Title: Anakinra for the Treatment of Chronically Inflamed White Matter Lesions in Multiple Sclerosis

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Précis:

- *Objective.* The overall goal of this study is to determine the safety, tolerability, and radiological efficacy of up to 12 weeks of subcutaneous injection of anakinra in people with multiple sclerosis and evidence, by magnetic resonance imaging (MRI), of chronic active (also known as “smoldering”) lesions in the white matter.
- *Study population.* 5 people with progressive or stable MS, at least one paramagnetic rim lesion on 7-tesla MRI, and no new white matter lesion formation for at least 3 months or clinical relapse for at least 12 months, will complete the study.
- *Design.* In this open label, dose escalation study, participants will receive up to 12 weeks of subcutaneous anakinra with initial dose of 100 mg daily up to a target dose of 300 mg daily. Study visits will occur every 4 weeks while on treatment, with 2 follow-up visits at 4 and 12 weeks after treatment discontinuation.
- *Outcome measures.* 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, clinical and radiological outcomes. Exploratory serological and CSF measures will also be obtained to investigate mechanism of action of anakinra and for biomarker development.

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1. Contents

List of Abbreviations

AE .....adverse event
ANC .....absolute neutrophil count
ALT .....alanine aminotransferase
AST ..... aspartate aminotransferase
BBB ..... blood-brain barrier
CBC .....complete blood count
CDC .....centers for disease control and prevention
CNS .....central nervous system
CSF .....cerebrospinal fluid
DLT .....dose-limiting toxicity
EAE .....experimental allergic encephalomyelitis
E.coli .....Escherichia coli
EDSS .....Expanded Disability Status Scale
EGFR .....estimated glomerular filtration rate

GVHD .....graft versus host disease
HDS .....human data sharing
IL .....interleukin
IL-1 .....interleukin-1
IL-1α- .....interleukin-1 α (alfa)
IL-1β- .....interleukin-1 β (beta)
IL-1Ra .....interleukin-1 receptor antagonist
rhIL-1ra .....recombinant human interleukin-1 receptor antagonist
sIL-1R .....soluble mouse recombinant interleukin-1 receptor
IP-10/CXCL10 .....interferon-gamma-induced protein 10/C-X-C motif chemokine 10
IND ..... investigational new drug
IFNγ..... interferon gamma
IRB .....institutional review board
IRIS .....Integrated Research Information System
ISM .....independent safety monitor
IV .....intravenous
KDa..... kilo Dalton
LP .....lumbar puncture
MRI .....magnetic resonance imaging
MS ..... multiple sclerosis
MSFC .....MS Functional Composite
NOMID .....Neonatal-Onset Multisystem Inflammatory Disease
NMR .....nuclear magnetic resonance
OCT ..... ocular coherence tomography
PET .....positron emission tomography

PMN ……polymorphonuclear neutrophils
QA…… quality assurance
RA ..... rheumatoid arthritis
SAE .....serious adverse event
SC .....subcutaneous
SDMT ……Symbol Digit Modalities Test
T ..... tesla
TB ……tuberculosis
TNF ……tumor necrosis factor
TEAE .....treatment-emergent adverse event
TUG ……..timed up-and-go
ULN .....upper limit of normal
WHO ……..world health organization
9HPT ……9-hole peg test

1. Introduction and Background

The purpose of this protocol is to test whether short-term treatment with anakinra, a recombinant, nonglycosylated form of the human interleukin-1 (IL-1) receptor antagonist (IL-1Ra), affects an imaging marker (the "paramagnetic rim") associated with chronically inflamed ("chronic active" or "smoldering") white matter lesions in multiple sclerosis (MS).

Section 0 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 1.2, we discuss the role of IL-1 and microglia in chronic active lesions. Section 1.3 describes anakinra, Section 1.4 the study rationale, Section 1.5 the risks and benefits based on preclinical and prior clinical testing, and Section 1.6 the proposed dosing. Finally, Section 1.7 provides the rationale for our exploratory outcome measures.

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 recognized 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 MRI contrast agent, usually gadolinium-based, into the parenchyma surrounding the inflamed venule. In our former 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) (6, 7).

The working hypothesis of the Translational Neuroradiology Section 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 be 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 age (Fig. 1) (11), consistent with laboratory data suggesting that inflammatory cell-mediated remyelination becomes progressively less successful with age (12, 13). 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 iron deposition within activated macrophages/microglia (11). Further, we have shown that this lesion outcome is associated with a more pro-inflammatory peripheral blood cytokine and chemokine profile at baseline.

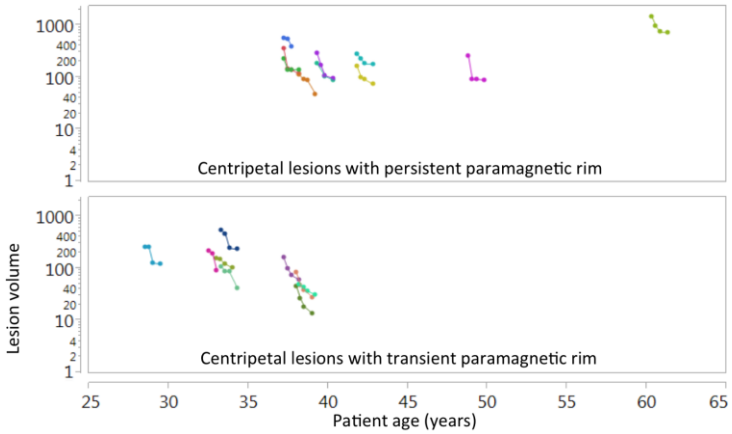

Figure 1. Semi logarithmic plot of the longitudinal volume for each lesion according to participant age and lesion group (centripetal with persistent paramagnetic rim and centripetal with transient paramagnetic rim) (Absinta et al., 2016b).

Over the past decade, results from several labs have converged to indicate that the pathological basis of the paramagnetic rim is in fact iron accumulation within activated macrophages/microglia, which is visible on 7T MRI (11, 14, 15). These rims can be long-lasting (16) (potentially for several decades), and pathologically they mark the class of chronically inflamed lesions known, variously, as “chronic active,” “slowly expanding,” or “smoldering.” Such lesions make up approximately 10–30% of white matter lesions in MS and are substantially more common in longstanding, clinically progressive disease (17). Pathologically, they 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.

Role of IL-1 in chronic active lesions in MS

IL-1β production is induced in response to inflammatory stimuli and mediates various physiologic responses, including inflammatory and immunological responses. Early in vitro studies showed that IL-1β in brain is produced mainly by ameboid microglia (18). In studies, evaluating postmortem brain material of MS patients, IL-1 was found to be expressed mostly by macrophages within the lesion center and by microglia at the lesion edge and beyond it (19, 20). More recent studies, using combined immunocytochemistry and in situ hybridization, confirmed the cellular source of IL-1β to be primarily microglia (21).

Further studies, showed, that the chronic expression of IL-1 leads to the recruitment of PMN to the brain parenchyma and induces the activation of microglia and astrocytes, BBB breakdown, and reversible demyelination (22).

In an animal model of MS (chronic relapsing experimental autoimmune encephalomyelitis), IL-1β and IL-1ra mRNA and protein were found in white and grey matter areas during early clinical phases of the disease. The cells, that expressed IL-1β and IL-1Ra mRNA, were macrophages or activated microglia (23).

Cerebrospinal fluid (CSF) analysis in MS has demonstrated elevated levels of members of IL-1 family, in particular IL-1β and IL-1Ra, with further elevations in IL-1Ra after high-dose corticosteroid therapy (24). Another study determined that detection of IL-1β in CSF correlated with higher cortical lesion volume and number, but there was no correlation with the presence of acute gadolinium-enhancing lesions (25).

In preclinical studies in EAE, injections of IL-1α resulted in more severe disease, compared to controls. In addition soluble mouse recombinant IL-1R (sIL-1R), administered as an IL-1 antagonist, led to suppression of clinical paralysis and reduced wasting (26). Finally, IL-1β inhibition has not been previously investigated in MS, but, in subsequent studies, it was determined that intraventricular anakinra delays the repopulation of the microglia after their initial depletion (27). In addition, an early study with in vivo treatment with recombinant human IL-1 receptor antagonist (rhIL-1ra) showed suppression of clinical paralysis and reduction in level of wasting secondary to EAE, compared to control groups (28).

Since the paramagnetic rim of chronic active lesions in MS is due to iron accumulation within activated macrophages/microglia, and since IL-1β is produced mainly by microglia, we hypothesize that antagonization of IL-1β by anakinra could inhibit microglial proliferation and possibly lead to resolution of the chronic active lesions in MS. This would be detectable by elimination, or perhaps reduction, of the paramagnetic rim around such lesions.

Anakinra

Anakinra is a recombinant, nonglycosylated form of the human interleukin-1 (IL-1) receptor antagonist (IL-1Ra), produced by recombinant DNA technology using an *E. coli* bacterial expression system. Anakinra consists of 153 amino acids and has a molecular weight of 17.3 kDa. Anakinra belongs to the pharmacological class of IL-1 inhibitors: it blocks the biological activity of IL-1β by competitively inhibiting IL-1β binding to the interleukin-1 type I receptor (IL-1RI), expressed in a wide variety of tissues and organs. Anakinra is FDA-approved for use in rheumatoid arthritis (RA) and the cryopyrin-associated periodic syndrome Neonatal-Onset Multisystem Inflammatory Disease (NOMID) (29).

Pharmacokinetics:

The absolute bioavailability of anakinra after a 70-mg subcutaneous (SC) bolus injection in healthy individuals (n=11) is 95%. In people with RA, maximum plasma concentrations of anakinra occurred 3–7

hours after SC administration of anakinra at clinically relevant doses (1 to 2 mg/kg; n=18); the terminal half-life ranged from 4–6 hours. In RA patients, no unexpected accumulation of anakinra was observed after daily SC doses for up to 24 weeks.

The estimated anakinra clearance increases with increasing creatinine clearance and body weight, as established in the pharmacokinetic analysis encompassing 341 patients receiving daily SC anakinra injection at doses of 30, 75, and 150 mg for maximum of 24 weeks (29). In individuals with severe or end stage renal disease, the mean plasma clearance of Anakinra decreased by 70–75%. In patients with hepatic impairment, no formal studies have been conducted.

Five clinical studies of anakinra in treatment of rheumatoid arthritis, with a total of 3055 patients, have been conducted. Collectively, these studies have shown significant improvement in clinical, laboratory, and radiographic parameters in the treated groups, although there was no additional benefit of anakinra in combination with a TNF inhibitor, etanercept (30-34). Safety data from these trials show that anakinra has been generally well-tolerated, with no difference in serious adverse events (SAEs) compared to placebo group. Concurrent administration of anakinra and etanercept for up to 24 weeks resulted in a 7% rate of serious infections, vs. 0% in the etanercept-only group (0%); 2% of the concurrent therapy group patients developed neutropenia (absolute neutrophil count $<10^9$/L) (32, 34). Therefore, use of anakinra combined with a TNF-blocking agent is not recommended.

Anakinra has also been approved by the FDA for treatment of neonatal onset multisystem inflammatory disease (NOMID), having produced improvement in neurological and systemic manifestations of disease, correlating with improvement in laboratory parameters; indeed, most NOMID patients have achieved sustained disease control (35). Further evidence suggests a benefit of IL-1β inhibition in other autoimmune disorders, including Schnitzler's syndrome, Behçet's disease, adult-onset Still disease, systemic juvenile idiopathic arthritis, gout, etc. (36).

Study rationale

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 (17). Activated macrophages/microglia are the primary inflammatory cell type in chronic active lesions, and, as such, short-term depletion of these cells might prevent ongoing tissue damage and potentially allow endogenous repair mechanisms to commence. As described above, IL-1Ra is a natural inhibitor of IL-1, produced in the brain primarily by microglia; animal studies further provide evidence of clinical improvement with use of an IL-1β antagonist (26, 28).

Thus, we postulate that using recombinant human IL-1Ra, or anakinra, will inhibit proliferation of activated macrophages/microglia at the edge of chronic active lesions. Restriction of the study to participants with clinically progressive MS, or stable MS with at least mild disability, reflects the current

lack of good treatment options to arrest or reverse disability in MS, with the goal of ultimately forestalling impaired mobility and cognition.

As there are no prior data by which to gauge the efficacy of anakinra on chronic active lesions in MS, we will initiate treatment at the FDA-approved dose of 100 mg/d SC and increase the dosage by 100 mg/d every 4 weeks to achieve a target dose of 300 mg/d. Dosing will increase if well tolerated by the patients or until the resolution of the paramagnetic rim lesions. Safety and tolerability measures, as well as pharmacokinetic and pharmacodynamic markers of IL-1Ra action in blood and CSF, will also be obtained as part of this study.

Dosing Considerations

FDA-approved dosage of anakinra for the treatment of RA is 100 mg/day subcutaneous injection. Doses up to 200 mg/day have shown to be safe in RA, and there are reports of safe administration of doses up to 300 mg/day in various rheumatological indications (37). In graft versus host disease (GVHD), doses from 400–3200 mg/d, by Q24H (every 24 hrs) continuous infusion for 7 days, have been attempted, and showed no significant side effects at the highest doses (the only toxicity was transient elevation of transaminases in two patients) (38). We therefore will initiate treatment with anakinra at a dose of 100 mg/day SC, with dose escalation every 4 weeks to a maximum dose of 300 mg/day, unless endpoints (Section 8.4, Section 9.1) are met earlier.

A study comparing anakinra at 3 mg/kg and 10 mg/kg doses showed that the CSF penetration of anakinra in nonhuman primates is 0.2–0.3%. CSF anakinra concentration peaked 1–2 hours post dose. The decline in CSF concentration of anakinra was slower than in serum, such that by the last measurable time point the CSF anakinra concentration was 56% of the serum concentration. Comparing the two doses, exposure in both serum and CSF increased by 3.7-fold at the higher dose, and the CSF penetration was similar (39).

That this degree of brain penetration is potentially effective in clinical situations is demonstrated by the example of NOMID, which is characterized by substantial neuroinflammation. Study evaluating CSF cytokines levels in NOMID patients treated with anakinra showed, that cytokines elevated at baseline, including IL-6, decreased in both plasma and CSF, whereas IP-10/CXCL10 decreased only in CSF (baseline blood levels being similar to controls) (40).

Rationale for outcome measures

7-tesla MRI. As described in Section 0, 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. The availability of this outcome measure makes it possible to assess the effects of novel therapies on such lesions.

Since multiple sclerosis is an inflammatory disease of the nervous system, and active CNS inflammation can only be detected by leakage of gadolinium contrast by MRI, the majority of the scans performed under this protocol will require gadolinium contrast.

Safety and tolerability. Safety and tolerability will be assessed through patient interview and logging of adverse events (AEs), as well as by lab work and imaging. Since anakinra has not previously been used in the treatment of multiple sclerosis and it is not known if this drug could unexpectedly exacerbate disease inflammatory activity, safety monitoring by imaging will require gadolinium contrast for all scans under this protocol except the Month 24 post treatment follow-up scan; at this latter time-point, gadolinium will only be administered if clinically indicated.

Blood and CSF biomarkers. We will collect blood and CSF samples for exploratory analysis of other potential biomarkers of anakinra's efficacy. Techniques to be used may include (but are not limited to) flow cytometry immunophenotyping and cytokine/protein assessments. Specific molecules to be assessed may include (but are not limited to); IL-1β, IL-1Ra, IL-2, IL-6, IL-10, IL-12, IL-18, TNF, IFNγ, IP-10/CXCL10, neopterin, and neurofilament light chain.

2. Study Objectives

Primary objectives

The primary objective of this protocol is to evaluate the effects of anakinra treatment on the paramagnetic rim of chronically inflamed white matter lesions, as seen on 7-tesla MRI.

Secondary objectives

The secondary objectives of this protocol are to collect and analyze additional clinical, imaging, and biological data relevant to the safety and tolerability of anakinra, as well as its mechanism of action.

3. Subjects

Description of study populations

This protocol will enroll up to 10 individuals with progressive or stable MS, evidence of chronic active lesions in the white matter (by MRI), and no evidence of new lesion formation in the prior 3 months or clinical relapse in the prior 12 months (by analysis of prior off-protocol MRI scans). We aim to achieve 5 study completers. NIH employees, regardless of their status as subordinates, relatives, or coworkers of investigators, are eligible.

Inclusion criteria

Inclusion criteria:

- Age ≥18
- Ability to give informed consent
- If fertile, agreement to use an effective method of birth control during the study and for up to 3 months after the last dose of the study drug
- Agreement not to participate in any other interventional study while participating in this protocol
- Diagnosis of MS, either stable or clinically progressive
- Prior 7-tesla MRI scan, with high image quality in the judgment of the study neuroradiologist, demonstrating at least one white matter lesion with a paramagnetic rim (41)

Exclusion criteria

General exclusion criteria:

- Pregnancy or current breastfeeding
- Use of another investigational agent within 1 month of screening
- Active infection and or neutropenia (ANC < 1000 cells/µL)
- History of lymphoma
- Known hypersensitivity to administration of anakinra
- Previous treatment with anakinra and/or TNF-receptor inhibitor
- History of asthma
- QuantiFERON-TB gold positive
- Prior treatment with anti-CD20 agent (ocrelizumab, rituximab)
- Prior treatment with anti-CD52 agent (alemtuzumab)
- History or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial or interfere with participation for the full duration of the trial; or not in the best interest of the subject to participate, in the opinion of the treating investigator
- Renal dysfunction, as defined by Clinical Center guidelines for administration of gadolinium
- Liver dysfunction, as indicated by baseline aspartate aminotransferase (AST) or alanine aminotransferase (ALT) greater than 1.5 times the upper limit of normal
- Clinical relapse in the 12 months prior to dosing
- New lesion formation (by comparison of screening MRI to a previous MRI of sufficient quality) in the 3 months prior to dosing
- One or more gadolinium-enhancing lesions on the screening scan
- Change in disease-modifying therapy in the 6 months prior to dosing
- Medical contraindication for 7-tesla MRI (including, but not limited to, any non-organic implant or other device such as a cardiac pacemaker or infusion pump or other metallic implants, objects, or body piercings, that are not MRI-compatible or cannot be removed)

- Psychological contraindication for 7-tesla MRI (e.g., claustrophobia)
- Contraindication to gadolinium administration.
- Active neoplastic disease or any medical condition, other than MS, that requires concurrent immunosuppression or immunomodulation

4. Study Design and Methods

Study overview

Screening will be done under an existing Neuroimmunology Clinic protocol that allows collection of data required for determining eligibility. Prospective participants who meet eligibility requirements will then provide informed consent and undergo baseline procedures. Following training on self-administration of subcutaneous injections, subjects will be started on initial dose of study drug of 100 mg/d. Participants will have a scheduled follow-up phone call at 24–48 hours following the first dose to assess for any adverse reactions or concerns. Subsequent routine assessment of tolerability and safety will be made at the time of scheduled study visits. For the duration of the study, including dose escalation and follow-up phases, participants will undergo assessments at NIH. Telehealth may be used for conducting routine visits to the NIH Clinical Center. Participants will return at weeks 4 and 8, at which time they will undergo clinic visit, lab work, and 7T brain MRI. As long as no treatment-limiting toxicity is encountered (see "safety endpoints," Section 8.4) AND the study endpoint is not met (see "efficacy study endpoint," primary outcome, section 9), the dose will be escalated every 4 weeks to a target dose of 300 mg/day, which will be administered for a further 4 weeks. The final on-treatment study visit will be at week 12. At that time, the drug will be discontinued, with off-treatment visits at weeks 16 and 24 (4 and 12 weeks following treatment discontinuation). The study timeline is in **Error! Reference source not found.**.

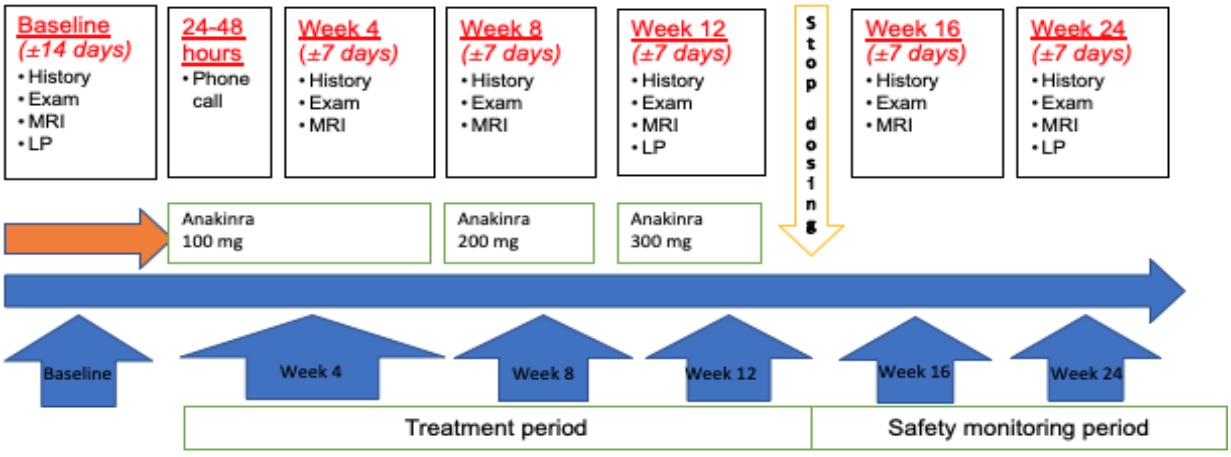

Fig. 2

- **MRI at week 24 will be done without Gadolinium, unless clinically indicated.**

Should early drug discontinuation occur, either due to dose-limiting toxicity or to achievement of efficacy endpoint, off-treatment follow-up visits will proceed 4 and 12 weeks after treatment interruption. If a 7T scan cannot be obtained within window at any particular study visit, a 3T MRI can substitute. Leftover biological samples will be stored for future analysis.

All study-related visits are expected to take place in outpatient facilities of the NIH Clinical Center and may last up to one full day, depending on the number of procedures to be performed. If more convenient for the participant or necessary for scheduling, visits may be split over several days (within the prescribed visit window). Participants may be hospitalized and treated for conditions that develop while on-protocol if medically necessary or for convenience. Data will be monitored in real time and fully analyzed once all 5 participants have completed all study procedures.

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 for new subjects and existing subjects. 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. 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. In the event that subjects are unable to come to the Clinical Center because of safety issues or travel restrictions, required in person procedures, physical exams, neurologic exams, research blood draws, lumbar punctures and MRIs, will be conducted as part of a make-up study visit at the next planned study visit time point or as soon as is safe and feasible.

Since this is an open-label study, results of research procedures may be discussed with the participants.

Recruitment

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.*
- *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, recruitment is anticipated to be straightforward. Should recruitment prove unexpectedly challenging, the study team will reach out to clinicians in the local community 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. Prior to enrollment, NIH employees will be asked to review the "Leave Policy for NIH Employees Participating in NIH Medical Research Studies" (NIH Policy Manual 2300-630-3).

Screening

Candidates will be screened under one or more Neuroimmunology Clinic protocols (e.g., 89-N-0045, 15-N-0158, 16-N-0055).

Study procedures

All procedures in this protocol are for research and are required; however, protocol deviations will only be filed for missed procedures if those procedures are necessary for primary outcome measures, or if more than 15% of procedures relating to secondary outcomes are missed. Missed collection of exploratory outcome measures will not be reported as study deviations. Data and leftover samples collected under this protocol may be used for other protocols on which the participant is enrolled, if allowed by the other protocol. Data and leftover samples collected under another protocol, if sufficient for study visits under this protocol, may be used.

We may review the participant's NIH medical records to obtain results of procedures conducted under another protocol which are consistent with the study procedures and time window outlined in Section 4, to minimize repeating procedures/tests, including medical history, clinical testing, biospecimen collection, and imaging.

For women of childbearing age, urine or serum pregnancy testing will be performed prior to initial study drug dosing and to all MRI scans. MRI scans will include intravenous administration of an FDA-approved, macrocyclic, gadolinium-based MRI contrast agent. The gadolinium is needed in order to detect active CNS inflammation; at baseline, this would exclude a patient from study participation (see Section 3,

Exclusion Criteria), while at Weeks 4, 8, 12 and 16 this is needed for safety monitoring. The Week 24 MRI will be performed without gadolinium, unless clinically indicated. Unless otherwise specified in the protocol, contrast agents will be used at FDA-approved doses. NIH Clinical Center Radiology and Imaging Sciences guidelines for gadolinium administration will be followed. If indicated by Clinical Center radiology guidelines, eGFR testing will be performed prior to MRI scans with gadolinium contrast. Any clinical testing required, including pregnancy testing and eGFR, will be done at the NIH Clinical Center.

An FDA approved gadolinium guide is available on the FDA website: https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-fda-warns-gadolinium-based-contrast-agents-gbcas-are-retained-body . We will give participants a copy of the gadolinium guide.

An MRI evaluations of the brain may be performed at a field strength of 7T, under conditions designated by the FDA as constituting nonsignificant risk under 21 CFR 812.2(b)(1). Unless specifically designated below, MRI scans are performed on FDA-approved scanners and their use conforms to the corresponding FDA labels. Acquisition parameters may be modified within range. These studies may involve software modifications, including research pulse sequences. All research pulse sequences will conform to the FDA's Criteria for Significant Risk Investigations of Magnetic Resonance Diagnostic Devices.

The following non-FDA-approved systems are 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 (Criteria for Significant Risk Investigations of Magnetic Resonance Imaging Devices) are operational:

- MAGNETOM 7T scanner (manufactured by Siemens)

For the purposes of this protocol, research postprocessing software is exempt from the IDE regulations under 21 CFR 812.2(c) because it is noninvasive, does not require an invasive sampling procedure that presents significant risk, and does not by design or intention introduce energy into a subject. It is unavoidable that results obtained with such software will sometimes have diagnostic implications in the context of this study. However, the diagnosis is always confirmed by another, medically established diagnostic product or procedure.

The study is expected to be completed within 30 months of first dosing. Each of the first two participants will complete at least 4 weeks of dosing prior to the next participant being dosed; thereafter, participants may be enrolled and dosed concurrently.

Additional clinical procedures may be ordered at any study visit, or at unscheduled follow-up visits, based on clinical judgment and would be obtained for clinical purposes.

Specific procedures:

- ***Baseline.*** The baseline visit (which may occur over a period lasting up to 2 weeks) will include an interview to confirm that eligibility criteria are met, informed consent, urine or serum pregnancy testing for women of childbearing age, 7T MRI, bloodwork (to include clinical laboratory studies required for establishment of baseline values as well as pre-treatment research samples for exploratory outcomes detailed in Section 9), and lumbar puncture (LP, under fluoroscopy if required for scheduling or if judged by the study team to be in the best interest of the participant). We may collect up to 172.8 ml of blood at the screening/baseline visit. Data and samples collected at screening and sufficient for baseline procedures may be used if collected within one month of dosing and if there is no change in clinical history, but they may also be repeated at this time at the discretion of the PI. If the LP is not tolerated, the participant will be withdrawn and replaced. Clinical scales may include, but may not be limited to, Expanded Disability Status Scale (EDSS), MS Functional Composite (MSFC), Symbol Digit Modalities Test (SDMT), and timed up-and-go (TUG).
- ***Dosing.*** Within one week of completion of baseline procedures, participants will be given their first supply of anakinra, to be self-administered at a dose of 100 mg/day by SC injection. Subjects will be provided with a sharps box to dispose of empty syringes and instructed to keep all used drug vials. After the first dose, participants will be contacted by telephone to assess tolerability. The dose will be escalated by 100 mg every 4 weeks, up to a target dose 300 mg/day, if tolerated well and as long as safety and efficacy end-points are not yet met. Participants will be instructed that missed doses should not be administered as a double dose at the next dosing time point. 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.
- ***Weeks 4, 8, and 12.*** At each visit, sharps boxes and empty vials will be collected. Participants will undergo a brief clinical interview/exam, lab work (to include clinical laboratory studies required for safety monitoring such as CBC with differential and blood collection for research purposes as detailed in Section 9, Exploratory Outcome Measures), clinical scales and LP (week 12 only), and 7T MRI (with gadolinium, if not contraindicated). We may collect up to 175 mL of blood at the week 4, week 8, and week 12 visit not to exceed the Clinical Center's policy for limits of blood drawn for research purposes. The window for these visits will be ±7 days.
- ***Week 16 (or 4 weeks after dosing discontinuation).*** Participants will undergo a brief clinical interview, lab work (clinical and research), 7T MRI (with gadolinium, if not contraindicated). We may collect up to 155 ml of blood at the week 16 visit. The window for this visit will be ±7 days.
- ***Week 24 (or 12 weeks after dosing discontinuation).*** Participants will undergo a brief clinical interview, lab work (clinical and research), clinical scales, LP, and 7T MRI (without gadolinium, unless clinically indicated). We may collect up to 175 mL of blood at the week 24 visit. The window for this visit will be ± 7 days.

Criteria for Early Discontinuation of Dose Escalation

Dose escalation will be discontinued in the following situations:

- Dose Limiting Toxicity is observed, as defined in Section 8.
- Efficacy Study Endpoint is achieved, as defined in Section 9.

- The patient is found to be pregnant.
- The patient, for any reason, is unwilling to continue treatment with study drug.

In the event of early discontinuation of dose escalation, the patient will proceed directly to the off-treatment follow-up phase of this study.

End of participation

Following completion of this protocol, study participants who so desire, and who meet eligibility criteria, may be followed under another Neuroimmunology Clinic protocol. They will also continue in the care of their primary outside clinicians. No specific medical care will be offered at completion of study procedures. At the discretion of the study team, selected data collected under this protocol may be shared with participants and/or their clinicians (following participant request).

5. Management of Data and Samples

Storage

At study completion, all leftover samples and data will be transferred to an appropriate repository protocol (11-N-0210 or a successor protocol) and may be pooled with similar data/samples collected under another protocol for exploratory analysis. Access to data and samples will be restricted to investigators on the repository protocol, or to appropriate designees. Loss or destruction of data and samples will be reported to the IRB per requirements of the repository protocol.

Data and sample sharing plan

The NIH Genomic Data Sharing policy does not apply to this protocol. Clinical, biological, and imaging data may be shared with other NIH protocols, other investigators, or databases/repositories, under the following guidelines:

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 with prospective IRB approval and informed consent from the subject. We may share existing identifiable data and/or specimens with other protocols when subjects are co-enrolled to minimize subjects having to repeat any of the procedures outlined or to pool data for analyses allowed on the other 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. Details of shared identified samples and data, and related agreements, will be reported to the IRB each year at the time of continuing review.

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"). When coded samples and/or data are shared, the key to the code will not be provided to collaborators, but will remain at NIH. Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

Some analysis of coded data will be conducted at Johns Hopkins University (JHU) under a reliance agreement. Data analysis will be conducted by Dr. Martina Absinta, a prior NIH employee who previously had access to identifiable data for this study. While no identifiers will be available to Dr. Absinta at JHU, the subject-level data may still be identifiable to her given the small sample size associated with this protocol.

Patients will not be consented to the study or participate in study interventions/procedures at JHU.

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.

This study is subject to the NIH Human Data Sharing (HDS) policy. De-identified study data may be shared with data repositories as described above. Furthermore, applicable (e.g., generated via CRIS) identified data may be shared via BTRIS following standard Clinical Center operating procedures including BTRIS data access policies. Data may be shared before the time of publication.

6. Additional Considerations

Research with investigational drugs or devices

Anakinra is FDA-approved for the treatment of rheumatoid arthritis (100mg/day) and NOMID (8mg/kg/day maximum). Its use in MS will be investigational. Since dose and route to be used in this study do not differ from those for FDA-approved indications, an IND exemption has been approved by the FDA.

The Clinical Center Pharmacy Department will be responsible for the receipt, storage, dispensing, and disposition of anakinra.

Gene therapy

No gene therapy will be used.

7. Risks and Discomforts

Risks of study drug

The following information is quoted (with minor changes) from the Kineret® (anakinra) package insert.

The most common (incidence >5%) adverse reactions to anakinra are injection-site reaction (seen in 71% of anakinra-treated patients vs. 29% in placebo; of these, 73% were mild, 24% moderate, and 3% severe), upper respiratory tract infection (see below), headache (12% vs. 9%), nausea (8% vs. 7%), diarrhea (7% vs. 5%). Sinusitis, arthralgia, flu-like symptoms, and abdominal pain were observed with the same frequency among people treated with anakinra or placebo. Among NOMID patients, typically treated with a higher dose of anakinra (up to 8mg/kg/day), the most common adverse reactions during the first 6 months of treatment (incidence >10%) are injection-site reaction, headache, vomiting, arthralgia, pyrexia, and nasopharyngitis.

Infections: Anakinra has been associated with an increased incidence of serious infections (2% vs <1% on placebo) in clinical trials in RA. People with asthma appear to be at higher risk of developing serious infections (4.5% vs. 0% on placebo). In patients receiving both anakinra and etanercept, the incidence of serious infections was 7%. The manufacturer recommends that health care providers follow current CDC guidelines to evaluate for and treat latent tuberculosis before initiating treatment with anakinra.

Malignancies: Among 5300 RA patients treated with anakinra, a 3.6-fold higher rate of lymphoma was observed than expected in the general population. An increased rate of lymphoma has been previously reported in RA, and the specific role (if any) of IL-1β blockers in the development of malignancy is not known. Thirty-seven malignancies other than lymphoma were observed. Of these, the most common were in the breast and respiratory, digestive systems and melanoma.

Hematological events: A decrease in total white blood cell counts (WHO toxicity grade I) is reported to occur in 8% of anakinra-treat patients vs. 2% on placebo. Only 0.4% of anakinra-treated patients developed neutropenia with absolute neutrophil count < 10^9/L; 9% presented hypereosinophilia (WHO toxicity grade I); and 2% developed thrombocytopenia (WHO toxicity grade I).

Immunogenicity: 49% of patients tested positive for anti-anakinra antibodies; no correlation was observed between antibody development and AEs.

<u>Contraindications, Drug Interactions, Warnings, and Precautions</u>

Anakinra is contraindicated in patients with known hypersensitivity to *E. coli*-derived proteins, anakinra, or any components of the product. Hypersensitivity reactions, including anaphylactic reactions and angioedema have been reported with anakinra.

TNF-blocking agents: The use of anakinra in combination with TNF blocking agents is not recommended due to heightened risk of serious infections. In a 24-week study of concurrent anakinra and etanercept (a TNF-blocking agent) therapy in adults with rheumatoid arthritis, the rate of serious infections in the combination arm (7%) was higher than with etanercept alone (0%).

Vaccinations/Immunizations: In a placebo-controlled clinical trial (n=126), no difference was detected in anti-tetanus antibody response between the anakinra and placebo treatment groups when the tetanus/diphtheria toxoids vaccine was administered concurrently with Kineret. No data are available on the effects of vaccination with other inactivated antigens in patients receiving Kineret. No data are available on either the effects of live vaccination or the secondary transmission of infection by live vaccines in patients receiving anakinra.

Pregnancy/Breast-feeding mothers: There are no adequate and well-controlled studies of anakinra in pregnant women. Animal studies reveal no evidence of impaired fertility or harm to the fetus due to anakinra. Anakinra should not be used during pregnancy unless clearly needed. It is not known whether anakinra is secreted in human milk, and, therefore, it should be used with caution in nursing women.

During the study, if the use of any concomitant treatment becomes necessary (e.g., for treatment of an AE), the treatment will be recorded in the electronic medical record, including the reason for treatment, name of the drug, dosage, route, and date of administration.

Risks of study procedures

- Risks of medical history, clinical examination, and clinical scale collection
  - There is minimal medical risk or discomfort from the clinical examination and clinical scale collection. The physical exam is for research purposes only. It does not replace any exam participants may receive from their own physicians.
- Risks of blood draws and intravenous lines
  - There may be some discomfort and bruising from the needle insertion. Some people feel light-headed or faint.
  - The risks of an intravenous catheter also include bleeding, infection, or inflammation of the skin and vein with pain and swelling. These will be treated if they occur.
- Risks of MRI
  - People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye, of

which they may be unaware. All subjects will be screened for these conditions prior to the study, and if they have any, they will not receive an MRI scan.
    - It is not known if MRI is completely safe for a developing fetus. Therefore, all women of childbearing potential will have a pregnancy test performed no more than 24 hours before each MRI scan. The scan will not be done if the pregnancy test is positive.
    - People with fear of confined spaces may become anxious during an MRI. Participants may be offered an anxiolytic prior to scans if they express anxiety about undergoing MRI (see below). Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. Everyone having a research MRI scan will be fitted with hearing protection. If the hearing protection comes loose during the scan, they will be instructed to let the technologist know right away. Subjects will be asked to notify the investigators if they have hearing or ear problems. Subjects will be asked to complete an MRI screening form for each MRI scan they have. There are no known long-term risks of MRI scans.
    - Anxiolytic agents, such as lorazepam, cause sedation. Other possible side effects include confusion, dizziness and agitation. Participants who receive an anxiolytic will be asked to stay in the clinic after the scan until an investigator determines it is safe for them to leave NIH. They will not be allowed to drive themselves home and will need to arrange for a driver; alternative, we will provide a taxi to take them home.
    - In this study, we will use an MRI scanner that has a strong 7-tesla magnet. Most people do not have any ill effects from the strong magnetic field, but some people have brief periods of muscle twitching, eye discomfort, dizziness, mild nausea, headache, a metallic taste in their mouth or a sensation of flashing lights. Many of these are from moving too rapidly in the magnetic field so the subject will be asked to walk slowly to the MRI table. Once on the table, the patient's head will be secured with cushions or the subject will be given a bar to bite on to prevent his/her head from moving. The table will then be slowly moved into the scanner. The subject will be informed that the scan can be stopped at any time should he/she be too uncomfortable. There is no evidence that scanning at high magnetic field strengths is dangerous, but long-term effects are not known.
    - During MRI procedures, the NIH code team with full medical coverage is available for any emergency. NIH NMR Center medical coverage policies will be followed. Individuals being scanned can be observed directly with a video surveillance system displayed in the control rooms and can be removed immediately upon request or in case of emergency. Steps to minimize risk also include the use of hearing protection and the testing for pregnancy in female participants with childbearing potential.
- Risks of gadolinium injection
    - Gadolinium is a contrast agent approved for use with MRI. The risks of an IV catheter include bleeding, infection, or inflammation of the skin and vein with pain and swelling. Symptoms from the contrast infusion are usually mild and may include feeling hot, burning or coldness in the arm during the injection, a metallic taste, headache, allergic reactions and nausea. In an extremely small number of individuals, more severe symptoms have been reported including shortness of breath, wheezing, hives, and lowering of blood pressure. Unless specifically allowed

by the protocol, participants will not receive gadolinium-based contrast agents for research purposes if they have previously had an allergic reaction to them. Individuals with a history of anaphylaxis to other agents or chronic asthma requiring treatment will not receive gadolinium under this protocol unless they have previously received gadolinium and tolerated it well. Participants will be asked about such allergic reactions and history of asthma before a contrast agent is administered.

- People with kidney disease are at risk for a serious reaction to gadolinium contrast called "nephrogenic systemic fibrosis," which has resulted in a very small number of deaths. If subjects are 60 years old or greater or have diabetes, kidney disease or liver disease, blood work to assess kidney function will be performed within 4 weeks before any MRI scan with gadolinium contrast. Participants may not receive gadolinium for a research MRI scan if kidney function is not normal. There is no evidence for the potential of gadolinium-related toxicity in people with normal kidney function. This protocol follows NIH Clinical Center guidelines for kidney-function screening related to gadolinium administration.
- Most of the gadolinium contrast is eliminated in the urine. However, recent studies have found very small amounts of residual gadolinium in the body, including the brain, by imaging and at autopsy. There is presently no evidence that the retained gadolinium is associated with any adverse effects.

- Risks of lumbar puncture
  - Adverse effects associated with lumbar punctures include brief pain or tingling paresthesias radiating down the lower extremity due to the needle brushing against a nerve. Should this occur, the needle can be repositioned. Mild lower back pain at the site of needle insertion following the procedure can occur; this can be managed with over the counter non-steroidal anti-inflammatory agents if needed. In approximately one third of patients, a post-dural puncture headache may develop and persist for a few days; in one in 50 to 200 lumbar punctures the post-dural puncture headache can last longer than 7 days. Generally, this headache is not severe and resolves spontaneously within days, but it can sometimes take up to 2 weeks. Should the headache persist or be severe, a blood patch can be performed. The volume of CSF collected per procedure represents negligible risk. In humans, the rate of CSF synthesis is approximately 21.5 ml/hour or approximately 500 ml/24 hours, which represents roughly 4 times the total volume of CSF in an adult patient. Therefore, the volume of 20–30 ml of CSF that would be collected for both diagnostic and research purposes will be replenished in its entirety within approximately 1–1.5 hours after collection. Lumbar puncture may be done in the radiology department under fluoroscopic guidance for patient medical or scheduling needs. The radiation exposure during this procedure is 0.046 rem, which is well below the guideline of 5 rem per year allowed for research subjects by the NIH Radiation Safety Committee.
- Risks of urine collection
  - There is minimal medical risk or discomfort associated with giving a urine sample.

8. Subject Safety Monitoring

Monitors for individual subjects during participation in study procedures

The NIH PI will be responsible for the safety of the participants and for monitoring and reporting any unanticipated problems or AEs. The study personnel will closely monitor patient safety during study procedures. Credentialed clinical staff will monitor all contrast-enhanced MRI scans in accordance with NIH NMR Center policies.

Each patient will be contacted within 24-48 hours following the first dose of anakinra to assess tolerability. At each subsequent visit to NIH, prior to dose escalation, tolerability will be assessed; any subject found to meet dose-limiting toxicity criteria, will discontinue treatment and proceed into the off-treatment follow-up phase of the study.

Parameters to be monitored

Parameters to be monitored include vital signs; clinical bloodwork (CBC and chemistry panel to include hepatic panel); neurological exam; brain MRI. Bloodwork will be performed for the purpose of safety monitoring only.

Toxicity tables/criteria to be used

Toxicity will be assessed according to the most recent version of the NCI Common Terminology Criteria for Adverse Events (CTCAE).

Criteria for individual subject withdrawal

Patients may be discontinued from the study at the discretion of the Investigator for medical, administrative or non-compliance issues. Pregnancy will constitute reason for individual subject withdrawal. Furthermore, any subject who wishes to discontinue study drug treatment has the right to withdraw from the study at any time and for any reason. Such participants will be asked to undergo a single concluding visit once off treatment, addressing only safety outcomes.

The following specific criteria will be considered dose-limiting toxicity (DLT) and will define criteria for discontinuation of study drug:

- *Tolerability criteria*. At the time of scheduled dose escalation, subjects will be asked if they are willing to initiate the next higher dosing regimen. Refusal to escalate based on poor tolerability will define the tolerability criteria and will constitute reason for withdrawal of individual patients from

the study. Tolerability criteria will further be met if subjects who escalated to the higher scheduled dose refuse to continue taking this dose due to poor tolerability.

- *General safety criteria.* Development of any treatment-related SAE will constitute reason for withdrawal of individual patients from the study.
- *Neurological safety criteria.* An increase in EDSS of ≥1 point and/or a decline of ≥20% on the 25-foot timed walk or 9-hole peg test (components of the MSFC) will prompt an extra follow up visit at 2 weeks +/- 3 days. The extra follow up visit would include clinical scales assessment, and may include neurological examination and an additional MRI brain and/or spinal cord, per the discretion of the clinician. Any confirmed sustained decline over the 2 clinic visits will constitute a reason for withdrawal of individual patients from the study. Changes will be measured with respect to average scores obtained during the screening phase. Relapses may constitute reason for withdrawal from the study; relationship to the study drug will be determined by the Independent Study Monitor.
- *Imaging safety criteria.* Appearance of new contrast-enhancing lesions or obvious enlargement of existing lesions on any single scan, in the opinion of the study neuroradiologist, will constitute reason for withdrawal of individual patients from the study. While new contrast enhancement could simply be due to the relapsing-remitting nature of this disease, it could also indicate unintended and unexpected exacerbation of CNS inflammation resulting directly or indirectly from modulation of the immune network by the study drug.

The reasons for discontinuation of the study drug will be recorded in the participant's Clinical Center patient file. These subjects will remain in the study and complete follow-up study visits (4 and 12 weeks following dosing discontinuation).

If discontinuation was because of an AE, the participant will be followed until the event is resolved or stabilized. If the discontinuation was because of pregnancy, the participant will be followed until after delivery.

9. Outcome Measures

Primary outcome measures

The primary outcome measure is the presence or absence of a paramagnetic rim around chronic active white matter MS lesions, assessed at the end of the dosing period.

Definition of efficacy endpoint: disappearance of one or all paramagnetic phase rims. At each study visit, paramagnetic phase rims will be assessed. For the purposes of dose escalation, if at any on-treatment scan, all paramagnetic rims that were present at baseline are no longer seen, dose escalation will end and the subject will proceed into the post-treatment follow-up phase of the study.

Secondary outcome measures

- Safety and tolerability: AE tables
- Clinical
  - Expanded Disability Status Scale (EDSS)
  - 9-hole peg test (9HPT)
  - Symbol digit modalities test (SDMT)
- MRI:
  - Proportion of paramagnetic rim lesions in which the rim has diminished or disappeared at any time point
  - Changes in T1 relaxation time within paramagnetic rim lesions at all time points, relative to non-rim lesions
  - Changes in size of paramagnetic rim lesions at all time points, relative to non-rim lesions

Exploratory outcome measures

Exploratory outcome measures will be derived from clinical scales (may include those mentioned as secondary outcome measures and others, e.g. 25-foot timed walk, paced auditory serial addition test, and Scripps Neurological Rating Scale), MRI parameters ((characteristics/persistence of paramagnetic rims at all time points (qualitative)), and stored biofluids, with focus on immune cell populations (e.g. macrophages, lymphocytes, and subtypes thereof), cytokines (e.g., IL-1ß, IL-1Ra, IL-6), and also on protein and other biomarkers of neurodegeneration (e.g. neurofilament light chain). Additional assays (e.g. for anti-anakinra antibodies) may also be performed. These analyses will be performed in NIH labs or through collaboration, either intramural or extramural.

10. Statistical Analysis

Analysis of data/study outcomes

The primary outcome is the per-patient proportion of lesions in which the paramagnetic rim has been modulated at the end of the dosing period. Proportion of diminished/resolved paramagnetic rims will be analyzed by investigators blinded to time point, reported descriptively, and compared to the assumption of no change off therapy (11, 16) — a reasonable assumption since we have never observed complete disappearance of a chronic rim — via Binomial exact tests.

For the secondary outcome measures, safety and tolerability will be assessed qualitatively; AEs will be tabulated and compared to prior published and unpublished (to the extent possible) data on anakinra. Quantification will include the proportion of participants who start anakinra therapy and complete the full dosing. MRI outcomes will be reduced to scalar measures at various time points. Longitudinal changes in rim presence/absence, T1 relaxation time, and lesion volume will be analyzed by analysis of variance or mixed-effects models (which naturally handle missing data), the latter two in comparison to

non-rim lesions. Analyses will be performed both in completers and on an intent-to-treat basis. There will be no interim analysis.

Analysis methods for exploratory outcome measures will be determined by the study team and collaborators at a later time point.

Power analysis

Given the previous natural history data, which indicates that only 1 out of 29 individuals experienced subtle changes in the paramagnetic rim over the long term (a period of several years), we would expect that very few to no untreated participants will experience changes in the short time period observed in this study. Thus, even 1 participant experiencing short term changes would potentially be clinically meaningful. Using an estimated null proportion of individuals with lesion changes of 0.01, a cohort of 5 participants achieves 80% power to detect a difference of 0.27 using a one-sided binomial exact test with a significance level of 0.05. The null hypothesis will be rejected if 1 individual experiences at least one lesion change.

Participants who drop out or in whom anakinra is stopped will be replaced, up to the accrual ceiling or until there are 5 completers — whichever comes first.

11. Human Subjects Protection

Subject selection

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. For this reason, in this initial pilot study, pediatric patients will be excluded.

Justification for exclusion of other vulnerable subjects

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.

Pregnant and lactating women will be excluded as the risk of anakinra in pregnancy and lactation is not known, and as the risk of MRI — a key outcome measure — would not outweigh the potential benefits of the study.

Justification for inclusion of employees

NIH staff will not be solicited for participation, but will not be excluded if they express the desire to enroll. Employees who participate in this protocol during work hours will be informed that they must obtain their supervisor's permission.

Justification for sensitive procedures

Not applicable.

Safeguards for vulnerable populations and sensitive procedures

An approved method of birth control will be required of all fertile participants (male or female) during the study and for up to 3 months after the last dose of the study drug. Prospective participants who are pregnant will be evaluated clinically, and previously acquired diagnostic studies and clinical documentation will be reviewed. Such individuals will be invited to return for additional evaluation following delivery, if the study is still recruiting. Within 24 hours and prior to undergoing any MRI, urine or serum specimens from women with childbearing potential will be collected, and pregnancy tests will be performed.

Protections for employees and staff participating in this study include: (1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the participant's employment, training or position at the NIH; (2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent; (3) 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.; and (4) requesting them to review the Leave Policy for NIH Employees Participating in NIH Medical Research Studies (NIH Policy Manual 2300-630-3) prior to enrollment; and (5) assuring that there will be no direct solicitation of employees or staff.

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 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.

12. Anticipated Benefit

Participants may benefit from participation if anakinra is able to decrease or resolve chronic inflammation in MS, as such inflammation may play an important role in disease progression (17). In addition, 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.

13. Consent Documents and Process

Designation of those obtaining consent

All study investigators obtaining informed consent have completed the NIMH Human Subjects Protection Unit "Elements of Successful Informed Consent" training.

Consent procedures

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.

If a non-English speaking participant is eligible for enrollment, the Clinical Center standard short written consent form in the appropriate language and a written summary of what the Investigator will say to the participant will be used as part of an oral consent process. The IRB-approved English written consent form will serve as the written summary if the short form process is used. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is independent of the participant (i.e., not a family member). Interpreters provided by the Clinical Center will be used whenever possible. The interpreters will translate the current IRB-approved English version of the consent verbatim and facilitate discussion between the participant and investigator.

The written summary will be signed by the investigator obtaining consent and a witness to the oral presentation. The short written consent form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent documents as the witness and, in this case, will note “interpreter” under the signature line. A copy of the signed form will be provided to the participant to take home.

Consent documents

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.

14. Data and Safety Monitoring

Data and safety monitor and monitoring plan

Data and safety will be monitored by the Principal investigator and by the Independent Safety Monitor (ISM), Dr. Peter Grayson. Data collection and participant safety is discussed and monitored on a weekly basis at the NINDS Neuroimmunology Clinic’s clinical care meetings when a subject is actively enrolled. The ISM’s responsibility will be to provide independent medical monitoring in a timely fashion. The ISM will be notified of all SAEs and serious unanticipated problems immediately, but not more than 7 days, after the PI first learns of the event, and will follow them up through resolution. The ISM will evaluate individual and cumulative participant data when making recommendations regarding the safe continuation of the study. Every 3 months, the ISM , Dr. Grayson, will be provided with reports on safety and enrollment as well as all protocol revisions. If no interval data were collected, the monitor will be informed, and a report will not be required. These reports will include information on laboratory tests and all AEs. The ISM will not have direct involvement in the conduct of the study.

Criteria for stopping the study or suspending enrollment or procedures

Enrollment will be suspended, until ISM assessment is completed, if 2 participants develop definite or probable treatment-related SAEs. Depending on the nature of the SAEs and their potential relationship to the study medication, the ISM may request additional clinical information and will decide on further action. If the SAEs are determined to be treatment-related, the study will be stopped. In this event, all enrolled participants still receiving treatment will have treatment discontinued and will proceed into the post-treatment follow-up.

15. Quality Assurance (QA)

Quality assurance monitor

This study is classified as “more than minimal risk” and thus will be audited with a target frequency of once during the first year following IRB approval, and then approximately every three years thereafter.

Quality assurance plan

Data obtained under this protocol will be reviewed by the study investigators and designees on a regular basis, and to the extent possible immediately after the data are collected. NINDS and NIH personnel with the appropriate authority may audit this protocol and associated data. Additionally, every 3 months, the ISM , Dr Grayson, will be provided with reports on safety and enrollment as well as all protocol revisions.

16. Reporting of Unanticipated Problems, Adverse Events and Protocol Deviations

Reportable events will be tracked and submitted to the IRB as outlined in Policy 801.

17. Alternative Therapies

There are currently no therapies approved to treat chronic active lesions in MS. Participants are not required to forego approved disease-modifying therapy for MS (which is designed to limit new lesion and relapse development, not resolution of chronic active lesions). Anakinra, the study drug, is FDA-approved for treatment of RA and NOMID, and may be obtained off-label by the patient’s treating physician.

18. Privacy

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

19. Confidentiality

For research data and investigator medical records

Data and investigator medical records will be stored using codes that we assign; some data may also be stored in the NIH medical record system. The data will be kept on password-protected computers. Only

study investigators, or designees in the investigators' groups who have completed appropriate requirements of the Office of Human Subjects Research Protection, will have access to the data, unless the data are shared according to the provisions of this protocol. Monitors and auditors may have access to identified information. De-identified data will be posted on clinicaltrials.gov per NIH policy.

For stored samples

Samples will be stored using codes that we assign and will be kept in locked storage. Only study investigators will have access to the samples, unless the samples are shared according to the provisions of this protocol.

Special precautions

Not applicable.

20. Conflict of Interest

Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

Conflict of interest

There are no conflicts-of-interest to report for NIH investigators. Non-NIH investigators will abide by the conflict-of-interest policies of their own institutions/organizations.

Role of a commercial company or sponsor

Not applicable.

21. Technology Transfer

Sample and data derived from this protocol may be analyzed in conjunction with outside collaborators, and in these instances materials and data transfer agreements will be obtained, or existing agreements will be followed. If we obtain such agreements for use with this protocol, we will provide details to the IRB at the time of continuing review, together with information about samples and data that were shared. This is not sponsored research.

22. Research and Travel Compensation

Participants will not be compensated for time and research-related inconveniences. Travel/lodging compensation may be provided for out-of-town participants or for those for whom traveling to NIH is a hardship. In such cases, an escort fee may be provided.

NIH employees or staff who participate during work hours must have permission from their supervisor. NIH employees or staff must either participate outside of work hours or take leave in order to receive compensation.

23. Attachments/ Appendices

Appendix 1. Eligibility checklist

Inclusion Criteria

- ☐ Age ≥18
- ☐ Ability to give informed consent
- ☐ If fertile, agreement to use an effective method of birth control during the study and for up to 3 months after the last dose of the study drug
- ☐ Agreement not to participate in any other interventional study while participating in this protocol
- ☐ Diagnosis of MS, either stable or clinically progressive
- ☐ Prior 7-tesla MRI scan, with high image quality in the judgment of the study neuroradiologist, demonstrating at least one white matter lesion with a paramagnetic rim (41)

Exclusion Criteria

- ☐ Pregnancy or current breastfeeding
- ☐ Use of another investigational agent within 1 month of screening
- ☐ Active infection and or neutropenia (ANC < 1000 cells/µL)
- ☐ History of lymphoma
- ☐ Known hypersensitivity to administration of anakinra
- ☐ Previous treatment with anakinra and/or TNF-receptor inhibitor
- ☐ History of asthma
- ☐ QuantiFERON-TB gold positive
- ☐ Prior treatment with anti-CD20 agent (ocrelizumab, rituximab)
- ☐ Prior treatment with anti-CD52 agent (alemtuzumab)
- ☐ History or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial or interfere with participation for the full duration of the trial; or not in the best interest of the subject to participate, in the opinion of the treating investigator

- ☐ Renal dysfunction, as defined by Clinical Center guidelines for administration of gadolinium
- ☐ Liver dysfunction, as indicated by baseline aspartate aminotransferase (AST) or alanine aminotransferase (ALT) greater than 1.5 times the upper limit of normal
- ☐ Clinical relapse in the 12 months prior to dosing
- ☐ New lesion formation (by comparison of screening MRI to a previous MRI of sufficient quality) in the 3 months prior to dosing
- ☐ One or more gadolinium-enhancing lesions on the screening scan
- ☐ Change in disease-modifying therapy in the 6 months prior to dosing
- ☐ Medical contraindication for 7-tesla MRI (including, but not limited to, any non-organic implant or other device such as a cardiac pacemaker or infusion pump or other metallic implants, objects, or body piercings, that are not MRI-compatible or cannot be removed)
- ☐ Psychological contraindication for 7-tesla MRI (e.g., claustrophobia)
- ☐ Contraindication to gadolinium administration.
- ☐ Active neoplastic disease or any medical condition, other than MS, that requires concurrent immunosuppression or immunomodulation

Appendix 2. Expanded Disability Status Scale (EDSS)

Kurtzke Expanded Disability Status Scale (EDSS)

❑ 0.0 - Normal neurological exam (all grade 0 in all Functional System (FS) scores*).

❑ 1.0 - No disability, minimal signs in one FS* (i.e., grade 1).

❑ 1.5 - No disability, minimal signs in more than one FS* (more than 1 FS grade 1).

❑ 2.0 - Minimal disability in one FS (one FS grade 2, others 0 or 1).

❑ 2.5 - Minimal disability in two FS (two FS grade 2, others 0 or 1).

❑ 3.0 - Moderate disability in one FS (one FS grade 3, others 0 or 1) or mild disability in three or four FS (three or four FS grade 2, others 0 or 1) though fully ambulatory.

❑ 3.5 - Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3 (others 0 or 1) or five grade 2 (others 0 or 1).

❑ 4.0 - Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combination of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 500 meters.

❑ 4.5 - Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability usually consisting of one FS grade 4 (others or 1) or combinations of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 300 meters.

❑ 5.0 - Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (e.g., to work a full day without special provisions); (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0).

❑ 5.5 - Ambulatory without aid for about 100 meters; disability severe enough to preclude full daily activities; (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combination of lesser grades usually exceeding those for step 4.0).

❑ 6.0 - Intermittent or unilateral constant assistance (cane, crutch, brace) required to walk about 100 meters with or without resting; (Usual FS equivalents are combinations with more than two FS grade 3+).

Appendix 3. Drug diary

PATIENT'S NAME: _____

Anakinra Self-Administration Study Drug Diary

This section to be completed by study team:

Dose (Please circle):	100 mg/ day	Month 1
	200 mg/ day	Month 2
	300 mg/ day	Month 3

Completed by: Date:

This section to be completed by patient:

Day	Date	Time Administered	Comments
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			

If you miss a dose of anakinra, you must inform the study team. You will restart the next day with the same dose. You should never inject a higher dose or more than one dose per day.

Appendix 4. Instructions for preparing the correct dose of Anakinra

INSTRUCTIONS FOR PREPARING THE CORRECT DOSE OF ANAKINRA

Anakinra comes prepared in a pre-filled syringe for subcutaneous injection. One pre-filled syringe contains 100 mg. Please see below for instructions dependent on the dose you have been instructed to self-administer. Please reach out to the study team at 301-496-3825 if you have any questions.

DOSE

100 mg/day

Please use 1 pre-filled syringe for injecting 100 mg subcutaneously. Please ensure you rotate injection sites.

200 mg/day

You will use 2 pre-filled syringes to inject 200 mg subcutaneously.

1. Transfer the contents of the first pre-filled syringe into the provided 3 mL syringe.
2. Transfer the contents of the second pre-filled syringe into the same 3 mL syringe.
3. Open the provided package for the needle and attach it to the 3mL syringe.
4. Administer the medication subcutaneously. Please ensure you rotate injection sites.

300 mg/day

You will use 3 pre-filled syringes to injection 300 mg subcutaneously.

1. Transfer the contents of the first pre-filled syringe into the provided 3 mL syringe.
2. Transfer the contents of the second pre-filled syringe into the same 3 mL syringe.
3. Transfer the contents of the third pre-filled syringe into the same 3 mL syringe.
4. Open the provided package for the needle and attach it to the 3mL syringe.
5. Administer the medication subcutaneously. Please ensure you rotate injection sites.

Please return any unused medication and your locked sharps-container at each visit.

24. Consent Forms

- Adult subject

25. References

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