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of survival status data was performed out to Day 180/Month 6 regardless of whether the subject was prematurely withdrawn from the study for any reason.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[6,7]. 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[9]. 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

	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)

	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%)

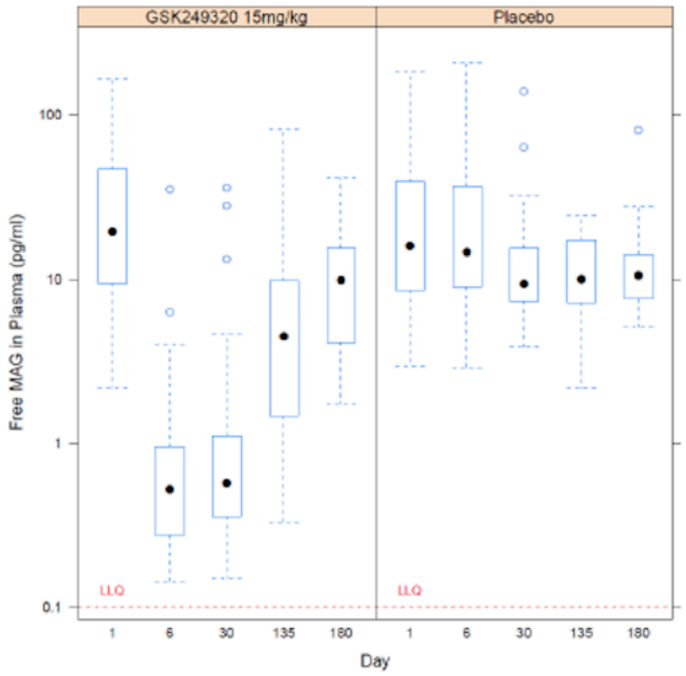
The number of subjects reporting a fall between baseline and end of the study was 18 (26%) in the placebo group and 16 (25%) in the GSK249320 group.

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%.

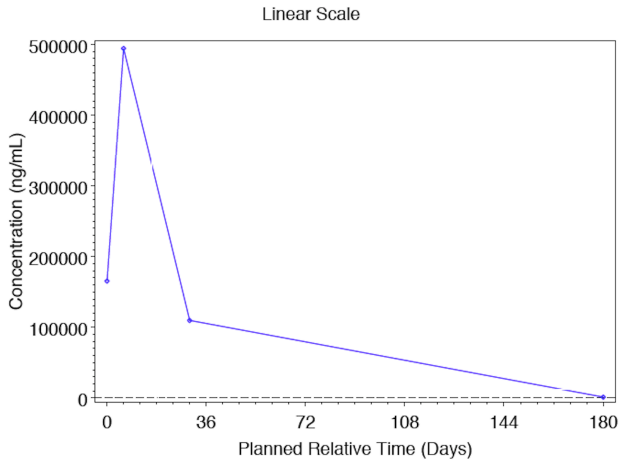
Supplementary Table III. Summary of Events Common to Stroke (Safety Population)

	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

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