The attached includes:

Protocol: A Phase 2 clinical trial of tolebrutinib, a brain-penetrant Bruton's tyrosine kinase inhibitor, for the modulation of chronically inflamed white matter lesions in multiple sclerosis

- Statistical Analysis Plan included within the protocol

NCT#: 04742400

Document Date: November 3, 2023

Abbreviated Title: BRaKe-MS

NIH IRB #: 21N0010

Version Date: 11/3/2023

Title: A Phase 2 clinical trial of tolebrutinib, a brain-penetrant Bruton's tyrosine kinase inhibitor, for the modulation of chronically inflamed white matter lesions in multiple sclerosis

Principal Investigator:

Daniel Reich, MD, PhD

National Institute of Neurological Disorders and Stroke

Building 10, Rm 5C103

9000 Rockville Pike

Bethesda, MD 20892

Phone: 301-496-1801

E-mail: daniel.reich@nih.gov

Investigational Agent:

Drug Name:	tolebrutinib
IND Number:	153292
Sponsor:	NINDS
Manufacturer:	Sanofi Genzyme/TDR17022

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: A Phase 2 trial of the effect of tolebrutinib, a brain-penetrant Bruton's tyrosine kinase inhibitor, on chronically inflamed white matter lesions in multiple sclerosis

Study Description: The primary goal of this protocol is to test whether 48 weeks of treatment with tolebrutinib, an investigational, orally available, brain-penetrant, Bruton's tyrosine kinase (BTK) inhibitor, affects an imaging marker (the "paramagnetic rim") associated with chronically inflamed white matter lesions in multiple sclerosis (MS). In this rater-blinded but otherwise open- label study, 16 adults with MS who are on stable disease-modifying treatment with anti-CD20 antibody therapy and are within 6 months of their most recent dose, have at least one paramagnetic rim lesion on 7-tesla magnetic resonance imaging (MRI), and have developed no new white matter lesions or clinical relapses for at least 6 months, will initiate treatment with tolebrutinib and agree to forego further anti-CD20 or other disease-modifying therapy for the duration of the trial.

An initial 7 enrolled study participants started tolebrutinib at 60 mg/day ("Initial Cohort"). Of the Initial Cohort, participants (n=3), who had initiated tolebrutinib 60 mg will remain at 60 mg and not be escalated to 120 mg/day. The remaining participants (n=4) consented to Cohort A, who had initiated tolebrutinib 60 mg and previously escalated to 120 mg/day will remain at that dose.

Radiological, clinical, and biological outcomes are measured at 24, 48, 72, 96, and 144 (Cohort A) weeks, with additional interspersed visits for safety monitoring. Participants may subsequently continue treatment until tolebrutinib is marketed or commercial development halted. A comparison group of 10 participants who meet enrollment criteria but choose to stay on anti-CD20 therapy will also be enrolled. The primary outcome measure is disappearance of one or more paramagnetic rims from white matter lesions identified at baseline. Secondary outcomes include safety and tolerability and additional radiological outcomes. Exploratory clinical, radiological, and laboratory investigations are planned to study the mechanism of action of tolebrutinib and for biomarker development, and to compare the tolebrutinib and anti-CD20 cohorts.

Objectives: *Primary Objective:* To evaluate the effects of 48 weeks of tolebrutinib 60 mg/day treatment on the paramagnetic rim of chronically inflamed white matter lesions, as seen on 7-tesla MRI.

Secondary Objectives: (1) To assess safety and tolerability of 48 weeks of treatment with tolebrutinib 60 mg and 96 weeks of treatment with tolebrutinib 120 mg (Cohort A) all following anti-CD20 antibody therapy. (2) To assess the possible repair of chronically inflamed white matter lesions in which inflammation at the lesion edge has been modulated by tolebrutinib.

Endpoints: *Primary Endpoint:* Per-patient proportion of lesions in which the paramagnetic rim has disappeared at the end of 48 weeks of tolebrutinib 60 mg.

Secondary Endpoints: (1) Adverse event tables. (2) Changes in T1 relaxation time within paramagnetic rim lesions at the end of 96 weeks of tolebrutinib 120 mg, relative to non-rim lesions. (3) Changes in size of paramagnetic rim lesions at the end of 96 weeks of tolebrutinib 120 mg, relative to non-rim lesions.

Study Population: Up to 10 adults with multiple sclerosis, targeting at least 7 participants who complete 48 weeks of therapy with tolebrutinib 60 mg in Cohort A. Up to 10 adults with multiple sclerosis who meet inclusion criteria but choose to stay on anti-CD20 therapy.

Phase: 2

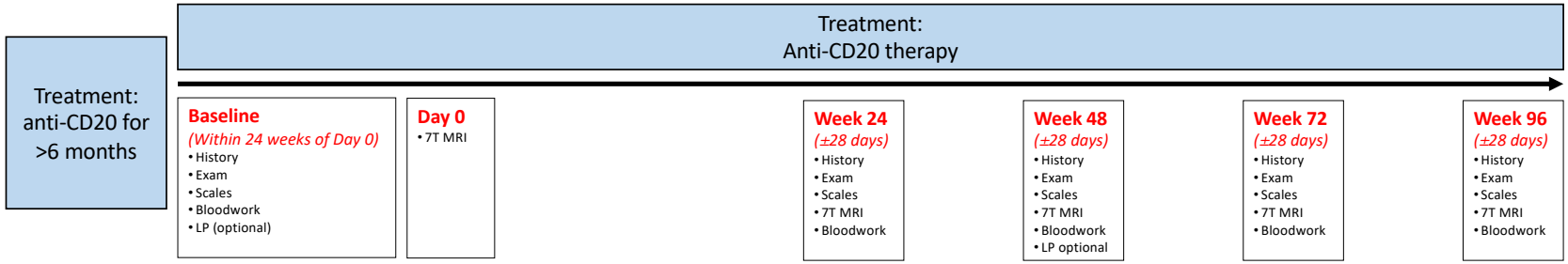
Description of Study Intervention: Oral tolebrutinib 60 mg per day for 48 weeks (Initial Cohort), and a subgroup of patients already escalated to 120 mg (Cohort A), with optional long-term extension and follow-up.

Study Duration: 5 years

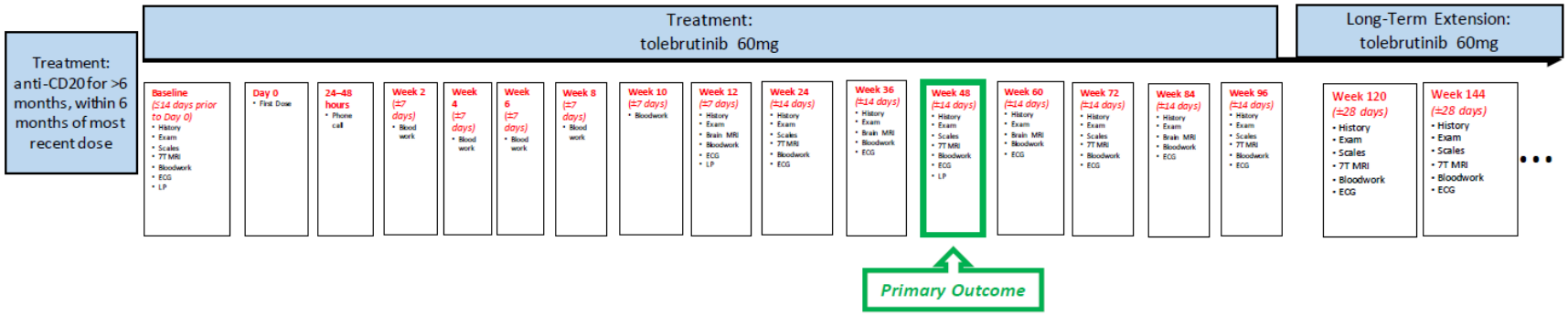
Participant Duration: 144 weeks (Cohort A) for primary and secondary outcomes, with optional long-term extension and follow-up.

1.2 SCHEMA

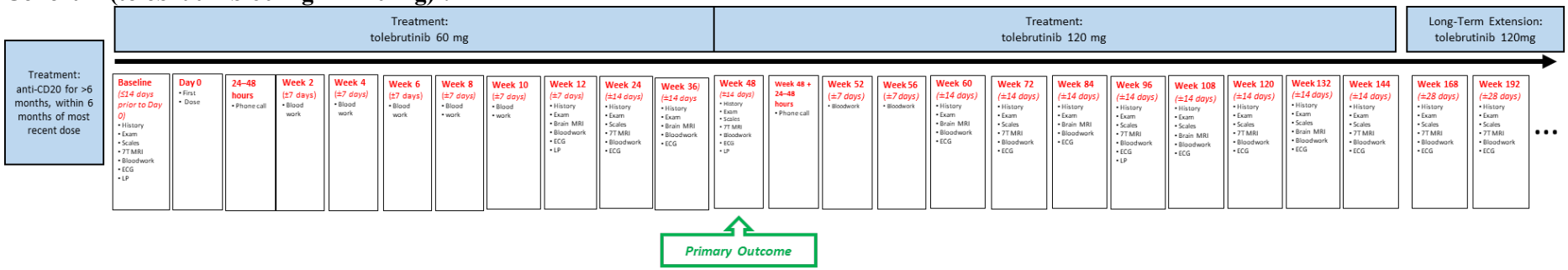
Control:



Initial Cohort (tolebrutinib 60 mg)



Cohort A (tolebrutinib 60 mg → 120 mg)[#]:



See section 6.1.2.5. Participants (n=4) in cohort A, who had initiated tolebrutinib 60mg and previously escalated to 120 mg/ day will remain at that dose and the 3 remaining participants, in the initial cohort, who initiated tolebrutinib 60mg will remain at 60mg and not be escalated to 120mg/day.

1.3 SCHEDULE OF ACTIVITIES (SOA)

Initial cohort (participants who choose not to escalate dose from tolebrutinib 60 mg):

Procedures	Baseline (≤14 days prior to Day 0)	Day 0	24 – 48 Hours Follow-Up	Week 2 (±7 days)	Week 4 (±7 days)	Week 6 (±7 days)	Week 8 (±7 days)	Week 10 (±7 days)	Week 12 (±7 days)	Week 24 (±14 days)	Week 36 (±14 days)	Week 48 (±14 days)	Week 60 (±14 days)	Week 72 (±14 days)	Week 84 (±14 days)	Week 96 (±14 days)	Every 24 weeks (±28 days)*
Informed consent	X																
Medical history	X																
Interval history/neurological exam	X								X	X	X	X	X	X	X	X	X
Vital signs	X	X							X	X	X	X	X	X	X	X	X
Clinical measures (EDSS, 9HPT, 25FTW, SNRS, SDMT)	X									X		X		X		X	X
Clinical labs	X			X[2]	X[1]	X[2]	X[1]	X[2]	X[2]	X	X[2]	X	X[2]	X	X[2]	X	X
Research labs	X								X	X	X	X	X	X	X	X	X
Pregnancy test (if applicable)	X	X							X	X	X	X	X	X	X	X	X
Brain MRI (7-tesla)	X									X		X		X		X	X
Brain MRI									X		X		X		X		
ECG	X								X	X	X	X	X	X	X	X	X
Lumbar puncture	X								X			X					
Tolebrutinib first dose		X															
Follow-up phone call			X														
Tolebrutinib study drug dispensed		X							X	X	X	X	X	X	X	X	X

*Long term extension until tolebrutinib is marketed or until its development is halted.

[1]Safety labs.

[2]Liver-specific safety labs (AST, ALT, alkaline phosphatase, total bilirubin)

Cohort A (tolebrutinib 60 mg → 120 mg)[#]:

Procedures	Baseline (≤14 days	Day 0	24 – 48 Hours Follow-Up	Week 2 (±7 days)	Week 4 (±7 days)	Week 6 (±7 days)	Week 8 (±7 days)	Week 10 (±7 days)	Week 12 (±7 days)	Week 24 (±14 days)	Week 36 (±14 days)	Week 48 (±14 days)	Week 48+24-48 hours	Week 52 (±7 days)	Week 56 (±7 days)	Week 60 (±14 days)	Week 72 (±14 days)	Week 84 (±14 days)	Week 96 (±14 days)	Week 108 (±14 days)	Week 120 (±14 days)	Week 132 (±14	Week 144 (±14	Every 24 weeks (±28 *
Informed consent	X																							
Medical history	X																							
Interval history/neurological exam	X								X	X	X	X				X	X	X	X	X	X	X	X	X
Vital signs	X	X							X	X	X	X				X	X	X	X	X	X	X	X	X
Clinical measures (EDSS, 9HPT, 25FTW, SNRS, SDMT)	X									X		X					X		X	X	X	X	X	X
Clinical labs	X			X[2]	X[1]	X[2]	X[1]	X[2]	X[1]	X	X[1]	X		X[1]	X[1]	X[1]	X	X[1]	X	X[1]	X	X[1]	X	X
Research labs	X								X	X	X	X				X	X	X	X	X	X	X	X	X
Pregnancy test (if applicable)	X	X							X	X	X	X				X	X	X	X	X	X	X	X	X
Brain MRI (7-tesla)	X									X		X					X		X		X		X	X
Brain MRI									X		X					X		X		X		X		
ECG	X								X	X	X	X				X	X	X	X	X	X	X	X	X
Lumbar puncture	X								X			X							X					
Tolebrutinib first dose at each dose level		X										X												
Follow-up phone call			X										X											
Tolebrutinib study drug dispensed		X							X	X	X	X				X	X	X	X	X	X	X	X	X

*Long term extension until tolebrutinib is marketed or until its development is halted.

[#] See section 6.1.2.5. Participants (n=4) in cohort A, who had initiated tolebrutinib 60mg and previously escalated to 120 mg/ day will remain at that dose and the 3 remaining participants in the initial cohort, who initiated tolebrutinib 60mg will remain at 60mg and not be escalated to 120mg/day.

[1]Safety labs.

[2]Liver-specific safety labs (AST, ALT, alkaline phosphatase, total bilirubin)

Comparison cohort (anti-CD20 therapy only):

Procedures	Baseline (≤14 days prior to Day 0)	Day 0	Week 24 (±14 days)	Week 48 (±14 days)	Week 72 (±14 days)	Week 96 (±14 days)
Informed consent	X					
Medical history	X					
Interval history/neurological exam	X		X	X	X	X
Vital signs	X	X	X	X	X	X
Clinical measures (EDSS, 9HPT, 25FTW, SNRS, SDMT)	X		X	X	X	X
Clinical labs	X		X	X	X	X
Research labs	X		X	X	X	X
Pregnancy test (if applicable)		X	X	X	X	X
Brain MRI (7-tesla)		X	X	X	X	X
Lumbar puncture	X			X		

2 INTRODUCTION

2.1 STUDY RATIONALE

The purpose of this protocol is to test whether short-term treatment with tolebrutinib (previously SAR442168), an investigational, orally available, brain-penetrant, Bruton's tyrosine kinase (BTK) inhibitor, affects an imaging marker (the "paramagnetic rim") associated with chronically inflamed ("chronic active" or "smoldering") white matter lesions in multiple sclerosis (MS). Clinical, radiological, and neuropathological data have indicated that such lesions are associated with, and potentially cause, long-term disability progression in MS, an aspect of the disease for which current treatments are gravely inadequate.

2.2 BACKGROUND

Section 2.2.1 begins with a general overview of the rationale for studying chronic active MS lesions and the advantages of approaching this study with ultra-high-field (7T) MRI. In Section 2.2.2, we discuss the role of BTK in MS pathogenesis. Section 2.2.3 discusses BTK inhibitors as a therapeutic class, Section 2.2.4 the BTK inhibitor tolebrutinib, Section 2.2.5 the co-treatment with anti-CD20 monoclonal antibodies, Section 2.2.6 the importance of this study, and Section 2.2.7 the rationale for our exploratory outcome measures.

2.2.1 Rationale for studying chronic active MS lesions

MS is a chronic immune-mediated disorder of the brain, spinal cord, and optic nerves.[1] Since the early 1980s,[2] MRI has been used as a valuable tool for diagnosing and monitoring MS.[3,4] Moreover, the pathognomonic focal demyelinating plaques, also termed "lesions," have been the subject of intense clinical and imaging research interest in MS.

It is widely accepted in the scientific community that newly forming demyelinating lesions are associated with inflammation and the short-term, abrupt opening of the blood-brain barrier (BBB) at the level of medium and small-caliber parenchymal veins.[5] The imaging correlate of these changes is the leakage of a peripherally injected gadolinium-based MRI contrast agent into the parenchyma surrounding the inflamed venule. In our earlier studies, we were able to image this dynamic process at high resolution, and we termed it "centrifugal enhancement."[6,7] We described subsequent changes in the BBB in capillaries and venules at the lesion's leading edge as "centripetal enhancement" (previously termed "ring" or "rim enhancement" in non-dynamic MRI acquisitions).

A reasonable hypothesis based on available data is that centripetal contrast enhancement is best considered as a manifestation of the immune response to focal inflammatory demyelination in new MS plaques. Metaphorically, this process can be thought of as a "controlled burn," in which inflammation is used to counter inflammation as part of normal wound healing.[8,9] However, such controlled inflammation might also turn detrimental. Indeed, we have evidence that inflammation of this type can sometimes result in a persistent paramagnetic rim at the lesion edge, visualizable on MRI scans generated from the phase of the signal obtained via a T2*-weighted gradient-echo pulse sequence at 7T.[10] Persistent paramagnetic rims develop more commonly in lesions that form in older people (Figure 1),[11] consistent with laboratory data suggesting that inflammatory cell- mediated remyelination becomes progressively less successful with age.[12,13] They are typically established within 3–6 months of initial demyelination and BBB opening and can be distinguished radiologically from lesions that repair. Indeed, through our in vivo and postmortem investigations, we have found that paramagnetic-rim lesions are associated with extensive intralesional tissue damage (based on measurement of the T1 relaxation time by MRI), failed repair, apparently permanent demyelination, and a more pro-inflammatory peripheral blood cytokine and chemokine profile at baseline.[11]

Over the past decade, data from our group and several additional labs have converged to indicate that the pathological basis of the paramagnetic rim is iron accumulation within activated macrophages and/or microglia at the lesion rim, which is visible on 7T MRI.[11,14,15] These rims can be long-lasting[16,17] (potentially for several decades), and pathologically they mark the class of chronically inflamed lesions known, variously, as "chronic active," "slowly expanding," or

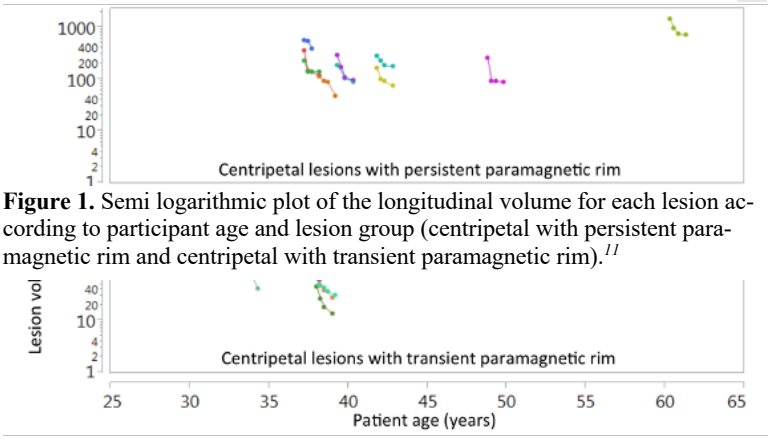
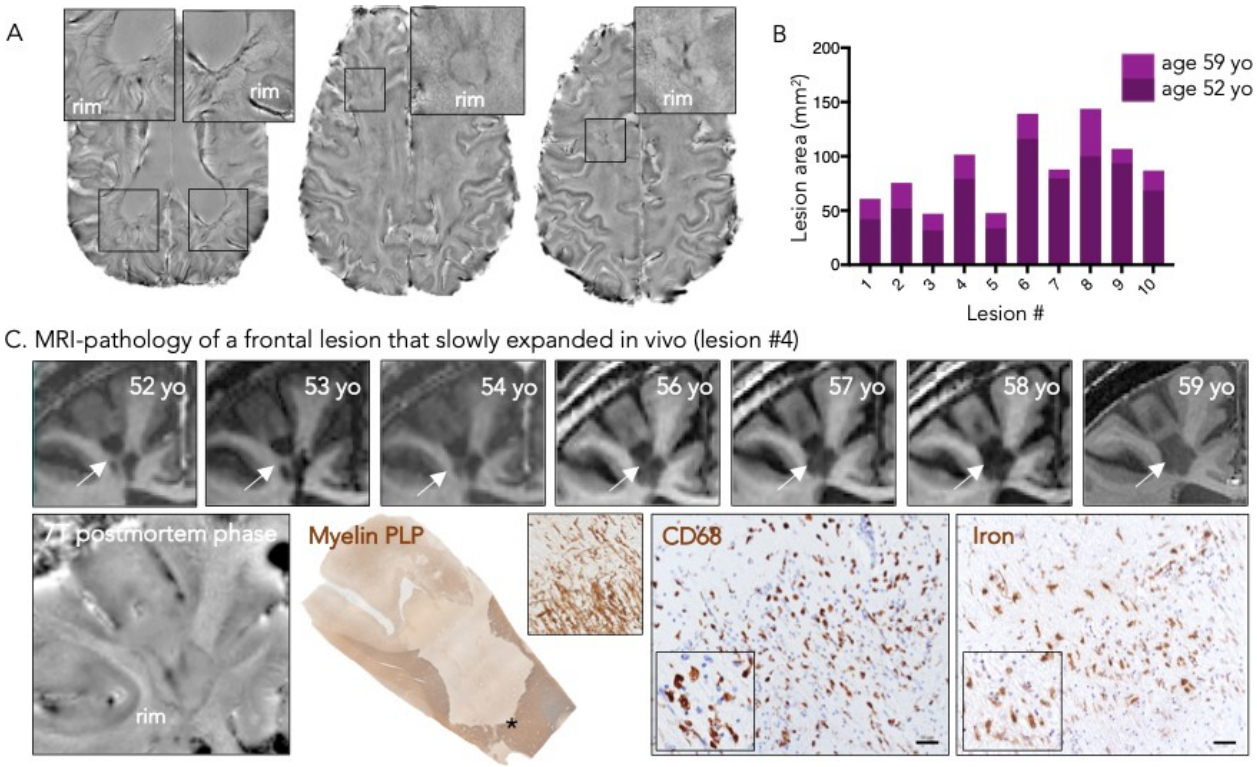


Figure 1. Semi logarithmic plot of the longitudinal volume for each lesion according to participant age and lesion group (centripetal with persistent paramagnetic rim and centripetal with transient paramagnetic rim).[11]



"smoldering" (Figure 2). Such lesions make up approximately 15–25% of white matter lesions in MS, and while they may occur even in cases of asymptomatic MS, they are substantially more common in longstanding, clinically progressive disease.[18]

Figure 2. A, In vivo 7T axial phase magnetic resonance imaging (MRI) acquired at age 59 years showing representative supratentorial rim lesions; magnified views are in the insets. B, Histogram of the 10 rim lesions analyzed by MRI and histopathology. The lesion area was measured on in vivo coregistered T1-weighted scans at ages 52 and 59 years. All 10-rim lesions expanded over 7 years. C, MRI-histopathology comparison of individual rim lesion evolution and pathology. Serial in vivo coronal T1-weighted MRI scans and relative patient age are shown in the first row of each section. Clearly expanding confluent areas are indicated by arrowheads. All expanding rim lesions were chronic-active by pathology results. An accumulation of iron-laden phagocytes (cluster of differentiation [CD] 68 and iron staining) was seen at the lesion edge (asterisks on myelin proteolipid protein [PLP] immunohistochemistry). From Absinta et al. (2019).[20]

At 7T, the intra-rater and inter-rater agreement is on the order of 85–90%, with Cohen's κ on the order of 0.75.[19] Indeed, in our recent clinical data, we showed that the majority of adults with MS have at least one rim lesion, and that those with 4 or more rim lesions reach motor and cognitive disability at an earlier age.

Moreover, whereas rimless lesions shrink over time (−3.6%/year), rim lesions are stable in size or expanded (2.2%/year; p<0.001).[20] Pathologically, rim lesions show evidence of ongoing myelin destruction at the lesion edge, even in lesions that may have been present for decades. Stopping the adverse inflammation in such lesions, potentially opening the door for remyelination, is therefore an important treatment target, for which none of the existing MS drugs is known to be effective.

2.2.2 Role of BTK in MS pathogenesis

BTK is expressed in several cell types that are known to be relevant in MS pathogenesis, most prominently B cells and macrophages.[21] Although MS and its animal model, experimental autoimmune encephalomyelitis (EAE), are thought by most to be driven by T cells (which do not express BTK), B cells have relatively recently been recognized as essential in the genesis of inflammatory demyelination, most likely by presenting antigen to T cells and/or cytokine production rather than through antibody production per se. Indeed, depleting B cells via the CD20 receptor is among the most potent MS therapies available today. Anti-CD20 therapy with ocrelizumab, a humanized anti-CD 20 monoclonal antibody, prevents >90% of new inflammatory demyelinating white matter lesions and can slow disability progression in some cases of primary progressive MS.[22,23] Moreover, studies in both EAE[24] and MS,[25] including our group's work using single-cell transcriptomic approaches,[17] have confirmed that BTK inhibition is a promising therapeutic strategy.

Immunologically, BTK is involved in cell signaling downstream of the B cell and Fcγ receptors, and the latter function is thought to be important for the action of BTK in microglia, the resident immune cells of the central nervous system (CNS) parenchyma.[26] Hence, BTK inhibition by a CNS-penetrant small molecule is a reasonable strategy for curtailing deleterious microglial activation, which occurs in chronic active white matter lesions (see Section 2.2.1). Indeed, preclinical studies, including in animal models of MS, show that BTK inhibition can alter the microglial transcriptional signature.[27] Moreover, immunohistochemical evaluation shows that some microglia at the edge of chronic active lesions express BTK (Figure 3A) as well as elevated levels of the gene encoding Fcγ receptors.[17]

In this study, the effects of BTK inhibition on B cells are potentially less relevant than the effects on myeloid cells and microglia, since we are studying patients who have been taking anti-CD20 antibody therapy and have not shown evidence of new white matter lesion formation, for at least 6 months. However, as anti-CD20 antibody therapy (which in intravenous form is dosed every 6 months) effectively depletes B cells in the periphery but is not sustained in the CNS,[28] the action of tolebrutinib on CNS B cells may also prove highly relevant. Such B cells can indeed be found in perivascular spaces surrounding chronic active lesions (Figure 3B).

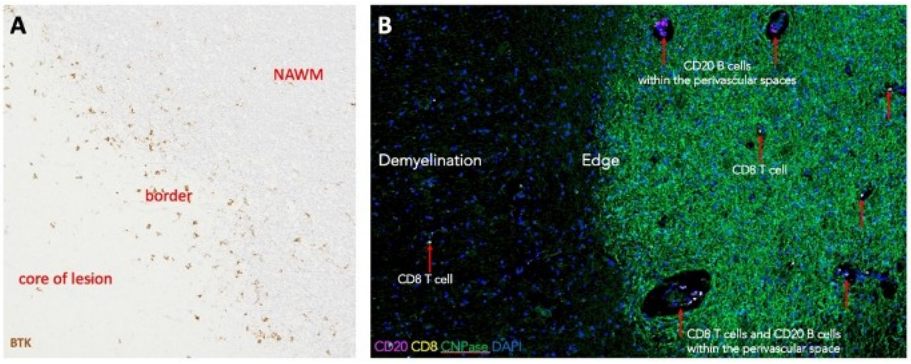


Figure 3. (A) Chromogenic immunohistochemistry showing cells at the inflammatory edge of achronic active lesion, with morphology of activated microglia, that express BTK. NAWM, normal appearing white matter. (B) Multiplex immunofluorescence at the edge of a chronic active lesion showing the presence of CD20+ B cells in perivascular spaces, mostly just outside the demyelinated area. Unpublished data, Translational Neuroradiology Section, NINDS.

Interestingly, both B cells and microglia appear to be relevant for the induction and sustenance of organized or semi-organized inflammatory foci in the leptomeninges, in part by contributing to the extensive subpial cortical demyelination that is found in many cases at biopsy and autopsy, and on MRI.[29–31] These foci are also thought to play a role in progressive MS, but they are not as well seen on MRI as

chronic active white matter lesions, and as such they will only be evaluated in an exploratory fashion in this study. Nonetheless, it is important to remember that centrally acting BTK inhibitors may have an impact on leptomeningeal foci.

2.2.3 BTK inhibitors

Existing BTK inhibitors limit activation, maturation, and proliferation of B cells and other BTK- expressing immune cells by interfering with critical signaling cascades, as discussed in Section 2.2.2. They also interfere with differentiation of B cells into antibody-secreting plasma cells. Because BTK is constantly synthesized and BTK inhibition does not deplete B cells, treatment with BTK inhibitors — even covalently binding inhibitors such as tolebrutinib — is reversible (unlike anti-CD20 antibody treatment). BTK inhibitors have received FDA approval in oncology (chronic lymphocytic leukemia, mantle cell lymphoma, and Waldenström's macroglobulinemia for ibrutinib, a first-generation BTK inhibitor, and mantle cell leukemia for acalabrutinib, a second-generation BTK inhibitor).

Well-known adverse reactions for both first- and second-generation BTK inhibitors include hemorrhage (grade 3 or higher in 2–3% of patients, thought to be related to platelet inhibition), infection (grade 3 or higher in 15–30% of patients), cytopenias (grade 3 or higher in up to 30% of patients), and atrial arrhythmias (3–4% of patients per year). It is possible that some of these adverse reactions are related to off-target effects on other kinases.

No BTK inhibitors have yet been approved for treatment of MS. In 2019, results of a double-blind, placebo-controlled Phase 2 trial in relapsing MS of a third-generation BTK inhibitor, evobrutinib, were reported.[25] The results suggested that this therapeutic strategy has similar efficacy to an FDA- approved oral disease-modifying treatment, dimethyl fumarate, with respect to reduction of MS relapses and new white matter lesions. However, unlike tolebrutinib, evobrutinib does not cross the blood-brain barrier, so its therapeutic effect most likely resulted from direct effects on adaptive immunity, and in particular B cell functioning, in the periphery. The evobrutinib trial also showed asymptomatic and reversible elevation of hepatic and pancreatic enzymes. As discussed in Section 2.2.4 , no treatment-emergent serious adverse reactions have been observed in individuals treated with tolebrutinib, despite specific monitoring.

2.2.4 Tolebrutinib

The material in this section is largely abstracted from the tolebrutinib (previously SAR442168) Investigator's Brochure (IB), 11th Edition (March 2, 2023), which is provided as an appendix to this protocol. Unless otherwise specified, quotes are taken directly from the IB.

"Tolebrutinib is an investigational, orally administered, small molecule that binds covalently to Bruton's tyrosine kinase (BTK)…. Tolebrutinib demonstrates selectivity for BTK and a few related kinases that contain a homologous cysteine residue (Cys-481)… Tolebrutinib inhibits BTK, forming an irreversible bond near the ATP-binding site, rendering the kinase non-reactive with respect to signaling…. Tolebrutinib 60 mg tablets should be administered orally once per day with food…"

2.2.4.1 *Preclinical studies*

Extensive preclinical work evaluated its safety and pharmacokinetic (PK) properties in a number of animal models, including mice, rats, dogs, and rabbits; these data are summarized on pp. 13–17 of the IB. Notable points are the following:

- Dose-dependent activity in two inflammatory models (Arthus reaction model in rats and experimental autoimmune encephalomyelitis in mice).
- No cardiovascular or respiratory effects at the highest doses in dogs.
- C_{max} reached in ~30 min in rats and ~90 min in dogs, bioavailability in the range of 20– 85%, volume of distribution on the order of 1.65 L/kg, and protein binding on the order of 85–95%.
- CSF-to-plasma ratio of ~0.3 in rats.
- Rapid metabolism with seven metabolites in all species tested (rats, dogs, humans).

- No-adverse-event-effect levels (NOAEL) at the highest doses tested in 14-day and 13-week studies in rats and dogs, 28-day studies in rats, and 9-month studies in dogs.
- In a 6-month rat study, a low-observed-adverse-effect level (LOAEL) at an exposure ~12 times higher than the human exposure at 60 mg/day in a subsequent multiple-ascending dose (MAD) trial.
- Adverse events at high doses in both rats and dogs that were consistent with known adverse effects of other BTK inhibitors and/or considered mild.
- Variable effects on pregnancy and embryos.

2.2.4.2 *Phase 1 clinical studies*

Seven Phase 1 clinical studies had been completed as of September 11, 2022. The first used an oral solution and was comprised of both single-ascending dose (SAD; N=30; doses ranging from 5–120 mg) and MAD (N=40; doses ranging from 7.5–90 mg) components. A second used a 15-mg pill formulation that was later adopted for the first Phase 2 study. A third was performed in healthy East Asian men (N=6; doses ranging from 7.5–60 mg). Findings are summarized on pp. 17–23 of the IB, with key points as follows:

- Extensive metabolism of the parent drug, with primary excretion in feces.
- Two metabolites (M2 and M5/M5a) with activity against BTK.
- Similar bioavailability of the Phase 3 tablet compared to the Phase 2b product.
- "A positive food effect was observed on tolebrutinib and M2 pharmacokinetics (PK) in moderate- and high-fat conditions with both tablets…"
- "After repeated doses of up to 240 mg under fed conditions (moderate fat), C_{max} and AUC_{last} values for tolebrutinib and M2 increased in proportion to dose, with no major deviation from dose proportionality. Overall, tolebrutinib exhibited linear PK, according to dose and time."
- At 2 hours, CSF-to-total plasma ratio of ~0.15, and CSF-to-free plasma ratio of ~2.25.
- "The most commonly reported AEs were mild diarrhea and headache… no clinically significant drug-related changes in vital signs, electrocardiography (ECG) parameters, or laboratory values…" except for a reversible ~25% decrease in platelet count in a single participant in the 60-mg MAD cohort whose platelet count was 145 x 109/L at baseline (slightly below the lower limits of normal).
- Predicted moderate PK interactions with CYP3A4 and CYP2C8 inhibitors and inducers, and ~50% reduction in tolebrutinib exposure when administered with gastric-acid reducing agents.
- Similar pharmacokinetics and safety results in East Asian men as in Western participants.

TDR16862 is a randomized, double blind, placebo-controlled study of the tolerability and pharmacokinetics of ascending 14-day repeated oral dose of tolebrutinib in healthy adult participants. Nine participants received 120 mg daily for 14 days. There were no serious adverse events or significant safety concerns at this dose, and no indication that drug-drug interactions should be modified relative to recommendations for 60 mg/day dosing.

2.2.4.3 *Phase 2 clinical study*

DRI15928 (NCT03889639) was a multicenter Phase 2b study to test the efficacy of tolebrutinib on reducing gadolinium-enhancing MRI lesions in relapsing MS. The design was double-blind, randomized, and placebo-controlled, with 4 dose levels (5, 15, 30, and 60 mg/day) of tolebrutinib for 12 weeks, which were either followed (Cohort 1) or preceded (Cohort 2) by 4 weeks of placebo. The study enrolled 130 participants ages 18–55 who had 1 relapse in the prior year, 2 or more relapses in the prior 2 years, or at least 1 gadolinium-enhancing MRI lesion in the prior 6 months.

As published,[32] and according to the IB: "The study met its primary objective, demonstrating a dose response relationship for tolebrutinib as evidenced by a reduction in the number of new active Gd-enhancing T1 hyperintense brain lesions detected by brain MRI after 12 weeks of treatment, with a statistically significant difference in the tolebrutinib 60 mg group as compared to placebo (relative risk [RR] 85.02%;

[95% CI: 28.02%, 96.88%]). Differences in the other tolebrutinib treatment groups were not statistically significant as compared to placebo. Consistently, efficacy on disease activity was also demonstrated by the reduction in the number of new and enlarging T2 lesions detected by brain MRI after 12 weeks of 60 mg tolebrutinib treatment (RR 89.34%; [95% CI: 68.39%, 96.41%]), but not so for the 5, 15, and 30 mg tolebrutinib doses. However, the data did not show a statistically significant reduction in the total count of Gd enhancing T1-hyperintense lesions after 12 weeks of tolebrutinib treatment whatever the tested dose."

In the Phase 2b, there were no deaths, and "one treatment-emergent SAE [serious adverse event] was reported in a participant in Cohort 1 treated with 60 mg SAR442168. All treatment- emergent AEs reported were of mild or moderate intensity except for one severe, treatment-emergent AE reported in the 60 mg tolebrutinib group."

Note that Dr. Daniel Reich, Principal Investigator of this protocol, was involved in discussions with Sanofi-Genzyme around the design and analysis of the Phase 2 trial of tolebrutinib, reported its results at the 2020 "virtual" annual meeting of the European Academy of Neurology (May 2020), and was the lead author of the primary article.[32] These discussions were approved as an official-duty activity in his role as Senior Investigator in the NINDS Intramural Research Program, and he has not received any remuneration for these activities. Dr. Reich's travel to one or more scientific meetings to present results from the Phase 2 study will be sponsored by Sanofi-Genzyme, consistent with NINDS procedures.

A long-term safety extension study (LTS16004; NCT03996291) is ongoing, in which participants were initially maintained on their randomized dose from the Phase 2b and subsequently migrated to the 60 mg dose used in the phase 3 clinical studies. Initial results from this study were presented at the 2021 "virtual" annual meeting of the European Committee on Treatment and Research in MS (ECTRIMS; October 2021), and Dr. Reich was the lead author on the report of MRI data. Further data have been presented at subsequent meetings, most recently the 2.5-year data at the 2023 Americas Committee on Treatment and Research in MS (ACTRIMS) Forum. These data indicate continued efficacy of the 60 mg dose against new gadolinium-enhancing lesions and general stability of paramagnetic rim lesions in the 20% of participants in whom this imaging biomarker could be measured. Note that these measurements were performed on a variety of scanners on less sensitive 3T MRI systems, so results at 7T MRI with the advanced sequences used in this protocol cannot be fully extrapolated.

2.2.4.4 *Phase 3 clinical studies*

Sanofi-Genzyme is sponsoring four Phase 3 multicenter registrational clinical trials of tolebrutinib, two in relapsing-remitting MS (GEMINI 1, NCT04410978; GEMINI 2, NCT04410991) and two in progressive MS (HERCULES, NCT04411641; PERSEUS, NCT04458051). Dr. Reich serves on the steering committee for HERCULES and PERSEUS as part of his official duties as Senior Investigator in the NINDS Intramural Research Program. He has not received, and will not receive, any remuneration for these activities.

2.2.5 Co-treatment with anti-CD20 monoclonal antibodies

Since tolebrutinib treatment will be initiated within 6 months of each participant's most recent infusion of anti-CD20 monoclonal antibodies, which are normally given every 6 months and cause B cell depletion that persists even longer, there will be a period, lasting approximately 3–6 months, during which where both treatments are effectively combined. The effects of anti-CD20 are expected to gradually wane, such that by the second half of the 96-week duration of the primary study, participants will for all intents and purposes be taking tolebrutinib monotherapy. Samples for B cell counts are collected at various time points during this protocol.

Although no prior studies have examined specific co-treatment of tolebrutinib with anti-CD20 monoclonal antibodies, there is substantial published data that co-treatment with first-generation BTK inhibitors (such as ibrutinib) and anti-CD20 monoclonal antibodies (in particular, rituximab) is safe.

- In a Phase 2 study (NCT01520519) of 40 patients with high-risk chronic lymphocytic leukemia

(CLL), 6 cycles of ibrutinib 420 mg plus rituximab 375 mg/m2, followed by continuous ibrutinib, 13% had grade 3 infections, none had grade 4 or grade 5 infections, and only 1 had grade 4 neutropenia.[33]

- A Phase 3 study (NCT01611090) of 578 patients with CLL or small lymphocytic leukemia (SLL) treated with a standard-of-care therapy, rituximab together with bendamustine, with the addition of ibrutinib 420 mg or placebo. At the 17-month interim analysis, progression- free survival was improved in the ibrutinib group (hazard ratio 0.23, $p<0.0001$), and safety signals were similar in both groups.[34]
- A similar study (NCT02048813) subsequently randomized 529 patients with previously untreated CLL or SLL to either the same regimen of ibrutinib plus rituximab followed by ibrutinib alone or standard-of-care fludarabine, cyclophosphamide, and rituximab chemoimmunotherapy, again showing improved progression-free survival (hazard ratio 0.35, $p<0.001$) similar overall adverse event rate in the two groups, and fewer grade 3 infections in the ibrutinib plus rituximab group.[35]
- Another study (NCT02007044) compared 420 mg of ibrutinib to ibrutinib together with rituximab in 208 CLL, with no difference in efficacy and similar rates of grade 3/4 treatment-emergent adverse events in both groups.[36]
- Most recently, a Phase 2 study (NCT02689141) enrolled 66 patients with CLL who were treated with bendamustine followed by ibrutinib together with ofatumumab, a humanized monoclonal anti-CD20 antibody that is also under investigation for treatment of MS; no unexpected safety signals were reported in this open-label study.[37]
- On the basis of NCT02048813, the FDA on April 20, 2020 approved the combination of ibrutinib and rituximab for the treatment of CLL or SLL.

2.2.6 Importance of this study

Currently, no existing treatment for MS is known to impact chronically inflamed (also known as "chronic active," "slowly expanding," or "smoldering") white matter lesions. In these lesions, in which inflammation may persist for decades, there is substantial destruction of the neuropil, and, as such, these lesions have been proposed to be part of the basis for clinical progression.[20,38] Activated macrophages/microglia are the primary inflammatory cell type in chronic active lesions, and modulation of these cells might therefore allow a resetting of wound repair mechanisms, thus preventing ongoing tissue damage and potentially allowing endogenous remyelination.

Thus, we postulate that tolebrutinib will inhibit the pathogenic activities of macrophages/microglia at the edge of chronic active lesions, thereby arresting further tissue damage within these lesions. Based on published data (discussed above) that co-treatment with first-generation BTK inhibitors and anti-CD20 monoclonal antibodies is safe, we will enroll patients who have been treated with an FDA-approved, anti-CD20 therapy for at least 6 months and are within 6 months of their most recent dose. We will also enroll a comparison cohort of participants who meet inclusion criteria but choose to stay on anti-CD20 therapy, and who are followed at similar time points. Finally, we will restrict the study to patients with clinically progressive MS, or stable MS, which reflects the current lack of good treatment options to arrest or reverse disability in MS. Data from this study will also provide novel information on safety and tolerability of long-term (96 week) dosing of tolebrutinib 120 mg daily[*], as previously only 14-day dosing has been trialed. Vaccination- and COVID-related treatment gaps in anti-CD20 therapy prior to enrollment are not exclusionary. In addition to monitoring the effect of tolebrutinib on chronic active lesions, we will collect safety and tolerability measures, as well as pharmacodynamic markers of macrophage/microglial modulation in blood and CSF.

[*] See Section 6.1.2.5

2.2.7 Rationale for outcome measures

7-tesla MRI. As described in Section 2.2.1, 7T MRI can detect iron deposition within activated macrophages/microglia at the edge of chronic active white matter lesions, which are the ultimate target of the therapy studied in this protocol. 7T MRI is also highly sensitive to the degree of parenchymal damage within lesions, and high-resolution data acquisition allows measurement of lesion size that is more accurate than at lower magnetic field strengths. The availability of 7T MRI therefore makes it possible to assess the effects of novel therapies on such lesions.

Safety and tolerability. Safety and tolerability will be assessed through patient interview and logging of adverse events (AE), as well as by lab work and imaging. Since tolebrutinib has not previously been used jointly with anti-CD20 antibody therapy in the treatment of MS, and it is not known if this combination could unexpectedly exacerbate disease inflammatory activity or predispose to development of opportunistic infections, safety monitoring by MRI will be interspersed between the 7T visits, with gradually decreasing frequency.

Blood and CSF biomarkers. We will collect blood and CSF samples for exploratory analysis of other potential biomarkers of tolebrutinib's efficacy. Techniques to be used may include (but are not limited to) flow cytometry immunophenotyping, cytokine/protein assessments, and single- cell RNA sequencing. Specific molecules to be assessed may include (but are not limited to); IL- 1β, IL-2, IL-6, IL-10, IL-12, IL-18, TNF, IFNγ, IP-10/CXCL10, soluble CD27, CHI3L1/YKL-40, neopterin, and neurofilament light chain. Lumbar puncture is optional in the anti-CD20-only cohort.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 Known Potential Risks

The indented material is quoted from edition 11 of the IB for tolebrutinib:

> The safety profile is characterized based on tolebrutinib nonclinical data, Phase 1 results from healthy participants, Phase 2b results from adult participants with RMS, the ongoing Phase 3 studies in RMS, nr-SPMS, PPMS, and gMG, and from published data of an evobrutinib clinical study in the RMS indication.[25] It also takes into account 3 approved BTK inhibitors (acalabrutnib, ibrutinib, and zanubrutinib) in oncology indications, such as mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL)…. However, it is unclear whether the risks identified in patients with hematologic malignancies (eg, hemorrhage, infection, cytopenia, and atrial arrhythmia) will be applicable in the MS or gMG populations given the higher selectivity of tolebrutinib and the differences between the MS/gMG and oncology populations.
>
> At the current stage of clinical development, tolebrutinib is considered to have the potential risks of bleeding, infections, cytopenia (e.g., thrombocytopenia), atrial arrhythmias (atrial fibrillation and atrial flutter) and DILI including severe DILI (risk of liver transplantation or death) as an identified risk.
>
> Investigators should adhere to inclusion/exclusion criteria and participant monitoring detailed in study protocols to manage these potential risks.
>
> Data supporting the assessment of these risks as identified and potential risks for tolebrutinib are presented below.
>
> **Drug-induced liver injury as an identified risk:**
>
> Tolebrutinib nonclinical: No liver toxicity has been observed at any tested dose in any species.
>
> Tolebrutinib clinical:
>
> - Phase 1 trials: Overall, no safety signal was observed. In the drug-drug interaction study INT16385, one participant had an AESI of an elevated ALT >2 × ULN after being

administered tolebrutinib + pantoprazole which led to permanent treatment discontinuation. This participant had abnormal ALT values at screening and baseline (65 and 45 IU/L, respectively [ULN = 41 IU/L]), which rose to 94 IU/L at the end of Period 2 (tolebrutinib + pantoprazole) and peaked below 3 × ULN (117 IU/L: 2.85 × ULN). He had no associated symptoms. This Grade 2 AESI was considered related to tolebrutinib and pantoprazole by the Investigator and was noted as recovering/resolving without corrective treatment/therapy. He also had an increase in AST <2 × ULN, reported as a Grade 1 AE and an increase in blood CPK, reported as a Grade 1 AE.

- Phase 2b trials: One participant in each of the tolebrutinib 30 and 60 mg groups had a moderate (not related to the study drug) and mild (related to the study drug) event of ALT increased >3 × ULN. In both cases, the liver enzymes elevation was transient, the IMP was continued, liver enzymes returned to normal levels, and the participant completed the study. The event in the participant receiving 30 mg was assessed as moderate with no concomitant symptoms. The event in the participant receiving 60 mg was assessed as mild by the Investigator and with concomitant pruritus reported; the participant had ALT levels above the ULN (34 IU/L) at screening and randomization (48 and 50 IU/L, respectively), and ALT >3 × ULN started during the placebo run-in period (Cohort 2 participant); the ALT levels did not increase further when the participant crossed over to the tolebrutinib 60 mg group and decreased over time while the participant continued on tolebrutinib 60 mg.
- In the unblinded extension trial (LTS16004), 1 participant in the open-label period (tolebrutinib 60 mg) had an ALT increase >8 × ULN associated with an AST increase >5 × ULN and abdominal pain. Tolebrutinib was permanently discontinued, and the liver enzymes values returned progressively to normal ranges. Additional investigations were performed to rule out viral and autoimmune causes. Anti-smooth muscle antibody (ASMA) was weakly positive; however, the test was not repeated. The other tests were negative or showed past infection. This AE was assessed as related to tolebrutinib by the Investigator.
- Phase 3 trials are blinded and ongoing. Drug-induced liver injury is an identified risk in the MS population for tolebrutinib: Four probable DILI cases characterized by liver enzyme elevations of >3 × ULN accompanied by TBILI >2 × ULN have been identified. The cases were detected and managed according to protocol monitoring and increased ALT algorithm guidelines. Symptoms and laboratory abnormalities were reversible upon tolebrutinib discontinuation.
- Potential confounding factors have been identified in 3 of the 4 cases.
- A late-breaking case of severe DILI which resulted in liver transplantation and death has occurred. The IMP was discontinued on Day 43. The participant presented on Day 44 with liver transaminase and TBILI elevations with a concurrent increase in ALP and subsequently developed elevated INR, eventually leading to liver transplantation on Day 97. The disease course was further complicated by renal failure and infectious processes, ultimately leading to death on Day 153. Potential confounders include obesity and a positive ASMA (low titer); repeat ASMA testing was negative. Of note, the initial liver biopsy revealed an underlying inflammatory etiology, and an initial improvement in INR values was observed during steroid administration (Days 70 through 96).

Evobrutinib: In the published data for the Phase 2b study, a few participants with elevations in ALT and AST were asymptomatic, the elevations were reversible, and no cases met the criteria of Hy's law for DILI, as defined by the Food and Drug Administration. Most of the discontinuations (12 of 213 [5.6%]) were due to protocol-mandated withdrawals for elevations in levels of ALT and AST, across evobrutinib-treated patients.

Zanubrutinib: There is a case report of the first case of severe liver injury due to zanubrutinib, a BTK inhibitor that has been recently licensed in refractory mantle cell lymphoma and under assessment in Phase 3 clinical trials for other B-cell malignancies. A participant aged between 18 and 65 years with a history of relapsed lymphoplasmacytic lymphoma was admitted to hospital with new onset jaundice, coluria, and pruritus for 10 days. The participant had been receiving zanubrutinib as part of a clinical trial for 30 months. The participant's blood profile showed a severe hepatocellular injury with jaundice (ALT 2474 IU/L and TBILI 141 μmol/L with mild coagulopathy). The participant had an extensive work up including virology, autoimmune, and metabolic profiles in addition to abdominal ultrasound with no alternative explanation found for the liver injury. Zanubrutinib-induced liver injury was suspected, and causality assessment by the updated Roussel Uclaf Causality Assessment Method score showed a probable causal relationship with zanubrutinib. The participant's liver histology was also consistent with DILI. The participant's liver biochemistry improved following cessation of zanubrutinib and normalized after 8 weeks. This case raises clinical awareness regarding zanubrutinib-induced liver toxicity and the importance of drug withdrawal in the event of liver injury.

Bleeding (hemorrhage):

Tolebrutinib nonclinical: Hemorrhage in the eye, pancreas, nasal cavity, and mesenteric lymph nodes was observed in rats at ≥2 mg/kg/day for 6 months. Minimal hemorrhage in the retropharyngeal lymph nodes, stomach, colon, ileum, testis and/or kidney was frequently associated with pigment consistent with hemosiderin was observed in dogs at ≥1 mg/kg/day for 9 months. The effect in dogs was not considered adverse due to the mild severity of findings, and the lack of impact on the health and wellbeing of the animals. There were no corresponding effects on erythron parameters or platelet counts in either species.

Tolebrutinib clinical:

- Phase 1 trials: Two events of vascular access site bruising (1 in the tolebrutinib 60 mg group and 1 in the tolebrutinib 90 mg group) and 2 events of petechiae in the tolebrutinib 60 mg group were reported in the MAD study. No hemorrhagic events were reported in the Study PRN2246-002 or in the other completed Phase 1 studies (TDU16117 and BEX16018).
- Phase 2b trial: One event of mild petechia in a female participant (at Week 8 in the tolebrutinib 30 mg group) and 2 events of mild microscopic hematuria in 2 male participants (1 event at Week 16 in the tolebrutinib 30 mg group and 1 event on Day 1 in the tolebrutinib 60 mg group, with occult blood noted in urine) were reported during the treatment period in the tolebrutinib Phase 2b DRI15928 trial. The hematology results were clinically insignificant for all 3 participants from the onset of the events. The participant with mild petechia had benign pigmentary lesions noted during screening, and the event was assessed as related to the study by the Investigator. Details of the anatomic location and clinical presentation of the 2 skin conditions were unavailable. The 2 events of mild microscopic hematuria were assessed as unrelated to the study drug. All 3 events resolved spontaneously.
- Phase 3 trials (ongoing): No pattern of bleeding events has been identified during the MS and MG Phase 3 trials. Nine serious total bleeding events of 3399 (0.26%) in the Phase 3 program were considered unrelated to the IMP by the Investigator. For MS participants' non-serious bleeding events, the majority of events were petechiae, ecchymoses, and menstrual bleeding disorders. For the gMG study, 1 non-serious case of bruising was assessed as related to background prednisolone therapy. Importantly, no bleeding event is related to decreased liver synthetic function or thrombocytopenia.

Evobrutinib (development stage BTK inhibitor): No hemorrhagic events have been reported in the

published data of Phase 2b clinical trial in the RMS indication.

Acalabrutnib: Serious hemorrhagic events, including fatal events, have occurred in the combined safety database of 612 patients with hematologic malignancies treated with acalabrutinib monotherapy. Grade 3 or higher bleeding events, including gastrointestinal, intracranial, and epistaxis, have been reported in 2% of patients. Overall, bleeding events, including bruising and petechiae of any grade, occurred in approximately 50% of patients with hematological malignancies.

Ibrutinib: Fatal bleeding events have occurred in patients treated with ibrutinib. Grade 3 or higher bleeding events (intracranial hemorrhage [including subdural hematoma], gastrointestinal bleeding, hematuria, and post procedural hemorrhage) have occurred in 6% of patients, with fatalities occurring in 0.3% of 1124 participants exposed to ibrutinib in clinical trials. Bleeding events of any grade, including bruising and petechiae, occurred in 44% of patients treated with ibrutinib.

Zanubrutinib: Fatal and serious hemorrhagic events have occurred in patients with hematological malignancies treated with zanubrutinib monotherapy. Grade 3 or higher hemorrhage including intracranial and gastrointestinal hemorrhage, hematuria and hemothorax have been reported in 3.4% of patients treated with zanubrutinib monotherapy. Hemorrhage events of any grade, excluding purpura and petechiae, occurred in 35% of patients.

[39]The mechanism for the bleeding events in this class of products is not well understood, with a potential mechanism being the inhibition of collagen-mediated platelet activation, spreading, and aggregation in vitro; however, prolonged bleeding was not observed in a model of bleeding.[40]

To mitigate this risk, selection criteria exclude participants with low platelet levels. In addition, antiplatelet or anticoagulant therapies are not allowed at inclusion and in concomitant use during the studies. Platelet counts will be monitored regularly. Treatment with IMP is to be withheld before and after surgery.

Infections:

Tolebrutinib is an immunomodulator of B cells and innate immune cells, which could increase the risk of infection.

Tolebrutinib nonclinical: No infection was observed at any tested doses in any species. In a 6-month toxicity study in rats, TDAR findings of decreased IgG and IgM response to antigen were observed at ≥2 mg/kg/day tolebrutinib.

Tolebrutinib clinical:

- Phase 1 trials: No significant infection occurred. Common cold occurred with no apparent relationship to dose in Study PRN2246-001. No significant infections were reported in the PRN2246-002 Study.
- Phase 2b trial: No significant infections occurred. The most frequently reported infections (≥3 events total) during the tolebrutinib treatment period were upper respiratory tract infection (2 in the tolebrutinib 5 mg group, 2 in the tolebrutinib 15 mg group, 1 in the tolebrutinib 30 mg group, and 1 in the tolebrutinib 60 mg group), nasopharyngitis (1 in the tolebrutinib 5 mg group, 1 in the tolebrutinib 30 mg group, and 3 in the tolebrutinib 60 mg group), gastroenteritis (1 in the tolebrutinib 5 mg group and 2 in the tolebrutinib 60 mg group), and respiratory tract infection (1 in the tolebrutinib 15 mg group, 1 in the tolebrutinib 30 mg group, and 1 in the tolebrutinib 60 mg group). Total infection incidence was below 30% without evidence of dose dependence.
- Phase 3 trials (ongoing): No pattern of infection safety events has been identified during the ongoing blinded MS and gMG Phase 3 trials. The overall incidence of infection events for the tolebrutinib-treated MS population was 14 of estimated 1956 (by randomization

ratio, 0.7%). Importantly, 6 of these 14 events (ie, 6 of 1956 [0.3%]) were COVID-19 related during the pandemic. In comparison, review of blinded infection events reveals 57 of 3548 (1.6%) with various bacterial, fungal, and viral infections (serious and non-serious), of which 26 (0.8%) were again COVID-19 related. Therefore, preliminary data do not indicate an increased risk of infection or COVID-19 outcomes during the tolebrutinib MS development program. Furthermore, no incidence of hepatitis-B-virus (HBV)-reactivation-induced liver injury has been reported.

Evobrutinib: Nasopharyngitis was reported as the most frequent AE in the Phase 2b study. Some cases of upper respiratory and urinary infections were reported. Incidence of all infection was 20% to 30% with no evidence of dose dependence.

Ibrutinib: Fatal infections have occurred. Grade 3 or greater infections occurred in 14% to 29% of patients. Progressive multifocal leukoencephalopathy (PML) and Pneumocystis jiroveci pneumonia have occurred.

Acalabrutnib: Grade 3 or higher infections occurred in 18% of patients, most frequently reported event was pneumonia. Hepatitis B virus reactivation and PML have occurred.

Zanubrutinib: Fatal and serious infections (including bacterial, viral, or fungal) and opportunistic infections have occurred in patients with hematological malignancies treated with zanubrutinib monotherapy. Grade 3 or higher infections occurred in 27% of patients, most commonly pneumonia. Infections due to HBV reactivation have occurred.

Collection of AEs throughout the study is considered adequate to detect infections. Participants will be informed of this risk while taking informed consent and will be instructed to contact Investigators immediately in case of symptoms.

Cytopenia including thrombocytopenia:

Tolebrutinib nonclinical: No cytopenia has been observed at any tested dose in any species.

Tolebrutinib clinical:

- Phase 1 trials: One participant in the 60 mg Phase 1 MAD cohort of Study PRN2246-001 developed decrease platelet counts that led to the unblinding of the treatment. The participant's platelet count was decreased from 145 × 10^9/L at baseline (reference range: 150 to 450 × 10^9/L) to 122 × 10^9/L at Day 7 after dosing, and subsequently decreased to 105 × 10^9/L at Day 9. The participant did not receive the study treatment at Day 10, and platelet level was 118 × 10^9/L at Day 10. The event of decreased platelet count was resolved, and platelet levels recovered 7 days after the last dose with platelet counts of 142 × 10^9/L. No bleeding AEs were reported in this participant. Other AEs reported in this participant included bloating, headache, abrasion of abdomen, abdominal bloating, and redness on sites of ECG electrodes. No significant cytopenias, including thrombocytopenia and neutropenia were reported in the PRN2246-002 Study.
- Phase 2b trial: No significant cytopenia including thrombocytopenia and neutropenia were reported or detected through lab results review.
- Phase 3 trials (ongoing): No evidence of significant cytopenias (including thrombocytopenia, neutropenia, and lymphopenia) has been identified during the MS and gMG Phase 3 trials. Mitigation includes clinical hematology laboratory assessments the thrombocytopenia algorithm in each protocol.

Evobrutinib: Low lymphocyte count was reported in 1 participant from the 75 mg twice daily group in the Phase 2b study.

Ibrutinib: Grade 3 or 4 cytopenias occurred including neutropenia (13% to 29%), thrombocytopenia (5% to 17%), anemia (0% to 13%).

Acalabrutinib: Grade 3 or 4 cytopenias occurred, including neutropenia (23%), anemia (11%) and thrombocytopenia (8%) based on lab measurements.

Zanubrutinib: Grade 3 or 4 cytopenias, including neutropenia (26%), thrombocytopenia (11%) and anemia (8%) based on laboratory measurements, developed in patients treated with zanubrutinib monotherapy. Grade 4 neutropenia occurred in 13% of patients, and Grade 4 thrombocytopenia occurred in 3.6% of patients.

[41]Regular hematology monitoring will be performed during the study to mitigate cytopenia risks.

Atrial arrhythmia (atrial fibrillation and atrial flutter):

Tolebrutinib nonclinical: No clinically relevant signals. Tolebrutinib inhibited hERG potassium channel tail currents with an IC_{50} of 9.1 μM. For context, the C_{max} for tolebrutinib at the highest clinical dose tested to date (120 mg) was 71 nM. No effect on the cardiovascular system was observed in a telemetered study in conscious dogs.

Tolebrutinib clinical:

- Phase 1 and Phase 2b trials: No clinically significant cardiac arrhythmias were observed. PCSAs [potentially clinically significant abnormalities] related to ECGs (eg, heart rate <50 beats/min, heart rate >90 beats/min, heart rate >100 beats/min, PR >200 msec, QRS >110 msec, QTc Bazett >450 msec, QTc Fridericia >450 msec) were reported in a small number of participants. These PCSAs were isolated occurrences and no dose relationship was observed.
- Phase 3 trials (ongoing): No pattern of atrial arrythmia safety events has been seen in the MS and gMG Phase 3 trials. To date, only 1 blinded case with atrial arrythmia has been identified at Day 5 post-seizure…. A participant aged between 18 and 64 years with history of RMS and seizure disorder presented on Day 5 to an emergency room with dizziness, sudden seizure, and atrial flutter (CTCAE Grade 2) noted on ECG. The participant was prescribed diltiazem for atrial flutter event. The participant returned on Day 18 in normal sinus rhythm. The Investigator deemed the atrial flutter event as nonserious, an AESI, and not related to IMP. The IMP was continued without interruption.

Evobrutinib: In the Phase 2b study, no cardiac arrhythmia was observed.

Ibrutinib: In a pooled data review,[42] atrial fibrillation was the most common AE that led to discontinuation. In pivotal trials atrial fibrillation was observed in 5% to 7% of ibrutinib patients, compared <1% to 2% of the comparator groups. Meta-analysis showed that the atrial fibrillation rate was 3.3/100 person-year over a median follow up of 26 months (control: 84/100 person-years). Prevalence over 10-year retrospective data was 6.1%.

Acalabrutinib: Atrial fibrillation/flutter of any grade occurred in 3% of patients, and Grade 3 atrial fibrillation/flutter occurred in 1% of patients.

Zanubrutinib: Atrial fibrillation and atrial flutter were reported in 3.2% of patients treated with zanubrutinib monotherapy. Patients with cardiac risk factors, hypertension and acute infections may be at increased risk. Grade 3 or higher events were reported in 1.1% of patients treated with zanubrutinib monotherapy.

The mechanism of atrial arrhythmia is poorly understood. No clinically significant cardiac arrhythmias have been observed to date with tolebrutinib. Monitoring with regular ECGs is scheduled. Furthermore, to mitigate the risk of cardiac arrhythmia, the selection criteria exclude participants

with abnormal ECG values that are judged of clinical significance for participation in this study.

[2525]With respect to drug interactions, the following material is quoted from edition 11 of the IB:

Effects of interacting drugs on tolebrutinib for CYP inhibitors/inducers

Based on the PK results from the drug-drug interaction studies INT16726 and INT16385 with tolebrutinib and M2 data and based on their corresponding PK exposure in the MAD study TDR16862 at up to 240 mg under fed conditions with corresponding satisfactory safety/tolerability data, concomitant use of tolebrutinib at 60 mg daily under fed conditions with drugs is updated as follows:

- All CYP3A inhibitors are allowed, because the interaction of the potent CYP3A4 inhibitor itraconazole produced a mild interaction on tolebrutinib and its active metabolite M2, and the exposures of tolebrutinib and M2 after co-administration of the 60 mg Phase 3 tablet under fed conditions with itraconazole remained below their exposures at 240 mg once daily, a safe and well tolerated dose.
- Coadministered potent CYP2C8 inhibitors should be avoided in order that tolebrutinib exposures remain below their exposures at 240 mg once daily. Moderate and weak CYP2C8 inhibitors are allowed.
- Potent and moderate CYP3A inducers (being also CYP2C8 inducers) should be avoided, as they may decrease the combined exposures of active moieties tolebrutinib and M2 by more than 50%.

Recommendations for comedication with gastric pH modifiers (proton pump inhibitors, H2-receptor blockers, and antacids)

Based on the drug interaction of pantoprazole (40 mg twice daily) toward tolebrutinib (60 mg under fed conditions) showing no effect on AUC, with a mild decrease on C_{max} (34% to 39%), coadministration of pump proton inhibitors, H2-receptor blockers, and antacids are allowed.

The mild decrease in C_{max} is considered to be not clinically relevant. The cumulative AUC up to t_{max} when tolebrutinib was co-administered with a proton pump inhibitor was similar to that of tolebrutinib alone, as the ratios of cumulated AUC up to t_{max} were close to 1 for tolebrutinib and M2.

Effects of tolebrutinib on interacting drugs

The risks of interaction of tolebrutinib as a perpetrator by CYP inhibition and induction and transporter inhibition were assessed for tolebrutinib and its main metabolites (M2, M5/M5a, M8, M10, and M18, for which exposure of the metabolites represented >100% of the exposure of the parent compound. The risk of interaction was assessed using a static approach and PBPK modeling following the FDA Guidance on in vitro Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions (January 2020)/(EMA Guideline on the investigation of drug interactions, 2012). The drug-drug interaction risk assessment of tolebrutinib, taking into account abundant metabolites compared to parent drug has been completed, and no PK interactions toward other drugs are predicted in vivo.

With respect to carcinogenesis, the following material is quoted from edition 11 of the IB:

Carcinogenicity studies have not been conducted to date.

> Tolebrutinib was negative for genotoxic potential in the Ames test and the in vivo micronucleus test in rats. Increased structural aberrations were observed in the in vitro chromosome aberration test at concentrations that produced substantial cytotoxicity.
>
> Completed developmental and reproductive toxicity studies provide support for use of tolebrutinib at doses up to 60 mg. There were no effects on fertility or early reproductive performance in rats. tolebrutinib exposure ratios at the embryo-fetal NOAEL were 74 times higher in rats and 193 times higher in rabbits compared to the human exposure at 60 mg in DRI15928 (49.8 ng.h/mL).

With respect to overdose, the following material is quoted from edition 11 of the IB:

> No symptomatic overdose was reported in the completed or ongoing clinical trials as of the data cutoff date.

Although dual therapy with earlier BTK inhibitors and anti-CD20 antibodies has been studied in oncology, there are no prior reports of this strategy in MS. Because anti-CD20 therapy depletes B cells in the periphery, the BTK inhibition in this study will mostly affect myeloid cells, at least initially after the treatment switch. The potential risk from the dual therapy in MS is unknown.

There is unknown risk of chronic dosing with tolebrutinib 120 mg/day, as this dose has only been given previously for up to 14 days. The lack of serious adverse events for 14-day dosing and the ongoing safety data from longer-term dosing at 60 mg, together with monitoring every 12 weeks in this protocol, mitigate this risk.

Participants who elect not to participate in the study will receive ongoing routine care for their MS. They can continue to be followed in NIH natural history protocols and may be eligible for other clinical trials at NIH or elsewhere.

2.3.2 Known Potential Benefits

Participants (i.e., adults who become unable to consent and participants who receive tolebrutinib) may benefit from participation if tolebrutinib at 60 or 120 mg daily is able to decrease or resolve chronic inflammation in MS, as such inflammation may play an important role in disease progression. They may also benefit through protection against development of new lesions, as suggested in the Phase IIb study in relapsing MS and its long-term extension. Participants in the anti-CD20-only arm may benefit from closer disease monitoring and surveillance. Although the number of participants is small, and clinical measures of MS progression are highly variable, MS relapse and disability scale data will be collected and analyzed. If we learn information during this study that may be important for a specific participant's health, we will share that information with the participant and possibly with the participant's outside medical provider(s). Finally, data from this study may be useful for designing longer-term and larger studies of higher-dose tolebrutinib in MS.

2.3.3 Assessment of Potential Risks and Benefits

Risks to participants, including those that derive from the novel dual treatment with anti-CD20 antibody therapy and BTK inhibition, are mitigated by strict adherence to the inclusion/exclusion criteria, frequent monitoring using MRI scans, laboratory work, and ECGs, as well as the DILI monitoring plan and hepatology assessment committee (Appendix 2). With these measures in place, the safety profile of tolebrutinib in prior clinical studies is promising. Therefore, since there are no approved treatments for control of chronic neuroinflammation in MS, nor are there studies of the effect of BTK inhibition on chronic active MS lesions assessed using 7T MRI, the potential benefits outweigh these risks.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To evaluate the effects of 48 weeks of tolebrutinib 60 mg treatment on the paramagnetic rim of chronically inflamed white matter lesions, as seen on 7-tesla MRI.	Per-participant proportion of lesions in which the paramagnetic rim has disappeared at the end of 48 weeks of tolebrutinib 60 mg treatment.	Paramagnetic rims indicate the presence of inflammation and ongoing demyelination and axonal transection at the lesion edge. We have never observed complete disappearance of a chronic rim over the course of months, and the lesions under study will have been present for at least 6 months.
Secondary		
To assess safety and tolerability of 96 weeks of tolebrutinib 60 mg (initial cohort), or 48 weeks of treatment with tolebrutinib 60 mg and 96 weeks of treatment with tolebrutinib 120 mg (Cohort A), all following anti-CD20 antibody therapy.	Tabulation of adverse events.	There are no prior data regarding the specific combination of tolebrutinib and anti-CD20 antibodies, or of BTK inhibitors and anti-CD20 antibodies in MS.
To assess the possible repair of chronically inflamed white matter lesions in which inflammation at the lesion edge has been modulated by tolebrutinib.	Changes in T1 relaxation time within paramagnetic rim lesions at the end of 96 weeks of tolebrutinib 120 mg, relative to non-rim lesions. The analysis of T1 relaxation time changes will be performed by analysis of variance or mixed-effects models in study completers. A threshold p-value of 0.025 will be taken as a significant change.	A reduction of the T1 relaxation time would be compatible with lesion repair.
To assess the possible repair of chronically inflamed white matter lesions in which inflammation at the lesion edge has been modulated by tolebrutinib.	Changes in size of paramagnetic rim lesions at the end of 96 weeks, relative to non-rim lesions. The analysis will be performed by analysis of variance or mixed-effects models in study completers. A threshold p-value of 0.025 will be taken as a significant change.	A reduction in lesion size could indicate lesion repair.
Tertiary/Exploratory		

To assess the time course of an effect of tolebrutinib on the paramagnetic rims of chronically inflamed white matter lesions.	Proportion of paramagnetic rim lesions in which the rim has diminished or disappeared at any time point, assessed by analysis of variance or mixed-effects models, which naturally handle missing data.	The time course of any effect of tolebrutinib on chronic neuroinflammation is unknown.
To obtain clinical data relevant to the efficacy of tolebrutinib.	Expanded Disability Status Scale (EDSS), 9-hole peg test (9HPT), 25-foot timed walk (25FTW), Scripps Neurological Rating Scale (SNRS), and symbol digit modalities test (SDMT), at baseline and every 24 weeks thereafter.	These are standard disability scales that are used in the assessment of clinical progression in MS.
To obtain additional imaging data relevant to the efficacy of tolebrutinib.	Characteristics, including persistence, of paramagnetic rims at all time points (qualitative). Additional MRI measures to be determined. These analyses will be performed in NIH labs or through collaboration, either intramural or extramural.	To assess imaging correlates of reduced chronic neuroinflammation and/or tissue repair.
To obtain laboratory data relevant to the safety and efficacy of tolebrutinib.	Immune cell populations (e.g., macrophages, lymphocytes, and subtypes thereof), cytokines (e.g., IL- 1ß, IL-6), and protein and other biomarkers of neuroinflammation and neurodegeneration (e.g., neurofilament light chain, chitinase-3-like-1). Additional assays may also be developed and performed, such as the rate of repopulation of CD19+ cells. These analyses will be performed in NIH labs or through collaboration, either intramural or extramural.	To assess laboratory correlates of reduced chronic neuroinflammation and/or tissue repair.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a single-site, Phase 2, two-arm, baseline-to-treatment trial to test the hypothesis that 48 weeks of treatment with tolebrutinib 60 mg, and a subgroup of patients escalated to 120mg, on an investigational, orally available, brain-penetrant, BTK inhibitor, can modulate an imaging marker (the "paramagnetic rim") associated with chronically inflamed ("chronic active" or "smoldering") white matter lesions in MS patients under stable treatment with anti-CD20 antibodies. The trial is open-label, and bias will be minimized by analysis of the primary and MRI-based secondary outcomes blinded to time point. No interim analysis, stratification, or sub-study is planned due to the small size of this study and the value of obtaining a complete dataset for exploratory analysis and guiding of future therapeutic development and clinical trial design. A comparison cohort of individuals who meet enrollment criteria (other than tolebrutinib contraindications) but choose not to take tolebrutinib will be enrolled and followed. Enrollment of the first 2 participants in the tolebrutinib cohort will be staggered; subsequent enrollment can proceed in parallel.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Natural history data (both published and unpublished) indicate that in the absence of treatment, including treatment with anti-CD20 antibodies, paramagnetic rims are stable over periods substantially longer than the 96-week treatment duration in this study. Indeed, in unpublished work from the NIH, only 1 out of 29 individuals experienced subtle changes in the paramagnetic rim over the medium term (a period of ~5 years), meaning that we expect no untreated participants to experience changes in the shorter time period of observation in this study. The baseline-to- treatment design of this study, with a primary MRI outcome, is therefore justified.

Disease Community Engagement:

Investigators have ongoing relationships and participate actively with community and patient advocacy groups focused on multiple sclerosis, most notably the National Multiple Sclerosis Society. Investigators routinely present knowledge and data at support groups, conferences, and patient educational events. Summary results from this study may be communicated through presentations at meetings, publications in peer-reviewed journals and/or if appropriate, press releases. Publications may be shared and discussed with participants as requested.

Patients who have participated in other Neuroimmunology Clinic protocols are actively engaged in our research efforts and provide ongoing feedback about the design of our research protocols, including procedures that may be especially taxing or impede participation particularly in the setting of high disability. Patients are the driving force to clinical studies not only with their participation but also with their active voice to improve their quality of life in multiple sclerosis. Research subject perspectives were included in defining clinical outcome measures through direct meetings with patients, caregivers, and support group representatives. As we do not expect to modulate clinical outcomes, we did not formally consult with organized patient groups about the design of this short study.

4.3 JUSTIFICATION FOR DOSE

The following is quoted from the protocol of Sanofi-Genzyme's protocol DRI15928, "A Phase 2b dose-finding study for SAR442168, a Bruton's tyrosine kinase inhibitor, in participants with relapsing multiple sclerosis" (NCT03889639):

First, allometric modeling intended to translate BTK occupancy by SAR442168 in preclinical animals (mouse, rat, and dog) predicts an optimal dose range between 1 and 100 mg once daily in humans. Second, Phase 1 multiple ascending- -dose measurements of BTK occupancy in human peripheral blood mononuclear cells (PBMCs) show an asymptotic approach to saturation of the receptor by SAR442168 at the 7.5 mg once daily dose with a more rapid approach to saturation at higher doses. Finally, measurements of absolute CD19+ B cell counts show a dose- dependent increase (observed maximally at Day 4) of up to 80% relative to baseline. The BTK- induced increase in circulating B cells is predicted from the literature, as BTK inhibition alters expression of cell surface adhesion molecules leading to egress from lymph nodes.[43] The dose-response relationship for this effect is maximal at approximately 30 mg once daily.

However, published results of NCT03889639 suggest that of the doses tested, peak efficacy in relapsing MS, with no increase in adverse reactions, was instead achieved at 60 mg once daily for 12 weeks, which was the highest dose tested.[32] This prompted choice of the 60-mg dose for the ongoing multicenter Phase 3 trials of tolebrutinib (to be taken with food due to a moderate food effect in the Phase 1 studies), but also led Sanofi to test higher doses in TDR16862, a randomized, double blind, placebo-controlled study of the tolerability and pharmacokinetics of ascending 14-day repeated oral dose of tolebrutinib in healthy adult participants. Nine participants received 120 mg daily for 14 days. There were no serious adverse events or significant safety concerns at this dose, and no indication that drug-drug interactions should be modified relative to recommendations for 60 mg/day dosing. Additionally, initial results from the long-term safety study of the Phase 2b cohort, LTS16004, indicate general stability of paramagnetic rim lesions in the 20% of participants in whom this imaging biomarker could be measured. Note that these measurements were

performed on a variety of scanners on less sensitive 3T MRI systems, so results at 7T MRI with the advanced sequences used in this protocol cannot be fully extrapolated. The lack of a strong efficacy signal even in the preliminary analysis of LTS16004, along with good tolerability of the 120-mg dose in TDR16862, justified escalation of the treatment dose for participants in this protocol.

Since no drug has yet been shown to resolve chronic active white matter lesions, and since disappearance of paramagnetic rim lesions has not previously been used as an outcome measure in clinical trials, the timing of the study visits at which efficacy outcomes are measured is somewhat arbitrary. As mentioned above, chronic active lesions are established on the order of 3–6 months following initial demyelination, so that 48 weeks at the target dose is a reasonable time point at which to measure the primary outcome. Additional measurements will be taken at 24, 72, 96, 120, and 144 weeks (the last two in Cohort A) to determine the time course of rim disappearance and any associated promotion of lesion repair. Following the initial 96 weeks of tolebrutinib 60 or 120 mg therapy, participants will be eligible to continue therapy, with monitoring visits every 24 weeks (or more frequently if clinically necessary), either until tolebrutinib is marketed or until its development is halted.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

5.1.1 Tolebrutinib Cohorts Inclusion Criteria

1. Able to provide informed consent
2. Willingness to comply with all study procedures and availability for the duration of the study
3. Male or female, aged ≥18
4. Diagnosed with multiple sclerosis according to the 2017 revision of the McDonald diagnostic criteria,[4] with no new lesion formation by comparison of baseline MRI scan with a historical MRI scan at least 6 months prior
5. On anti-CD20 antibody treatment for at least 6 months, with the most recent dose at most 6 months prior to enrollment.
6. Willing to forego further anti-CD20 antibody treatment for the duration of the study
7. Fully vaccinated against SARS-CoV-2 by Day 0. At the time of this writing, fully vaccinated is defined as:
    - Two weeks out from the second dose of a two-dose vaccine series (Moderna, Pfizer-BioNTech); or
    - Two weeks out from a single-dose vaccine (Johnson & Johnson/Janssen)

    Note: Should guidelines change, we will amend these inclusion criteria accordingly.
8. Has a prior 7-tesla MRI scan, no more than 1 year prior to enrollment, demonstrating at least one white matter lesion with a paramagnetic rim[44]
9. For females of reproductive potential: agrees to use highly effective contraception for at least **1 month** prior to dosing and to use such a method during study participation and for an additional 12 weeks after the end of tolebrutinib administration
10. For males of reproductive potential: agrees to use condoms or other methods to ensure effective contraception with partner; agrees not to donate sperm from the inclusion up to 12 weeks after the last dose
11. QuantiFERON-TB Gold negative; skin testing (e.g., tuberculin skin test) will be allowed if blood testing is not available or the blood test result is indeterminate
12. Agrees to adhere to Lifestyle Considerations (see section 5.4) throughout study duration
13. Agrees not to participate in any other interventional study while participating in this protocol

5.1.2 Control Cohort Inclusion Criteria

1. Able to provide informed consent
2. Willingness to comply with all study procedures and availability for the duration of the study
3. Male or female, aged ≥18
4. Diagnosed with multiple sclerosis according to the 2017 revision of the McDonald diagnostic criteria,[4] with no new lesion formation by comparison of baseline MRI scan with a historical MRI scan at least 6 months prior
5. On anti-CD20 antibody treatment for at least 6 months, with the most recent dose at most 6 months prior to enrollment. (Participants in this cohort should remain on their baseline anti-CD20 treatment at least through week 48.)
6. Has a prior 7-tesla MRI scan, no more than 1 year prior to enrollment, demonstrating at least one white matter lesion with a paramagnetic rim[44]
7. For females of reproductive potential: agrees to use highly effective contraception during study participation
8. Agrees not to participate in any other interventional study while participating in this protocol

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

5.2.1 Tolebrutinib Cohorts Exclusion Criteria

1. Pregnancy or lactation
2. MS relapse in the 6 months prior to dosing
3. Febrile illness within 4 weeks prior to dosing, or persistent chronic or active infection requiring treatment with systemic antibiotics, antivirals, or antifungals.
4. Treatment with another investigational drug or other investigational intervention within 3 months prior to baseline.
5. Contraindications for 7-tesla MRI
6. Presence of screening laboratory or ECG values outside normal limits that are considered in the PI or MAI's judgment to be clinically significant, including but not limited to:
    a. Presence of liver injury defined as underlying hepatobiliary disease or screening alanine aminotransferase (ALT) more than 1.5 times the upper limit of normal (ULN)
    b. At screening, positive for hepatitis B surface antigen and/or hepatitis B core antibody and/or positive for hepatitis C antibody
    c. Any of the following:
        i. Bleeding disorder or known platelet dysfunction at any time prior to the first screening visit
        ii. Platelet count less than 150,000/μL at the screening visit
    d. Lymphocyte count less than 1000 cells/dL at the screening visit
7. Is HIV-positive
8. Has received any live (attenuated) vaccine (including but not limited to varicella zoster, oral polio, and nasal influenza) within 2 months before dosing
9. Has received any of the following medications/treatments within the specified time frame before baseline assessment:

Medication	**Exclusionary if used/used within required wash-out period**
Systemic corticosteroids, adrenocorticotropic hormone (other than used for premedication)	1 month prior to baseline MRI scan
Dimethyl fumarate	6 months prior to dosing
Intravenous immunoglobulin, fingolimod,	6 months prior to dosing

natalizumab	
Teriflunomide	2 years prior to dosing or 1 month prior to dosing if participant undergoes an accelerated elimination procedure and has documented teriflunomide plasma level below 0.02 mg/L before dosing
Mildly to moderately immunosuppressive/chemo-therapeutic medications such azathioprine and methotrexate	6 months prior to dosing
Highly immunosuppressive/chemotherapeutic medications: mitoxantrone up to 120 mg/m2 body surface area, cyclophosphamide, cladribine	2 years prior to dosing
Alemtuzumab	4 years prior to dosing
Lymphoid irradiation, bone marrow transplanta-tion, mitoxantrone (with evidence of cardiotoxi-city following treatment, or cumulative lifetime dose >120 mg/m2), other strongly immunosup-pressive treatments with very long-lasting effects	Any time
Any BTK inhibitor	Any time

10. Is receiving potent and moderate inducers and inhibitors of cytochrome P450 3A (CYP3A) or potent inhibitors of CYP2C8 hepatic enzymes as listed in Appendix 1.

11. Is receiving anticoagulant/antiplatelet therapies, including:
    a. Acetylsalicylic acid (aspirin); half-life elimination: Parent drug: Plasma concentration: 15 to 20 minutes; Salicylates (dose dependent): 3 hours at lower doses (300 to 600 mg), 5 to 6 hours (after 1 g), 10 hours with higher doses
    b. Antiplatelet drugs (eg, clopidogrel); half-life: 6 hours
    c. Warfarin (vitamin K antagonist); half-life: 20-60 hours
    d. Heparin, including low molecular weight heparin (antithrombin agents); half-life: 60-90 minutes
    e. Dabigatran (direct thrombin inhibitor); half-life:12-17 hours
    f. Apixaban (IV half-life: approximately 5 hours, oral half-life: approximately 12 hours), edoxaban (half-life: 10-14 hours), rivaroxaban (half-life: 5-9 or 11-13 hours in younger or elderly individuals, respectively) (direct factor Xa inhibitors)

Note: All above drugs need to be stopped at least 5 half-lives before study drug administration except for aspirin, which needs to be stopped at least 8 days before.

12. Has a history or presence of significant other concomitant illness that, according to the PI or MAI's judgment, would adversely affect participation in this study; examples include but are not limited to clinically significant cardiovascular, renal, hepatic, or metabolic disease.
    - Acute liver disease, cirrhosis, chronic liver disease (unless considered stable for >6 months)
    - Has untreated hepatitis C
    - Has chronic hepatitis B unless stable on oral suppression and/or followed by a local hepatol-ogist to monitor for reactivation
    - Has active alcohol use disorder
    - Has an alcohol intake greater than 2 drink per day for men, and greater than 1 drink per day for women (1 drink = approximately 14 grams of alcohol = 350 ml beer = 140 mL wine = 40 mL of spirits)
    - Has aspartate transaminase (AST) or alanine transaminase (ALT) levels greater than 1.5x

ULN
- Has a total bilirubin level greater than 1.5x ULN unless due to Gilbert's or non-liver related disorder
- Has an alkaline phosphatase level greater than 2x ULN unless caused by non-liver related disorder or explained by a stable chronic liver disorder
- At baseline, elevated transferrin saturation (>50% in males and >40% in females) and/or with elevated ferritin levels >500 μg/L.

13. Unwilling to allow coded samples to be processed offsite
14. Unwilling to have coded samples and/or data saved or used in other studies.

5.2.2 Control Cohort Exclusion Criteria

1. Pregnancy or lactation
2. MS relapse in the 6 months prior to baseline
3. Treatment with another investigational drug or other investigational intervention within 3 months prior to baseline
4. Contraindications for 7-tesla MRI
5. Has received any of the following medications/treatments within the specified time frame before baseline assessment:

Medication	**Exclusionary if used/used within required wash-out period**
Systemic corticosteroids, adrenocorticotropic hormone (other than used for premedication)	1 month prior to baseline MRI scan
Dimethyl fumarate	6 months prior to dosing
Intravenous immunoglobulin, fingolimod, natalizumab	6 months prior to dosing
Teriflunomide	2 years prior to dosing or 1 month prior to dosing if participant undergoes an accelerated elimination procedure and has documented teriflunomide plasma level below 0.02 mg/L before dosing
Mildly to moderately immunosuppressive/chemotherapeutic medications such azathioprine and methotrexate	6 months prior to dosing
Highly immunosuppressive/chemotherapeutic medications: mitoxantrone up to 120 mg/m2 body surface area, cyclophosphamide, cladribine	2 years prior to dosing
Alemtuzumab	4 years prior to dosing
Lymphoid irradiation, bone marrow transplantation, mitoxantrone (with evidence of cardiotoxicity following treatment, or cumulative lifetime dose >120 mg/m2), other strongly immunosuppressive treatments with very long-lasting effects	Any time
Any BTK inhibitor	Any time

6. Has a history or presence of significant other concomitant illness that, according to the PI or MAI's judgment, would adversely affect participation in this study; examples include but are not limited to clinically significant cardiovascular, renal, hepatic, or metabolic disease.
7. Unwilling to allow coded samples to be processed offsite
8. Unwilling to have coded samples and/or data saved or used in other studies

5.3 INCLUSION OF VULNERABLE PARTICIPANTS

Selection will be equitable among eligible participants.

Justification for exclusion of children: Participants under the age of 18 are excluded as progressive MS is exceedingly rare in this group, as is the development of rim lesions, suggesting a different biology from adult patients.

Justification for non-inclusion of prisoners: NIH policy does not allow research that is more than minimal risk to be conducted in prisoners.

Justification for exclusion of decisionally impaired adults: Individuals without consent capacity at the time of enrollment are not eligible to enroll in this study, as the study requires subjects to complete a large number of procedures over a relatively short time period. Patients with multiple sclerosis may lose consent capacity with disease progression, and such participants will be allowed to remain in the study. This is justified, given the prospect of direct benefit from research participation (Section 2.3.3) and that, at the time of enrollment the participants had capacity, agreed to full participation, and chose a surrogate to speak on their behalf should they lose capacity. This participant appointed DPA will be invoked should it be determined the subject no longer has consent capacity. If participants who receive tolebrutinib permanently lose the capacity to provide on-going consent subsequent to giving initial consent, they may continue to participate in the study if they have a Durable Power of Attorney (DPA) for health care.

5.3.1 Participation of NIH Staff of family members of study team members

NIH employees and staff will not be solicited for participation but will not be excluded if they express the desire to enroll. NIH staff and family members of study team members may be enrolled in this study as this population meets the study entry criteria. Employees and staff who participate in this protocol during work hours will be informed that they must obtain their supervisor's permission. Neither participation nor refusal to participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's employment, training or position at NIH. Protections for employees and staff participating in this study include: (1) providing them with the NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research and request that they review the FAQ document; (2) assuring that there will be no direct solicitation of employees or staff; and (3) monitoring of independent consent monitoring by the NIH Human Subjects Protection Unit or Clinical Center Department of Bioethics. Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable an unidentifiable manner. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures.

The NIH Information Sheet on NIH Staff Research Participation will be made available. Please see section **10.1.3** for consent of NIH Staff.

5.4 INCLUSION OF PREGNANT WOMEN, FETUSES OR NEONATES

Pregnant and lactating women are excluded as the risk of tolebrutinib in pregnancy and lactation is not known, and as the risk of MRI — the primary and key secondary outcome measures — would not outweigh the potential benefits of the study. Within 24 hours and prior to undergoing any MRI, urine or serum specimens from women with childbearing potential will be collected, and pregnancy testing will be performed.

5.5 LIFESTYLE CONSIDERATIONS

During this study, participants are asked to:

- *Tolebrutinib cohorts only.*
    - For males of reproductive potential, use condoms or other methods to ensure effective contraception, and not donate sperm from baseline until 12 weeks after the last dose.
    - During the entire study, participants will be advised not to consume substantial quantities of alcohol, defined as >14 grams (1 standard drink) per day in female participants or >28

grams (2 standard drinks) per day in male participants on a regular basis.

- *All cohorts*. For females of reproductive potential, use highly effective contraception. For the tolebrutinib cohorts, contraception use should last from at least 1 month prior to dosing until 12 weeks after the end of tolebrutinib administration.

5.6 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) may be rescreened. Rescreened participants should be assigned the same participant number used for the initial screening.

5.7 STRATEGIES FOR RECRUITMENT AND RETENTION

Candidates for participation may enter this protocol in the following ways:

Self-referral. Advertisements and notifications may be placed on NIH or outside websites, either through established Clinical Center mechanisms or following specific approval by the IRB.

- *The Clinical Center Recruitment Office and NIH Clinical Research Volunteer Program.* Recruitment may be conducted by the NIH Office of Patient Recruitment (OPR). The OPR may distribute recruitment information via the internet to neurology clinics, patient support groups, relevant websites, or other institutions or individuals. Recruitment strategies to be utilized by the OPR will include flyers, a Public Service Announcement (PSA), NIH newsletters, ResearchMatch, Clinical Center Facebook, Twitter, CC News/NIH Record, and LISTSERVs.

- *Through ongoing Neuroimmunology Clinic protocols.* In this case, individuals with MS thought by the study team to meet eligibility criteria will be recruited by direct inquiry.

Given the small sample size and short duration of this study, as well as the current unavailability of effective treatments for chronic inflammation in MS and the high number (>50) of MS patients taking anti-CD20 therapy in the Neuroimmunology Clinic, recruitment is anticipated to be straightforward and to accrue over the course of 18–24 months or sooner. The study team may reach out to clinicians who treat people with MS. Subject to IRB rules and specific approval, study information may also be placed in locations, such as National MS Society newsletters and websites, where it may be seen by prospective participants. In this event, any materials prepared for recruitment purposes will be submitted to the IRB for review and approval.

NIH employees may also be recruited. NIH employees and staff will not be directly solicited by supervisors. Co-workers of investigators will not be directly solicited. NIH employees are required to abide by NIH Policy Manual 2300-630-3 “Leave Policy for NIH Employees Participating in NIH Medical Research Studies.”

5.7.1 Costs

The participant is not expected to be responsible for any costs due to participation in this research. Ongoing treatment with anti-CD20 antibody therapy, and any monitoring associated with standard of care for this treatment not already performed as a study procedure, will not be covered by the NIH.

5.7.2 Compensation

There is no compensation for participation in this study. Travel/accommodations for the participant may be provided for out-of-town participants or for those for whom traveling to NIH is a hardship. In such

cases, travel/lodging for the participant and one traveling caregiver may also be provided. If unexpected travel related expenses occur, participants may be provided with a voucher.

Employees and staff who participate during work hours must have permission from their supervisor and cannot receive compensation. NIH employees must either participate outside of work hours or take leave in order to receive compensation. NIH employees must take leave in order to accompany family members to appointments for research participation during their duty hours.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTIONS(S) ADMINISTRATION

6.1.1 Study Intervention Description

This study intervention includes an investigational medical product (IMP), a non-investigational medicinal product (NIMP), and potentially an investigational MRI scanner.

6.1.1.1 *Investigational medicinal product*

The IMP is tolebrutinib, provided in the form of 60-mg tablets.

6.1.1.2 *Non-investigational medicinal product*

Approved anti-CD20 antibody therapy for participants not in the tolebrutinib cohort. Participants who wish to do so may, in consultation with their primary non-NIH clinician, switch to a different anti-CD20 therapy after week 48.

6.1.1.3 *Devices*

The primary 7-tesla MRI scanner and relevant accessories, as well as the pulse sequences used in this study, are FDA-approved, and their use conforms to the corresponding FDA labels.

If for any reason the primary 7-tesla MRI scanner is not available, another 7-tesla or (less preferred) 3-tesla scanner may be used under conditions designated by the FDA as constituting nonsignificant risk under 21 CFR 812.2(b)(1). Acquisition parameters may be modified within the permitted range. These studies may also involve image reconstruction and analysis software, including research pulse sequences. In addition, the following non-FDA-approved systems may be used in this study under conditions designated by the FDA as constituting nonsignificant risk, with assurances from the manufacturer that all measures found in FDA-approved MRI are operational.

Justification for MRI, image reconstruction and analysis software, and head coil non-significant risk (NSR) designation

- Magnetic resonance imaging system (7T and 3T MRI) operating in research mode and with research pulse sequences
- MAGNETOM 7T scanner (manufactured by Siemens)
- Image reconstruction and analysis software
- 1Tx 32Rx Head Coil (manufactured by Nova Medical; tested and supplied by Siemens)

According to 21 CFR 812.3 (m) and FDA "Information Sheet Guidance for IRBs, Clinical Investigators, and Sponsors: Significant Risk and Nonsignificant Risk and Nonsignificant Risk Medical Device Studies January 2006," the use of these devices in the study are a Non-significant Risk study. 21 CFR 812.3(m) enumerates four criteria for a Significant Risk Device Study; none of these apply to this study:

- is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject.
  None of the devices are implants.
- is purported or represented to be for a use in supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject.

None of the devices are for use in supporting or sustaining human life.

- is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject.
  The investigational use of the devices is not of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health.
- otherwise presents a potential for serious risk to the health, safety or welfare of a subject.

The protocol will comply with the abbreviated IDE requirements under 21 CFR 812.2(b).

6.1.2 Dosing and Administration

6.1.2.1 *Dose Escalation*

As of January 20, 2023, participants (n=4) who started tolebrutinib 60 mg/day and previously escalated to 120 mg/day[†] at Week 48 will remain at that dose. Participants (n=3) who initiated tolebrutinib 60 mg/day and did not escalate to 120 mg/day due to the partial clinical hold will be followed on study for an additional 48 weeks, according to the schedule of the Initial Cohort. They will furthermore be eligible to continue taking tolebrutinib 60 mg/day until tolebrutinib becomes FDA-approved or the clinical development program is terminated.

6.1.2.2 *Dose Limiting Toxicity*

Dose-limiting toxicity has not been established.

6.1.2.3 *Dose Modifications*

Should tolebrutinib 120 mg/day prove intolerable, the dose may temporarily or permanently be reduced to 60 mg/day. Participants will contribute to subsequent data analyses even if the dose is reduced. Treatment might need to be interrupted or permanently discontinued if deemed necessary due to an adverse event (Sections 7 and 8.4).

6.1.2.4 *Drug Administration*

As of January 20, 2023, participants (n=3) who have received one 60-mg tablet of tolebrutinib per day will no longer have the option to increase to 120 mg (2 tablets) per day at Week 48[1]. The tablet(s) should be taken orally with food. The time of day should be as consistent as possible throughout the study. Missed doses will not be made up.

Study interventions will be dispensed at regular site visits. Tolebrutinib dosing will continue for the duration of the study and may be continued thereafter until tolebrutinib is either marketed or its development halted.

6.1.2.5 *Partial Clinical Hold*

On June 29, 2022, we were informed by Sanofi that as of June 27, 2022, new enrollment was being halted across all 5 of their US phase 3 trials of tolebrutinib, and that all participants dosed after April 29, 2022 in the US would be required to stop tolebrutinib but would continue for observation on the trials. Sanofi staff detailed a call earlier in the week, on which the FDA verbally communicated a partial clinical hold to the Sponsor. The partial clinical hold was enacted after internal discussions regarding four participants with evidence of drug-induced liver injury (DILI), and the FDA outlined specific steps that Sanofi could take to support lifting the hold.

On July 12, 2022, the FDA issued a partial clinical hold to the NINDS IND as well, with identical stipulations to the Sanofi hold. As such, until the hold is lifted, we will not initiate new subjects on tolebrutinib. However, as no participants in our study had been taking tolebrutinib for less than 60 days at the time of

[†] See Section 6.1.2.5

the FDA partial clinical hold, the FDA does not require that we stop tolebrutinib for any participant in our trial. Furthermore, although not specifically stipulated by the FDA, we have elected to defer dose escalation from 60 to 120 mg (the "week 48" time point) until the partial clinical hold is lifted or modified; once the dose is escalated, however, the trial clock will restart.

On January 20, 2023, the FDA requested a revision to the protocol to remove the potential for dose escalation. Participants who have initiated tolebrutinib 60 mg and escalated to 120 mg/day will remain at that dose. The 3 remaining participants who initiated tolebrutinib 60 mg will remain at 60 mg and not be escalated to 120 mg/day.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 Acquisition and Accountability

Sanofi-Genzyme will ship tolebrutinib 60-mg tablets directly to the NIH clinical Center Pharmacy.

6.2.2 Formulation, Appearance, Packaging, and Labeling

Tolebrutinib tablets will be packaged in blister packs protected by a wallet. Each wallet contains 20 tablets which are dispensed in sufficient amount to cover patient treatment until the next study visit. Each wallet will be labeled. The packaging may be updated during the course of the study.

6.2.3 Product Storage and Stability

Tolebrutinib kits must be stored between 36°F and 86°F. Procedures for temperature monitoring and excursion management will be followed as per Sanofi's pharmacy manual. Tolebrutinib kits will be stored in a secure location in accordance with local regulations, labeling specifications, policies and procedures.

6.2.4 Preparation

Tolebrutinib will be supplied as a 60-mg oral tablets and will not require any preparation.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Participants will not be randomized in this study. Up to 10 adults with MS who are on stable disease modifying treatment with anti-CD20 antibody therapy and are within 6 months of their most recent dose, have at least one paramagnetic rim lesion on 7-tesla magnetic resonance imaging (MRI), and have developed no new white matter lesions or clinical relapses for at least 6 months, will receive treatment with tolebrutinib 60 mg/day or escalating to 120 mg/day and agree to forego further anti-CD20 or other disease-modifying therapy for the duration of the trial.[‡] A comparison cohort of individuals who meet enrollment criteria (other than tolebrutinib contraindications) but choose not to take tolebrutinib will continue to receive their current anti-CD20 therapy.

The study is unblinded for the participant and investigators, except for the investigators performing analysis of the raw MRI data for the primary and secondary outcomes, who will be blinded to the time point at which the data were acquired. If these investigators become unblinded, their analysis will be scrapped and redone by blinded investigators.

6.4 STUDY INTERVENTION COMPLIANCE

At the end of the baseline visit (Day 0), the participant will receive the study intervention for the following 144 weeks (Cohort A) before entering the extension phase. At each following visit, a new kit will be dispensed, and the participant will self-administer the medication. Participants will be asked to track daily dosage either electronically or in a written format per their preference. They will be provided with a template drug diary, which they may opt to use. Remaining pills will be counted at each follow-up visit to assess overall drug compliance.

6.5 CONCOMITANT THERAPY

[‡] See Section 6.1.2.5

Concomitant therapies (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) will be reviewed at each study visit and documented in the clinical chart.

Use of certain medications will be restricted during study participation, as detailed in Section 5.2 and below.

The following medications will be prohibited throughout study participation in the tolebrutinib cohorts:

- Other MS disease-modifying treatments
- Acetylsalicylic acid (aspirin)
- Anti-platelet drugs (e.g., clopidogrel)
- Anticoagulants, including:
  - Warfarin
  - Heparin, including low-molecular-weight heparins
  - Dabigatran
  - Apixaban, edoxaban, rivaroxaban
- Potent and moderate inducers and inhibitors of cytochrome P450 3A (CYP3A) or potent inhibitors of CYP2C8 hepatic enzymes as listed in Appendix 1.

In the tolebrutinib cohorts, short courses (up to 5 days) of NSAIDs (other than acetylsalicylic acid) at the recommended dose may be used if clinically indicated. Standard treatment of MS relapse with high dose glucocorticoids is permitted.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Participants are free to withdraw from participation in the study at any time upon request.

The PI or MAI may discontinue or withdraw a participant from the study for any of the following reasons:

- Disease activity or progression that requires discontinuation of the study intervention
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Investigator judgment for safety, behavioral, compliance, or administrative reasons
- Positive pregnancy test

The reason for withdrawal or discontinuation will be documented in the medical record. Participants will be asked to return for a follow-up visit within 3 months, and ideally within 1 month after discontinuation of the intervention.

Events of Special Interest (Section 8.4.7) will follow the algorithm outlined in Appendix 2.

7.1.1 Definitive discontinuation

Definitive discontinuation is any IMP discontinuation associated with the definitive decision of the PI not to re-expose the participant to the IMP at any time during the study, or from the participant not to be re-exposed to the IMP, whatever the reason.

Definitive discontinuation will ensue for any confirmed treatment-related serious adverse event.

If discontinuation is due to adverse event preventing further exposure to the IMP, the participant will be followed up to recovery or stabilization of the event.

If possible, and after the definitive discontinuation of intervention, participants will be assessed using the

procedure normally planned for Week 24 if prior to Week 24, or the next protocol timepoint if they drop out at any later time. Details are provided in the Schedule of Activities (Section 1.3).

7.1.2 Temporary discontinuation

Temporary IMP discontinuation will ensue during evaluation of any suspected IMP-related serious adverse event. For all temporary IMP discontinuations, the duration of the discontinuation should be recorded by the PI or designee in the appropriate pages of the study record. Temporary discontinuation of study drug will be discussed with the IMM. Temporary IMP discontinuation decided by the PI or MAI corresponds to >1 dose not administered to the participant.

The current NIH treatment guidelines recommend against the use of BTK inhibitors in the treatment of COVID-19 unless a patient is enrolled in a clinical trial studying that indication. The guidelines are being updated regularly. If a trial participant on tolebrutinib treatment is hospitalized for COVID-19, the PI and MAI will discuss the case with the treating physician and appropriate experts to determine whether IMP will be continued or halted based on patient safety. Participants who are off of tolebrutinib for more than 12 weeks will not contribute to the primary analysis, although they can resume IMP and trial participation when determined by the PI, MAI, and treating physician.

7.1.2.1 *Rechallenge*

Re-initiation of intervention with tolebrutinib may be considered under close and appropriate clinical/and or laboratory monitoring if the PI or MAI determine in conjunction with either the study HAC or DSMB (see Section 10.6 and Appendix 2), according to his/her best medical judgment, that the responsibility of tolebrutinib in the occurrence of the concerned event was unlikely and if the selection criteria for the study are still met.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

The reason and date of any study withdrawal will be documented in the study record.

Participants who sign the informed consent form but do not receive tolebrutinib may be replaced and followed in the non-tolebrutinib cohort.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for two scheduled visits and is unable to be contacted by the study site staff after 3 attempts.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- An investigator or designee will attempt to contact the participant within 1 month to reschedule the missed visit, counsel the participant on the importance of maintaining the assigned visit schedule, and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant. These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCREENING PROCEDURES

Candidates for this study are screened under the natural history protocol 89-N-0045. Data collected during screening may be used for baseline measurements and/or combined with data collected in this protocol for exploratory analyses. Screening procedures/evaluations must be performed within 6 months prior to enrollment. Screening will occur only after the participant has signed the consent for 89-N-0045.

8.2 EFFICACY ASSESSMENTS

Assessments are performed according to the Schedule of Activities, Section 1.3. In the event that data or samples are found to be technically inadequate for a particular procedure, procedures in Section 8.2 may be repeated twice.

8.2.1 Clinical Evaluations

History and physical. This is a targeted history and neurological examination to allow calculation of the Expanded Disability Status Scale and Scripps Neurological Rating Scale and must be performed by a credentialed provider.

Clinical disability scales. These include, but are not limited to, 9-hole peg test (9HPT), timed 25- foot walk (T25W), and symbol digit modalities test (SDMT). The paced auditory serial addition test (PASAT) and timed up-and-go (TUG) tests may also be performed.

Magnetic resonance imaging. Details regarding the use of MRI are further described in Section 6.1.1. For women of childbearing age, urine or serum pregnancy testing is performed prior to initial study drug dosing and to all MRI scans.

8.2.2 Biospecimen Evaluations

Not applicable.

8.2.3 Correlative Studies for Research

Research blood work, cerebrospinal fluid, and urine testing are used for exploratory outcomes in this study.

Research blood work and urine. In addition to samples sent to the NIH Clinical Center lab for safety evaluation (Section 8.3), up to 150 ml of whole blood, as well as a urine sample, may be collected at each study visit for research purposes and processed in the NINDS Viral Immunology Section and/or Translational Neuroradiology Section. The amount of blood that may be drawn for research purposes will not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any 8-week period.

Research lumbar puncture. Lumbar puncture may be performed at the bedside or under fluoroscopy if required for scheduling or if judged by the study team to be in the best interest of the participant. In addition to samples sent to the NIH Clinical Center lab for safety evaluation (Section 8.3), up to 25 ml of CSF are collected for research purposes and processed in the NINDS Viral Immunology Section and/or Translational Neuroradiology Section.

Research assays are focused primarily on immune-cell subsets and their products and may include, but are not limited to, flow cytometry and protein measurements (e.g. ELISA, MesoScale, Single Molecule Array, mass spectrometry). Bulk and single-cell and/or single-nucleus RNA sequencing assays, as well as epigenetic assays (e.g. ATAC-Seq), may also be performed. Assays to assess products of glia and neurons (e.g. neurofilament light chain) may also be developed and performed. Specimens are preserved and patched for subsequent analysis whenever possible, but some assays (e.g. flow cytometry and single-cell RNA sequencing) may require use of fresh samples. Cell lines are not expected to be created as part of this protocol, but they may be created for further exploratory analyses.

8.2.4 Samples for Genetic/Genomic Analysis

Not applicable.

8.3 SAFETY AND OTHER ASSESSMENTS

History and physical. Interval history is obtained at visits over the course of the study to assess for adverse events. Focused neurological and additional portions of the physical examination are performed if clinically warranted or to follow up on any issues reported. These procedures are performed by a credentialed provider.

Vital signs. Temperature, pulse, blood pressure, and respiratory rate are assessed and recorded in the study record.

Electrocardiogram (ECG). A 12-lead ECG is obtained using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. At least one longer rhythm monitoring recording should be part of each ECG testing.

Magnetic resonance imaging. MRI is obtained without intravenous administration of an FDA-approved gadolinium- based contrast agent. However, an FDA-approved, gadolinium-based contrast agent may be given for clinical purposes (for example to investigate new neurological symptoms), if judged warranted by the evaluating clinician and provided that there are no contraindications for the subject to receive a contrast agent. Details regarding the use of MRI are further described in Section 6.1.1. For women of childbearing age, urine or serum pregnancy testing is performed prior to initial study drug dosing and to all MRI scans. MRI scans will be read by a neuroradiologist and a report entered into the NIH clinical system.

Laboratory work. Blood and urine samples will be sent to the NIH Clinical Center lab as per study schedule, or they may be evaluated at an outside facility. For the tolebrutinib cohorts, lab work should include complete blood count with differential (including lymphocyte subsets), blood chemistry, to include liver function tests, and ferritin, transferrin, amylase, lipase, JCV Ab, JCV PCR (blood and urine), and quantitative immunoglobulins. Serum iron panel (iron, ferritin, transferrin saturation, TIBC – total iron-binding capacity), QuantiFERON® TB Gold Test, serology for hepatitis and/or confirmatory testing, HIV and other infectious diseases to confirm inclusion and exclusionary conditions may be performed at baseline and throughout the protocol as required. TBNK assays may be performed at the time of 7T MRI for the tolebrutinib cohorts. For the control cohort, lab work should include complete blood count with differential (including lymphocyte subsets), blood chemistry as needed for MRI, JCV Ab, JCV PCR (blood and urine), and quantitative immunoglobulins. “Safety labs,” as designated in the Schedule of Activities, include complete blood count and blood chemistry including liver function tests. “Liver-specific safety labs” refer to liver function tests (AST, ALT, alkaline phosphatase, and total bilirubin). Additional labs may be ordered when clinically indicated.

Lumbar puncture. Lumbar puncture may be performed at the bedside or under fluoroscopy if required for scheduling or if judged by the study team to be in the best interest of the participant. Samples are sent to the NIH Clinical Center lab for cell count, protein, and glucose.

Assessment of study intervention adherence. See Study Intervention Compliance, Section 6.4.

Assessment of adverse events. Adverse events, including serious adverse events, will be followed up as clinically warranted, including possibly by telephone or other method of communication and additional visits to the NIH.

Clinically relevant results of laboratory or radiological evaluations performed in this protocol will be provided to participants, along with a recommendation for next step (including potential follow- up at NIH or elsewhere).

In the event that circumstances beyond our control preclude the travel of one or more of the study participants to the NIH Clinical Center for a visit, or otherwise would put their health at greater risk (i.e. epidemic or pandemic), we will endeavor to establish capabilities for remote visits (using NIH approved platforms). Specifically, for safety visits, the study team will arrange for local laboratory studies to be done, and conduct a telehealth visit with the participant. Unscheduled visits, due to safety, may also be conducted remotely through telehealth and/or offsite laboratory and radiologic studies and/or in conjunction with a local physician with the guidance of the study PI and medically responsible investigator. The laboratory studies may be performed through LabCorp, Quest, or with the local physician, and results will be sent to the study team for safety monitoring and oversight. Radiology studies will be arranged through a local, non-study physician, or directly by the study team, at a site close to the participants home area if

travel to the NIH is not possible.

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.4.1 Definition of Adverse Event

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting.

8.4.2 Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life- threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.4.3 Classification of an Adverse Event

8.4.3.1 *Severity of Event*

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious."

8.4.3.2 *Relationship to Study Intervention*

All adverse events (AEs) must have their relationship to study intervention assessed by the investigator who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to

concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.
- **Unlikely to Be Related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.4.3.3 *Expectedness*

An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention in the protocol or IB, according to FDA guidance (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/safety-reporting-requirements-inds-investigational-new-drug-applications-and-babe). Events described in the literature, but not in the protocol or IB, will be considered unexpected for the purpose of this reporting. The PI will make the initial determination, along with the IMM. The NINDS DSMB will make final decisions of expectedness and relatedness in conjunction with the Sponsor, normally at the time of routine DSMB review. For all SAEs and high-grade AEs (AE grade 3 or above), the DSMB will come to a determination of expectedness and relatedness within ideally within 48 hours, but no more than within 7 days, of notification by the PI.

All SAEs/SUSARS will be reported to the Study Sponsor or his delegate. Form 3500 (MedWatch) may be used for all potential SUSARs submitted to the Study Sponsor.

8.4.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The investigator will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.4.5 Adverse Event Reporting

The Principal Investigator or delegated AI is responsible for detecting, documenting, and reporting unanticipated problems, adverse events (AEs), including serious adverse events (SAEs), and deviations in accordance with NIH policy, IRB requirements, and federal regulations. Relatedness to the research of all serious adverse events will initially be determined by the PI or delegated AI in consultation with the IMM. Final determinations will be made by the DSMB in conjunction with the sponsor. All high-grade AEs (grade 3 and above) and all SAEs will be forwarded to the IMM and the DSMB within 2 business days, regardless of initial determination.

The IMM and DSMB should receive the written initial determination and justification from the PI at the time of initial reporting. The IMM and DSMB will receive updates from the PI regarding potential changes to expectedness or relatedness, and safety, within 48 hours, but not later than 7 days.

All AEs (serious and non-serious) that are either related or possibly related to research procedures; protocol deviations; and unanticipated problems will be summarized and reported at the time of continuing review.

8.4.6 Serious Adverse Event Reporting

The study investigator will immediately (but no later than 24 hours) report to the sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the study sponsor and should be provided as soon as possible.

The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

8.4.7 Events of Special Interest

The following are defined as Events of Special Interest (ESI). Once identified, they follow the algorithm outlined in Appendix 2 and separate HAC charter.

- Increase in alanine transaminase (ALT) > 3xULN
- Increase in aspartate transaminase (AST) > 3xULN
- Increase in total bilirubin Level (TBL) > 2xULN

Expedited (<15 days) reports will be sent to the FDA for any cases with transaminases >8x ULN or >3x ULN with total bilirubin >2x ULN.

8.4.8 Reporting of Pregnancy

Pregnancy of a female participant as well as pregnancy occurring in a female partner of a male participant is reported as an AE and is qualified and reported as an SAE only if it fulfills one of the seriousness criteria (Section 8.4.2). In the event of pregnancy in a female participant, tolebrutinib should be discontinued. Follow up of the pregnancy in a female participant or in a female partner of a male participant is mandatory until the outcome has been determined.

8.5 UNANTICIPATED PROBLEMS

8.5.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

8.5.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801.

8.5.3 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NIH Intramural IRB.

9 STATISTICAL CONSIDERATIONS

No separate Statistical Analysis Plan has been developed for this protocol.

9.1 STATISTICAL HYPOTHESIS

- Primary Endpoint: The per-participant proportion of lesions in which the paramagnetic rim has disappeared at the end of 48 weeks of tolebrutinib 60 mg treatment is >0.01.
- Secondary Endpoints:
    - Tolebrutinib 60 and 120 mg doses are safe and tolerable in the study population.
    - The T1 relaxation time has decreased in paramagnetic rim lesions at the end of 96 weeks of tolebrutinib 120 mg, relative to non-rim lesions.
    - The size of paramagnetic rim lesions has diminished at the end of 96 weeks of tolebrutinib 120 mg, relative to non-rim lesions.

9.2 SAMPLE SIZE DETERMINATION

Given our previous natural history data, which indicates that only 1 out of 29 individuals experienced subtle changes in at least one paramagnetic rim lesion over a period of ~5 years, we expect that very few to no participants in the non-tolebrutinib cohort will experience changes in this study and conservatively

estimate this null proportion to be 0.01. Although even a single tolebrutinib-cohort participant experiencing short-term changes would potentially be clinically meaningful, we have powered this study to detect disappearance of at least one paramagnetic rim lesion in 2 out of 7 completers (expected proportion: 0.29). In this scenario, the interval of (0.0367, 0.7096) will have 95% probability to include the true proportion. Thus, the null hypothesis will be rejected if 2 participants experience at least one lesion change.

To recruit 7 study completers in tolebrutinib Cohort A, we expected to enroll 10 prospective participants into that cohort. As of May 2023, the first 7 participants had completed 48 weeks of tolebrutinib 60 mg, so no additional participants will be recruited into this Cohort. Participants in the non-tolebrutinib comparison cohort (up to 10) will not be replaced if they drop out. We expect the total study duration, including the long-term extension (beyond Week 144 for Cohort A, to be approximately 5 years from the time of first dosing.

9.3 POPULATIONS FOR ANALYSES

The analysis for the primary and MRI-based secondary outcomes will be performed on all study completers. The analysis for the safety and tolerability secondary outcome will be performed on all participants who take at least one dose of tolebrutinib. Populations for exploratory post-hoc analyses will be defined at a later date.

9.3.1 Evaluable for toxicity

All participants will be evaluable for toxicity from the time of their first treatment with tolebrutinib.

9.3.2 Evaluable for objective response

Only those participants who have received at least one dose of therapy and have had their disease re-evaluated will be considered evaluable for response. These participants will have their response classified according to the definitions in section 9.4.

9.3.3 Evaluable Non-Target Disease Response

Not applicable.

9.4 STATISTICAL ANALYSES

9.4.1 General Approach

9.4.2 Analysis of the Primary Endpoint

The primary outcome is the per-patient proportion of lesions in which the paramagnetic rim has disappeared at the end of 48 weeks of tolebrutinib 60 mg/day, in comparison to the scan just prior to initiation of tolebrutinib dosing. Such participants can be in Cohort A or the Initial Cohort (combined analysis). Proportion of diminished/resolved paramagnetic rims is analyzed by investigators blinded to time point, reported descriptively, and compared to the assumption of no change off therapy[10,11,15,16,20] via a binomial exact test. The analysis will be performed in study completers, and by definition no data will be missing. A threshold p-value of 0.05 will be taken as a significant change.

9.4.3 Analysis of the Secondary Endpoints

To assess the possible repair of chronically inflamed white matter lesions in which inflammation at the lesion edge has been modulated by tolebrutinib 120 mg/day, changes in T1 relaxation time within paramagnetic rim lesions, relative to non-rim lesions, will be determined by analysis of variance or mixed-effects models. Comparisons are relative to the scan taken just prior to initiation of 120 mg dosing. A threshold p-value of 0.025 will be taken as a significant change.

To assess the possible repair of chronically inflamed white matter lesions in which inflammation at the lesion edge has been modulated by tolebrutinib 120 mg/day, changes in size of paramagnetic rim lesions, relative to non-rim lesions, will be determined by analysis of variance or mixed-effects models. Comparisons are relative to the scan taken just prior to initiation of 120 mg dosing. A threshold p-value of 0.025

will be taken as a significant change.

9.4.4 Safety Analyses

To assess safety and tolerability, adverse events will be tabulated.

9.4.5 Baseline Descriptive Statistics

Not applicable.

9.4.6 Planned Interim Analyses

The study may be repowered based on analysis of the per-patient proportion of lesions in which the paramagnetic rim has disappeared at the end of 48 weeks of tolebrutinib 60 mg/day. If no changes are observed, powering will not change.

Given the FDA partial clinical hold and the inability to recruit additional participants into the tolebrutinib cohort, there is no need for an interim analysis as of May 2023.

9.4.7 Subgroup Analyses

This is a small study, and subgroup analyses would be meaningless. Changes observed in individual participants will be described in the context of the demographic information of those participants.

9.4.8 Tabulation of Individual Participant Data

Individual participant data will be listed by measure and time point.

9.4.9 Exploratory Analyses

Exploratory analyses based on data collected during this study are considered post-hoc and will be defined at a later time point.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 INFORMED CONSENT PROCESS

10.1.1 Consent/Assent Procedures and Documentation

The informed consent document will be provided as a physical or electronic document to the participant for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss with friends, family members and/or other advisers, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to any research activities taking place.

In the event that circumstances beyond our control preclude the travel to the NIH Clinical Center for a visit, or otherwise would put their health at greater risk (i.e. epidemic or pandemic), we will endeavor to establish capabilities for remote consent. The initial consent process as well as reconsent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator with the agreement of the participant.

A physical copy of the consent form may be sent to the subject if a telephone consent process is used. The consent interview may then be conducted when the subject can read the consent form during the discussion. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. If the consent process is occurring remotely, participants and investigators will view individual copies of the approved consent document on screens at their respective locations; the same screen may be used when both the investigator and the participant are

co-located but this is not required.

Note: When required, the witness signature will be obtained similarly as described for the investigator and participant below.

Consent will be documented with required signatures on the physical copy of the consent (which includes the printout of an electronic document sent to the participant), or on the electronic document. The process for documenting signatures on an electronic document is described below.

When an electronic document is used for the documentation of consent, this study will use the iMedConsent platform which is 21 CFR, Part 11 compliant to obtain the required signatures. During the consent process, participants and investigators will view the same document simultaneously in their respective locations.

The identity of the participant will be determined by a prompt which will require the provision of information from an official identification document, prior to obtaining the signature. Both the investigator and the participant will sign the electronic document using a finger, stylus or mouse.

A copy of the informed consent document signed and dated by the subject are given to the subject. Confirmation of a subject's informed consent is documented in the subject's medical records prior to any testing under this protocol.

When possible, consent will be obtained by an individual in a non-supervisory relationship with the subject. When consent is conducted, a third party (e.g., a consent monitor) will be present to observe the consent process. This may be achieved by one of the following methods:

- A consent monitor from the CC Department of Bioethics Consultation Service or by a Clinical Research Advocate from the NIMH Human Subjects Protection Unit (HSPU), or
- Another party independent of the research team (such as an IC monitor), or
- If a consent monitor is not available, the consent process will be observed by another qualified investigator on the study who is independent of the NIH staff member's work unit and not a supervisor to the NIH staff member. If no such person exists, consent observation may be performed by a qualified investigator on the study.

The consent form contains all required elements. A single consent document, for the patient population, is submitted with this protocol. No special documents are needed.

10.1.2 Consent for minors when they reach the age of majority

Not applicable.

10.1.3 Considerations for Consent of NIH staff, or family members of study team members

Consent for NIH staff will be obtained as detailed above with following additional protections:

Consent from staff members will be obtained by an individual independent of the staff member's team whenever possible. Otherwise, the consent procedure will be independently monitored by the CC Department of Bioethics Consultation Service in order to minimize the risk of undue pressure on the staff member.

10.1.4 Consent of subjects who are, or become, decisionally impaired

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section 2.3.3), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research

Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP Policy 403 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if the sponsor, IMP manufacturer, IRB, and/or FDA communicate new safety data regarding the IMP that requires adjustment of study risk, or if multiple participants are required to discontinue the study due to similar IMP-related serious adverse events. Decisions will be made in consultation with the sponsor and the Data and Safety Monitoring Board. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, the IND sponsor, and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform study participants, the IRB, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Additional circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or FDA.

10.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the NINDS Neuroimmunology Clinic and affiliated labs in the NINDS Division of Neuroimmunology and Neurovirology for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NINDS Clinical Trials Unit. This, as well as data and samples shared with third parties that carry out various analyses under appropriate materials and data sharing agreements,

will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number, the key to which is kept in the NINDS Neuroimmunology Clinic. The study data entry and study management systems used by the NINDS Neuroimmunology Clinic and Clinical Trials Unit research staff, as well as third parties, will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NINDS Neuroimmunology Clinic.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data and samples collected in this study may be sent to a repository for indefinite storage and may be released for research purposes. Participants' identifying information will not be on such samples or data, but the samples and data may be coded with the key kept in a secure area on password- protected computers in the NINDS Neuroimmunology Clinic. The samples and data may be used for other research projects, including those that are not related to MS. Genetic testing will not be performed from samples obtained solely for this study. Participants who do not want samples and data to be used for other projects should not participate in this study. Participants who withdraw from this research study before it is completed may ask that their remaining samples, which can be identified as theirs, be destroyed. Data obtained before those participants withdraw will be kept.

10.5 SAFETY OVERSIGHT

Safety oversight will be under the direction of an Independent Medical Monitor (IMM) and a Data and Safety Monitoring Board (DSMB). Additionally, an Independent Hepatology Assessment Committee (HAC) will assess liver safety for participants during the course of this trial. The IMM, DSMB, and HAC should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. Individuals with the appropriate expertise will be selected to serve as the IMM, or as a member of the DSMB. Safety and date integrity reviews will be conducted as described in section 10.6.

10.6 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

Monitoring for this study will be performed by an Independent Medical Monitor (IMM) and Data and Safety Monitoring Board (DSMB).

Study investigators will evaluate the safety of study subjects throughout the conduct of the study and respond to adverse events (AEs) in a timely manner. The PI will review AEs with the study team at Weekly Event Reporting Meetings.

The IMM's responsibility is to provide independent medical monitoring in a timely fashion. The IMM evaluates individual and cumulative participant data when making recommendations regarding the safe continuation of the study. The IMM is also provided with reports on safety and enrollment as well as all protocol revisions and other pertinent documents relating to the study. If no interval data are collected, a

report is not required. The IMM does not have direct involvement in the conduct of the study. The IMM is not responsible for analyzing the primary or secondary outcomes of the study. The IMM's recommendations need not be based on data provided regarding those outcomes, though he/she may take such data into account. However, it is expected that the IMM's primary recommendations are based on review of the raw study data and participant records.

The DSMB will serve as the sponsor's medical monitor and will make the final determinations of relatedness on behalf of the IND sponsor (NINDS). The DSMB will be charged with reviewing all safety and efficacy data at a twice-yearly meeting. This meeting may take place in person, as a teleconference, or via email. The DSMB may be consulted in person and as needed to discuss clinical issues. The DSMB will operate under the rules of an approved charter that will be reviewed during the first DSMB meeting. After the twice-yearly interim meetings, the DSMB chair will provide a report to the study PI and NINDS Office of the Clinical Director.

10.6.1 Independent Hepatology Assessment Committee (HAC)

An expert committee of independent hepatologists will review all cases of potential DILI and will provide guidance on case evaluation and risk mitigation. The Hepatology Assessment Committee recommendations will be made available to the DSMB. Details of the responsibilities of the Independent Hepatology Assessment Committee and its workflow will be described in a separate charter.

10.7 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management. A Contract Research Organization (CRO) will perform quality assurance monitoring for this study. This study will be monitored in accord with the NINDS Monitoring SOP.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

A Contract Research Organization (CRO) will provide on-site monitoring of this protocol. The study team, the Sponsor and the CRO monitor will determine the frequency of monitoring visits, which will be described in the QA Monitoring Plan. The frequency of visits will include, at a minimum, annual monitoring visits until the protocol has undergone a close-out visit, unless otherwise indicated by the Sponsor. The Sponsor, via the CRO, will be responsible for providing adequate oversight of the investigation to ensure adequate protection of the rights, welfare, and safety of human subjects and the quality and integrity of the resulting data.

10.8 DATA HANDLING AND RECORD KEEPING

10.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of

data.

Study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into CRIS, a 21 CFR Part 11-compliant data capture system provided by the NIH Clinical Center. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.8.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention, and as per the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.9 PROTOCOL DEVIATIONS AND NON-COMPLIANCE

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations to the NIH Institutional Review Board as per Policy 801 and per NINDS Event Reporting SOP. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.9.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

10.10 PUBLICATION AND DATA SHARING POLICY

10.10.1 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH- Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. De-identified study data may be shared with data repositories as described below. Furthermore, applicable (e.g., generated via the NIH Clinical Center Clinical Research Information System [CRIS]) identified data may be shared via the NIH Biomedical Translational Research Information System (BTRIS) following standard NIH Clinical Center operating procedures, including BTRIS data access policies. Data may be shared before the time of publication. Finally, data from this study may be requested

from other researchers up to 2 years after the completion of the primary endpoint by contacting the Principal Investigator.

The following specific provisions apply to this protocol:

Sharing of identified samples and data. Identified samples and data obtained under this protocol, in participants who are also enrolled in other research protocols at NIH or outside NIH, may be shared with those protocols' investigators. This sharing will enable pooling of often-scarce resources and will also reduce demands on participants' time and effort. Provisions for sharing of identified samples and data in this fashion are included in the consent form for this protocol. Required institutional approval, including materials/data transfer and collaboration agreements, will apply in all cases. Materials/data will be shipped/transferred in accordance with NIH and federal regulations.

Sharing of coded and unlinked samples and data. Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). Coded samples and/or data may be shared; the key to the code will not be provided to collaborators but will remain at NIH. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval. Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

Fellows, students, or other trainees in the investigators' labs may work with coded samples and data while at NIH and after leaving NIH, if needed to complete a project or prepare data for publication. Those who have left NIH and who are not associate investigators on this study will not have access to identifying information or to the key to the data code. Departing fellows, students, and trainees will sign an agreement with NINDS requiring that coded study data will be maintained on password-protected computers, used only for the specified purposes of the protocol, not shared, and destroyed once the project is completed. Before sharing identifiable data with departing investigators, all necessary agreements will be obtained.

10.10.2 Genomic Data Sharing Plan

Not applicable.

10.11 COLLABORATIVE AGREEMENTS

10.11.1 Agreement Type

This study is performed under a Cooperative Research and Development Agreement (CRADA) with Sanofi-Genzyme.

10.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership, in conjunction with the NINDS, has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

9HPT	9-Hole Peg Test
AE	Adverse Event
AESI	Adverse Event of Special Interest
BBB	Blood-Brain Barrier

BRaKe-MS	BRuton's tyrosine KinasE in Multiple Sclerosis
BTK	Bruton's Tyrosine Kinase
BTRIS	Biomedical Translational Research Information System
CD	Cluster of Differentiation
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CNS	Central Nervous System
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CFR	Code of Federal Regulations
CRF	Case Report Form
CRIS	Clinical Research Information System
CRO	Contract Research Organization
CSF	Cerebrospinal Fluid
CSSRS	Columbia Suicide Severity Rating Scale
DSMB	Data and Safety Monitoring Board
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DILI	Drug-Induced Liver Injury
DPA	Durable Power of Attorney
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EAE	Experimental Autoimmune Encephalomyelitis
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Forms
EDSS	Expanded Disability Status Scale
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
gMG	Generalized Myasthenia Gravis
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HAC	Hepatology Assessment Committee
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IMM	Independent Medical Monitor
IMP	Investigational Medicinal Product

IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
IV	Intravenous
LLN	Lower Limit of Normal
LOAEL	Lower Adverse Event Effect Level
LSMEANS	Least-squares Means
MAD	Multiple Ascending Dose
MAI	Medical Advisory Investigator
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
NIMP	Non-Investigational Medical Product
NINDS	National Institute of Neurological Disorders and Stroke
NOAEL	No Adverse Event Effect Level
nr-SPMS	Nonrelapsing Secondary Progressive Multiple Sclerosis
NSAID	Non-Steroidal Anti-Inflammatory Drug
OHRP	Office for Human Research Protections
PASAT	Paced Auditory Serial Addition Test
PBMC	Peripheral Blood Mononuclear Cell
PCSA	Potentially Clinically Significant Abnormality
PI	Principal Investigator
PK	Pharmacokinetic
PML	Progressive Multifocal Leukoencephalopathy
PPMS	Primary Progressive Multiple Sclerosis
QA	Quality Assurance
QC	Quality Control
QTcF	QT Interval corrected using Fridericia's formula
RMS	Relapsing Multiple Sclerosis
RNA	Ribonucleic Acid
SAD	Single Ascending Dose
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SDMT	Symbol Digit Modalities Test
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
T	Tesla

T25W	Timed 25-foot Walk
TUG	Timed Up-and-Go
ULN	Upper Limit of Normal
UP	Unanticipated Problem
US	United States

12 APPENDIX

12.1 APPENDIX 1: MEDICATIONS THAT CANNOT BE TAKEN WITH TOLEBRUTINIB

Certain medications cannot be taken while taking tolebrutinib. These include strong CYP3A inducers or CYP2C8 inhibitors or anticoagulant/antiplatelet therapies. Antacids can be used. Please see below for a list or medications that cannot be taken with tolebrutinib.

Strong CYP3A Inducers or CYP2C8 Inhibitors

Generic name	Brand name	Therapeutic class
metamizole (dipyrone)	Novalgin	Analgesics
apalutamide	Erleada	Antiandrogens
enzalutamide	Xtandi	Antiandrogens
rifampicin	Rifampin	Antibiotics
rifapentine	Priftin	Antibiotics
rifabutin	Mycobutin	Antibiotics
nafcillin	Nallpen, Unipen	Antibiotics
phenytoin	Dilantin	Anticonvulsants
carbamazepine	Tegretol	Anticonvulsants
phenobarbital	Luminal Sodium, Solfoton	Anticonvulsants
cenobamate	Xcopri, Ontozry	Anticonvulsants
telotristat ethyl	Xermelo	Antidiarrheals
lesinurad	Zurampic	Antigout and uricosuric agents
mitotane	Lysodren	Antineoplastics
thioridazine	Mellaril	Antipsychotics
asunaprevir/beclabuvir/ daclatasvir	Daklinza	Antivirals
ivosidenib	Tibsovo	Cancer treatments
lumacaftor	Orkambi	Cystic fibrosis treatments
bosentan	Tracleer	Endothelin receptor antagonists
gemfibrozil	Lopid	Fibric acid derivatives
elagolix	Orilissa	Gonadotropin-releasing hormone antagonist
St John's wort extract		Herbal medications
dabrafenib	Tafinlar	Kinase inhibitors
lorlatinib	Lorbrena	Kinase inhibitors

pexidartinib	Turalio	Kinase inhibitors
efavirenz	Sustiva	Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
etravirine	Intelence	Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
lopinavir/ritonavir	Kaletra	Protease inhibitors
tipranavir / ritonavir	Aptivus	Protease inhibitors

CYP3A4 Inhibitors

boceprevir	cobicistat	danoprevir and ritonavir
elvitegravir and ritonavir	grapefruit juice	indinavir and ritonavir
itraconazole	ketoconazole	lopinavir and ritonavir
paritaprevir and ritonavir and (ombitasvir and/or dasabuvir)	posaconazole	ritonavir
saquinavir and ritonavir	telaprevir	tipranavir and ritonavir
telithromycin	troleandomycin	voriconazole
clarithromycin	idelalisib	nefazodone
nelfinavir		

Anticoagulant/Antiplatelet Therapies

Acetylsalicylic acid (aspirin)

Antiplatelet drugs (e.g., clopidogrel)

Vitamin K antagonist (warfarin)

Antithrombin agents (heparin, including low molecular weight heparin)

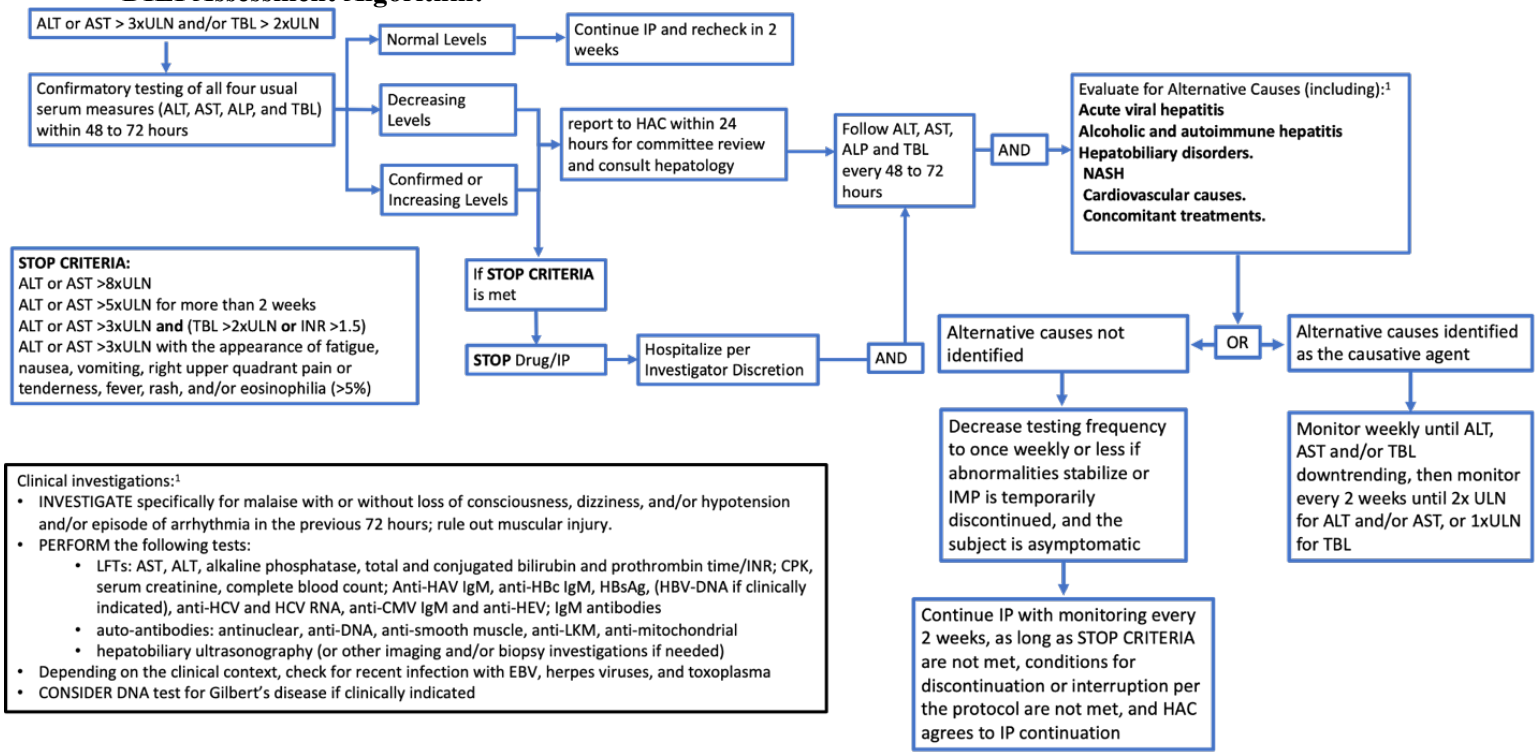
Direct thrombin inhibitor (dabigatran)

Direct factor Xa inhibitors (apixaban, edoxaban, rivaroxaban)

Note: All above drugs need to be stopped at least 5 half-lives before study drug administration except for aspirin, which needs to be stopped at least 8 days before.

12.2 APPENDIX 2: Adverse Events of Special Interest (AESI)

DILI Assessment Algorithm:



In ANY CASE REQUIRING HAC REPORTING, INFORM the PI, HAC and Sponsor and follow the instructions below:

- INVESTIGATE specifically for malaise with or without loss of consciousness, dizziness, and/or hypotension and/or episode of arrhythmia in the previous 72 hours; rule out muscular injury
- EVALUATE for alternative causes
- CONSULT with a hepatologist
- PERFORM the following tests:
    - LFTs: AST, ALT, alkaline phosphatase, total and conjugated bilirubin, GGT and pro-thrombin time/ INR
    - CPK, serum creatinine, complete blood count
    - Anti-HAV lgM, anti-HBc lgM, HBsAg, (HBV-DNA if clinically indicated), anti-HCV and HCV RNA, and anti-HEV lgM antibodies
    - Auto-antibodies: antinuclear, ANCA, anti-dsDNA, anti-smooth muscle, anti-LKM, anti-mitochondrial
    - Hepatobiliary ultrasonography (or other imaging and/or biopsy investigations if needed)
- DEPENDING on the clinical context, check for recent infection with EBV, CMV, herpes viruses, and toxoplasma
- CONSIDER iron, ferritin, transferrin saturation
- CONSIDER, DNA test for Gilbert's disease if clinically indicated
- CONSIDER patient hospitalization if INR>2 (or PT<50%) and/or central nervous system disturb-ances suggesting hepatic encephalopathy
- MONITOR transaminases and total bilirubin levels as closely as possible every 48 hours to every week until transaminases and/or total bilirubin levels are down-trending, then monitor every 2

weeks until 2xULN for transaminases and 1xULN for total bilirubin and then monitor every scheduled visit.

- SEND expedited (<15 days) reports to the FDA of any cases with transaminases >8x ULN or >3x ULN with total bilirubin >2x ULN.

RECHALLENGE: Re-initiation of the study drug can only be considered after discussion with the HAC, PI, DSMB and study Sponsor once the ALT/AST decrease below 2xULN and/or TBL decrease below 1xULN and there is no clinical contraindication. In case it is agreed to re-start the study drug, ALT, ALT, TBL and ALP will be assessed weekly for the 1st month and, then, monthly for the 2nd and the 3rd month. The occurrence of new elevations above 3xULN for the ALT/AST values will lead to permanent discontinuation of the study drug.

(Optional) FREEZE serum sample (5ml x 2)

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