomized across all four participating countries, 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 iSRC to make and communicate the decision to stop the study. Of the 133 that received investigational product, 64 (48%) subjects completed the study and 69 (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 (75%) subjects were in the study for more than 90 days. A total of 116 (87%) subjects received both infusions of IP; one subject received no IP infusions, 10 subjects received only one IP infusion, two subjects received an incorrect dose for one infusion due to incorrect preparation of the dose, and three subjects received less than the full 100mL volume of IP for at least one infusion. Overall, protocol deviations were reported for 109 (81%) subjects, most of which were minor and did not require exclusion from the PP population (Supplementary Table I); 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 ITT 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 (Figure 2)

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, 0.199) and the posterior probability that true treatment difference was greater than 0 was 0.621, which was lower than the predefined futility cutoff of 0.70. Analysis (1) of the PP population, and (2) using the final database including subject data for those subjects with an early withdrawal visit due to study termination, were concordant (Supplemental Material).

Gait velocity data described the proportion of subjects in each gait impairment category (0, >0 to < 0.4m/s, 0.4m/s to 0.8m/s, and >0.8m/s) over time. Most subjects were non-ambulatory at baseline and progressed to some level of ambulation by Day 180, but a review of summary statistics for the secondary endpoints (change in gait impairment category, change in Box & Blocks score, distribution of modified Rankin Scale scores, total NIHSS score) suggests no obvious differences between treatment groups (Table 4).

Analysis of safety

The two 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 due to AEs (Supplementary Table II). 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 (Supplementary Table III). Adverse events were reported in 57 (84%) of subjects in the placebo group, and 49 (75%) of subjects 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 due to an AE occurred in two subjects in the placebo group and no subjects in the GSK249320 group.

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

Immunogenicity

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

GSK249320 reduced free serum MAG levels

Prior to administration of IP, soluble free MAG plasma levels were similar between placebo and GSK249320 groups (33.0±42.0 vs. 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 1 and 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 (Supplementary Figure I). 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 (Supplementary Figure II).

Discussion

The current study hypothesized that GSK249320, administered as two IV infusions beginning 24–72 hours post-stroke 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 predefined 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[14, 15]. Another advantage is that it measures function (i.e., disability, activities limitations), rather than impairment, and can be directly linked with participation level (i.e., handicap)[15, 16, 18, 19]. As a modality-specific outcome measure, gait velocity has potential advantages over global outcomes for understanding recovery such as granularity of assessment[20]. 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[16, 21, 22]. The value of gait velocity as primary endpoint 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/sec), 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/sec and patients in whom gait velocity could not be assessed. Another potential disadvantage of gait as the primary endpoint 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/sec) was more than three-fold greater compared to placebo group of the prior phase II GSK249320 trial (0.18 m/sec)[13], a difference possibly due to 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 prior trial compared to 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 less than 15 mm maximum diameter or 4cc volume[23], were excluded given their comparatively favorable prognosis[24, 25]. Study entry required total NIHSS score 3–21 and leg motor score 1–4. 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 end of the second infusion (maximum concentration) was lower herein as compared to subjects receiving the same dose in the prior study[20] in which the second infusion was administered 9±1 days apart (median 494.5 versus 723.0 mcg/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 conformed to published recommendations[17]. Preclinical studies in rodents[10] and primates[11] suggested efficacy. The antibody was found to be safe in 37 healthy subjects, who received a single IV infusion up to 25 mg/kg[12], and in a phase II study of 42 patients 24–72 hours after ischemic stroke, among whom 25 subjects received two IV infusions up to 15 mg/kg[13]; significant benefit over placebo was found over time for gait velocity, an endpoint well aligned with preclinical behavioral endpoints.

Other issues relevant to current results pertain to translation from animals to humans. Behavioral recovery[26, 27] and neural plasticity[28–30] after stroke are accelerated in rodents compared to humans. On this basis, time of first infusion in animals (24 hours post-stroke) 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 post-stroke and peak at 2 weeks post-stroke[2] but it is uncertain whether this is true in humans. White matter constitutes 14% of rodent vs. 50% of human brain volume[31, 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 patients face after stroke such as depression, caregiver support, and financial stressors[33].

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[12]. Neutralizing antibodies were not detected and so did not contribute to current findings.

The experience of translating therapies targeting acute ischemic stroke has provided a number of lessons[34] 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[33] within the context of topics such as endpoint 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[1, 35].

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 to placebo. As above, a number of possible reasons might have contributed to these findings, including using an endpoint with too large a floor effect at baseline, enrolling patients with too severe a level deficit, using too low an antibody dose, inter-species 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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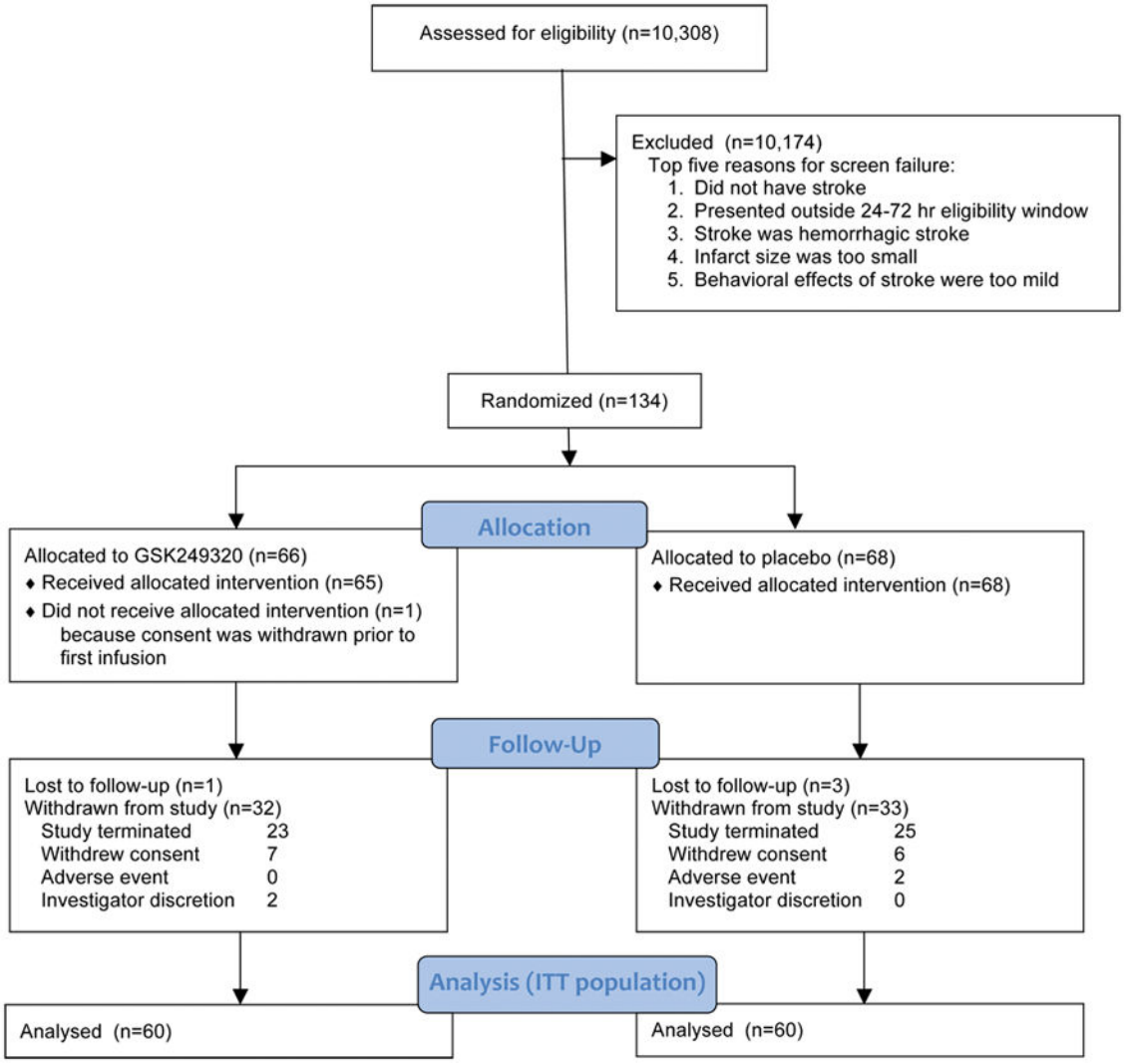

Figure 1.
CONSORT diagram.

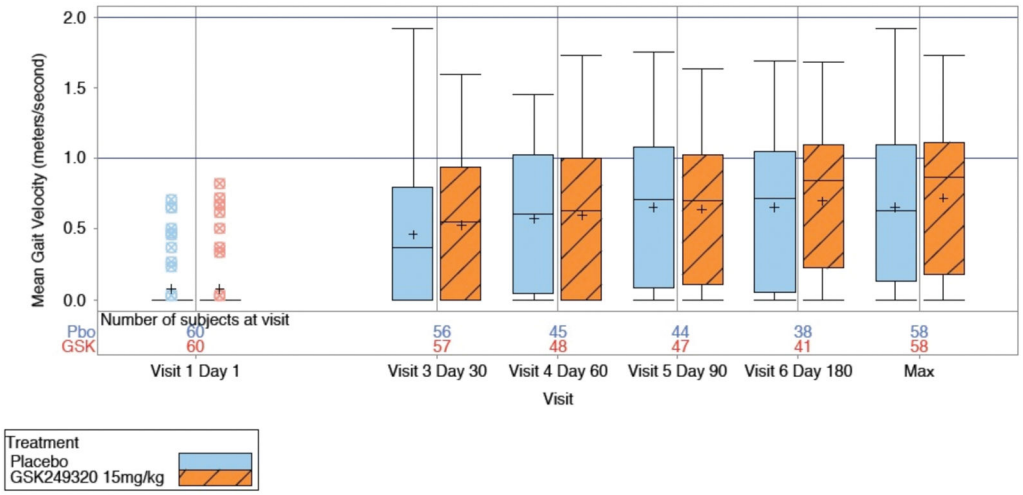

Figure 2.

Box-and-whisker plots of gait velocity change over time and maximum value for the two treatment arms (ITT group).

Table 1

Key entry and exclusion criteria

Entry criteria
Radiologic confirmed supratentorial ischemic stroke; non-lacunar (either >15mm diameter in one direction or >4cc volume)
Stroke onset within 24–72 hours of IP infusion
NIHSS score 3–21
Leg motor deficit: NIHSS Q6 score 1–4
Impaired walking ability: gait velocity≤0.8m/s
Aged 18–90 years
Expectation subject will receive standard physical, occupational and speech rehabilitation therapy as indicated for post-stroke deficits.
Exclusion criteria
Ability to walk>0.8m/s per Gait Velocity assessment.
Symptomatic stroke <3 months before study entry
Significant pre-stroke disability: Rankin score>2 before index stroke
Poorly responsive: NIHSS Q1a score 2 or 3
Significant aphasia
Pre-existing significant gait deficit, chronic liver disease, or prolonged QTc interval
Pre-existing active poorly controlled neurological or psychiatric disease
Expected death due to index stroke or other pre-existing condition
Participation in another investigational study targeting stroke recovery during study
MRI contraindication
Pregnant/lactating

Table 2

Baseline Clinical Measures and Demographics

	Placebo ***(N=68)***	**GSK249320** ***(N=65)***
Age (years) *	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
Ethnicity		
Hispanic/Latino	0	1
Not Hispanic/Latino	68	64
Race		
White	62	62
African-American/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 etiology	2	2
Ischemic stroke undetermined etiology	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, Day 1 *	2.4±1.20	2.1±1.09

	Placebo ***(N=68)***	**GSK249320** ***(N=65)***
NIHSS Q5 Arm Deficit, Day 1 *	2.7±1.29	2.4±1.34
Box & Blocks Score, Day 1 *		
Stroke-affected arm	3.2±7.7	4.2±8.4
Non-stroke arm	25.1±13.3	23.3±12.4
Hours, Stroke Onset-First IP Infusion *	52.7±14.4	52.4±13.3

Values are for Safety population, except Box & Blocks Score Day 1=Per Protocol population.

*mean±SD

Table 3

Therapy Provided to Enrollees for the Duration of Study Participation

	Placebo *(N=52)*	**GSK249320 *(N=52)***
Physical Therapy	1,422 [0–10,003]	1,610 [92–11,285]
Occupational Therapy	771 [0–10,003]	1,312 [0–11,415]
Total Therapy	2,241 [0–20,006]	3,264 [184–22,700]

Results are PP population, median (range), and are minutes of therapy

Table 4

Study outcomes

	Placebo	GSK249320
Change in gait velocity, baseline-Day 90, mean±SD (ITT)	*n=44* 0.56±0.50	*n=47* 0.55±0.46
Change in gait velocity, baseline-Day 180, mean±SD (ITT)	*n=38* 0.56±0.48	*n=41* 0.60±0.44
Change in Box & Blocks score, baseline-Day 90, mean±SD (PP)	*n=41*	*n=40*
Stroke-affected arm	17.1±19.1	14.9±16.5
Non-stroke arm	18.6±15.2	14.6±16.4
Subjects falling to Day 90 (Safety)	15	12
modified Rankin Scale score, Day 90(PP)	*n=46*	*n=45*
0	0	2
1	7	6
2	13	11
3	10	11
4	14	14
5	2	1
NIH Stroke Scale score, Day 90, median (IQR) (PP)	4 (1.25, 8.75)	4 (1,7)

Values provided for the population indicated. Gait velocity is in m/sec. ITT was used for the primary efficacy analysis of the primary endpoint (gait velocity), PP was used for secondary endpoints, and the Safety population was used for data on falls.