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PROTOCOL TITLE: A Multicenter, Double-Blind, Placebo-Controlled, Randomized, Parallel-Group, Dose-Ranging Study to Evaluate the Safety and Efficacy of Intravenous Natalizumab (BG00002) in Acute Ischemic Stroke

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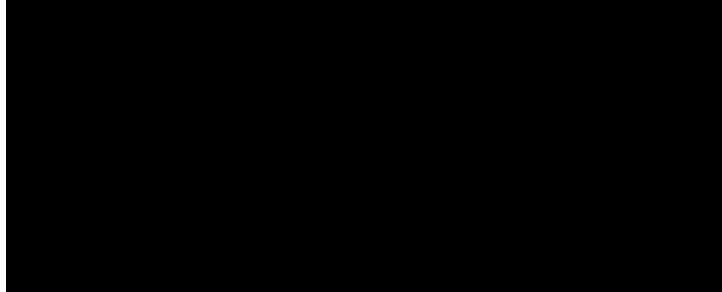
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Protocol 101SK202 was approved by:



19 May 17

Date

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1. SPONSOR INFORMATION

Biogen MA Inc.	Biogen Idec Research Limited
250 Binney Street	Innovation House
Cambridge, MA 02142	70 Norden Road
United States	Maidenhead, Berkshire
	SL6 4AY
	United Kingdom

For urgent medical issues in which the study's Medical Monitor should be contacted, please refer to the Study Reference Manual's Official Study Contact List for complete contact information.

Biogen may transfer any or all of its study-related responsibilities to a contract research organization (CRO) and other third parties; however, Biogen retains overall accountability for these activities.

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2. LIST OF ABBREVIATIONS

AE	adverse event
AUC	area under the serum concentration versus time curve
AUC ₀₋₁₂₀	area under the serum concentration versus time curve from dosing (time=0) to 120 hours after dosing
AUC ₀₋₂₁₆₀	area under the serum concentration versus time curve from dosing (time=0) to 2160 hours after dosing
AUC ₀₋₆₇₂	area under the serum concentration versus time curve from dosing (time=0) to 672 hours after dosing
AUC _{0-last}	area under the serum concentration versus time curve, from dosing (time=0) to last measurable concentration
BDI-2	Beck Depression Inventory 2
BI	Barthel Index
CD	Crohn's disease
CI	confidence interval
C _{max}	maximum serum concentration
CRO	contract research organization
CT	computed tomography
DAMPs	damage associated molecular patterns
DHA	Directions for Handling and Administration
DSMC	Data Safety Monitoring Committee
EC	ethics committee
eCRF	electronic case report form
FIM	Functional Independence Measure
FSS	Fatigue Severity Scale
GCP	Good Clinical Practice
HRU	health resource utilization
ICF	Informed Consent Form
ICH	International Council on Harmonisation
IRB	institutional review board
IRT	interactive response technology
IV	intravenous
IXRS	Interactive Voice/Web Response System
JCV	John Cunningham virus
LKN	last known normal
MoCA	Montreal Cognitive Assessment
MRI	magnetic resonance imaging
mRS	modified Rankin Scale
MS	multiple sclerosis
NIHSS	National Institute of Health Stroke Scale

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OR	odds ratio
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PML	progressive multifocal leukoencephalopathy
RDC	remote data capture
rtPA	recombinant tissue plasminogen activator
SAE	serious adverse event
SAP	statistical analysis plan
SDMT	Symbol-Digits Modalities Test
SE	standard error
SIS-16	Stroke Impact Scale-16
SUSAR	suspected unexpected serious adverse reaction
$t_{1/2}$	half-life
t_{last}	time of last measurable concentration
t_{max}	time to C_{max}
tPA	tissue plasminogen activator
US	United States
VCAM-1	vascular cell adhesion molecule-1
VLA-4	$\alpha 4\beta 1$ integrin, also known as very late antigen-4

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3. SYNOPSIS

Protocol Number:	101SK202
Protocol Title:	A Multicenter, Double-Blind, Placebo-Controlled, Randomized, Parallel-Group, Dose-Ranging Study to Evaluate the Safety and Efficacy of Intravenous Natalizumab (BG00002) in Acute Ischemic Stroke
Version Number	3.0
Name of Study Treatment:	BG00002 (natalizumab; Tysabri)
Study Indication:	Acute Ischemic Stroke
Study Rationale	<p>Stroke is a leading cause of mortality and serious long-term disability. There is a substantial unmet medical need for new therapies that can improve outcomes in acute stroke.</p> <p>Experimental and pathologic data suggest that peri-infarct inflammation contributes to secondary injury after brain ischemia and that blocking inflammation reduces neuronal injury and improves clinical outcomes.</p> <p>Natalizumab is a humanized monoclonal antibody approved for the treatment of both relapsing multiple sclerosis and Crohn's disease that blocks $\alpha 4\beta 1$- or $\alpha 4\beta 7$-integrin-mediated adhesion of leukocytes to vascular endothelial cells and inhibits the transmigration of leukocytes into inflamed parenchymal tissue.</p> <p>In Study 101SK201 (ACTION), while treatment with natalizumab did not reduce magnetic resonance imaging-defined infarct volume, there was evidence that it resulted in clinical benefit in patients with acute ischemic stroke, and no safety concerns were identified.</p> <p>This study (101SK202) will assess dose response and overall safety and efficacy of natalizumab in subjects with acute ischemic stroke. Time dependency of treatment effects will also be assessed through a treatment window of up to 24 hours from last known normal (LKN).</p>
Phase of Development:	2

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Study Objectives and
Endpoints:

The primary objective of the study is to assess the effects of natalizumab versus placebo in acute ischemic stroke on clinical measures of independence and activities of daily life.

The primary efficacy endpoint is a composite global measure of functional disability based on a score of 0 or 1 on the modified Rankin scale (mRS) and a score of ≥ 95 on the Barthel Index (BI) at Day 90 [Tilley 1996].

The secondary objective of the study is to explore dose and exposure response, time dependency of the treatment effect, and the clinical treatment effects of natalizumab versus placebo in acute ischemic stroke on the following measures of independence, activities of daily living, neurologic function, quality of life, and cognition and safety and tolerability.

The secondary endpoints are:

- mRS score at Day 90
- BI score at Day 90
- Stroke Impact Scale-16 score at Day 90
- Montreal Cognitive Assessment score at Day 90
- Safety (incidence and proportion of adverse events and serious adverse events)
- National Institutes of Health Stroke Scale (NIHSS) score at Day 90

The exploratory objective of this study is to evaluate the effect of natalizumab on measures of function, cognition, fatigue, depression, quality of life, and pharmacokinetic/pharmacodynamic relationships over time.

The exploratory endpoints are:

- Functional Independence Measure score at Day 90
- Symbol-Digits Modalities Test score at Day 90
- Fatigue Severity Scale score at Day 90

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- Beck Depression Inventory 2 score
- Serum concentrations of natalizumab at selected times after dosing
- Blood biomarkers of natalizumab
- Subject direct resource use (assessed using a health resource utilization questionnaire)
- EuroQoL EQ-5D-3L (questionnaire)

Study Design: This is a Phase 2 multicenter, double-blind, placebo-controlled, randomized, 3-arm, dose-ranging study.

Study Location: Approximately 67 sites in the United States and Europe are planned.

Number of Planned Subjects: Approximately 270 subjects are expected to be enrolled. Of these, no more than 90 subjects will be treated between >9 and ≤ 24 hours from LKN.

Study Population: This study will be conducted in subjects aged 18 to 80 years with acute ischemic stroke defined by LKN at ≤ 24 hours prior to study treatment initiation.

Detailed criteria are described in Section 8.

Treatment Groups: All subjects will receive 1 dose of study treatment at Screening according to their randomization in a 1:1:1 ratio to 1 of the following 3 treatment groups:

- Natalizumab 600 mg intravenous (IV) in approximately 90 subjects
- Natalizumab 300 mg IV in approximately 90 subjects
- Placebo IV in approximately 90 subjects

Randomization will occur separately within each treatment window. For subjects in the ≤ 9 hour treatment window, randomization will be stratified by baseline NIHSS category (NIHSS scores from 5 to 15 or 16 to 23), tissue plasminogen activator (tPA) use (yes or no), and region; for subjects in the >9 to ≤ 24 hour window, randomization will be stratified

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by tPA use (yes or no) and region.

Duration of Treatment and
Follow-up:

Subjects will participate in this study for approximately
90 days.

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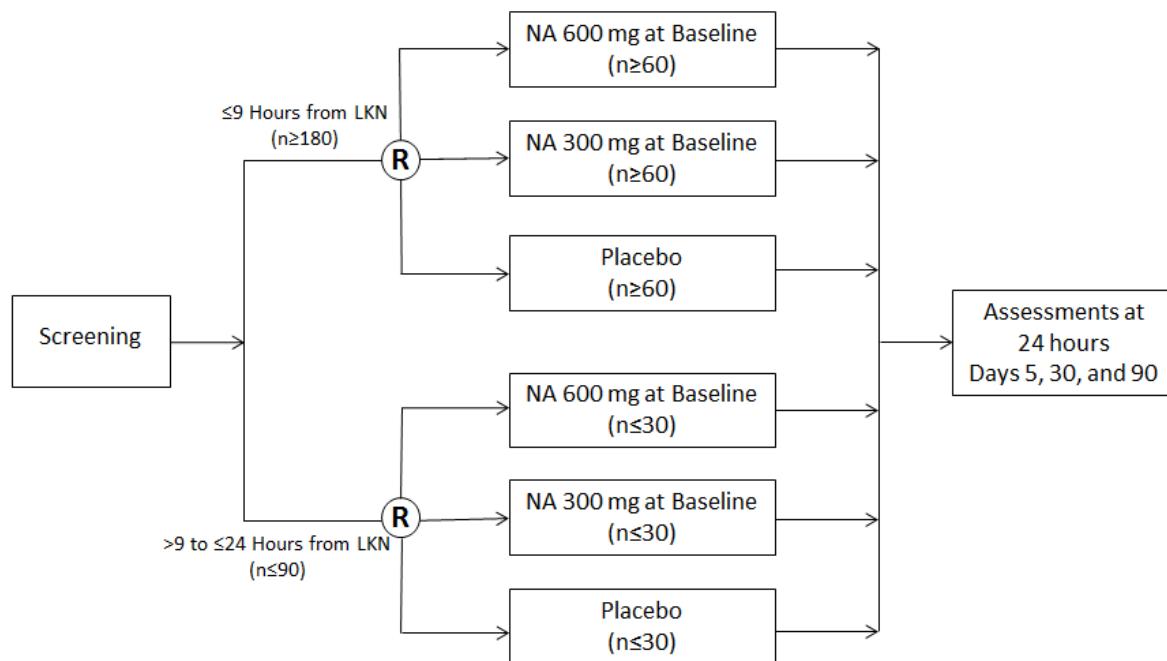
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4. STUDY SCHEMATIC AND SCHEDULE OF ACTIVITIES FOR STUDY 101SK202

4.1. Study Schematic

The study design is displayed in [Figure 1](#).

Figure 1: Study Design



LKN = last known normal; NA = natalizumab; R = randomization.

Note: PK/PD/biomarkers are performed within 1 hour after the end of the infusion.

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4.2. Schedule of Activities

Table 1: Schedule of Activities for Study 101SK202

Tests and Assessments	Screening ¹	0 Hours ² (Day 1)	Within 1 Hour After End of Infusion	12 Hours ±3 Hours	24 Hours ±6 Hours	Day 5 ³	Day 30 ⁴ ±5 Days	Day 90 Follow-up ⁴ / Early Termination ⁴ ±5 Days
Informed consent	X							
Confirm eligibility	X							
Demographics and medical history ⁵	X							
Physical and neurological examination	X				X	X	X	X
Vital signs ⁶	X	X	X		X	X	X	X
Height (if available) and weight				X (anytime from Screening to Day 5 Visit)				
Urine pregnancy test ⁷	X							
Hematology and blood chemistry	X			X	X	X	X	X
Serum biomarkers	X			X	X	X	X	X
PD sampling	X		X	X	X	X	X	X
PK sampling	X		X	X	X	X	X	X
Blood sample for anti-natalizumab antibodies	X						X	X
NIHSS	X	X ²			X	X	X	X
mRS						X ⁸	X	X
BI						X	X	X
SIS-16						X ⁹	X	X
MoCA						X	X	X
Functional Independence Measure							X	X
SDMT						X	X	X
Fatigue Severity Scale						X ⁹	X	X
Beck Depression Inventory 2						X ⁹	X	X
HRU questionnaire						X	X	X
EQ-5D-3L						X	X	X
Study treatment administration ¹⁰		X						
Stroke subtype classification ¹¹						X		
SAE reporting								Record as per Section 15.3.2 of the protocol

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Tests and Assessments	Screening ¹	0 Hours ² (Day 1)	Within 1 Hour After End of Infusion	12 Hours ±3 Hours	24 Hours ±6 Hours	Day 5 ³	Day 30 ⁴ ±5 Days	Day 90 Follow-up ⁴ / Early Termination ⁴ ±5 Days
AE reporting						Record as per Section 15.3.1 of the protocol		
Concomitant medications						Record as per Section 11.5 of the protocol		
Concomitant procedures						Record as per Section 11.5 of the protocol		

AE = adverse event; BI = Barthel Index; BDI-2 = Beck Depression Inventory 2; CT = computed tomography; ECG = electrocardiogram; FIM = Functional Independence Measure; FSS = Fatigue Severity Scale; HRU = health resource utilization; LKN = last known normal; MoCA = Montreal Cognitive Assessment; MRI = magnetic resonance imaging; mRS = modified Rankin Scale; NIHSS = National Institute of Health Stroke Scale; PD = pharmacodynamic; PK = pharmacokinetic; SAE = serious adverse event; SDMT = Symbol-Digits Modalities Test; SIS-16 = Stroke Impact Scale-16.

Note: All timepoints, except “within 1 hour after the end of the infusion”, are relative to start of study treatment administration.

¹ All Screening assessments must be performed prior to infusion. Screening assessments that are performed as standard of care do not need to be repeated for the study, provided that Biogen has access to the information and data are recorded in subject’s case report form. Data collected for the Screening assessments will be used for patient baseline analysis in this study. The ECG and CT or MRI assessments results should already be available at Screening (as they are performed as part of the standard of care), and a copy of the images and report should be filed with the subject’s study records.

² The 0 Hours vital signs assessments are to be performed within 15 minutes prior to study treatment administration. If the Screening NIHSS is performed more than 1 hour prior to study treatment administration, it must be repeated during the visit at 0 Hours before study treatment administration.

³ Day 5 assessments are to occur on Day 5 or earlier if discharged, but must occur prior to discharge.

⁴ The subject will be asked to complete the Day 30 and Day 90 Follow-Up or Early Termination assessments in person (see Section 7.2.3 and Section 10.2). If the subject is unable to return to the study center to complete the Day 30 or Day 90 (Follow-up or Early Termination) assessments in person, safety information will be collected by telephone or remotely, as local regulations allow and pending medical monitor approval, and will include the collection of AEs, SAEs, FIM, mRS, BI, BDI-2, SIS-16, EQ-5D-3L, physical/neurological examination, vital signs, hematology/blood chemistry, serum biomarkers, PD sampling, PK sampling, anti-natalizumab antibodies, NIHSS, MoCA, SDMT, FSS, HRU, and concomitant medications.

⁵ Medical history should include an assessment of prior substance abuse.

⁶ Vital signs collected at each of the specified timepoints include temperature, blood pressure, pulse or heart rate, and respiratory rate. Vital signs collected as part of the subject’s standard of care and that fall within 30 minutes of a study visit do not need to be repeated for the study, provided that Biogen has access to the information and data are recorded in the subject’s case report form.

⁷ Required only for women of childbearing potential. If a serum beta human chorionic gonadotropin test was performed as part of the subject’s standard of care, the results will be accepted in lieu of the urine pregnancy test.

⁸ An mRS assessment will be done on Day 5, or earlier if discharged.

⁹ Per instructions on the SIS-16, BDI-2, and FSS forms, the subject or proxy should provide responses as it applies to the prior 2 week period. When completing the SIS-16, BDI-2, and FSS assessments during the Day 5 Visit, the subject or proxy should consider only the time period starting between the initial stroke (LKN) and the Day 5 Visit.

¹⁰ The subject must be observed for 1 hour after completion of infusion.

¹¹ The stroke etiology will be assessed based on the subject’s standard of care and diagnostic testing. This should be completed at Day 5 or earlier if discharged, but if results are incomplete, it may be completed at Day 30.

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5. INTRODUCTION

Natalizumab is a recombinant humanized monoclonal antibody approved in the United States (US), European Union, and multiple other countries for the treatment of relapsing multiple sclerosis (MS). It is also approved for the treatment of Crohn's disease (CD) in the US.

Natalizumab is produced in a murine myeloma cell line (NS/0) and binds to the $\alpha 4$ subunit of human integrin, which is expressed at high levels on all circulating human leukocytes except polymorphonuclear leukocytes.

Natalizumab binding blocks the interaction of $\alpha 4\beta 1$ integrin (also known as very late antigen-4 [VLA-4]) on leukocytes with its counter receptor, vascular cell adhesion molecule-1 (VCAM-1) and fibronectin, located on endothelial cells. Likewise, natalizumab blocks the interaction of $\alpha 4\beta 7$ integrin expressed on leukocytes with mucosal addressin cell adhesion molecule-1.

Disruption of these cell adhesion molecule interactions prevents trafficking of mononuclear leukocytes across the endothelium and into parenchymal tissue. This is believed to be the basis of its efficacy in MS and CD, and in clinical studies, natalizumab has demonstrated reduction in MS relapse rates by approximately 70%.

Experimental and pathologic data suggest that peri-infarct inflammation in patients with ischemic stroke contributes to secondary injury after brain ischemia, and blocking inflammation reduces the volume of brain infarction and improves clinical outcomes. The Phase 2a Study 101SK201 demonstrated that although natalizumab did not reduce infarct volume, it was associated with improved clinical outcomes, and no safety risks were observed. The observed clinical benefits warranted further investigation.

5.1. Overview of Acute Ischemic Stroke

Stroke occurs when there is an interruption of blood flow to the brain, causing death of neural tissue and focal neurological deficits. The signs and symptoms vary with the location and extent of the stroke. There are nearly 800,000 strokes of all types per year in the US, of which ischemic stroke accounts for approximately 80% [Roger 2011]. In Europe, the estimated annual incidence of stroke is over 1.1 million, with a similar percentage of these (approximately 80%) being ischemic strokes [Heuschmann 2009; Truelson 2006].

Guidelines for the evaluation and treatment of acute stroke patients focus on reperfusion therapies and controlling factors that may exacerbate stroke or complicate the clinical course. The diagnosis of acute ischemic stroke is made through a combination of a history and physical examination that is consistent with focal ischemia and a resulting neurologic deficit. Brain imaging, obtained through either computed tomography (CT) or magnetic resonance imaging (MRI), is used to exclude hemorrhage and other focal pathologies and document early signs of ischemia.

Regardless of the mechanism of ischemia, inflammation is thought to contribute to the progression of brain injury. Inflammation was long considered to be merely a reaction to

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damaged brain tissue, but recent evidence suggests that inflammation is a key part of ischemic injury that stems from both responses in the vasculature and the release of damage associated molecular patterns (DAMPs) from ischemic brain tissue [Shichita 2012].

5.2. Current Therapies for Acute Ischemic Stroke

Recombinant tissue plasminogen activator (rtPA) is the only approved pharmacological therapy for acute ischemic stroke. It is approved for use within 3 hours of stroke onset in the US and within 4.5 hours in many European countries. Current American Heart Association guidelines also suggest use up to 4.5 hours after stroke onset, although treatment effects diminish over time and risk of hemorrhage increases [Jauch 2013]. Because of the narrow time window, it is estimated that only 3% of patients with stroke received rtPA within the treatment window, and there is a clear need for therapies that may be more widely used. In addition to rtPA, intra-arterial thrombectomy has also been shown to improve clinical outcomes in stroke patients [Berkhemer 2015; Goyal 2015; Jovin 2015]. Participation in this study will not impact the ability of subjects to receive current standards of care for acute stroke treatment.

5.3. Profile of Previous Experience With Natalizumab

5.3.1. Nonclinical Experience

See the Investigator's Brochure for detailed information on nonclinical studies.

5.3.2. Clinical Experience

Natalizumab has been used extensively in the setting of relapsing remitting MS and CD, both in clinical studies involving over 5500 subjects, as well as in the postmarketing setting, with more than 9 years of experience and a cumulative exposure of over 200,000 person-years. As of 31 December 2012, approximately 112,200 patients have been treated with natalizumab worldwide. Therefore, the safety of natalizumab, when given as a 300 mg intravenous (IV) dose every 4 weeks, has been well characterized in these specific patient populations.

The efficacy of natalizumab in relapsing MS patients has been established in 3 controlled studies: a Phase 2 dose-comparison study as well as 2 Phase 3 efficacy and safety studies. In both Phase 3 studies, treatment with natalizumab delayed the time to sustained progression in disability, substantially reduced the frequency of relapses, and markedly attenuated brain MRI measures of inflammation and tissue destruction in subjects with relapsing MS. Natalizumab as monotherapy significantly reduced the risk of disability progression by 42% relative to placebo.

The most notable complication of treatment is the risk of progressive multifocal leukoencephalopathy (PML). PML is an opportunistic viral infection of the central nervous system caused by the John Cunningham virus (JC virus). Among the patients who developed PML, natalizumab exposure ranged from 8 to 90 months, with the majority having received >24 months of treatment. Three established risk factors for the development of PML have been identified: presence of anti-JC virus antibodies, longer treatment duration of natalizumab (especially ≥ 2 years), and prior use of immunosuppressant therapy before initiation of natalizumab.

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Other risks of natalizumab treatment include a risk for hypersensitivity reactions, infections, and hepatic injury. Serious hypersensitivity reactions (e.g., anaphylaxis) have occurred at an incidence of <1%.

For Phase 2a experience, see Section 5.4.3.

5.4. Study Rationale

Stroke is a leading cause of mortality and serious long-term disability. There is a substantial unmet medical need for new therapies that can improve the outcome of acute ischemic stroke.

5.4.1. Role of Inflammation in Stroke

The inflammatory response is closely coupled to the initial ischemia and activates both the innate and adaptive immune systems. It begins in the intravascular compartment within the first 6 hours (hyperacute period). The production of reactive oxygen species activates platelets and endothelial cells. Oxidative stress reduces the vasodilatory effects of nitric oxide, which is a potent inhibitor of platelet aggregation and leukocyte adhesion to the vascular wall. Intravascular leukocytes loosely adhere to P-selectin, which is upregulated minutes after the onset of ischemia, and subsequently firmly binds to adhesion molecules, such as intercellular adhesion molecule-1 and VCAM-1. The activation of matrix metalloproteinase and expression of proteases promote blood-brain barrier breakdown, allowing for further influx of leukocytes. After the hyperacute period, DAMPs released from injured neural tissue, including nuclear and cytoplasmic neural proteins, trigger cells of the innate and adaptive immune systems. These endogenous danger signals are sensed through pattern recognition receptors, such as toll-like receptors. This post-ischemic inflammatory reaction has been shown to increase infarct volume and worsen functional outcomes in preclinical stroke models [Gelderblom 2009].

5.4.2. Preclinical Rationale

The key role of mononuclear leukocyte-mediated damage in acute stroke is supported by the fact that depletion of these cells or blocking $\alpha 4$ with the rodent-homologue antibody in experimental models reduces brain infiltration of leukocytes, attenuates expression of cytokines such as interferon- γ and interleukin-6 in brain tissue, reduces stroke size, and improves behavior in the rodent model [Liesz 2011]. In 1 study, the reduction in stroke volume due to blocking $\alpha 4\beta 1$ was greater at Day 7 than Day 1, which is consistent with the delayed damage induced by peri-infarct inflammation [Liesz 2011]. In animal models of experimental brain ischemia, monoclonal antibodies targeting $\alpha 4$ integrin have reduced infarct volume and improved functional outcomes by approximately 30% compared to placebo. This effect has been observed in models of transient ischemia with treatment administered around the time of reperfusion [Becker 2001; Relton 2001]. In a preclinical randomized controlled study involving 6 centers, treatment with anti-CD49 (targeting $\alpha 4$ integrin) reduced leukocyte invasion and infarct volume in a permanent distal middle cerebral artery occlusion model [Llovera 2015].

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5.4.3. Clinical Rationale

Natalizumab has been evaluated in a Phase 2a study assessing patients with acute ischemic stroke. Study 101SK201 explored the efficacy and safety of a single dose of 300 mg natalizumab IV administered at ≤ 6 hours or at >6 to ≤ 9 hours from when the subjects were last known normal (LKN). In this study, 161 subjects (79 subjects in the natalizumab group and 82 subjects in the placebo group) were randomized in the study. The primary efficacy analysis demonstrated that natalizumab did not decrease acute infarct volume growth defined by MRI. However, on prespecified secondary and tertiary clinical endpoints, natalizumab treatment was associated with improved clinical outcomes. More patients had an “excellent” outcome on the modified Rankin Scale (mRS) (defined as a score of 0 or 1) with natalizumab than with placebo at Days 30 (odds ratio [OR] 2.88; 90% confidence interval [CI] 1.20 to 6.93) and 90 (OR 1.48; 90% CI 0.74 to 2.98). More patients had an “excellent” outcome on the Barthel Index (BI; defined as a score of ≥ 95) at Day 90 with natalizumab (OR 1.91; 90% CI 1.07 to 3.41) than placebo. There was no evidence of benefit on the National Institute of Health Stroke Scale (NIHSS). Tertiary clinical endpoints (Stroke Impact Scale-16 [SIS-16], Montreal Cognitive Assessment [MoCA]) also supported improved outcomes in the natalizumab group. In both treatment groups, the incidences of death (18% natalizumab versus 16% placebo) and serious adverse events (SAEs; 46% natalizumab versus 46% placebo) were similar, and no increase in infections or infusion-related reactions were observed in the natalizumab group. Overall, the safety profile was consistent with its use in the postmarketing setting.

5.5. Rationale for the Treatment Window of ≤ 24 Hours From LKN

In experimental rodent models, immune cell infiltration in the brain parenchyma following an ischemic stroke occurs predominantly after 24 hours of lesion onset, reaching its peak at approximately 3 days after the index event [Gelderblom 2009]. Congruently, blockage of maladaptive immune responses through immune modulatory treatments as far as 5 days after ischemia onset has been demonstrated to yield significant beneficial effects in rodent models, suggesting that therapies targeting lymphocyte infiltration after ischemia may have a prolonged therapeutic window [Doyle 2015; Shichita 2009].

A stratified randomization process was adopted in Study 101SK201 to evaluate the potential time dependency of treatment effect when administering 300 mg of natalizumab in a 9-hour window as compared to placebo. In this study, half of the subjects in the intent-to-treat population were assigned to receive study treatment at ≤ 6 hours from LKN, while the other half had study treatment administered within the >6 to ≤ 9 hour window. Results from Study 101SK201 indicated that estimates of treatment effect favored natalizumab against placebo at both treatment windows, with no evidence of time dependency for the treatment benefit. Likewise, the safety profile of natalizumab treatment was comparable in the ≤ 6 hour and the >6 to ≤ 9 hour treatment windows, with a small decrease in the proportion of deaths (21% and 15%, respectively) and SAEs (47% and 45%, respectively) being reported among subjects treated in the later treatment window.

Based on these observations, the therapeutic window for natalizumab therapy appears to be greater than 9 hours. Therefore, in addition to evaluating a ≤ 9 hour treatment window,

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Study 101SK202 will further explore the time dependency of the treatment effect of natalizumab in patients with acute ischemic stroke by enrolling a limited number of subjects within the treatment window of >9 to \leq 24 hours from LKN.

5.6. Rationale for Dosing Regimen

Study 101SK201 indicated that a dose of 300 mg natalizumab was associated with improved clinical outcomes after stroke. Post hoc analyses demonstrated that those subjects with greater area under the serum concentration versus time curve (AUC; but not maximum serum concentration [C_{max}]) following administration of 300 mg) were more likely to have better clinical and MRI defined outcomes compared to placebo treated subjects and subjects with lower AUC following 300 mg. For example, higher natalizumab AUC after the 300 mg IV infusion was associated with lower MRI-defined infarct volume growth at Day 30 ($p = 0.033$), and subjects in the top tertile of natalizumab AUC had the highest OR for excellent outcome on the mRS at Day 90 (OR: 3.02; 95% CI: 0.89 to 10.75) and BI at Day 90 (OR: 3.00; 95% CI: 0.97 to 9.99). Exposure-response modeling predicted that doses in the range of 450 to 600 mg may be associated with improved outcomes relative to the 300-mg dose.

Therefore, Study 101SK202 will explore this relationship by evaluating 300-mg and 600-mg doses administered at 0 Hours in comparison with placebo administered at 0 Hours. Dosing instructions and details regarding administration will be provided in the Directions for Handling and Administration (DHA).

Doses as high as 6 mg/kg of natalizumab have been studied previously in subjects with MS with the highest dose of 697 mg given monthly. The Phase 2b Study AN100226-231 evaluated the safety, tolerability, and efficacy of multiple administrations of natalizumab (3 mg/kg versus 6 mg/kg) given every 28 days for 6 months in subjects with MS. Monthly infusions of natalizumab at doses of 3 and 6 mg/kg were well tolerated and were associated with a safety profile similar to that of placebo. There was no significant difference in the incidence of adverse events (AEs) between treatment groups. The majority of AEs in each treatment group were reported by the Investigator as mild or moderate in severity and not related to study drug.

5.6.1. Rationale for Comparator/Reference Product or Placebo

The use of placebo is justified because subjects will be receiving the standard of care in addition to study treatment.

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6. STUDY OBJECTIVES AND ENDPOINTS

6.1. Primary Objective and Endpoint

The primary objective of the study is to assess the clinical effects of natalizumab versus placebo in acute ischemic stroke on clinical measures of functional independence and activities of daily living.

The primary efficacy endpoint that relates to this objective is a composite global measure of functional disability based on a score of 0 or 1 on the mRS and a score of ≥ 95 on the BI at Day 90 [Tilley 1996].

6.2. Secondary Objectives and Endpoints

The secondary objective of the study is to explore dose and exposure response, time dependency of the treatment effect, and the clinical treatment effects of natalizumab versus placebo in acute ischemic stroke on the following measures of independence, activities of daily living, neurologic function, quality of life, cognition, and safety and tolerability.

The secondary endpoints that relate to this objective are:

- mRS score at Day 90
- BI score at Day 90
- SIS-16 score at Day 90
- MoCA score at Day 90
- Safety (incidence and proportion of AEs and SAEs)
- NIHSS score at Day 90

6.3. Exploratory Objectives and Endpoints

The exploratory objective of this study is to evaluate the effect of natalizumab on measures of function, cognition, fatigue, depression, quality of life, and pharmacokinetic (PK)/pharmacodynamic (PD) relationships over time.

The exploratory endpoints that relate to this objective are:

- Functional Independence Measure (FIM) score at Day 90
- Symbol-Digits Modalities Test (SDMT) score at Day 90

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- Fatigue Severity Scale (FSS) score at Day 90
- Beck Depression Inventory 2 (BDI-2) score
- Serum concentrations of natalizumab at selected times after dosing
- Blood biomarkers of natalizumab
- Subject direct resource use (assessed using a health resource utilization [HRU] questionnaire)
- EuroQoL EQ-5D-3L (questionnaire)

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7. STUDY DESIGN

7.1. Study Overview

See [Figure 1](#) for a schematic of the study design.

This is a Phase 2, multicenter, double-blind, placebo-controlled, randomized, dose-ranging, 3-arm study of natalizumab administered \leq 24 hours from when the subject was LKN in subjects with acute ischemic stroke. This study will evaluate the efficacy and safety of natalizumab over a 90-day period. The study will be conducted at approximately 67 sites in the United States and Europe.

After completing screening assessments and fulfilling the criteria for study entry, eligible subjects in each of 2 treatment windows (\leq 9 hours and >9 to \leq 24 hours from LKN) will be randomized in a 1:1:1 ratio to 1 of 3 treatment groups, each of which will receive a single dose at 0 Hours (Day 1) according to one of the following 3 regimens: 600 mg IV natalizumab, 300 mg IV natalizumab, or placebo IV. Post-treatment assessments will be performed at the following timepoints: within 1 hour after the end of the infusion, 12 ± 3 hours after the start of the infusion, 24 ± 6 hours after the start of the infusion, Day 5, Day 30 (± 5 days), and the Day 90 Follow-Up visit (± 5 days).

Participation in this study will not influence the subject's ability to receive standard of care treatment for stroke, as deemed necessary by the Investigator.

7.2. Overall Study Duration and Follow-Up

The study period will consist of Screening, Randomization, Treatment, and Safety Follow-up periods. Subjects will participate in this study for approximately 90 days.

7.2.1. Screening

Subject eligibility for the study will be determined at the time of acute ischemic stroke diagnosis.

7.2.2. Treatment

Eligible subjects will report to the study site to receive study treatment \leq 24 hours from their LKN, as described in Section [7.1](#). Approximately one-third of the subjects will be treated in each treatment group.

7.2.3. Follow-Up

Subjects are to return to the study site for a follow-up visit on Day 30 and Day 90 (Follow-Up Visit; the final study visit). If the subject is unable to return to the study center to complete a visit in person, the Day 30 or the Day 90 Follow-Up visits can be completed over the telephone or remotely, as local regulations allow and pending medical monitor approval.

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The final study visit will be the Day 90 Follow-Up and will consist of the collection of AEs, SAEs, FIM, mRS, BI, BDI-2, SIS-16, EQ-5D-3L, physical/neurological examination, vital signs, hematology/blood chemistry, serum biomarkers, PD sampling, PK sampling, anti-natalizumab antibodies, NIHSS, MoCA, SDMT, FSS, HRU, and concomitant medication information.

7.3. Study Stopping Rules

Biogen may terminate this study at any time after informing Investigators. Investigators will be notified by Biogen or designee if the study is placed on hold, completed, or closed. There are no prespecified stopping rules. An independent Data Safety Monitoring Committee (DSMC) will be formed and will review safety data regularly. Details of the DSMC responsibilities will be provided in the DSMC charter.

7.4. End of Study

The end of study is last subject, last visit for final collection of data.

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8. SELECTION OF SUBJECTS

8.1. Inclusion Criteria

To be eligible to participate in this study, candidates must meet the following eligibility criteria at Screening or at the timepoint specified in the individual eligibility criterion listed. All eligibility assessments must be completed in time for dosing within ≤ 9 hours or >9 to ≤ 24 hours of the subject's LKN:

1. Ability to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use protected health information in accordance with national and local subject privacy regulations OR consent provided by an independent physician where local regulation allows, and/or provision of informed consent by the subject's representative in accordance with all local and national regulations OR according to the local institutional review board's (IRB's)/ethics committee's (EC's) guidelines OR by another process compliant with applicable national laws and regulations and IRB/EC requirements
2. Aged 18 to 80 years, inclusive, at the time of informed consent
3. Clinical diagnosis of supratentorial acute ischemic stroke defined by LKN ≤ 24 hours prior to study treatment initiation. Note: An acute brain CT or MRI scan must be available from the patient's history to assess eligibility for the study and be consistent with the diagnosis of acute ischemic stroke

Note: The number of subjects enrolling in the treatment window of >9 to ≤ 24 hours from LKN will be limited to no more than 90.

4. Score of 5 to 23 points, inclusive, on the NIHSS at Screening for subjects initiating treatment ≤ 9 hours from LKN. Note: NIHSS eligibility must be confirmed within 60 minutes prior to randomization.
5. Score of 5 to 15 points, inclusive, on the NIHSS at Screening for subjects initiating treatment >9 to ≤ 24 hours from LKN. Note: NIHSS eligibility must be confirmed within 60 minutes prior to randomization.
6. Prior to index stroke, patient was able to perform the following basic activities of daily living without assistance: dressing, eating, walking, bathing, and using the toilet
7. For those subjects who underwent a cranial MRI, there is at least 1 acute infarct with a diameter of ≥ 2 cm on baseline brain diffusion-weighted imaging
8. Subjects of childbearing potential must be willing and able to practice effective contraception during the study

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9. All women of childbearing potential and all men must practice effective contraception during the study and for at least 3 months after their last dose of study treatment. For further details of contraceptive requirements for this study, please refer to Section [15.5](#).

8.2. Exclusion Criteria

Candidates will be excluded from study entry if any of the following exclusion criteria exist at Screening, or at the timepoint specified in the individual criterion listed:

1. Lacunar or isolated brainstem or cerebellar stroke based on clinical assessment and available acute imaging studies performed under the standard of care.
2. Presence of acute intracranial hemorrhage on acute brain CT or MRI. However, petechial hemorrhages of ≤ 1 cm are not exclusionary.
3. Severe stroke defined by imaging criteria based on either one of the following
 - a. ASPECTS score of 0 to 4 based on head CT OR
 - b. Acute infarct volume on MRI diffusion weighted imaging ≥ 70 mL (cc)
4. Seizure at the onset of stroke
5. Hypotension requiring the use of IV vasopressor support or systolic blood pressure <90 mmHg at the time of randomization
6. Known history of prior treatment with natalizumab
7. Immunocompromised subjects as determined by the Investigator, based on medical history, physical examination, or laboratory testing, or due to prior or current immunosuppressive or immunomodulating treatment
8. Known history of positive test result for human immunodeficiency virus (HIV)
9. Known history of active viral hepatitis B or C.
10. Signs of active herpes simplex type 1 and 2 or varicella within 4 weeks prior to randomization
11. Known history of chronic, recurrent, or recent serious infection (e.g., pneumonia, septicemia) as determined by the Investigator within 6 months of randomization
12. Signs and symptoms of active or acute infection
13. Abnormal laboratory values indicative of or known history of significant medical, neurologic (other than stroke), or psychiatric disorders or known history of substance abuse that might preclude safe participation in the study in the opinion of the Investigator

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14. Known history of malignant disease within the last 5 years, including solid tumors and hematological malignancies (with the exception of basal cell and squamous cell carcinomas of the skin that have been completely excised and are considered cured)
15. Inability to comply with study requirements
16. Previous registration in this study
17. Other unspecified reasons that, in the opinion of the Investigator and/or Biogen, make the subject unsuitable for enrollment
18. Nursing or pregnant females or females planning to become pregnant during study participation
19. Known history of participation in any other investigational study that involved treatment with an investigational drug within 6 months prior to enrollment
20. Hypersensitivity reaction to present tissue plasminogen activator treatment

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9. ENROLLMENT, REGISTRATION, AND RANDOMIZATION

9.1. Screening and Enrollment

Subjects (or their legally authorized representative [e.g., parent or legal guardian], where applicable) must provide informed consent before any screening tests are performed (see Section 17.3). When a subject signs the Informed Consent Form (ICF), that subject is considered to be enrolled in the study. Subjects will not be eligible for rescreening.

Participating study sites are required to document all screened candidates initially considered for inclusion in this study. If a subject is excluded from the study, the reasons for exclusion will be documented in the subject's source documents and on the screening log.

9.2. Randomization and Registration of Subjects

Two separate randomization schemes will be used for subjects in the 2 treatment windows (i.e., ≤ 9 hours from LKN and >9 to ≤ 24 hours from LKN). Within each treatment window, subjects will be randomized and registered by an Interactive Voice/Web Response System (IXRS) at Screening, after all Screening assessments have been completed and after the Investigator has verified that the subjects are eligible per criteria in Sections 8.1 and 8.2. No subject may begin treatment prior to randomization and assignment of a unique subject identification number. Any subject identification numbers that are assigned will not be reused even if the subject does not receive treatment.

Subjects in each treatment window will be randomized to receive natalizumab 300 mg, 600 mg, or placebo in a 1:1:1 ratio (see Figure 1). For subjects in the ≤ 9 hour treatment window, randomization will be stratified by baseline NIHSS category (NIHSS score 5 to 15 or 16 to 23), tPA use (yes or no), and region; for subjects in the >9 to ≤ 24 hour treatment window, randomization will be stratified by tPA use (yes or no) and region.

Refer to the Study Reference Manual for details on registration and randomization.

As confirmation, IXRS will provide the Investigator with written verification of the subject's registration by e-mail or fax. Study treatment preparation and administration should commence as soon as confirmation of randomization and assignment of a drug kit number is received.

9.3. Blinding Procedures

This is a randomized, double-blinded, placebo-controlled study.

All study staff will be blinded to the subject treatment assignments. To maintain the study blind, it is imperative that subject treatment assignments are not shared with the subjects, their families, or any member of the study team, either at the study site or at Biogen.

Matching placebo vials will be provided.

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10. DISCONTINUATION OF STUDY TREATMENT AND/OR WITHDRAWAL OF SUBJECTS FROM THE STUDY

10.1. Discontinuation of Study Treatment

Infusion of study treatment must be stopped immediately and permanently discontinued for any of the following reasons:

- The subject is found to be pregnant. Study treatment must be discontinued immediately. Report the pregnancy according to the instructions in Section [15.4.1](#).
- The subject experiences a hypersensitivity or suspected allergic reaction to study treatment
- Subject consent for participation is withdrawn
- The subject experiences a medical emergency that necessitates discontinuation of study treatment
- The subject experiences a medical emergency that necessitates unblinding of the subject's treatment assignment
- At the discretion of the Investigator or the Sponsor (Biogen) for medical reasons

The reason for discontinuation of study treatment must be recorded in the subject's electronic case report form (eCRF). For all subjects who discontinue study treatment, safety data will be collected at the Day 90 Follow-Up visit.

10.2. Withdrawal of Subjects From Study

Subjects must be withdrawn from the study for any of the following reasons:

- Subject consent for participation is withdrawn.
- The subject enrolls into another interventional clinical study in which an investigational treatment or approved therapy for investigational use is administered.
- The subject is unwilling or unable to comply with the protocol.

The reason for the subject's withdrawal from the study must be recorded in the subject's eCRF. The subject will be asked to complete early termination assessments in person. If the subject cannot complete early termination assessments in person, a follow-up phone call or remote visit will be made to all subjects who withdraw from the study for purposes of collecting information on AEs/SAEs, FIM, mRS, BI, BDI-2, SIS-16, EQ-5D-3L, physical/neurological examination,

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vital signs, hematology/blood chemistry, serum biomarkers, PD sampling, PK sampling, anti-natalizumab antibodies, NIHSS, MoCA, SDMT, FSS, HRU, and concomitant therapy.

For all subjects who withdrew from the study, safety data will be collected at the Day 90 Follow-Up or Early Termination visit.

Subjects who withdraw from the study will not be replaced.

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11. STUDY TREATMENT USE

11.1. Regimen

Refer to and follow the DHA.

11.2. Modification of Dose and/or Treatment Schedule

The dose should be followed as described in Section [7.1](#). No modifications of dose are allowed.

11.3. Precautions

Subjects will be observed for 1 hour after the study treatment infusion is complete to allow monitoring for hypersensitivity reactions.

11.4. Compliance

Compliance with treatment dosing is to be monitored and recorded by site staff.

11.5. Concomitant Therapy and Procedures

11.5.1. Concomitant Therapy

A concomitant therapy is any drug or substance administered between time of informed consent or authorization to enroll and completion of assessments at the Day 90 Follow-Up visit (as required by the protocol). All thrombolytic treatments (i.e., IV and intra-arterial rtPA) and mechanical thrombectomy that a subject receives are to be recorded as concomitant therapy regardless of whether they are given before or after the time of informed consent.

11.5.1.1. Allowed Concomitant Therapy

Concomitant therapy with any of the following is allowed as long as the exclusion criteria described in Section [8.2](#) are observed:

- Medications necessary for treatment of AEs according to the discretion of the Investigator.
- Medications used in standard of care to treat stroke, cardiovascular risk factors, or other comorbid conditions, except for disallowed concomitant therapy.
- Corticosteroids that are administered by non-systemic routes (e.g., topical or inhaled). Acute use of systemic corticosteroids is allowed in this study.

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11.5.1.2. Disallowed Concomitant Therapy

Concomitant therapy with any investigational product is not allowed.

11.5.2. Concomitant Procedures

A concomitant procedure is any therapeutic intervention (e.g., surgery/biopsy, physical therapy) or diagnostic assessment (e.g., blood gas measurement, bacterial cultures) performed between the time the subject is enrolled in the study and Safety Follow-Up. All thrombolytic treatments and mechanical thrombectomy that a subject receives are to be recorded as concomitant procedures regardless of whether they are given before or after the time of informed consent.

The use of concomitant therapies or procedures defined above must be recorded on the subject's eCRF, according to instructions for eCRF completion. All AEs following the administration of these therapies or procedures must be documented on the appropriate eCRF, regardless of relatedness.

11.6. Continuation of Treatment

No further provisions are made for access to the study treatment. If natalizumab is proven to be beneficial, all regulatory requirements regarding post-study access will be met.

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12. STUDY TREATMENT MANAGEMENT

Study site staff should follow the DHA for specific instructions on the handling, preparation, administration, and disposal of the study treatment. The DHA supersedes all other references (e.g., protocol).

Study treatment must be dispensed only by a pharmacist or appropriately qualified staff. Study treatment is to be dispensed only to subjects enrolled in this study. Once study treatment is prepared for a subject, it can be administered only to that subject. Study treatment vials are for one-time use only; do not use any study treatment remaining in the vial for another subject.

12.1. Natalizumab

Natalizumab is supplied as a liquid in 15-mL vials containing 300 mg of natalizumab per vial. Natalizumab drug product contains recombinant humanized anti- α 4 integrin antibody and excipient materials (sodium phosphate monobasic monohydrate, sodium phosphate dibasic heptahydrate, sodium chloride, and polysorbate 80).

Natalizumab is manufactured by Biogen.

The contents of the natalizumab label will be in accordance with all applicable regulatory requirements. At a minimum, the label will include a study reference code, study treatment identifier, quantity of dosage units, lot number, and other pertinent information in accordance with local law. The expiry or use-by date is stored in the interactive response technology (IRT) system, and printable assignment reports are available to site personnel. Study treatment should not be used after the expiration, expiry, or use-by date.

12.1.1. Natalizumab Preparation

The individual preparing natalizumab should carefully review the instructions provided in the DHA.

If the packaging is damaged, or if there is anything unusual about the appearance or attributes of the vials or study treatment, do not use the study treatment. The vials in question should be saved at the study site and the problem immediately reported to Biogen.

12.1.2. Natalizumab Storage

Study treatment must be stored in a secure location.

Natalizumab is to be stored at 2°C to 8°C (36°F to 46°F), protected from light in a monitored and locked refrigerator with limited access. Study treatment is not to be frozen. For the most up-to-date storage requirements, follow the instructions provided in the DHA.

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12.1.3. Natalizumab Handling and Disposal

The Investigator must return all used and unused vials of natalizumab as instructed by Biogen, unless approved for onsite destruction.

If any study treatment supplies are to be destroyed at the study site, the institution or appropriate site personnel must obtain prior approval from Biogen by providing, in writing, the destruction policy or details of the method of destruction. After such destruction, Biogen must be notified, in writing, of the details of the study treatment destroyed (e.g., lot or kit numbers, quantities), the date of destruction, and proof of destruction.

12.1.4. Natalizumab Accountability

Accountability for study treatment is the responsibility of the Investigator. The study site must maintain accurate records demonstrating dates and amount of study treatment received, to whom study treatment was dispensed (subject-by-subject accounting), and accounts of any study treatment accidentally or deliberately destroyed or lost.

Unless otherwise notified, all vials both used and unused must be saved for study treatment accountability. At the end of the study, reconciliation must be made between the amount of study treatment supplied, dispensed, and subsequently destroyed, lost, or returned to Biogen. A written explanation must be provided for any discrepancies.

12.2. Placebo

Placebo is supplied as a liquid in 15-mL vials containing sodium phosphate monobasic monohydrate, sodium phosphate dibasic heptahydrate, sodium chloride, and polysorbate 80.

Placebo is manufactured by Biogen.

The label will include conditions for storage and other pertinent information required by local law, such as the lot/kit number and caution statement. Placebo should not be used after the expiration date.

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13. EFFICACY, PHARMACOKINETIC, AND PHARMACODYNAMIC ASSESSMENTS

See Section 4 for the timing of all assessments.

13.1. Clinical Efficacy Assessments

The following clinical tests/assessments will be performed to assess the efficacy of natalizumab. Designation of eligibility criteria for performing the assessments will be provided in the Study Reference Manual.

mRS: The mRS measures independence, rather than neurological function, with specific tasks pre- and poststroke [Bonita and Beaglehole 1988; Rankin 1957; Rankin 2003; van Swieten 1988]. The scale consists of 7 grades, from 0 to 6, with 0 corresponding to no symptoms and 6 corresponding to death. Raters will be required to be certified in the use of the mRS. The mRS will be completed by a telephone interview for any subjects who fail to attend their Day 90 Follow-Up or Early Termination visit. The mRS takes less than 5 minutes to administer.

BI: The BI consists of 10 items that measure a person's daily functioning, specifically the activities of daily living and mobility [Collin 1988; Mahoney and Barthel 1965; Wade and Collin 1988]. The items include feeding, moving from wheelchair to bed and returning, grooming, transferring to and from a toilet, bathing, walking on a level surface, going up and down stairs, dressing, and maintaining continence of bowels and bladder. The assessment can be used to determine a baseline level of functioning and can be used to monitor change in activities of daily living over time. The items are weighted according to a scheme developed by the authors. The person receives a score based on whether they have received help while doing the task. The scores for each of the items are summed to create a total score up to a maximum of 100. The higher the score, the more "independent" the person is. The BI takes less than 5 minutes to administer.

NIHSS: The NIHSS is a reliable tool for rapidly evaluating the effects of acute cerebral infarction [Lyden 1999]. Raters will be required to be certified in the use of the NIHSS. A trained observer rates the subject's ability to answer questions and perform activities relating to level of consciousness, language, visual-field loss, extraocular movement, motor strength, ataxia, dysarthria, sensory loss, and extinction and inattention (formerly neglect). There are 15 items. Ratings for each item are scored with 3 to 5 grades, with 0 as normal and a maximum possible total severity score of 42 for all items. There is an allowance for untestable items. The test takes approximately 10 minutes to complete.

SIS-16: The SIS-16 is a 16-item physical dimension instrument that was developed as a brief, stand-alone tool for measuring the physical aspects of stroke recovery [Duncan 2003]. The 16 physical aspects are rated on a 1 to 5 scale as follows: not difficult at all (5), a little difficult (4), somewhat difficult (3), very difficult (2), and could not do at all (1). The SIS-16

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takes 5 to 10 minutes to complete and is also available in a proxy version that can be used when patients are unable to answer themselves.

MoCA: The MoCA is a global cognitive screening test with favorable psychometric properties [Cumming 2013; Pasi 2013]; it has been shown to be more sensitive to executive impairment than the Mini-Mental State Examination. It screens 8 domains: visuospatial/executive, naming, memory, attention, language, abstraction, delayed recall, and orientation. The assessment takes approximately 10 minutes. The highest possible total score is 30 points, and the assessment is available in 31 languages.

FIM: The FIM instrument is a widely used functional performance measure developed specifically for the inpatient acute rehabilitation population. It has been recommended by the Agency for Health Care Policy and Research Post-Stroke Rehabilitation panel as a measure of activities of daily living after stroke. It is an 18-item instrument graded on a 7-point ordinal scale, with a maximum total score of 126. The 7-point ordinal scale indicates the burden of care associated with each aspect of function. The assessment is verbally administered by a certified rater to the patient or proxy and takes approximately 30 to 60 minutes to complete.

SDMT: The SDMT measures Informational Processing Speed in the visual modality. This is a 90-second timed test, patient respondent only. Patients are presented with a key that includes 9 numbers, each paired with a different symbol. Patients must then provide the correct numbers that accompany the symbols. Both written and oral response versions are used and the SDMT takes only a few minutes to administer (including task instructions). When used with the MoCA, SDMT has been found to improve accuracy in detecting post-stroke vascular cognitive impairment [Dong 2014]. Rater certification is required.

FSS: The FSS is a 9-item scale which measures the severity of fatigue and its effect on a person's activities and lifestyle in patients with a variety of disorders. The scale is self-reported or verbally administered and takes approximately 3 to 5 minutes to complete. The items are scored on a 7 point scale with 1 = strongly disagree and 7= strongly agree. The minimum total score is 9, and the maximum score possible is 63, with the higher the score indicating greater fatigue severity.

BDI-2: The BDI-2 quantifies the severity of depression that has been evaluated in the setting of stroke. It is a self-report inventory and can be self-administered or verbally administered. This instrument rates items on a 4 point scale that ranges from 0 to 3. Ratings are summed to provide a total score ranging from 0 to 63.

Subject direct resource use (HRU questionnaire): The HRU questionnaire quantifies the amount of time patients spent in various settings of care (e.g., intensive care unit, general medical ward, skilled nursing facility, long-term care, Home with Support, Home without Support, etc), the 30-day readmission rate, and rehabilitation utilization.

EQ-5D-3L: EQ-5D-3L is an instrument that evaluates the generic quality of life; it was developed in Europe and is widely used. The EQ-5D-3L descriptive system is a preference-based health-related quality of life measure with 1 question for each of the following 5

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dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. This scale can be self-reported, proxy-respondent, or verbally administered and takes approximately 10 minutes to complete.

13.2. Pharmacokinetic Assessments

The following tests will be performed to assess the PK of natalizumab:

- Serum concentrations of natalizumab at selected times after dosing

13.3. Pharmacodynamic Assessments

The following tests may be performed to assess the PD properties of natalizumab:

- Blood biomarkers of natalizumab target engagement
- Serum cytokines and other inflammatory markers of stroke

Serum samples collected during the study may be used for other potential or exploratory biomarkers related to efficacy or safety related outcomes and/or natalizumab-VLA-4 pharmacology.

The purpose of serum sample collection is to develop potential predictive biomarkers throughout the study that may predict response to treatment or understand disease course and to develop potential PD markers through characterization of changes in blood cell transcription induced by the study treatment or disease course. A combination of linear modeling and machine learning approaches may be used to analyze the data.

Blood samples may be stored for up to 15 years after study completion for analyses.

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14. SAFETY ASSESSMENTS

Refer to Section 4 for the timing of all safety assessments.

14.1. Clinical Safety Assessments

The following clinical assessments will be performed to evaluate the safety profile of natalizumab:

- Medical history
- Physical examinations and neurological assessments
- Vital sign measurements: temperature, pulse rate, systolic and diastolic blood pressure, and respiratory rate
- Height and weight measurements
- Concomitant therapy and procedure recording
- Monitoring of AEs and SAEs

14.2. Laboratory Safety Assessments

The following laboratory tests will be performed to evaluate the safety profile of natalizumab:

- Hematology: Complete blood count with differential and platelet count and absolute neutrophil count
- Blood chemistry: total protein, albumin, creatinine, blood urea nitrogen, uric acid, bilirubin (total and direct), alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl-transferase, glucose, calcium, phosphorus, bicarbonate, chloride, sodium, and potassium
- Anti-natalizumab antibodies

Duplicate samples for laboratory assessments will be collected as a backup in case the original sample is lost or not evaluable. All samples listed above will be sent to central laboratories. Standard of Care samples will be analyzed locally.

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15. SAFETY DEFINITIONS, RECORDING, REPORTING, AND RESPONSIBILITIES

Throughout the course of the study, every effort must be made to remain alert to possible AEs. If an AE occurs, the first concern should be for the safety of the subject. If necessary, appropriate medical intervention should be provided.

At the signing of the ICF, each subject or his/her legally authorized representative and/or main caregiver must be given the names and telephone numbers of study site staff for reporting AEs and medical emergencies.

15.1. Definitions

15.1.1. Serious Pretreatment Event

A serious pretreatment event is any event that meets the criteria for SAE reporting (as defined in Section 15.1.3) and occurs after the subject or the subject's representative signs the ICF or after enrollment is authorized by the site or by another process compliant with applicable national laws and regulations and IRB/EC requirements, but before administration of study treatment.

15.1.2. Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Determination of whether an abnormal laboratory value meets the definition of an AE will be made by the Investigator. Although abnormal laboratory values are typically not considered AEs, the following considerations may result in an abnormal laboratory value being considered an AE:

- A laboratory test result that meets the criteria for an SAE
- A laboratory test result that requires the subject to receive specific corrective therapy
- A laboratory abnormality that the Investigator considers to be clinically significant

15.1.3. Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death

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- In the view of the Investigator, places the subject at immediate risk of death (a life-threatening event); however, this does not include an event that, had it occurred in a more severe form, might have caused death
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect

An SAE may also be any other medically important event that, in the opinion of the Investigator, may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. (Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or convulsions occurring at home that do not require an inpatient hospitalization.)

15.1.4. Prescheduled or Elective Procedures or Routinely Scheduled Treatments

A prescheduled or elective procedure or a routinely scheduled treatment will not be considered an SAE, even if the subject is hospitalized. The study site must document all of the following:

- The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the subject's consent to participate in the study.
- The condition requiring the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the subject's consent to participate in the study and the time of the procedure or treatment.
- The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission.
 - If a subject is hospitalized due to local requirements for administration of the study treatment, the hospitalization should not be considered an SAE unless one of the requirements in Section [15.1.3](#) is met.

15.2. Safety Classifications

15.2.1. Investigator Assessment of Events

All events must be assessed to determine the following:

- If the event meets the criteria for an SAE as defined in Section [15.1.3](#).
- The relationship of the event to study treatment as defined in Section [15.2.2](#).

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- The severity of the event as defined in Section 15.2.3.

15.2.2. Relationship of Events to Study Treatment

The following definitions should be considered when evaluating the relationship of AEs and SAEs to the study treatment.

Relationship of Event to Study Treatment	
Not related	An AE will be considered “not related” to the use of the investigational drug if there is not a reasonable possibility that the event has been caused by the product under investigation. Factors pointing toward this assessment include but are not limited to: the lack of reasonable temporal relationship between administration of the drug and the event, the presence of a biologically implausible relationship between the product and the AE, or the presence of a more likely alternative explanation for the AE.
Related	An AE will be considered “related” to the use of the investigational drug if there is a reasonable possibility that the event may have been caused by the product under investigation. Factors that point toward this assessment include but are not limited to: a positive rechallenge, a reasonable temporal sequence between administration of the drug and the event, a known response pattern of the suspected drug, improvement following discontinuation or dose reduction, a biologically plausible relationship between the drug and the AE, or a lack of an alternative explanation for the AE.

15.2.3. Severity of Events

The following definitions should be considered when evaluating the severity of AEs and SAEs:

Severity of Event	
Mild	Symptoms barely noticeable to subject or does not make subject uncomfortable; does not influence performance or functioning; prescription drug not ordinarily needed for relief of symptoms but may be given because of personality of subject.
Moderate	Symptoms of a sufficient severity to make subject uncomfortable; performance of daily activity is influenced; subject is able to continue in study; treatment for symptoms may be needed.
Severe	Symptoms cause severe discomfort; symptoms cause incapacitation or significant impact on subject’s daily life; severity may cause cessation of treatment with study treatment; treatment for symptoms may be given and/or subject hospitalized.

15.2.4. Expectedness of Events

Expectedness of all AEs will be determined by Biogen according to the Investigator’s Brochure.

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15.3. Monitoring and Recording Events

15.3.1. Adverse Events

Any AE experienced by the subject between the time of first dose of study treatment (initiation of infusion [0 hour]) and the subject's last study visit is to be recorded on the eCRF, regardless of the severity of the event or its relationship to study treatment.

15.3.2. Serious Adverse Events

Any SAE experienced by the subject between the time of first dose of study treatment (initiation of infusion [0 hour]) and the subject's last study visit is to be recorded on an SAE form, regardless of the severity of the event or its relationship to study treatment. SAEs must be reported to [REDACTED] within 24 hours as described in Section 15.3.3. Follow-up information regarding an SAE also must be reported with 24 hours.

Subjects will be followed for all SAEs until the last study visit. Thereafter, the event should be reported to [REDACTED] only if the Investigator considers the SAE to be related to study treatment.

Any SAE that is ongoing when the subject completes or discontinues the study will be followed by the Investigator until the event has resolved, stabilized, or returned to baseline status. If, after the subject's last study visit, an SAE occurs in a subject, then the Investigator should report this event to the Sponsor as the Investigator becomes aware of each event.

15.3.3. Immediate Reporting of Serious Adverse Events

In order to adhere to all applicable laws and regulations for reporting an SAE, the study site must formally notify [REDACTED] within 24 hours of the study site staff becoming aware of the SAE. It is the Investigator's responsibility to ensure that the SAE reporting information and procedures are used and followed appropriately.

Reporting Information for SAEs

Any SAE that occurs between the time that the subject has signed the ICF and the subject's last visit must be reported to [REDACTED] within 24 hours of the study site staff becoming aware of the event. Thereafter, the event should be reported only if the Investigator considers it related to study treatment.

A report **must be submitted** to [REDACTED] regardless of the following:

- Whether or not the subject has undergone study-related procedures
- Whether or not the subject has received study treatment
- The severity of the event
- The relationship of the event to study treatment

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To report initial or follow-up information on an SAE, fax a completed SAE form; refer to the Study Reference Manual for complete contact information.

15.3.3.1. Deaths

Death is an outcome of an event. The event that resulted in death should be recorded on the appropriate eCRF. All causes of death must be reported as SAEs within 24 hours of the site becoming aware of the event. The Investigator should make every effort to obtain and send death certificates and autopsy reports to [REDACTED]. The term death should be reported as an SAE only if the cause of death is not known and cannot be determined.

15.3.4. Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are SAEs that are unexpected and judged by the Investigator or Biogen to be related to the study treatment administered.

Appropriate personnel at Biogen will unblind SUSARs for the purpose of regulatory reporting. Biogen will submit SUSARs (in blinded or unblinded fashion) to regulatory agencies according to local law. Biogen will submit SUSARs to Investigators in a blinded fashion.

15.4. Procedures for Handling Special Situations

15.4.1. Pregnancy

Subjects should not become pregnant during the study and up to 3 months after their last dose of study treatment. If a female subject becomes pregnant, study treatment must be discontinued *immediately*.

The Investigator must report a pregnancy by faxing the appropriate form to [REDACTED] within 24 hours of the study site staff becoming aware of the pregnancy at the fax number found in the Study Reference Manual. The Investigator or study site staff must report the outcome of the pregnancy to [REDACTED].

Congenital abnormalities and birth defects in the offspring of male or female subjects should be reported as an SAE if conception occurred during the study treatment period.

15.4.2. Overdose

An overdose is any dose of study treatment administered to a subject or taken by a subject that exceeds the dose assigned to the subject according to the protocol. Overdoses are not considered AEs and should not be recorded as an AE on the eCRF; however, all overdoses must be recorded on an Overdose form and faxed to [REDACTED] within 24 hours of the site becoming aware of the overdose (see Study Reference Manual for fax number). An overdose must be reported to [REDACTED] even if the overdose does not result in an AE. If an overdose results in an AE, the AE must be recorded on the eCRF. If an overdose results in an SAE, both the SAE and Overdose

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forms must be completed and faxed to [REDACTED]. All study treatment-related dosing information must be recorded on the dosing eCRF.

15.4.3. Medical Emergency

In a medical emergency requiring immediate attention, study site staff will apply appropriate medical intervention, according to current standards of care. The Investigator (or designee) should contact the study's Medical Monitor. Refer to the Study Reference Manual's Official Study Contact List for complete contact information.

15.4.3.1. Unblinding for Medical Emergency

In a medical emergency when knowledge of the subject's treatment assignment may influence the subject's clinical care, the Investigator and, if applicable, designated personnel at Biogen may access the subject's treatment assignment by IRT. The Investigator must document the reasons for unblinding in the subject's source documents. The Investigator is strongly advised not to divulge the subject's treatment assignment to any individual not directly involved in managing the medical emergency, nor to personnel involved with the analysis and conduct of the study. The Investigator can contact Biogen or designee to discuss such situations.

15.5. Contraception Requirements

All women of childbearing potential must practice effective contraception during the study and for at least 3 months after their last dose of study treatment.

For the purposes of this study, women who do not meet one of the following criteria listed below are considered to be physiologically capable of becoming pregnant and are, therefore, defined as women of childbearing potential:

- Postmenopausal
 - 12 months of natural (spontaneous) amenorrhea without an alternative medical cause
 - 6 weeks after surgical bilateral oophorectomy with or without hysterectomy
- Posthysterectomy
- Female surgical sterilization (e.g., bilateral tubal ligation)

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For the purposes of the study, highly effective contraception is defined as contraception that achieves a failure rate of less than 1% when used consistently and correctly, and includes the following:

For females:

- Established use of oral, intravaginal, or transdermal combined (estrogen and progestogen containing) hormonal methods of contraception associated with the inhibition of ovulation.
- Established use of oral, injected, or implanted progestogen-only hormonal methods of contraception associated with the inhibition of ovulation.
- Placement of an intrauterine device or intrauterine hormone-releasing system.
- Bilateral tubal occlusion.
- For female subjects participating in the study, male sexual partners must have undergone surgical sterilization with the appropriate postvasectomy documentation of the absence of sperm in the ejaculate.

For males:

- Effective male contraception includes a vasectomy with negative semen analysis at follow-up.

True abstinence, when this is consistent with the preferred and usual lifestyle of the subject, can be considered a highly effective method of contraception based on the evaluation of the Investigator who should also take into consideration the duration of the clinical study. Periodic abstinence (e.g., calendar, ovulation, symptothermal, postovulation methods) and withdrawal are not considered acceptable methods of contraception.

Pregnancy reporting is described in Section 15.4.1.

15.6. Safety Responsibilities

15.6.1. The Investigator

The Investigator's responsibilities include the following:

- Monitor and record all AEs, including SAEs, regardless of the severity or relationship to study treatment.
- Determine the seriousness, relationship, and severity of each event.
- Determine the onset and resolution dates of each event.

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- Monitor and record all pregnancies and follow up on the outcome of the pregnancy in female subjects.
- Complete an SAE form for each SAE and fax it to [REDACTED] within 24 hours of the study site staff becoming aware of the event.
- Pursue SAE follow-up information actively and persistently. Follow-up information must be reported to [REDACTED] within 24 hours of the study site staff becoming aware of new information.
- Ensure all AE and SAE reports are supported by documentation in the subjects' medical records.
- Pursue AE follow-up information, if possible, until the event has resolved or become stable.
- Report SAEs to local IRB/ECs, as required by local law.

15.6.2. Biogen

Biogen's responsibilities include the following:

- Before study site activation and subject enrollment, the Clinical Monitor is responsible for reviewing with study site staff the definitions of AE and SAE, as well as the instructions for monitoring, recording, and reporting AEs and SAEs.
- Biogen is to notify all appropriate regulatory authorities, IRBs, central ECs, and Investigators of SAEs, as required by local law, within required time frames.
- Safety data will be provided to the DSMC for review of all AEs and key laboratory tests for all subjects prior to completion of Study 101SK202.

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16. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The objectives of the study and the endpoints to be analyzed are listed in Section [6](#).

16.1. Efficacy

16.1.1. Analysis Population

The efficacy analysis will include subjects who are randomized and have received the entire infusion of study treatment.

16.1.2. Methods of Analysis

Unless otherwise specified, continuous variables will be summarized using summary statistics (mean, standard deviation, median, minimum, and maximum) by treatment group, and categorical variables will be presented using frequency distributions by timepoint and treatment group. Point estimates and 95% CIs will be provided where applicable.

Subgroup analysis by key factors (including baseline NIHSS category and tPA use) will be performed for selected efficacy endpoints.

16.1.2.1. Analysis of the Primary Endpoint

The primary endpoint is a global composite of an excellent outcome on the mRS (score of 0 or 1) and an excellent outcome on the BI (score of at least 95) at Day 90. The primary analysis will be based on a Generalized Estimating Equation model and logit link function assuming a common OR of natalizumab versus placebo across 2 components of the endpoint: the proportion of subjects with an excellent outcome on the mRS and the proportion of subjects with an excellent outcome on the BI. The model will include covariates of treatment window (≤ 9 hours from LKN vs. >9 to ≤ 24 hours from LKN), baseline NIHSS category, and tPA use. The interaction between treatment and treatment window will be assessed to determine whether the data of the 2 treatment windows can be pooled. If not, then data from the >9 to ≤ 24 hour window will be excluded from the analysis population of the primary endpoint and will be analyzed separately. The global OR of natalizumab (2 doses combined) and CIs will be provided.

Sensitivity analyses will be performed to evaluate the impact of missing data, and the missing data handling will be specified in the statistical analysis plan (SAP).

Similar analyses for other timepoints, key subgroups, and each dose versus placebo will be performed. The details will be provided in the SAP.

16.1.2.2. Analysis of the Secondary Endpoints

The same analysis population for the primary endpoint as described in Section [16.1.2.1](#) will apply to the secondary endpoints. The same covariates used in the final modeling of the primary

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endpoint will be adopted in all statistical models for the secondary endpoints described in this section.

16.1.2.2.1. mRS and BI at Day 90

The distribution of mRS at Day 90 will be analyzed by Van Elteren's test. The proportion of subjects who have excellent outcome in mRS (score of 0 or 1) at Day 90 will be analyzed by logistic regression.

The proportion of subjects with excellent outcome on mRS at other timepoints (Day 30, Day 5) will be analyzed similarly. The mRS at each visit will also be analyzed using a mixed-effects model for repeated measures with treatment and treatment by visit interaction as explanatory variables.

The proportion of subjects who have an excellent outcome on the BI (score of at least 95) at Day 90 will be analyzed by logistic regression.

The proportion of subjects with an excellent outcome on the BI at other timepoints (Day 30, Day 5) will be analyzed similarly. The BI at each visit will also be analyzed using a mixed-effects model for repeated measures with treatment and treatment-by-visit interaction as explanatory variables.

16.1.2.2.2. SIS-16 and MoCA at Day 90

Summary statistics of SIS-16 and MoCA at each visit will be presented by treatment group using observed data.

SIS-16 and MoCA at each visit will be analyzed by a mixed-effects model for repeated measures with treatment and treatment by visit interaction as explanatory variables. Treatment contrasts at Day 90 will be derived.

In addition, MoCA assessments will be presented using summary statistics for the following categories: <10 (severe cognitive impairment), 10 to 17 (moderate cognitive impairment), and ≥ 18 (mild cognitive impairment).

16.1.2.2.3. NIHSS at Day 90

The change in NIHSS score from baseline at each visit will be analyzed by a repeated-measures mixed model with treatment and treatment by visit interaction as explanatory variables. Treatment contrasts at Day 90 and other timepoints will be derived.

The proportion of subjects who have an NIHSS score of 0 or 1 or who have demonstrated at least an 8-point improvement from baseline at Day 90 will be analyzed by logistic regression, adjusting for baseline NIHSS category and tPA use.

NIHSS at other timepoints will be analyzed similarly.

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16.1.2.2.4. Dose-Response and Exposure-Response

In addition to the estimation of treatment effect of each dose versus placebo, trend testing [Armitage 1955; Cochran 1954] of a monotonically increasing dose response in proportion of excellent outcome on mRS and BI will be performed. Exposure-response models, including E_{max} model of natalizumab, will be explored. The details will be provided in the SAP.

16.1.2.3. Additional/Exploratory Endpoints Analysis

Analyses for exploratory efficacy endpoints will be provided in the SAP.

16.2. Pharmacokinetics

16.2.1. Analysis Population

Subjects who have received natalizumab at the 0 Hours Visit and had at least 1 measurable sample collected for the determination of natalizumab concentrations will be included in the analysis.

16.2.2. Methods of Analysis

Serial natalizumab concentrations will be measured during the study. Summary statistics for the concentration values will be calculated at each collection timepoint, and the mean concentrations (\pm standard error [SE]) will be plotted over time on both linear and logarithmic scales. Plots for individual investigational sites will also be produced.

PK parameters will be calculated using noncompartmental methods [REDACTED].

Noncompartmental estimations of the various parameters will be calculated using Model 202 (noncompartmental IV infusion) in the [REDACTED]. Calculations will be performed using a “linear up/log down” algorithm, with a 1-hour infusion assumed. Points for the determination of terminal rate constants will be selected manually. Summary statistics for each PK parameter will be calculated. The following PK parameters will be determined for this study:

- C_{max}
- Time to C_{max} (t_{max})
- Area under the serum concentration versus time curve from dosing (time=0) to 120 hours after dosing (AUC_{0-120})
- Area under the serum concentration versus time curve from dosing (time=0) to 672 hours after dosing (AUC_{0-672})
- Area under the serum concentration versus time curve from dosing (time=0) to 2160 hours after dosing (AUC_{0-2160})

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- Area under the serum concentration versus time curve, from dosing (time=0) to last measurable concentration (AUC_{0-last})
- Half-life (t_½)
- Time of last measurable concentration (t_{last})
- Volume of distribution (V)
- Clearance (CL)

Concentrations reported as below the limit of quantification at any time after dosing will be treated as missing. The actual times of sample collection (and not the protocol-specified times) will be used in the calculation of all parameters.

16.3. Pharmacodynamics

16.3.1. Analysis Population

Subjects who have received the entire infusion of study treatment and have at least 1 postbaseline assessment of the parameter being analyzed will be included in the PD analysis.

16.3.2. Methods of Analysis

Summary statistics for α 4-integrin saturation include the following:

- Maximum % saturation [R_{max}], time to R_{max} [TR_{max}]
- Percent saturation pre-dose [R_{pre-dose}] and percent saturation at the end of the dosing interval [R₆₇₂] were calculated.

The mean % saturation values (\pm SE) were plotted over time for both the entire study population and the individual dosing groups.

16.4. Safety

16.4.1. Analysis Population

The safety analysis will include subjects who are randomized and have received any portion of the infusion of study treatment.

16.4.2. Methods of Analysis

No formal statistical testing will be performed on the safety data.

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16.4.2.1. Adverse Events

The incidence of treatment-emergent AEs will be tabulated by treatment group, severity, and relationship to study treatment. The tabular summaries will include incidence by system organ class and by preferred term. SAEs and AEs resulting in study withdrawal will be summarized by treatment group.

Treatment-emergent AEs are those defined as having onset after the start of study treatment, or a sign, symptom, or diagnosis that worsens since the event was previously reported. For the analysis of incidence by severity, the occurrence of the AE with the greatest severity will be used, and a subject will be counted only once and only in the category of the greatest severity for each event. For the analysis of incidence by relationship to study treatment, the occurrence of the AE with the strongest relationship to study treatment will be used, and a subject is counted only once and only in the category of the strongest relationship to study treatment for each event.

16.4.2.2. Clinical Laboratory Results

Clinically relevant abnormalities for laboratory parameters will be identified by treatment group using shift tables and evaluated for their clinical relevance.

16.4.2.3. Vital Signs

The analysis of vital signs will focus on the incidence of clinically relevant abnormalities by treatment group.

16.5. Interim Analyses

An interim futility analysis may be performed after 50% of the study population has completed the Day 30 assessment. No interim stopping rules for superiority will be applied.

16.6. Sample Size Considerations

A sample size of 270 (90 per treatment group) will provide at least 88% probability for the point estimate of the OR for the primary comparison of natalizumab (dose groups combined) versus placebo on the global composite measure at Day 90 to exceed 1.3 in both treatment windows (i.e., same efficacy for subjects being treated \leq 9 hours and >9 to \leq 24 hours from LKN), assuming a true OR of 1.8 as observed in Study 101SK201. Adopting a conservative assumption of a 50% reduction in treatment efficacy in the >9 to \leq 24 hour treatment window when compared to the \leq 9 hour treatment window, this probability will be at least 80%. An OR of \geq 1.3 on the global outcome measure is considered to be clinically meaningful based on the effect of tPA in the 3- to 4.5-hour time window. At this sample size, the probability of observing a point estimate exceeding 1.3, if the true OR is \leq 1 (i.e., the false positive rate), is less than 0.2. In addition, if the true OR of the higher dose versus placebo is 3.0, as observed in the subgroup with exposure above the median in Study 101SK201, the probability of observing an OR of \geq 1.3 when comparing the 600- to 300-mg dose is at least 74%.

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17. ETHICAL REQUIREMENTS

Biogen and [REDACTED] and the Investigator must comply with all instructions, regulations, and agreements in this protocol and applicable International Council on Harmonisation (ICH) and Good Clinical Practice (GCP) guidelines and conduct the study according to local regulations.

The Investigator may delegate responsibilities for study-related tasks where appropriate to individuals sufficiently qualified by education, training, and experience, in accordance with applicable ICH and GCP guidelines. The Investigator should maintain a list of the appropriately qualified persons to whom significant study-related duties have been delegated.

17.1. Declaration of Helsinki

This study will be performed in alignment with the ethical principles outlined in the Declaration of Helsinki.

17.2. Ethics Committee

The Investigator must obtain EC approval of the protocol, ICF, and other required study documents prior to starting the study. The Sponsor or designee will submit documents on behalf of the investigational sites in countries other than the US.

If the Investigator makes any changes to the ICF, Biogen must approve the changes before the ICF is submitted to the EC. A copy of the approved ICF must be provided to Biogen. After approval, the ICF must not be altered without the agreement of the relevant EC and Biogen.

It is the responsibility of the Investigators to ensure that all aspects of institutional review are conducted in accordance with current applicable regulations.

Biogen must receive a letter documenting EC approval, which specifically identifies the protocol, protocol number, and ICF, prior to the initiation of the study. Protocol amendments will be subject to the same requirements as the original protocol.

A progress report must be submitted to the EC at required intervals and not less than annually.

At the completion or termination of the study, the investigational site must submit a close-out letter to the EC and Biogen.

17.3. Subject Information and Consent

Prior to performing any study-related activities under this protocol, including screening tests and assessments, written informed consent with the approved ICF must be obtained from the subject or subject's legally authorized representative (e.g., parent or legal guardian), as applicable, in accordance with local practice and regulations.

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The background of the proposed study, the procedures, the benefits and risks of the study, and that study participation is voluntary for the subject must be explained to the subject (or the subject's legally authorized representative). The subject must be given sufficient time to consider whether to participate in the study.

A copy of the signed and dated ICF must be given to the subject or the subject's legally authorized representative. The signed and dated ICF will be retained with the study records. Local regulations must be complied with in respect to the final disposition of the original (wet signature) and copies of the signed and dated ICFs.

Confirmation of informed consent must also be documented in the subject's medical record.

17.4. Subject Data Protection

Prior to any testing under this protocol, including screening tests and assessments, candidates must also provide all authorizations required by local law (e.g., Protected Health Information authorization in North America).

The subject will not be identified by name in the eCRF or in any study reports, and these reports will be used for research purposes only. Biogen, its partners and designees, ECs, and various government health agencies may inspect the records of this study. Every effort will be made to keep the subject's personal medical data confidential.

17.5. Compensation for Injury

Biogen maintains appropriate insurance coverage for clinical studies and will follow applicable local compensation laws.

17.6. Conflict of Interest

The Investigators should address any potential conflicts of interest (e.g., financial interest in Biogen or [REDACTED]) with the subject before the subject makes a decision to participate in the study.

17.7. Registration of Study and Disclosure of Study Results

Biogen will register the study and poststudy results regardless of outcome on a publicly accessible website in accordance with the applicable laws and regulations.

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18. ADMINISTRATIVE PROCEDURES

18.1. Study Site Initiation

The Investigator must not screen any subjects prior to completion of a study initiation visit, conducted by Biogen. This initiation visit will include a detailed review of the protocol and study procedures.

18.2. Quality Assurance

During and/or after completion of the study, quality assurance officers named by Biogen or the regulatory authorities may wish to perform onsite audits or inspections. The Investigator will be expected to cooperate with any audit or inspection and to provide assistance and documentation (including source data) as requested.

18.3. Monitoring of the Study

The Investigator must permit study-related monitoring by providing direct access to source data and to the subjects' medical histories.

The Clinical Monitor will visit the Investigator at regular intervals during the study and after the study has completed, as appropriate.

During these visits, eCRFs and supporting documentation related to the study will be reviewed and any discrepancies or omissions will be resolved.

Monitoring visits must be conducted according to the applicable ICH and GCP guidelines to ensure protocol adherence, quality of data, study treatment accountability, compliance with regulatory requirements, and continued adequacy of the investigational site and its facilities.

18.4. Study Funding

Biogen is the Sponsor of the study and is funding the study. All financial details are provided in the separate contracts between the institution, Investigator, and Biogen.

18.5. Publications

Details are included in the clinical study agreement for this study.

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19. FURTHER REQUIREMENTS AND GENERAL INFORMATION

19.1. External Contract Organizations

A CRO will be responsible for all administrative aspects of this study including but not limited to study initiation, monitoring, management of AEs, and data management.

19.1.1. Contract Research Organization

A CRO will be responsible for administrative aspects of the study including but not limited to study initiation, monitoring, and management of SAE reports and data management. Before subjects are screened at each study site, the CRO will review study responsibilities with the Investigators and other study site staff, as appropriate.

19.1.2. Interactive Response Technology

IRT will be used in this study. Before subjects are screened or enrolled, the IRT vendor will provide each study site with the necessary training, a user manual, and access rights to the system.

19.1.3. Remote Data Capture

Subject information will be captured and managed by study sites on eCRFs by a remote data capture (RDC) tool developed and supported by the RDC vendor and configured by Biogen.

19.1.4. Central Laboratories for Laboratory Assessments

A central laboratory has been selected by Biogen to analyze all hematology and blood chemistry, PK sampling, and immunogenicity samples collected for this study. Standard of Care samples will be analyzed locally.

Duplicate samples for laboratory assessments will be collected as a backup in case the original sample is lost or not evaluable.

19.1.5. Central Facility for Other Assessments

A central facility has been selected by Biogen to perform imaging assessments for this study.

19.2. Study Committees

19.2.1. Advisory Committee

An advisory committee will be formed to provide scientific and medical direction for the study and to oversee the administrative progress of the study. The advisory committee will meet at regular intervals to monitor subject accrual and to monitor compliance with the protocol at

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individual study sites. The advisory committee will be blinded to subject treatment assignments. The advisory committee will determine whether the study should be stopped or amended for reasons other than safety.

Members of the advisory committee will include the Medical Director, the Clinical Operations Lead, and the Project Statistician from Biogen (and/or their designees), as well as participating Investigators who will be appointed by Biogen based on relevant expertise. Biogen will designate one of the participating Investigators to be the chairperson of the advisory committee.

The Ongoing Data Review Committee will have an Ongoing Data Review Plan that is separate from the DSMC.

19.2.2. Independent Data Safety Monitoring Committee

An independent DSMC will be formed to review data regularly to assess safety and risk-benefit and recommend appropriate modification to the study or termination of the study. The DSMC receives reports of all SUSARs, AEs, and key laboratory tests and meets throughout the study at regular timepoints described in the DSMC charter.

19.3. Changes to Final Study Protocol

All protocol amendments must be submitted to the EC and regulatory authorities if required by local law. Protocol modifications that affect subject safety, the scope of the investigation, or the scientific quality of the study must be approved by the EC before implementation of such modifications to the conduct of the study. If required by local law, such modifications must also be approved by the appropriate regulatory agency prior to implementation.

However, Biogen may, at any time, amend this protocol to eliminate an apparent immediate hazard to a subject. In this case, the appropriate regulatory authorities will be notified subsequent to the modification.

In the event of a protocol modification, the ICF may require similar modifications (see Section 17).

19.4. Ethics Committee Notification of Study Completion or Termination

Where required, the regulatory authorities and ECs must be notified of completion or termination of this study, and sent a copy of the study synopsis in accordance with necessary timelines.

19.5. Retention of Study Data

The minimum retention time for study records will meet the strictest standard applicable to that site, as dictated by any institutional requirements or local laws or regulations. Prior to proceeding with destruction of records, the Investigator must notify Biogen in writing and receive written authorization from Biogen to destroy study records. In addition, the Investigator

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must notify Biogen of any changes in the archival arrangements including but not limited to archival at an offsite facility or transfer of ownership if the Investigator leaves the site.

19.6. Study Report Signatory

Biogen will designate one of the participating Study Investigators as a signatory for the study report. This determination will be made by several factors, including but not limited to, the Investigator's experience and reputation in the studied indication; the Investigator's contribution to the study in terms of design, management, and/or subject enrollment; or by other factors determined to be relevant by Biogen.

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21. SIGNED AGREEMENT OF THE STUDY PROTOCOL

I have read the foregoing protocol, "A Multicenter, Double-Blind, Placebo-Controlled, Randomized, Parallel-Group, Dose-Ranging Study to Evaluate the Safety and Efficacy of Intravenous Natalizumab (BG00002) in Acute Ischemic Stroke," and agree to conduct the study according to the protocol and the applicable ICH guidelines and GCP regulations, and to inform all who assist me in the conduct of this study of their responsibilities and obligations.

Investigator's Signature

Date

Investigator's Name (Print)

Study Site (Print)

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Biogen MA Inc.



Biogen MA Inc.
250 Binney Street
Cambridge, MA 02142
United States

Biogen Idec Research Limited
Innovation House
70 Norden Road
Maidenhead Berkshire
SL6 4AY
United Kingdom

AMENDMENT SUMMARY

Biogen Protocol 101SK202/NCT02730455

A Multicenter, Double-Blind, Placebo-Controlled, Randomized, Parallel-Group, Dose-Ranging Study to Evaluate the Safety and Efficacy of Intravenous Natalizumab (BG00002) in Acute Ischemic Stroke

Version 3.0

Date: 03 May 2017

EUDRA CT Number: 2015-004783-11

Version 3.0 of the protocol has been prepared for this amendment, which supersedes Version 2.0 (dated 15 December 2016).

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PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 101SK202 is to correct an error in the pharmacokinetic (PK)/pharmacodynamic (PD) sampling times. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

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Section 4.2, Schedule of Activities**Now reads:****Table 1: Schedule of Activities for Study 101SK202**

Tests and Assessments	Screening ^{1,2}	0 Hours ² (Day 1)	Within 1 Hour After Start End of Infusion	12 Hours ±3 Hours	24 Hours ±6 Hours	Day 5 ³	Day 30 ⁴ ±5 Days	Day 90 Follow-up ⁴ / Early Termination ⁴ ±5 Days
Informed consent	X							
Confirm eligibility	X							
Demographics and medical history ⁵	X							
Physical and neurological examination	X				X	X	X	X
Vital signs ⁶	X	X	X		X	X	X	X
Height (if available) and weight			X (anytime from Screening to Day 5 Visit)					
Urine pregnancy test ⁷	X							
Hematology and blood chemistry	X			X	X	X	X	X
Serum biomarkers	X			X	X	X	X	X
PD sampling	X		X	X	X	X	X	X
PK sampling	X		X	X	X	X	X	X
Blood sample for anti-natalizumab antibodies	X						X	X
NIHSS	X	X ²			X	X	X	X
mRS						X ⁸	X	X
BI						X	X	X
SIS-16						X ⁹	X	X
MoCA						X	X	X
Functional Independence Measure							X	X
SDMT						X	X	X
Fatigue Severity Scale						X ⁹	X	X
Beck Depression Inventory 2						X ⁹	X	X
HRU questionnaire						X	X	X
EQ-5D-3L						X	X	X
Study treatment administration ¹⁰		X						

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Tests and Assessments	Screening ^{1,2}	0 Hours ² (Day 1)	Within 1 Hour After Start End of Infusion	12 Hours ±3 Hours	24 Hours ±6 Hours	Day 5 ³	Day 30 ⁴ ±5 Days	Day 90 Follow-up ⁴ / Early Termination ⁴ ±5 Days
Stroke subtype classification ¹¹						X		
SAE reporting						Record as per Section 15.3.2 of the protocol		
AE reporting						Record as per Section 15.23.1 of the protocol		
Concomitant medications						Record as per Section 11.5 of the protocol		
Concomitant procedures						Record as per Section 11.5 of the protocol		

AE = adverse event; BI = Barthel Index; BDI-2 = Beck Depression Inventory 2; CT = computed tomography; ECG = electrocardiogram; FIM = Functional Independence Measure; FSS = Fatigue Severity Scale; HRU = health resource utilization; LKN = last known normal; MoCA = Montreal Cognitive Assessment; MRI = magnetic resonance imaging; mRS = modified Rankin Scale; NIHSS = National Institute of Health Stroke Scale; PD = pharmacodynamic; PK = pharmacokinetic; SAE = serious adverse event; SDMT = Symbol-Digits Modalities Test; SIS-16 = Stroke Impact Scale-16.

Note: All timepoints, **except “within 1 hour after the end of the infusion”**, are relative to start of study treatment administration.

¹ All Screening assessments must be performed prior to infusion. Screening assessments that are performed as standard of care do not need to be repeated for the study, provided that Biogen has access to the information and data are recorded in subject's case report form. Data collected for the Screening assessments will be used for patient baseline analysis in this study. The ECG and CT or MRI assessments results should already be available at Screening (as they are performed as part of the standard of care), and a copy of the images and report should be filed with the subject's study records.

² All Screening assessments must be performed prior to infusion. The 0 Hours vital signs assessments are to be performed within 15 minutes prior to study treatment administration. If the Screening NIHSS is performed more than 1 hour prior to study treatment administration, it must be repeated during the visit at 0 Hours before study treatment administration.

³ Day 5 assessments are to occur on Day 5 or earlier if discharged, but must occur prior to discharge.

⁴ The subject will be asked to complete the Day 30 and Day 90 Follow-Up or Early Termination assessments in person (see Section 7.2.3 and Section 10.2). If the subject is unable to return to the study center to complete the Day 30 or Day 90 (Follow-up or Early Termination) assessments in person, safety information will be collected by telephone or remotely, as local regulations allow and pending medical monitor approval, and will include the collection of AEs, SAEs, FIM, mRS, BI, BDI-2, SIS-16, EQ-5D-3L, physical/neurological examination, vital signs, hematology/blood chemistry, serum biomarkers, PD sampling, PK sampling, anti-natalizumab antibodies, NIHSS, MoCA, SDMT, FSS, HRU, and concomitant medications.

⁵ Medical history should include an assessment of prior substance abuse.

⁶ Vital signs collected at each of the specified timepoints include temperature, blood pressure, pulse or heart rate, and respiratory rate. Vital signs collected as part of the subject's standard of care and that fall within 30 minutes of a study visit do not need to be repeated for the study, provided that Biogen has access to the information and data are recorded in the subject's case report form.

⁷ Required only for women of childbearing potential. If a serum beta human chorionic gonadotropin test was performed as part of the subject's standard of care, the results will be accepted in lieu of the urine pregnancy test.

⁸ An mRS assessment will be done on Day 5, or earlier if discharged.

⁹ Per instructions on the SIS-16, BDI-2, and FSS forms, the subject or proxy should provide responses as it applies to the prior 2 week period. When completing the SIS-16, BDI-2, and FSS assessments during the Day 5 Visit, the subject or proxy should consider only the time period starting between the initial stroke (LKN) and the Day 5 Visit.

¹⁰ The subject must be observed for 1 hour after completion of infusion.¹¹ The stroke etiology will be assessed based on the subject's standard of care and diagnostic testing. This should be completed at Day 5 or earlier if discharged, but if results are incomplete, it may be completed at Day 30.

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Rationale: As natalizumab (both 300 mg and 600 mg) and placebo are given as infusions over 2 hours, the original wording for the collection of PK/PD samples would not be appropriate as the infusion would still be ongoing. Thus, a column heading in Table 1 was revised to ensure that the blood samples for PK/PD assessments, as well as vital sign measurements, are taken within 1 hour after the infusion has stopped.

In order to get appropriate PK/PD results, the blood samples need to be taken within 1 hour after the infusion has stopped.

This change also affects Section 7.1, Study Overview.

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SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

No other major changes were made to the protocol.

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SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- The version number and date of the superseded version of the protocol were updated.
- In Table 1, the Schedule of Activities, the Section numbers cited for serious adverse event and adverse event reporting were changed to the correct sections.
 - Changed the Note to include “within 1 hour after the end of the infusion”
 - The first sentence of footnote 2 was moved to the beginning of footnote 1
- In Figure 1, Study Schema, a footnote was added to state PK/PD/biomarker sample times are conducted within 1 hour after the end of the infusion.
- Minor format changes to Section 7.1 were made to ensure proper cross-referencing.
- Spelled out examination throughout text (previously ‘exam’)

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Biogen MA Inc.



Biogen MA Inc.
250 Binney Street
Cambridge, MA 02142
United States

Biogen Idec Research Limited
Innovation House
70 Norden Road
Maidenhead Berkshire
SL6 4AY
United Kingdom

AMENDMENT SUMMARY

Biogen Protocol 101SK202/NCT02730455

A Multicenter, Double-Blind, Placebo-Controlled, Randomized, Parallel-Group, Dose-Ranging Study to Evaluate the Safety and Efficacy of Intravenous Natalizumab (BG00002) in Acute Ischemic Stroke

Version 2.0

Date: 15 December 2016

EUDRA CT Number: 2015-004783-11

Version 2.0 of the protocol has been prepared for this amendment, which supersedes Version 1.0.

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PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 101SK202 is to remove unnecessary study procedures (i.e., 0 hour blood draw and the Functional Independence Measure at Day 5) and to add an expanded treatment window (>9 to ≤ 24 hours) in which subjects would be eligible for inclusion in the trial.

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 4.2, Schedule of Activities

Now reads:

See pages 4 and 5 for updated schedule of activities.

Rationale: To relieve the burden of too many procedures on the subjects and the study centers.

Section 7.1, Study Overview

Now reads:

This is a Phase 2, multicenter, double-blind, placebo-controlled, randomized, dose-ranging, 3-arm study of natalizumab administered ≤ 9 hours from when the subject was LKN in subjects with acute ischemic stroke. This study will evaluate the efficacy and safety of natalizumab over a 90-day period. This study will be conducted at approximately 67 sites in the United States and Europe.

After completing screening assessments and fulfilling the criteria for study entry, eligible subjects **in each of 2 treatment windows (≤ 9 hours and >9 to ≤ 24 hours from LKN)** will be randomized in a 1:1:1 ratio to 1 of 3 treatment groups, each of which will receive a single dose at 0 Hours (Day 1) according to one of the following 3 regimens: 600 mg IV natalizumab, 300 mg IV natalizumab, or placebo IV.

Rationale: In experimental rodent models, immune cell infiltration in the brain parenchyma following an ischemic stroke occurs predominantly after 24 hours of lesion onset, reaching its peak at approximately 3 days after the index event. Congruently, blockage of maladaptive immune responses through immune modulatory treatments as far as 5 days after ischemia onset has been demonstrated to yield significant beneficial effects in rodent models, suggesting that therapies targeting lymphocyte infiltration after ischemia may have a prolonged therapeutic window.

A stratified randomization process was adopted in Study 101SK201 to evaluate the potential time dependency of treatment effect when administering 300 mg of natalizumab in a 9-hour

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window as compared to placebo. In this study, half of the subjects in the intent-to-treat population were assigned to receive study treatment at ≤ 6 hours from last known normal (LKN), while the other half had study treatment administered within the >6 to ≤ 9 hour window. Results from Study 101SK201 indicated that estimates of treatment effect favored natalizumab against placebo at both treatment windows, with no evidence of time dependency for the treatment benefit. Likewise, the safety profile of natalizumab treatment was comparable in the ≤ 6 hour and the >6 to ≤ 9 hour treatment windows, with a small decrease in the proportion of deaths (21% and 15%, respectively) and serious adverse events (47% and 45%, respectively) being reported among subjects treated in the later treatment window.

Based on these observations, the therapeutic window for natalizumab therapy appears to be greater than 9 hours. Therefore, Study 101SK202 will further explore the time dependency of the treatment effect of natalizumab in patients with acute ischemic stroke by enrolling a limited number of subjects within the treatment window of >9 to ≤ 24 hours from LKN.

This change also affects Section 4.1, Study Schematic; Section 5.5, Rationale for the Treatment Window of ≤ 24 Hours from LKN; Section 6.2, Secondary Objectives and Endpoints; Section 7.2.2, Treatment; Section 8.1, Inclusion Criteria; Section 9.2, Randomization and Registration of Subjects; Section 16.1.2.1, Analysis of the Primary Endpoint; Section 16.1.2.2, Analysis of the Secondary Endpoints; Section 16.1.2.2.1, mRS and BI at Day 90; Section 16.1.2.2.2, SIS-16 and MoCA at Day 90; Section 16.1.2.2.3, NIHSS at Day 90; Section 16.6, Sample Size Considerations; and Section 20, References.

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Table 1: Schedule of Activities for Study 101SK202

Tests and Assessments	Screening ^{1,2}	0 Hours (Day 1)	Within 1 Hour After EndStart of Infusion	12 Hours ±3 Hours	24 Hours ±6 Hours	Day 5 ³	Day 30 ⁴ ±5 Days	Day 90 Follow-up ⁴ / Early Termination ⁵⁴ ±5 Days
Informed consent	X							
Confirm eligibility	X							
Demographics and medical history ⁵⁵	X							
Physical and neurological examination	X				X	X	X	X
Vital signs ⁵⁶	X	X	X		X	X	X	X
Height (if available) and weight			X (anytime from Screening to Day 5 Visit)					
Urine pregnancy test ⁵⁷	X							
Hematology and blood chemistry	X	X		X	X	X	X	X
Serum biomarkers	X			X	X	X	X	X
PD sampling	X		X	X	X	X	X	X
PK sampling	X		X	X	X	X	X	X
Blood sample for anti-natalizumab antibodies	X						X	X
NIHSS	X	X ²			X	X	X	X
mRS						X ⁹⁸	X	X
Bl						X	X	X
SIS-16						X ¹⁰⁹	X	X
MoCA						X	X	X
Functional Independence Measure						X	X	X
SDMT						X	X	X
Fatigue Severity Scale						X ¹⁰⁹	X	X
Beck Depression Inventory 2						X ¹⁰⁹	X	X
HRU questionnaire						X	X	X
EQ-5D-3L						X	X	X
Study treatment administration ^{10,10}		X						
Stroke subtype classification ^{12,11}						X		
SAE reporting			Record as per Section 15.2 of the protocol					

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Tests and Assessments	Screening ^{1,2}	0 Hours (Day 1)	Within 1 Hour After EndStart of Infusion	12 Hours ±3 Hours	24 Hours ±6 Hours	Day 5 ³	Day 30 ⁴ ±5 Days	Day 90 Follow-up ⁴ / Early Termination ^{5,6} ±5 Days
AE reporting						Record as per Section 15.2 of the protocol		
Concomitant medications						Record as per Section 11.5 of the protocol		
Concomitant procedures						Record as per Section 11.5 of the protocol		

AE = adverse event; BI = Barthel Index; BDI-2 = Beck Depression Inventory 2; CT = computed tomography; ECG = electrocardiogram; FIM = Functional Independence Measure; FSS = Fatigue Severity Scale; HRU = health resource utilization; LKN = last known normal; MoCA = Montreal Cognitive Assessment; MRI = magnetic resonance imaging; mRS = modified Rankin Scale; NIHSS = National Institute of Health Stroke Scale; PD = pharmacodynamic; PK = pharmacokinetic; SAE = serious adverse event; SDMT = Symbol-Digits Modalities Test; SIS-16 = Stroke Impact Scale-16.

Note: All timepoints are relative to start of study treatment administration.

¹ Screening assessments that are performed as standard of care do not need to be repeated for the study, provided that Biogen has access to the information and data are recorded in subject's case report form. Data collected for the Screening assessments will be used for patient baseline analysis in this study. The ECG and CT or MRI assessments results should already be available at Screening (as they are performed as part of the standard of care), and a copy of the images and report should be filed with the subject's study records.

² All Screening assessments must be performed prior to infusion. The 0 Hours vital signs assessments are to be performed within 15 minutes prior to study treatment administration. If the Screening NIHSS is performed more than 1 hour prior to study treatment administration, it must be repeated during the visit at 0 Hours before study treatment administration.

³ Day 5 assessments are to occur on Day 5 or earlier if discharged, but must occur prior to discharge.

⁴ ~~If the patient is unable to return to the study center to complete a visit in person, the Day 90 Follow Up can be completed over the telephone or remotely, as local regulations allow and pending medical monitor approval. The Day 90 Follow Up consists of the collection of AEs, SAEs, FIM, mRS, BI, BDI-2, SIS-16, EQ-5D-3L, physical/neurological exam, vital signs, hematology/blood chemistry, serum biomarkers, PD sampling, PK sampling, anti-natalizumab antibodies, NIHSS, MoCA, SDMT, FSS, HRU, and concomitant medications.~~

^{5,6} The subject will be asked to complete the Day 30 and Day 90 Follow-Up or Early Termination assessments in person (see Section 7.2.3 and Section 10.2). If the subject ~~does not~~ **unable to return to the study center to complete the Day 30 or Day 90 (Follow-up or Early Termination) assessments in person**, safety information will be collected by telephone or remotely, as local regulations allow and pending medical monitor approval, and will include the collection of AEs, SAEs, FIM, mRS, BI, BDI-2, SIS-16, EQ-5D-3L, physical/neurological exam, vital signs, hematology/blood chemistry, serum biomarkers, PD sampling, PK sampling, anti-natalizumab antibodies, NIHSS, MoCA, SDMT, FSS, HRU, and concomitant medications.

^{6,5} Medical history should include an assessment of prior substance abuse.

^{7,6} Vital signs collected at each of the specified timepoints include temperature, blood pressure, pulse or heart rate, and respiratory rate. Vital signs collected as part of the subject's standard of care and that fall within 30 minutes of a study visit do not need to be repeated for the study, provided that Biogen has access to the information and data are recorded in the subject's case report form.

^{8,7} Required only for women of childbearing potential. If a serum beta human chorionic gonadotropin test was performed as part of the subject's standard of care, the results will be accepted in lieu of the urine pregnancy test.

^{9,8} An mRS assessment will be done on Day 5, or earlier if discharged.

^{10,9} Per instructions on the SIS-16, BDI-2, and FSS forms, the subject or proxy should provide responses as it applies to the prior 2 week period. When completing the SIS-16, BDI-2, and FSS assessments during the Day 5 Visit, the subject or proxy should consider only the time period starting between the initial stroke (LKN) and the Day 5 Visit.

^{11,10} The subject must be observed for 1 hour after completion of infusion.

^{12,11} The stroke etiology will be assessed based on the subject's standard of care and diagnostic testing. This should be completed at Day 5 or earlier if discharged, but if results are incomplete, it may be completed at Day 30.

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SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 3, Synopsis

The Synopsis was revised to reflect changes made throughout the protocol.

Section 8.1, Inclusion Criteria

Change: A National Institute of Health Stroke Scale (NIHSS) Screening score for eligibility was added for the >9 to ≤ 24 hour treatment window, and NIHSS eligibility is to be confirmed within 60 minutes prior to randomization.

Now reads:

4. Score of 5 to 23 points, inclusive, on the NIHSS at Screening **for subjects initiating treatment ≤ 9 hours from LKN. Note: NIHSS eligibility must be confirmed within 60 minutes prior to randomization.**
5. **Score of 5 to 15 points, inclusive, on the NIHSS at Screening for subjects initiating treatment >9 to ≤ 24 hours from LKN. Note: NIHSS eligibility must be confirmed within 60 minutes prior to randomization.**

Rationale: The maximum NIHSS Screening score for subjects initiating treatment in the >9 to ≤ 24 hour window was set at 15 points because prognosis associated with higher NIHSS scores is worsened when present at later time points. The timeframe for confirming NIHSS eligibility was clarified to occur 60 minutes prior to randomization.

This change also affects Section 4.2, Schedule of Activities.

Section 8.2, Exclusion Criteria

Changes: (1) Patients with rapidly improving or minor stroke symptoms or (2) with petechial hemorrhages ≤ 1 cm are no longer excluded from the study. (3) The statement regarding performing brain magnetic resonance imaging (MRI) to assess subject eligibility was removed. The types of exclusionary strokes were clarified. (4) The exclusion criteria regarding hepatitis infections and bacterial, fungal, or viral infections were simplified.

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Now reads:

~~1. Rapidly improving or minor stroke symptoms~~

~~2.1. Lacunar or isolated brainstem or cerebellar stroke based on clinical assessment and available acute imaging studies performed under the standard of care. If it is unclear whether the subject has had a lacunar stroke based on clinical findings and head CT results, a brain MRI should be performed to assess eligibility, provided that all other inclusion criteria are met~~

~~3.2. Presence of acute intracranial hemorrhage on acute brain CT or MRI. However, petechial hemorrhages of ≤1 cm are not exclusionary.~~

[...]

~~10.9. Known history of active viral hepatitis B or C positive test result for hepatitis C virus antibody or current hepatitis B infection (defined as positive for hepatitis B surface antigen [HBsAg] and hepatitis B core antibody [HBcAb]). Subjects with immunity to hepatitis B from active vaccination or previous natural infection (defined as negative HBsAg, positive hepatitis B surface antibody immunoglobulin G, and negative HBcAb) are eligible to participate in the study (per the US Centers for Disease Control and Prevention's interpretation of the hepatitis B serology panel)~~

~~13.12. Signs and symptoms of bacterial, fungal, or viral infection (including upper respiratory tract infection) within 14 days prior to randomization~~**active or acute infection**

Rationale: (1) Subjects who experience rapid improvement in stroke symptoms may still be eligible to participate in the study based on their NIHSS score at the time of eligibility. Subjects with an NIHSS score of 5 or more, as specified in the inclusion criteria, would not have minor stroke symptoms. (2) A brain MRI is not a study procedure and should be performed according to standard of care. Cerebellar strokes are considered exclusionary. (3) Petechial hemorrhage is associated with baseline NIHSS and is commonly observed in moderate to severe ischemic strokes. Petechial hemorrhage is not associated with poor clinical outcome and is easily distinguishable from parenchymal hemorrhage in imaging studies. Results from Study 101SK201 supported that treatment with natalizumab had no impact on the rate of hemorrhagic transformation. Therefore, small petechial hemorrhages should not be considered exclusionary in this study. In addition, petechial hemorrhages are likely to be randomly well balanced among the treatment arms because the randomization process stratifies for stroke severity. (4) Laboratory testing for infections is not required as a study procedure, and the level of detail specified in the original exclusion criteria may be difficult to obtain from a patient's medical history. Furthermore, only active or acute infection events that are ongoing at the time of eligibility assessment should be considered exclusionary.

Section 11.5.1, Concomitant Therapy, and Section 11.5.2, Concomitant Procedures

Change: Thrombolytic treatments/procedures will be recorded as concomitant regardless of whether they are given before or after informed consent.

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Section 11.5.1 now reads:

A concomitant therapy is any drug or substance administered between time of informed consent or authorization to enroll and completion of assessments at the Day 90 Follow-Up visit (as required by the protocol). **All thrombolytic treatments (i.e., IV and intra-arterial rtPA) and mechanical thrombectomy that a subject receives are to be recorded as concomitant therapy regardless of whether they are given before or after the time of informed consent.**

Section 11.5.2 now reads:

A concomitant procedure is any therapeutic intervention (e.g., surgery/biopsy, physical therapy) or diagnostic assessment (e.g., blood gas measurement, bacterial cultures) performed between the time the subject is enrolled in the study and Safety Follow-Up. **All thrombolytic treatments and mechanical thrombectomy that a subject receives are to be recorded as concomitant procedures regardless of whether they are given before or after the time of informed consent.**

Rationale: All thrombolytic treatments need to be recorded.

Section 15.5, Contraception Requirements

Change: Contraception requirements were updated.

Now reads:

For the purposes of the study, highly effective contraception is defined as ~~use of 1 or more of contraception that achieves a failure rate of less than 1% when used consistently and correctly, and includes~~ the following:

For females:

- **Established use of oral, intravaginal, or transdermal combined (estrogen and progestogen containing) hormonal methods of contraception associated with the inhibition of ovulation.**
- Established use of oral, injected, or implanted **progestogen-only** hormonal methods of contraception **associated with the inhibition of ovulation.**
- Placement of an intrauterine device or intrauterine **hormone-releasing** system.
- **Bilateral tubal occlusion**
- ~~Barrier methods of contraception with use of a spermicide: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository. The use of barrier contraceptives should always be supplemented with the use of a spermicide.~~

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- ~~Male surgical sterilization (For female subjects participating in the study, male sexual partners must have undergone surgical sterilization with the appropriate postvasectomy documentation of the absence of sperm in the ejaculate). (For female subjects participating in the study, male sexual partners must have undergone surgical sterilization.)~~

For males:

- Effective male contraception includes a vasectomy with negative semen analysis at follow-up, ~~or the use of condoms with spermicide~~.

True abstinence, when this is consistent with the preferred and usual lifestyle of the subject, can be considered ~~an acceptable~~ **a highly effective** method of contraception based on the evaluation of the Investigator who should also take into consideration the duration of the clinical study.

Rationale: Contraception language was updated so that all subjects are instructed to use highly effective methods of contraception and so that the methods of highly effective contraception are defined consistently with the Clinical Trial Facilitation Group's recommendations.

Section 16.6, Sample Size Considerations

Change: The sample size was increased.

Now reads: A sample size of ~~225~~**270** (~~75~~**90** per **treatment** group) will provide at least ~~86~~**88**% probability for the point estimate of the OR for the primary comparison of natalizumab (dose groups combined) versus placebo on the global composite measure at Day 90 to exceed 1.3, **in both treatment windows (i.e., same efficacy for subjects being treated ≤ 9 hours and >9 to ≤ 24 hours from LKN)**, assuming a true OR of 1.8 as observed in Study 101SK201. **Adopting a conservative assumption of a 50% reduction in treatment efficacy in the >9 to ≤ 24 hour treatment window when compared to the ≤ 9 hour treatment window, this probability will be at least 80%.** An OR of ≥ 1.3 on the global outcome measure is considered to be clinically meaningful based on the effect of tPA in the 3- to 4.5-hour time window. At this sample size, the probability of observing a point estimate exceeding 1.3, if the true OR is ≤ 1 (i.e., the false positive rate), is less than 0.2. In addition, if the true OR of the higher dose versus placebo is 3.0, as observed in the subgroup with exposure above the median in Study 101SK201, the probability of observing an OR of ≥ 1.3 when comparing the 600- to 300-mg dose is at least ~~76~~**74**%. ~~To allow for approximately 6% discontinuation before Day 90 (as observed in Study 101SK201), 240 subjects will be randomized.~~

Rationale: The sample size was increased to account for the addition of a new treatment window (>9 to ≤ 24 hours from LKN).

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SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- A Sponsor signature page was added.
- The list of abbreviations was updated.
- In Section 1, Sponsor Information, the information on how to contact the study's Medical Monitor for urgent medical issues was updated. This change also affects Section 15.3.3, Immediate Reporting of Serious Adverse Events, and Section 15.4.3, Medical Emergency.
- In Section 7.1, Study Overview, clarification was made: the timing of post-treatment assessments are from the start of infusion and now reads as follows: Post-treatment assessments will be performed at the following timepoints after the start of study treatment administration: within 1 hour after the ~~end start~~ of the infusion, 12 ± 3 hours after the ~~end start~~ of the infusion, 24 ± 6 hours after the ~~end start~~ of the infusion, Day 5, Day 30 (± 5 days), and the Day 90 Follow-Up visit (± 5 days). This change also affects Section 4.2, Schedule of Activities.
- In Section 7.1, Study Overview, the following statement from the Synopsis was added: **The study will be conducted at approximately 67 sites in the United States and Europe.**
- In Section 7.2.3, Follow-Up, clarification was made that subjects are to return to the site only for the Day 30 and 90 assessments and if subjects are unable to return to the site, these assessments can be done over the phone. This section now reads: Subjects are to return to the study site for a follow-up visit on ~~Day 5~~, Day 30, and Day 90 (Follow-Up Visit; the final study visit). If the ~~patient~~subject is unable to return to the study center to complete a visit in person, the ~~Day 5~~, Day 30, or the Day 90 Follow-Up visits can be completed over the telephone or remotely, as local regulations allow and pending medical monitor approval. This change also affects Section 4.2, Footnote 4 of the Schedule of Activities.
- In Section 8.1, Inclusion Criteria, inclusion criteria were updated to identify the types of daily activities the patient was able to perform and now reads as follows: ~~56~~. Prior to index stroke, patient was able to perform **the following** basic activities of daily living without assistance: **dressing, eating, walking, bathing, and using the toilet.**
- In Section 10.2, Withdrawal of Subjects From Study, a statement was added to indicate that subjects who withdraw from the study will not be replaced. It was clarified that information collected for early termination assessments can also be collected by a remote visit.
- In Section 13.1, Clinical Efficacy Assessments, the reference to premorbid mRS was removed since it is not being collected.

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- In Section 14.2, Laboratory Safety Assessments, the reference to anti-JCV serology testing was removed. This also affects Section 19.1.4, Central Laboratories for Laboratory Assessments.
- In Section 16.5, Interim Analyses, it was clarified that an interim analysis may be performed after 50% of the study population has completed the Day 30 assessment.
- Typographical errors and formatting were corrected.

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