Research Protocol

Analysis of lymphatic fluid from the thoracic duct of healthy subjects and patients with MS before and during Ofatumumab treatment

Protocol Version 2.0

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List of abbreviations

ADCC	Antibody-Dependent Cell-mediated Cytotoxicity
AE	adverse event
AESI	adverse event of special interest
AIDS	Acquired Immunodeficiency Syndrome
ALT	alanine aminotransferase
ARR	Annual Relapse Rate
AST	aspartate aminotransferase
BCR	B cell receptor
CDC	Complement-Dependent Cytotoxicity
CRA	Collaborative Research Agreement
CRF	Case Report/Record Form (paper or electronic)
CSF	Cerebrospinal Fluid
CTC	Common Toxicity Criteria
DMT	Disease Modifying Therapy
DNA	Deoxyribonucleic Acid
EDSS	Expanded Disability Status Scale
EOS	End of Study
FAS	Full Analysis Set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
Gd	Gadolinium
GFAP	Glial Fibrillary Acidic Protein
h	hour
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	human immunodeficiency virus
IDS	Investigational Drug Service (Penn Research Pharmacy)
Ig	Immunoglobulin
IRB	Institutional Review Board
iv	Intravenous

LFT	Liver function test
MAB	Monoclonal Antibody
MedDRA	Medical dictionary for regulatory activities
mg	milligram(s)
ml	milliliter(s)
MS	Multiple Sclerosis
NfL	Neurofilament Light Chain
OMB	Ofatumumab
PCR	Protein-Creatinine Ratio
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PML	Progressive Multifocal Leukoencephalopathy
PT	Prothrombin Time
q4	Every 4
RBC	red blood cell(s)
RMS	Relapse Multiple Sclerosis
s.c.	subcutaneous
SAE	serious adverse event
sCR	serum creatinine
TBL	total bilirubin
ULN	upper limit of normal
Penn	University of Pennsylvania
UTI	Urinary Tract Infection
WBC	white blood cell(s)

Protocol summary

IRB Number	831944
Full Title	Analysis of lymphatic fluid from the thoracic duct of healthy people and people with MS before and after Ofatumumab treatment
Brief title	Immune profiles in MS patients and healthy volunteers through thoracic duct cannulation
Sponsor Clinical Phase	University of Pennsylvania 3450 Hamilton Walk Philadelphia, PA 19104
Investigation type	'In-and-out' Cannulation: Healthy Volunteers and MS patients Indwelling Cannulation: Healthy Volunteers, and early MS patients before and after treatment with Ofatumumab (OMB)
Methodology	Clinical trial
Study type	Interventional
Study Duration	Approximately 4 years
Purpose and rationale	The central nervous system (CNS) contains a system of lymphatic drainage which has been described both based on pathology and more recently through in vivo imaging in humans and non-human primates. It has also been demonstrated that B cells traffic bi-directionally between the CNS compartment and the periphery – in particular the deep cervical lymph nodes which drain the CNS lymphatic system. From the deep cervical lymphatics, cells and soluble factors drain further into the thoracic duct. To characterize immunologically cells and soluble molecules (markers of inflammation/injury/repair) in the thoracic duct fluid is of great biological interest to understand normal immune system, changes in the immune system with MS and effects of treatment onto this immune system with a CD20+ B cell depleting drug, Ofatumumab. Insights from this study will have major implications both to our understanding of peripheral and CNS-compartmentalized biologies of MS, potentially identifying new druggable targets, as well as enable entirely novel assessment of the impact of OMB on disease-relevant immune responses in distinct compartments.
Primary Objective(s)	The study is designed to evaluate the safety and feasibility of collection of lymphoid fluid via thoracic duct cannulation in healthy people and people with MS.
Secondary Objective	Assess the impact of anti-CD20 OMB treatment in patients with MS on biological measures in the thoracic duct fluid and in the blood.
Exploratory Objectives	To compare immune cell profiles of blood and lymphatics between MS and healthy controls and in untreated MS patients before and following a B-cell depleting therapy (Ofatumumab).
Study design	Monocentric, single arm, open label study:

	1. 'In-and-out' catheterization: Safety and immune-cell profile of lymphatic fluid in MS patients with a single time-point sampling of lymphatic fluids and peripheral blood compared to healthy controls. **2. "Indwelling" catheterization**: immune-biology in people with MS before and during/after OMB treatment within thoracic duct and peripheral blood via indwelling catheter and multiple time-point sampling compared to healthy controls (without drug treatment). All MS patients undergoing 'in-and-out' or "indwelling" catheterization will be offered OMB treatment provided by the Research Collaborator for up to 2 years post-catheterization. OMB treatment is required for participation in the "indwelling" catheter procedure of MS patients.
Population	Total of 24 participants including: **'In-and-out' catheterization:** Two healthy controls and six patients with early MS (never treated or at least 90 days after discontinued treatment with glatiramer acetate or interferons), who consent to the 'In-and-out' catheter procedure. MS participants can also consent to OMB treatment with 2-year follow-up. **"indwelling" catheterization:** Twelve patients with early MS (never treated or at least 90 days after discontinued treatment with glatiramer acetate or interferons), who consent to treatment with OMB and to the indwelling catheter procedure with serial sampling and up to four healthy controls (no drug treatment)
Key Inclusion criteria	• Written informed consent must be obtained before any assessment is performed • Participants must be able to understand English and be able to review and comprehend the informed consent form independently or with the help of research staff • Participants aged between 18 and 55 years of age at the time of screening • No use of systemic glucocorticoids in the past 4 weeks For subjects with MS, following additional criteria apply: • Diagnosis of MS according to the 2017 Revised McDonald criteria • Disability status at screening defined by EDSS score of 0 to 4 (inclusive) • Treatment naive (never been treated) or interferons/glatiramer acetate only and drugs have been discontinued at least 90 days prior to intervention • Neurologically stable one month prior to first study drug administration
Key Exclusion criteria	• History of cardiovascular disease or uncontrolled hypertension • History of diabetes mellitus • History of cancer (other than localized skin cancer) in the previous 5 years • History of systemic connective tissue disease • Chronic HBV or HCV infection • Absolute neutrophil count (ANC) <750/mm3; • Hemoglobin <10.0 g/dL • Platelet count <100,000/mm3 • Prothrombin time (PT) > 2 x ULN • International normalized ration (INR) >1.5 x ULN

Study treatment where applicable	Ofatumumab 20 mg s.c. injections will be administered s.c. on treatment day 1, day 7 and day 14 and thereafter every 28 days. OMB also offered to MS patients participating undergoing “indwelling” cannulation, and will continue on for up to 2 years with follow-up. MS patients undergoing “in-and-out” may be OMB treated with 2-year post-procedure follow-up. OMB will be provided by the Research Collaborator as the FDA approved formulation. Treatment will commence approximately 48 hours after “indwelling” catheter or no sooner than 48 hours in those who undergo single “in-and-out” procedure, or, if present, once AEs have resolved.
Efficacy assessment	**Biological outcomes** will be described in terms of immune biology in healthy volunteers, people with MS, people with MS before and during early OMB treatment comparing central compartments (thoracic lymph) to peripheral (blood) **Exploratory clinical outcomes** for people with MS will be described in terms of: MRI (Gd+, T2 enlarging lesions, slowly evolving lesions, regional atrophy) MS relapse expressed as Annualized Relapse Rate (ARR) Expanded Disability Status Scale (EDSS) Neurofilament light chain (NfL) Glial fibrillary acidic protein (GFAP)
Key safety assessments	Adverse Events (AE) and Serious Adverse Events (SAE)
Data analysis	Analyses will be performed jointly by Penn and the Research Collaborator designates. Details of responsibilities and analyses will be outlined in the research collaboration agreement and the statistical analyses plan. This is an exploratory study. There is no data describing the distribution of populations of immune cells in the thoracic duct in healthy people, MS patients pre and post treatment with OMB compared to peripheral blood. Descriptive statistics will be used as well as nonparametric Mann Whitney U tests, the Wilcoxon matched pairs signed rank test or the Friedman test with Dunn’s multiple comparison post-test, as appropriate, to compare immune cell subset frequencies and antigen specific responses between thoracic duct and blood, between patients and controls, and prior to and following OMB treatment in each compartment. Correlation analyses will be analyzed using the nonparametric Spearman test. Differentially expressed genes between groups will be ranked using Smyth’s variance-moderated t-test. ‘In-and-out’ thoracic duct cannulation analyses will be based on all data collected from untreated healthy volunteers and early MS patients undergoing evaluating 1. Safety of procedure 2. Comparing immune biology of peripheral blood and thoracic duct fluid “indwelling” catheter intervention analyses will be based on all data collected from people with MS pre and post OMB and healthy controls 1. Safety of procedure 2. Indwelling catheter and immune profiling pre- and during OMB treatment for approximately 3 weeks – MS only

	All patients with MS will be offered up to two years of OMB to explore peripheral blood and clinical measures outcomes in people with early MS with findings obtained from thoracic duct samples.
Data and Safety Monitoring Plan	The three Penn investigators listed above will review the safety data after every 4 patients for "in-and-out" and every successive participant in "indwelling" for the first 4 participants and every 3 participants thereafter and will immediately review any cases of serious AE related to the study procedures following a reported event. All serious AEs will be reported to the Research Collaborator. Based on their assessment of risk/benefit of the procedure, by the Sponsor and the Research Collaborator, the study will progress as outlined or the study will be terminated.
Applicable Ethics, Policy and Procedure Statement	The following research study will be conducted according to the procedures and stipulations described in this protocol, and in full accordance with all applicable University of Pennsylvania Research Policies and Procedures and all applicable Federal and state laws and regulations including those regulations governing the protection of human participants (Human Participants Research, 45 CFR 46, US Department of Health and Human Services, and the Code of Federal Regulations 21 CFR Parts 50, 54, and 56).

1 Introduction

1.1 Background

1 Disease

Multiple sclerosis (MS) is the most common chronic inflammatory, demyelinating and neurodegenerative disease of the central nervous system in young adults. This disorder is a heterogeneous, multifactorial, immune-mediated disease that is influenced by both genetic and environmental factors. In most patients, reversible episodes of neurological dysfunction lasting several days or weeks characterize the initial stages of the disease (that is, clinically isolated syndrome and relapsing–remitting MS; RRMS). Over time, irreversible clinical and cognitive deficits develop.

The pathological hallmark of MS is the formation of demyelinating lesions in the brain and spinal cord, which can be associated with neuro-axonal damage. Focal lesions are thought to be caused by the infiltration of immune cells, including T cells, B cells and myeloid cells, into the central nervous system parenchyma, with associated injury.

In patients with MS, the most implicated T cell subset is the IL-17 expressing CD4+ T cell population (CD4+CD3+IL-17+ T cells). However, the effectiveness of B-cell depleting therapies has helped to broaden the view of MS being only a T-cell mediated disease to a view of an aberrant immune system that includes the interaction of multiple cell lines and signaling molecule located in the periphery and compartmentalized in the CNS; in particular, and as shown in our previous work, interactions between pro-inflammatory GM-CSF expressing B cells with the disease-implicated Th17 T cells are thought to be important drivers of the human disease (Li et al., 2015).

Much of our understanding of immunophenotypes of cells present in the CNS of patients with MS is by virtue of CSF analysis – to demonstrate oligoclonal bands - which has relevant limitations including the very small number of cells and their fragility which make functional assessments extremely challenging.

Additionally, though blood is considered a window into immune activity, recent work of the group demonstrated in people with HIV that studies in blood fail to capture the major component of HIV-specific CD8+ T cell responses resident in lymphatic tissues (Buggert et al, 2018). Immune cells in the lymphatic channels like the thoracic duct are unique compared to immune cells found in peripheral blood and study of these cell can provide a new window into the biology of diseases like HIV and potentially also in MS.

Additionally, it is now recognized that CNS cells and soluble factors can egress via lymphatics into deep cervical lymph nodes. The thoracic duct is an important gateway for trafficking of immune cells out of the CNS to the periphery through the deep draining lymph nodes and allows sampling of lymphatic tissues.

2 Thoracic duct lymph in disease

The TD represents a highly unique anatomic and physiologic compartment that is quite distinct from the peripheral blood. It is the major deep efferent lymphatic and, based on pioneering work at Penn exploring this previously inaccessible compartment in healthy controls and patients with HIV (Voillet V et al, JCI Insight. 2018; Buggert M et al, Sci Immunol. 2018; Vella LA, et al J Clin Invest 2019; Buggert M, et al Cell. 2020), is now known to harbor a different profile of cells compared to blood, as well as considerably higher concentrations of certain populations that are much more dilute in the blood. In addition to the considerable fundamental immunology interest, studying the TD is of particular interest in multiple sclerosis. The upper part of the thoracic duct drains half of the brain in most people and, as such, represents a site of enrichment of brain-draining cells. Accessing this level of the TD provides an opportunity to study disease-relevant CNS-autoreactive cells that may not be present in the blood or, if present in blood, are so dilute that it has not been possible to characterize them. The lower part of the thoracic duct drains the gut and is major interest now given the implication of an important 'gut-brain' axis that can modulate CNS inflammation. While leukapheresis provides larger numbers of cells than feasible in routine blood draw, their profile is in essence the same as circulating blood cells, hence leukapheresis cannot be used as an alternate method for this research.

Sampling of the cells and soluble factors in the thoracic duct will provide a unique opportunity to gain understanding of the following:

1. Immune biology in healthy volunteers and comparing immune biology of lymphatic fluid and in blood
2. Immune biology of people with early MS comparing immuno-profiles in blood to lymphatic fluids
3. Immune biology comparing immuno-profiles before and after treatment with Ofatumumab, in blood and lymphatic fluids, over a period for up to 25 days.

3 CNS Lymphatic Drainage

Lymphatic vessels have a key role as the afferent arm of the immune system for most organs in the body, thereby maintaining fluid homeostasis and acting as pathways for the passage of antigens and antigen-presenting cells (APCs) to regional lymph nodes.

The CNS parenchyma has no conventional lymphatic vessels, and although a well-regulated Blood Brain Barrier (BBB) controls the entry of solutes into the CNS, there is still a need to maintain homeostasis and effective afferent pathways to lymph nodes.

Two extracellular fluids are associated with the CNS: CSF in the ventricles and the Subarachnoid Space (SAS), and ISF (interstitial fluid) in the CNS parenchyma. Both fluids drain to cervical and lumbar lymph nodes, but by separate pathways. CSF drains along lymphatic vessels that pass through the cribriform plate and the dura mater, and this pathway allows for trafficking of immune cells.

In contrast, ISF and solutes from the CNS parenchyma drain to lymph nodes along 100–150-nm-thick basement membranes in the walls of cerebral capillaries and arteries. Only 15% of ISF draining from the cerebral hemispheres leaks into the CSF. In contrast to observations regarding the drainage pathways for CSF, no evidence exists showing that immune cells can traffic directly from the CNS parenchyma along the narrow intramural perivascular pathways to lymph nodes (Da Mesquite S et al., 2018).

Drainage of CSF via this route is largely to deep cervical lymph nodes, and the lymphatic channels allow the trafficking of CD4+ T cells, monocytes and dendritic cells from CSF to cervical lymph nodes. CSF drainage to cervical lymph nodes via dural lymphatics seems to occur mainly at the base of the skull. Cranial and spinal nerve roots also act as pathways for lymphatic drainage of CSF to cervical and lumbar lymph nodes. Direct drainage pathways to lymph nodes for APCs in the CSF could be a major reason that CSF spaces are immunologically competent and demonstrate rapid inflammatory responses.

Given the demonstration that *B cells traffic bi-directionally between the CNS compartment and the periphery* – in particular the deep cervical lymph nodes which drain the CNS lymphatic system, cannulating the thoracic duct provides a unique opportunity to assess, for the first time, the disease-relevant make-up and functional responses of CNS-draining immune cells and soluble factors in people with MS.

1.2 Thoracic Duct Cannulation

Cannulation of the thoracic duct is part of the interventional radiologic procedure most commonly performed to embolize the thoracic duct. Historically, leaks from the thoracic duct or its tributaries (which result in chylothorax; leakage of lymph fluid into the pleural space), typically caused by trauma in adults, were surgically corrected, with high rates of morbidity and mortality. The development of minimally invasive embolization of the thoracic duct following thoracic duct cannulation under fluoroscopy was first performed in humans in 1998, and has become the gold standard treatment for traumatic chylothorax (Itkin and Chen, 2011). Until recently, the technical success rate for catheterization of the thoracic duct was approximately 70% to 80% (Itkin and Chen, 2011). A technical limitation of these procedures was the need to

cannulate lymph vessels in the feet to perform a lymphangiogram, the initial step in this procedure.

The success rate of the procedure has improved following the innovation (developed by Max Itkin) to perform an ultrasound guided lymphangiogram following injection of dye into an inguinal lymph node, rather than injection of dye into lymph vessels in the feet. (Nadolski and Itkin, 2012) All thoracic duct cannulation and embolization procedures have used the inguinal intranodal lymphangiogram technique during the past three years, and this is now the standard of care for clinical procedures.

The procedure shows a low complication rate (see adverse related events chart below) of approximately 3%. The procedure that will be performed in this study will not include embolization of the thoracic duct or the use of glue, therefore none of the complications of embolization reported in prior series would be expected in this study.

Additionally, Max Itkin and colleagues have shown that externalization of the thoracic duct catheter can be safely performed in the setting of chronic drainage of lymphatic fluid.

4 Procedural Details for 'In-and-Out' Catheterization or "Indwelling" Catheter Placement

The procedure is performed in the following manner. Participants will have abstained from eating the night before the procedure. They will come to the interventional radiology suite, and an intravenous catheter will be placed in a vein in the arm, antibiotics will be administered, consisting of either ampicillin-sulbactam, or in the event of a history of penicillin allergy, the combination of levofloxacin and clindamycin. The areas of the groin and abdomen will be prepped and draped. Participants will be given conscious sedation with midazolam and fentanyl. An ultrasound of the groin will be performed to identify suitable lymph nodes. A 25- or 26-gauge spinal needle will be advanced into a lymph node and under fluoroscopic guidance 5 mL of oil-based contrast agent (Lipiodol; Guerbet Group, Princeton, NJ) will be injected into the lymph node at a rate of approximately 1 mL per minute. Lymph nodes on both sides of the groin will be injected as part of the intranodal lymphangiogram component of the procedure to enhance visualization of the lymphatic vessels. The infusion of contrast will be stopped when the lymphatics are visualized at approximately the level of the L3 lumbar spine. If the cisterna chyli is not visualized after the injection normal saline will be injected at the rate of approximately 1 mL per minute for 5 minutes to facilitate propagation of the contrast agent. After identification of the target lymphatic vessel (the cisterna chyli, thoracic duct, or a tributary of the thoracic duct), a 21- or 22-guage 15 to 20 cm Chiba needle will be passed into the vessel via a transabdominal approach. A 0.018 guidewire will be advanced through the needle into the lymph vessel and advanced into the thoracic duct. A microcatheter will then be advanced into the thoracic duct over the wire. The microcatheter will then be left in place and chyle (lymph) will be collected.

For a 'in-and-out' catheterization procedure, up to 100 mL will be collected from the catheter over a 30-minute collection period at two levels within the thoracic duct and the catheter will then be removed and sedation will be stopped.

For serial sampling, the cannulation will be done as described above followed by placement of an 'indwelling' catheter. After achieving the transabdominal access of the thoracic duct using the microcatheter, a guidewire will be placed through the catheter previously positioned within the thoracic duct and advanced into a subclavian vein. A vascular sheath will then be placed into a vein in the upper arm under ultrasound and fluoroscopy guidance. The wire in the subclavian vein will be then snared using a vascular snare through venous access sheath and pulled out from venous sheath. A 4 Fr catheter/sheath will then be threaded and advanced over the wire until its tip is in thoracic duct. The catheters from the thoracic duct will then be removed, leaving the 4 Fr catheter extending from the arm vein into the thoracic duct. Sedation will be stopped. The procedure will take approximately 2 hours from start to completion. The participant will then be monitored in the Interventional Radiology recovery area until he or she is able to return home. The 4 Fr catheter/sheath will be left in place until completion of the procurement of lymph fluid samples (up to 21 days). Participants will be provided education on the standard care of an indwelling line as well as any concerning signs to contact the study team or on-call physician with questions. Participants will have frequent follow-up visits to identify any problems related to the catheter or its procedure.

1.3 Experience with Thoracic Duct Cannulation

The Penn Interventional Radiology team collaborating on this study has tabulated the complications associated with thoracic duct embolization performed for clinically indicated reasons (see Section 16). They have performed 486 thoracic duct embolizations on 347 patients and have encountered 21 complications (complication rate of 4%). Among the 21 complications, 16 were due to the different steps of embolization procedure (mis-embolization by coils or by glue) that will not be a component of the procedures conducted in this trial. One complication was a result of a pedal lymphangiogram that will not be performed in this study, and another was a complication of a thoracentesis that will also not be performed as part of this study. In one patient the wire was sheared during thoracic duct access, with no consequences to the patient. Of the remaining two complications, one involved an episode of low oxygen saturation and low blood pressure that may have been due to over sedation; the other was documented in a patient who developed acute renal failure, and was also thought to be a consequence of embolization (rather than cannula insertion). In the HIV study, in which only cannulation was done as planned in this study (i.e. without embolization or glue), none of the 12 patients enrolled encountered complications.

1.3.1 Indwelling catheter and drainage:

A published meta-analysis of studies of external drainage of the thoracic duct reported on a total of 71 studies involving a total of 1160 patients, where this approach was used in solid organ transplantation, acute pancreatitis, neoplastic disease, sepsis, and autoimmune disease, for periods of up to and over 4 weeks (Meta analysis: Wang et. al Journal of Surgical Research. Vol 204. 2016). The most common complication was wound infection at the site of cannulation which occurred in 5-10% of participants. Other rare complications were accumulation of lymph in the neck, back pain, and edema of the face or arm. There was no reported mortality related to thoracic duct drainage. We were surprised to find that the meta-analysis included one small study in patients with MS (Ring et al. Lancet, 1974) where thoracic duct drainage was assessed as a potential therapeutic intervention (the study reported on immunological and neurological outcomes but did not report on complications).

It is important to note that many of the prior studies took place between the 1960s -1980s with techniques of thoracic duct cannulation and drainage that involved surgical incision of the neck and more invasive techniques to access the duct. The modernized IR techniques have become more refined with lower complication rates than past surgical approaches. Important differences are that the majority of the prior studies described in the meta-analysis employed larger catheters as well as continuous drainage, unlike our proposed approach which uses considerably finer catheters and only intermittent sampling of smaller lymph volumes.

To date, the IR team at Penn, led by Dr. Itkin has performed indwelling thoracic duct cannulation with drainage in 8 patients including 3 infants with anasarca and 5 adolescent patients with chylothorax. The duration of indwelling catheter and drainage **averaged 9.9 days (with a range of 2 to 43 days).** There were no intra-procedure complications, all 8 patients experienced symptomatic improvement and no line-infections developed this quite sick patient population. Our IR collaborators have a Penn IRB approved research protocol to perform thoracic duct cannulation with externalization and drainage in the context of sepsis (protocol :834599), though they have not yet initiated this trial.

Given our proposed use of the smaller bore catheters and intermittent sampling of much smaller volumes of lymph fluid **(50mL per sample versus the liters that are drained in the clinical setting)**, we anticipate any added risks of indwelling catheter in the generally healthy MS patients who do not have abnormal thoracic duct anatomy will be minimal, particularly over a period of several weeks. According to our IR team, the closest approximation of risk associated with the indwelling thoracic duct catheter in this generally healthy population is the risk associated with a peripherally inserted central catheter (PICC) line. PICC line cannulation of 3 weeks or longer is routinely done in the appropriate clinical setting. We will use a similar 4-5 Fr line to access the thoracic duct through left arm vein. The greatest potential complication is infection. The risk of PICC line associated infections in the heme/onc population such as bone marrow transplantation patients who are much more profoundly immune compromised is low. In a study of oncology patients, 165 BMT patients had PICC lines for an average of 88.3 days and an infection rate of 0.96 infections per thousand line-days (Moturu, A et al. Infection rates for PICC lines in oncology patients. Journal of Vascular and Interventional Radiology, 2017). We anticipate an even lower risk of infection given the shorter duration of indwelling catheter and the healthier MS population.

1.4 Ofatumumab

Ofatumumab, a fully human monoclonal antibody (mAb) that is FDA approved for relapsing MS, recognizes an epitope localized close to the cell membrane on the 2 extracellular domains of the CD20+ molecule. CD20-binding of ofatumumab induces effective B-cell lysis primarily through complement-dependent cytotoxicity (CDC) and ADCC.

Ofatumumab was evaluated in OMS112831 which was a randomized, placebo-controlled, dose-ranging, 48-week study (24-week double-blind treatment phase, then 24-week follow-up phase) that examined the efficacy and safety of repeat-dose *subcutaneous* ofatumumab in RRMS. Two hundred and thirty two patients were randomized (2:1:1:1:2) to placebo, ofatumumab 3 mg, 30 mg, or 60 mg every 12 (q12) weeks, or ofatumumab 60 mg every 4 (q4) weeks. The primary

endpoint was the cumulative number of new gadolinium-enhancing lesions during Weeks 0-12 on brain magnetic resonance imaging (MRI).

Ofatumumab reduced the mean cumulative number of new gadolinium-enhancing lesions by 65% vs placebo during Weeks 0-12 ($p < 0.001$), and by ≥ 90% during Weeks 4-12 vs placebo in a post hoc analysis of cumulative ofatumumab doses ≥ 30 mg ($p < 0.001$). Dose-dependent decreases were maintained up to Week 48. Ofatumumab reduced cumulative few/newly enlarged T2 lesions vs placebo during Weeks 0-12 (60-72%; $p \leq 0.002$). Between 24-48 weeks, new/newly enlarged T2 lesion counts were stable for all ofatumumab doses apart from the 3 mg dose group.

The results also demonstrated a rapid, dose and dose frequency dependent reduction in B cell counts. Monthly dosing showed no signs of B-cell repletion during the inter-dosing interval. Both 30 mg and 60 mg q12 weeks showed approximately 75% suppression of B-cells prior to re-dosing. Once dosing was ceased, all treatments showed similar rate of B-cell repopulation over 60 weeks of follow-up. Overall, ofatumumab was safe and well tolerated in patients with RRMS.

In total, 43 (64%) patients receiving placebo and 121 (74%) receiving ofatumumab (65%–81% across dose groups) experienced AEs during weeks 0 to 12. During weeks 12 to 24 and 24 to 48, the proportions of patients who experienced AEs across treatment groups were 45% to 62% and 47% to 55%, respectively. AEs were largely mild to moderate in severity, and no patients died. Incidences of serious AEs (SAEs) were 3%, <1%, 4%, and <1% in weeks 0 to 12, 12 to 24, and 24 to 48 and the individualized follow up (IFU) phase, respectively. The only SAEs to occur in ≥1 patient during the treatment phase were injection-related reactions (IRRs), occurring in 3 patients; all continued in the study, including 1 patient who reportedly experienced a cytokine-release syndrome within hours of the first ofatumumab (60 mg) dose. Other SAEs occurring in single patients were cholelithiasis and hypokalemia (both with 60 mg ofatumumab every 4 weeks) and angioedema and urticaria (both in the same patient receiving 3 mg ofatumumab). There was no pattern of SAEs in the 24-week FU phase. During the IFU, 2 (2%) patients, both in the ofatumumab 60 mg every 4 weeks group, reported a total of 2 SAEs: head injury and malignant melanoma stage IV. The latter was considered treatment related, and the patient recovered (as noted by the investigator).

Two multinational Phase 3 studies of ofatumumab (ASCLEPIOS: COMB157G2301 and COMB157G2302) are being conducted to demonstrate efficacy, safety and tolerability for s.c. injection of ofatumumab 20 mg q4 weeks compared to oral teriflunomide in patients with relapsing MS, with about 1800 patients randomized.

OMB received FDA approval for the treatment of Multiple Sclerosis including relapsing, active secondary progressive and clinically isolated syndrome in August 2020. Use of this drug in this study will be according to FDA label.

2 Objectives

The overall objectives of this study are to understand immune biology of the healthy, young immune system, to compare this to people with MS, understand composition in blood and thoracic duct and understand treatment effects of OMB on central and peripheral compartments.

2.1 Primary and secondary objectives

To evaluate the safety and feasibility of collection of lymphoid fluids & tissue via thoracic duct cannulation (both "in-and-out" and "indwelling" procedures) in healthy volunteers and in early MS patients and understand the effects of OMB on peripheral immune markers over 2 years compared to markers pre-OMB and shortly following OMB initiation in peripheral blood and thoracic duct lymph.

2.2 Exploratory objectives

To compare immune cell profiles of blood and lymphatics between MS and healthy controls and in untreated MS patients before and following a B-cell depleting therapy (Ofatumumab), including:

1. to compare cellular and soluble biomarkers in thoracic duct lymph with blood measures in untreated MS patients
2. to explore differences in cellular and soluble biomarkers in thoracic duct lymph and blood measures between healthy controls and people with MS without treatment
3. to explore changes in immune biology in people with MS before and during OMB treatment
4. compare effects of acute OMB treatment on immune biology to healthy controls

3 Study Assessments

Study measures will be assessed at a single time point for "in-and-out" catheterization and at multiple time points for indwelling catheter procedure combined with OMB treatment (details outlined below) for immunologically related endpoints.

Fluid sampling results will be assessed in both lymph fluid collected from the thoracic duct and blood. Analyses will be prioritized based upon the volume of lymph fluid obtained. In addition to the measures listed below, other cellular or soluble markers may be measured on residual material.

"In-and-out" procedure: The primary types are the percentage of patients with AE and/or SAEs related to study procedure ('in-and-out' cannulation of thoracic duct). Additional parameters will be explored:

1. To generate data on cell profiles of blood and lymphatics in untreated participants with MS
    a. To evaluate the phenotype, function, and transcriptional profiles of immune cell sub-populations (including T cell, B cell and innate cell subsets)
    b. To identify CNS-antigen specific responses of immune cells and profile B cell repertoires
    c. To quantify soluble measures of inflammation and CNS injury in lymph fluid compared to serum
    d. To investigate the humoral autoantibody repertoire at serological and cellular level

e. To document presence or absence of subclinical disease activity using brain MRI (+/- Gd) in order to better interpret immunological results (single MRI done within approximately one week of cannulation)

"Indwelling" procedure: The primary endpoints are the percentage of patients with AE and/or SAEs related to study procedure ('indwelling' cannulation of thoracic duct). Additional parameters will be explored:

5 To generate data on cell profiles of blood and lymphatics in participants with MS before and following a B-cell depleting therapy (OMB), and in healthy controls including:
   a. To evaluate the phenotype, function, and transcriptional profiles of immune cell sub-populations (including T cell, B cell and innate cell subsets)
   b. To identify CNS-antigen specific responses of immune cells and profile B cell repertoires
   c. To quantify soluble measures of inflammation and CNS injury in lymph fluid compared to serum
   d. To investigate the humoral autoantibody repertoire at serological and cellular level
   e. To document presence or absence of subclinical disease activity using brain MRI (+/- Gd) in order to better interpret immunological results (MRIs: first done within a week of catheterization, second done within 1 month following removal of indwelling catheter.

Patients with MS will be offered OMB treatment for up to 2 years free of charge, and be dosed according to FDA label, to evaluate clinical and biomarker outcomes in an early MS population and described effectiveness, safety, and tolerability. Additional testing will be including clinical, blood biomarkers, and imaging including standard clinical and additional research studies as described in detail (below).

3.1 Detailed Immunologic Assessments

In depth functional and phenotypic analysis of innate and adaptive immune compartments will be performed. The Bar-Or lab has established methods to identify phenotype and function of immune cell type in vitro using a different combination of stimulation, which is specific for different cell types present in blood circulation and thoracic duct. Specifically, the following analysis will be conducted:

a) Analysis of T cell functional subsets namely: CD4 naïve, central memory, effector memory, terminal differentiated effector memory (TEMRA), T follicular helper cells, regulatory T cell and CD8 naïve, central memory, effector memory and terminal differentiated effector memory. Chemokine receptors, activation as well as exhaustion markers will be analyzed on the different T cell subsets described above upon T cell specific stimulation according to established protocol.

b) Analysis of B cell functional subsets namely: transitional, naïve, IgM memory, IgG memory and plasma blasts/plasma cells will be enumerated. Chemokine receptors, activation markers, as well as antigen presentation markers (CD80, CD86, CD83) will be evaluated upon B cell specific stimulation according to established protocol.

c) A recent finding has described a new population of lymphocytes co-expressing BCR and TCR, expanded in T1D and having a potential role in modulating autoreactive T cells. Thus we

will evaluate whether this population exist in blood and thoracic duct of healthy and MS patients, using the following surface markers: CD19, CD5, IgM, IgD, TCR (Ahmed et al., 2019).

d) Analysis of the following myeloid subsets: monocytes according to the expression of CD14 and CD16, conventional dendritic cells and plasmacytoid dendritic cells. Chemokine receptors, activation markers as well as antigen presentation markers will be analysed upon myeloid specific activation according to established protocol.

Overall in order to accomplish the analysis described above a combination of the following surface markers will be used: Phenotypic and functional assessment of mononuclear cells as determined by multi-parametric FACS panels well-established in the Bar-Or lab including, but not necessarily limited to, the following cell surface or intracellular protein markers: CD3+, CD4+, CD8+, CCR2, CCR5, CXCR3, CXCR4, CXCR5, CCR6, PD-1, CD57, CD127, HLA-DR, CD38, Bcl-6, ICOS, CCR7, CD19, CD20, CD24, CD27, CD45RO, Ki67, CD95, CD28, CD103, CD150, CD11a, CD123, CD200, CD16, CD56, KLRG-1, LAG-3, TIGIT, Helios, IgM, IgD, CD80, CD86, CD83, CD69, TCR, etc. All markers will allow the identification of naive, central memory, effector memory, regulatory T cells and follicular helper T cells as well as naive and memory B cells and plasma cells.

The adaptive and innate immune cell subsets will also be characterized using a large panel of soluble and intracellular functional markers of cells and cell subsets, as determined by ELISA or ICS: IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-17, IL-21, IL-22, IL 23, IL 33, IL 35, and IL 1beta, IFNa, IFNg, TGF b.; granzyme B, perforin. Specifically, we will investigate cytokine secretion both pro-inflammatory as well as anti-inflammatory in B and T lymphocytes.

We will assess the magnitude and breadth of CNS antigen-specific reactivity using tetramer assays with established tetramer technology and/or Activation Induced Marker (AIM) assays, and flow cytometry in the Bar-Or lab.

Transcriptional profiles of sorted (T cell, B cell, innate cell) immune subsets including bulk profiling and single cell 10X genomics.

Repertoire analysis pre and post treatment with ofatumumab, with respect to: 1) public clones; 2) VH and Vb gene and allele usage; 3) alterations in clonal selection through the analysis of somatic hypermutations); 4) integration of BCR or TCR data with scRNA sequencing profiles in selected samples (this would be done computationally; Prak lab).

Functional B cell auto-antibodies repertoire analysis: for this specific end point, frozen lymph mononuclear cells isolated from peripheral blood as well as from the lymph fluid obtained from the thoracic duct will be shipped to the Research Collaborator site in Basel, Switzerland in order to perform a functional B cell receptor (BCR) repertoire analysis. Briefly IgM and IgG memory B cells will be isolated by FACS sorting and seeded at clonal level in 384 well plates. Human B cells will be expanded in vitro according to published protocol (Lindner et al., 2019), and a panel of autoantigens previously selected based on the serological and lymphatic fluid analysis will be used to screen for the presence of specific autoantibodies. Purified autoantibodies expressed in a mammalian system will be characterized for affinity and function and then eventually shipped back to the collaborator for further characterization and analysis.

The Bar-Or lab has been able to establish a technique which would allow the isolation of cytokine secreting B cells. Thus we plan to isolate by FACS cell sorting cytokine secreting B

cells and perform the functional specificity analysis of the BCR with the technique established in Traggiai's lab at Novartis Institute for Biomedical Research (Lindner et al., 2019).

3.2 Other soluble markers

A variety of other soluble markers will be examined depending on availability of fluids for analysis.

1. NfL and GFAP in peripheral blood
2. NfL and GFAP in thoracic lymphatic fluids
3. Other fluid based biomarkers under research
4. Potential Proteomic analysis
5. Potential Lipid analysis
6. Potential metabolomic analysis on fluid phase samples (in plasma and thoracic duct fluid) applying global metabolomics and weighted correlation network analysis (WGCNA) to first identify modules of correlated metabolites that change with treatment, followed by confirmation using targeted metabolomic assessment. Changes in metabolite modules and individual metabolites can be assessed in relation to changes in immune measurements
7. Autoantibody profiling in serum and lymphatic fluid: autoantibody profiling will be conducted using a high-throughput protein microarray chip developed by the Research Collaborator. This chip contains approximately 3000 self-secreted proteins. Known MS specific auto-antigens will be included in the printed collection. IgM, IgG and IgA sera and lymphatic fluid profile on self-protein chip will give us a unique opportunity to investigate new potential auto-antigens and their modification in MS patients upon treatment. (done by Novartis)

3.3 Hematology Assessments

Hematology assessments include: red blood cell (RBC) count, RBC morphology, white blood cell (WBC) count with absolute and differential (basophils, eosinophils, lymphocytes, monocytes, neutrophils, and segmented neutrophils), platelet count, hemoglobin, and HCT.

3.4 Clinical Outcomes

This study includes the following clinical outcome assessments:

- MS Relapse[1]
- Expanded Disability Status Scale (EDSS)
- MRI

[1] *MS relapse definition*: appearance of a new neurological abnormality or worsening of previously stable or improving pre-existing neurological abnormality, separated by at least 30 days from onset of a preceding clinical demyelinating event (McDonald et al. 2001). The abnormality must have been present for at least 24 hours and occurred in the absence of fever (< 37.5°C) or known infection.
Confirmation of MS relapse: the definition of a confirmed MS relapse is one accompanied by a clinically relevant change in the EDSS i.e. an increase of at least 0.5 points on the EDSS score, or an increase of 1 point on two functional scores (FSs) or 2 points on one FS, excluding changes involving bowel/bladder or cerebral FS compared to the previous available rating (the last EDSS rating that did not occur during a relapse).

An overview of each of these assessments is provided in the sections below and the details of these assessments will be provided in the site manuals.

4 Investigational Plan

4.1 Participant Recruitment

6 Healthy Volunteer Recruitment

The MS center has access to different options to recruit healthy volunteers. Below is a list of options which will be pursued:

a. Penn healthy volunteer pool to connect the community to research activities at Penn.

b. A research volunteer registry for healthy volunteers outside of Penn community which enables direct contact with people interested in participating in research.

Healthy volunteers identified via the above will be contacted via phone or email and, if interested, scheduled to come in for a screening visit, which will begin with informed consent. Any advertising will be IRB approved prior to use.

7 People with Multiple Sclerosis

Participants with MS will be recruited amongst the patients cared for within the MS Center practices of the University of Pennsylvania Health System. Patients will also be recruited via provider referral from outpatient clinic or inpatients at Hospital of the University of Pennsylvania. Over 5000 MS patients receive clinical care at the Penn MS center annually across the spectrum of disease. The clinic evaluates patients with first presentation of disease and provides long-term care for people with inflammatory diseases including MS.

8 Vulnerable Populations

No vulnerable populations will be included in this research. Penn employees and students will be neither targeted nor excluded from participating in this study, and appropriate protections are in place to ensure that these individuals are in no way coerced into participating. During the Informed Consent process (which is compliant with 21 CFR 50 Subpart B, 45 CRF 46.116-117, and federal and local laws), the study team will communicate that participation is voluntary and will have absolutely no effect on their employment, education, future care at Penn, or any other relationship with Penn. The study team will take proactive measures to ensure that educationally or economically disadvantaged persons are not coerced into participating. This will be accomplished by ensuring that each individual fully appreciates both the risks involved and the alternatives that exist, and can articulate a general interest in the study even prior to any mention of monetary compensation. To minimize the risk of attracting people who may be economically coerced to participate, we have chosen not to advertise for this study. Rather, our team of clinicians will gauge the appropriateness for possible participation among patients whom they know well. We believe these proactive steps will help to minimize financial incentives influencing participation in the study and would be happy to implement additional mechanisms that may be identified. The per-visit reimbursement will be nominal and provided in consideration of travel to Penn and the time commitment of the participant and an individual who accompanies the participant. The study team will remind the potential participants that

their decision regarding participation in the study will have no impact on the care they receive at Penn at the current time or in the future.

4.2 Screening Period

After signing the informed consent, participants will enter the screening phase. Participant eligibility will be determined based on the study inclusion and exclusion criteria and baseline assessments. Participants on DMT will be taken off interferons and glatiramer acetate should be discontinued for 90 days prior to inclusion into the study if medically appropriate. The screening period could thus last up to 120 days.

4.3 Procedure and Treatment Period

No B cell depleting therapy is provided to healthy participants.

9 4.3.1 "In-and-out" catheter with single time point lymph fluid procurement

Comprised of healthy controls and participants with MS who will undergo a single "in-and-out" catheterization of the thoracic duct with procurement of lymphatic fluid and peripheral blood. Eligible MS participants will be offered OMB treatment period to start no sooner than 48 hours after the 'in-and-out' catheter procedure. These participants will receive OMB provided by the Research Collaborator, as approved by the FDA, as an initial Autoinjector 20mg sc loading dose (20mg sc OMB starting dose, +2 to 14d after treatment initiation) and subsequent maintenance phase dosing consisting of 20mg s.c. ofatumumab every 4 weeks. OMB will be provided through the study free of charge for up to 2 years. Post-procedure follow-up study visits will occur per the visit schedule (Table 3).

10 4.3.2 "Indwelling" catheter with serial lymph fluid procurement

Comprised of healthy controls and participants with MS who will undergo thoracic duct cannulation and procurement of lymph fluid followed by conversion into an indwelling catheter with externalization. Thoracic duct lymph fluid sampling will be repeated through the external catheter at multiple time points as described in the visit schedule (Table 2). The external drain will be removed approximately 21 days later. Approximately 48 hours after the thoracic duct cannulation procedure, OMB will be initiated (as per FDA label, with initial Autoinjector 20mg sc loading dose, followed by sc injections a week later and again a week later, and subsequent maintenance phase dosing consisting of 20mg s.c. ofatumumab every 28 days or every 4 weeks. OMB will be provided free of charge through the study for up to 2 years. Post-procedure follow-up visits will occur per the visit schedule (Table 3).

4.3.3. 2-year OMB Follow-up

MS patients who undergo OMB treatment and 2-year post-procedure follow up will be treated with the FDA approved commercial supply medication for the treatment of MS, free of charge, through the study provided by the Research Collaborator. These participants will undergo clinical research monitoring, clinical research testing (i.e. relapse assessment, EDSS) as well as collections of peripheral research blood. This will allow for the study of the prolonged effects of OMB on the peripheral immune compartment in MS in the context of 2-year follow-up.

4.4 Rationale for study design

The proposed study adds new dimensions into our understanding of immune biology in young healthy participants as well the changes seen in participants with MS. As previously published, we identified differences in central and peripheral immune biology in people with HIV (Buggert et al., 2018) including identification of subsets of cells, which were present centrally, but not identifiable in peripheral compartments (blood).

In order to understand differences in immune biology between healthy individuals and participants with MS in the different compartments (e.g, blood and lymphatic fluids), the study will establish the safety and feasibility of the procedures ("in-and-out" and "indwelling" catheter procedures) in healthy controls and early MS, and generate understanding of the immune system profiles. Additionally, to understand the early treatment effects of OMB on peripheral blood and thoracic duct compartments.

The study is exploratory and a limited number of participants will be exposed to the procedure. Based on our recent experience with HIV, the number is likely to be sufficient to describe differences as outlined in the objective sections.

For MS patients, effects of B cell depletion over a 2-year period will be compared to thoracic duct lymph, peripheral blood, and clinical measures obtained pre- and early post-OMB treatment

4.5 Allocation to Interventional Group

This is an open label, single arm, study including healthy controls, and people with MS for in-and-out' thoracic duct catheterization with or without treatment with the FDA approved anti-CD20 MAB OMB or "indwelling" catheter procedures and treatment with OMB.
Allocation and recruiting will be based on a convenience sample from the Penn MS clinic and healthy volunteer recruiting pool. People will be assigned to interventional group based on their eligibility of the study and their informed consent.
Healthy controls are ascertained through described measures and will not receive any investigational drug.

4.6 Risk and Benefits

The procedure will be performed by highly experienced interventional neuroradiologist, Dr. Max Itkin, to minimize the risk of the procedure. There is no direct benefit to the healthy participants. The indirect benefit lies in the support of the understanding of immune biology in the healthy hence potentially supporting understanding of different diseases better.

For people with early MS participants have no direct benefit. Indirectly they support our scientific understanding of MS and will benefit of being systematically monitored over 2 years at regular follow-ups for safety, efficacy, and deep immune profiling of early treatment with OMB.

5 Study Population

Six healthy volunteers and 18 MS participants will be selected from the outpatient clinic or inpatients at Hospital of the University of Pennsylvania meeting all eligibility criteria as described below.

A 25% screen failure rate is expected, hence approximately 30-32 participants will be screened. Participants who discontinue within a week of indwelling catheter placement will be replaced (possibly requiring more patients to be screened). Participants who discontinue during the 2-year post-procedure follow up phase will not be replaced.

5.1 Inclusion criteria

Participants eligible for inclusion in this study must meet **all** of the following criteria:

1. Written informed consent must be obtained before any assessment is performed.
2. Adult participants aged 18 to 55 years (inclusive) at Screening.
3. Participants must be able to understand English and be able to review and comprehend the informed consent form independently or with the help of research staff

For **participants with MS following additional criteria need to be met**:

1. Diagnosis of MS according to the 2017 Revised McDonald criteria.
2. Ability to undergo several MRIs
3. Disability status at Screening with an EDSS score of 0 to 4 (inclusive)
4. MS treatment history restricted to Interferons and/glatiramer acetate treatment only or untreated to date
5. Neurologically stable within one month prior to interventional procedure.

5.2 Exclusion criteria

Participants meeting any of the following criteria are not eligible for inclusion in this study:

1. Participants suspected of not being able or willing to cooperate or comply with study protocol requirements in the opinion of the investigator.
2. Participants with primary progressive MS (Polman C et al., 2011)
3. Participants meeting criteria for neuromyelitis optica (Wingerchuck D et al., 2015)
4. Participants with disease duration of more than 10 years
5. Participants who are pregnant or nursing (lactating)
6. Participants with active chronic disease (or stable but treated with immune therapy) of the immune system other than MS (e.g. rheumatoid arthritis, scleroderma, Sjögren's syndrome, Crohn's disease, ulcerative colitis, etc.) or with immunodeficiency syndrome (hereditary immune deficiency, drug-induced immune deficiency)
7. Participants with active systemic bacterial, viral or fungal infections, or known to have acquired immunodeficiency syndrome (AIDS)

8. Participants with neurological symptoms consistent with PML or confirmed PML
9. Participants at risk of developing or having reactivation of syphilis or tuberculosis
10. History of cardiovascular disease or uncontrolled hypertension
11. History of diabetes mellitus
12. History of cancer (other than localized skin cancer) in the previous 5 years
13. Have received any live or live-attenuated vaccines (including for varicella-zoster virus or measles) within 2 months prior to first study drug administration
14. Immunization with non-live vaccines within 2 weeks of the study procedure
15. Participants at risk of developing or having reactivation of hepatitis: Positive results at Screening for serological markers for hepatitis (H) B and C indicating acute or chronic infection:
    a. HBs Ag and/or anti-HBc IgM and/or HB virus deoxyribonucleic acid (DNA)
    b. anti-HBs negative and Anti-HBc positive
    c. anti-HC IgG (if positive IgG, HCV-RNA PCR will be performed and if negative, participant can be enrolled)
16. Use of other investigational drugs at the time of enrollment (Screening) or within the prior 30 days, or five elimination half-lives, or until the expected pharmacodynamic effect has returned to baseline, whichever is longer
17. Absolute neutrophil count (ANC) <750/mm^3;
18. Hemoglobin <10.0 g/dL
19. Platelet count <100,000/mm^3
20. Prothrombin time (PT) or international normalized ration (INR) >1.5 x ULN
21. Use of systemic glucocorticoids in the 4 weeks prior to research biological sample acquisition

5.3 Study Procedures

All the below described study procedures and the procedure-associated medications are being performed for research purposes.

11 Screening Procedures & Visit(s)

All participants (including Healthy participants, participants with MS) will undergo screening evaluation.

The screening visit(s) will occur within 120 days of the scheduled date of the study procedure. Participants will be screened against inclusion and exclusion criteria of the protocol.

The screening visit(s) will include:

- Informed consent review. Participants will review and sign the informed consent document before any additional screening is performed.

- Medical record review. The participant's complete medical history (medical diagnoses, surgical procedures, psychiatric treatment history, medications taken within the past 6 months) will be obtained and reviewed.
- Vital signs
- Complete physical examination
- Laboratory Tests
- Cranial MRI for people with MS (within 4 weeks of cannulation event)
- If receiving OMB, additional testing including neurological measurements such as extended disability scoring scale (EDSS).
- If person with MS experiences at attack during screening, cannulation

All study procedures will be obtained at the convenience of study team and study participants and could include single or multiple visits within the Screening window.

Further details are listed in the Visit Schedule Tables.

For individuals with MS who develop a relapse during screening, research biological sample acquisition (blood and TD) should be deferred until at least 4 weeks after the relapse-onset or the use of systemic steroids (whichever is longer)

Individuals who undergo screening visits but do not complete their scheduled procedure within the 120-day window may undergo a second screening evaluation.

12 Visit Day 0: Thoracic Duct Cannulation and lymph fluid collection

5.3.1.1 Technique for thoracic duct cannulation: "In-and-out" procedure

The procedure will take approximately 2 hours from start to completion

1. Participants will have been instructed to abstain from eating starting at midnight the night before the procedure.
2. Participants will come to the Interventional Radiology Suite at HUP and have peripheral IVs placed.
3. They will be given moderate conscious **sedation** with midazolam and fentanyl or other appropriate drugs as determined by the patient status.
4. Prophylactic **antibiotics** will be administered, consisting of either ampicillin-sulbactam, or in the event of a history of penicillin allergy, the combination of levofloxacin and clindamycin.
5. The areas of the groin and abdomen and left arm will be sterilized with chlorhexidine-based cleanser.
6. An **intranodal lymphangiogram** and thoracic duct cannulation will be performed in standard clinical technique. Briefly, a 25- or 26-gauge spinal needle will be advanced into an inguinal lymph node under ultrasound guidance and 5-10 mL of oil-based contrast agent (Ethiodol; Savage Laboratories, Melville, New York) will be injected into the lymph node at a rate of approximately 1 mL per minute under fluoroscopic guidance. The infusion of

contrast will be stopped when the lymphatics are visualized at approximately the level of the L3 lumbar spine.

7. If the cisterna chyli is not visualized after the injection **normal saline** will be injected at the rate of approximately 1 mL per minute for 5 minutes to facilitate propagation of the contrast agent.
8. After identification of the target lymphatic vessel (the cisterna chyli, thoracic duct, or a tributary of the thoracic duct), a 21- or 22-guage 15 to 20 cm **Chiba needle** will be passed into the vessel via a transabdominal approach.
9. A 0.018 **guidewire** will be advanced through the needle into the lymph vessel and advanced into the thoracic duct.
10. A **microcatheter** will then be advanced into the thoracic duct over the wire. The wire will be removed, and contrast injected to confirm cannulation of the thoracic duct.
11. Approximately 100mL of lymphatic fluid will be removed through the catheter and may occur at multiple levels within the thoracic duct.
12. Approximately 100mL of peripheral blood will be removed through peripheral phlebotomy

The thoracic duct catheter will be removed and the participant will then be monitored in the Interventional Radiology recovery area until he or she is able to leave.

5.3.1.2 Technique for thoracic duct cannulation: "indwelling" procedure

All procedures as described above in the "in-and-out" procedure through step 12 will occur and include the following additional steps:

13. In order to externalize the thoracic duct catheter, the **guidewire** will be advanced though the microcatheter into the subclavian vein.
14. A **vascular sheath** will then be placed into a vein in upper arm under ultrasound and fluoroscopic guidance.
15. The wire in the subclavian vein will be then snared using a **vascular snare** through the venous access sheath and the wire will be pulled out through venous sheath to establish through and through access to the thoracic duct (the wire passing from the abdomen, up the thoracic duct and out the vein in the arm).
16. A 5 Fr or other appropriately size **catheter**/sheath will then be advanced over the wire until its tip is well down the thoracic duct. The catheter/wire from the thoracic duct will then be removed, leaving the 5 Fr catheter extending from the arm vein into the thoracic duct. The 5 Fr sheath will be then secured to the skin using monofilament sutures.
17. The participant will then be monitored in the Interventional Radiology recovery area until he or she is able to leave.

5.4 Ofatumumab treatment

Ofatumumab is FDA approved to treat relapsing remitting MS and is available to MS patients commercially outside of study participation. Participants will be informed that this drug is available to them regardless of study participation. Patients may incur some out of pocket costs in the form of co-pays, the amounts vary and are determined by insurance coverage plans.

13 Handling of study treatment

The Research Collaborator will provide FDA approved drug for sc OMB delivered as an auto-injector including appropriate labelling.

The Penn investigational drug service (IDS)pharmacy will store and dispense OMB. OMB will be treated as investigational medicinal product in accordance with all federal regulations by Penn IDS (shipment/disposition records, dispensing logs, e.g.).

OMB must be refrigerated at 2ºC to 8ºC (36ºF to 46ºF). The product will be kept in the original carton to protect from light until the time of use. Do not freeze. To avoid foaming, do not shake.

When dispensed from Penn IDS, the prescription label would indicate the federal caution label language of an IMP as this is hard-coded into the pharmacy's system even though OMB will be approved in its use by the Food and Drug Administration (FDA) at the time of its use.

14 Instruction for prescribing and taking study treatment

OMB will be dispensed at scheduled visits throughout the 2-year study period. Schedule is defined within the visit schedule table within the protocol.

In order to assess tolerability of the initial dose of study medication, participants will be assessed following administration for any reactions including injection related and post dose vital signs will be collected. A longer observational period may be required if these vital signs are not within reasonable limits of the participant's baseline values.

Following the first dose, subsequent dose two, and three may be injected under supervision by clinical nurse or other HCPs to ensure compliance, appropriate timing of fluid collection and proper technique and safety precautions. Subjects will be instructed to not inject OMB on the day of a visit, unless it is after the collection of research samples

If a participant is unable/unwilling to self-administer injections after the third dose, home-administration may be performed by another individual (e.g. partner, relative or a healthcare professional) who has accompanied the participant to the site and has been trained on and demonstrated ability to correctly administer the s.c. injections. The participant may also continue to have injections at the site if this is the participant's preference.

Participants should be instructed to record any missed doses and to inform the study staff of any missed s.c. injection(s). This can be done at the visits.

Participants who miss s.c. injections or temporarily interrupt study drug without discontinuing from the study or withdrawing consent, will be permitted to resume study drug if determined to be safe and appropriate. When resuming study drug, the timing of the next s.c. injection will be determined based on the original study schedule as followed:

- If one monthly injection is missed by 1 week or less, the participant should take injection as soon as possible. The next injection should then be administered according to the original schedule.
- If one monthly injection is missed by more than 1 week, the participant should skip the dose and take the next dose at the time when the next injection would be due according to the original schedule.

- If two or more consecutive monthly injections are missed, the study will be restarted based on clinical judgement.

A participant injection instruction leaflet will be provided which includes detailed information, precautions and instructions for administering s.c. injections using the autoinjectors. This information should be reviewed with the participant (and his/her partner/relative as applicable) to ensure that they understand the correct procedure for self/home administration.

Ofatumumab will be provided in auto-injectors for subcutaneous administration containing 20 mg ofatumumab (50 mg/ml, 0.4 ml content). Ofatumumab (OMB) is clear to opalescent, colorless to pale yellow, essentially particle-free liquid.

OMB will be supplied by the Research Collaborator and dispensed though Penn for up to 2 years.

Ofatumumab is started with a loading dose at 20mg sc at Day 1, Day 7, Day 14, , followed by a sc injection every 28 days or every 4 weeks.

For MS patients undergoing "in-and-out" cannulation: OMB is initiated no sooner than 48 hours after the procedure.

For MS patients undergoing "indwelling" cannulation: the first injection of OMB is given approximately 48 hours (day 2) after the "indwelling" catheterization procedure once fluids have been extracted from the indwelling catheter; in case of an ongoing AE, OMB application could be delayed until AE is resolved based on clinical judgement.

Afterwards, OMB is self-administered at home as sc injections every 28 days (or 4 weeks). Self-administration refers to patient self-treatment of the drug or by a trained caregiver regardless of whether dosing occurs at study site or at home. Instruction and training will be provided by site staff to patients/caregivers prior to treatment.

5.4.1.1 Premedication prior to s.c. injection

Premedication with acetaminophen and/or antihistamines (or equivalent) is optional and may be administered at the discretion of the Investigator. If investigators choose to administer premedication, it should be administered 30 to 60 minutes prior to study drug injection.

Application of systemic corticosteroids are at the discretion of the investigator and needs to be recorded in the CRF.

5.4.1.2 Concomitant therapy

Participant will report any new medications, procedures, and significant non-drug therapies he/she takes starting OMB treatment.

Each concomitant drug must be individually assessed against all exclusion criteria/prohibited medication.

5.4.1.3 Treatment duration

The planned duration of Ofatumumab treatment is up to 2 years. Participants may be discontinued from treatment earlier due to unacceptable adverse events, disease progression and/or if treatment is discontinued at the discretion of the investigator or the participant.

15 Other treatment(s)

5.4.1.4 Prohibited medication

Use of investigational new drugs are NOT allowed in combination with study drug. Use of excluded medications is not allowed while the participant is on study medication.

5.5 Treating the patient

16 Participant numbering

Each participant is identified in the study by a unique Participant Number (Participant No.), that is assigned when the participant is first enrolled for screening and is retained as the primary identifier for the participant throughout his/her entire participation in the trial. Upon signing the informed consent form, the participant is assigned to the next sequential Participant No. available.

If an enrolled participant fails to be treated for any reason, the reason will be entered on the Screening Participant Status CRF. If the participant is re-screened later on, a new Participant No. will be assigned.

17 Drug Interruptions

Conditions/events that may lead to study drug interruptions based on Investigator judgment and overall clinical assessment include:

- reported serious adverse event;
- emergency medical condition, unplanned hospitalization involving use of excluded concomitant medications;
- abnormal laboratory value(s) or abnormal test or examination result(s)

Should the participant interrupt the study drug for whatever reason, the re-start decision should be made on a case-by-case basis. Should the Investigator decide to re-initiate treatment with study drug, depending on the duration of the interruption, the first dose at re-start may need to take place at the study site to ensure observation in a similar manner as on first dose.

The reason for interruption of treatment and date of interruption should be appropriately documented in the source documents as well as in the appropriate CRF.

18 Additional treatment guidance

5.5.1.1 Transition period including washout from previous disease modifying therapy (DMT)

Participants with MS enrolled into the study will be treatment naive (i.e. never treated with DMT) or previously treated with interferon-B or glatiramer acetate only, with at least 90 day washout prior to procedure.

19 Treatment compliance

General instructions are provided to promote compliance e.g. by stating that compliance is necessary for the participant's safety. The participant must also be instructed to contact the

investigator if he/she is unable for any reason to take the study drug as prescribed. Compliance data will be captured in the source document at each visit. All study drug dispensed and returned will be recorded in the CRF.

Catheter Procedure and Follow up care: All participants will receive sedating medication and antibiotics during the procedure to minimize infections and burden.

5.6 Patient Monitoring

20 'In-and-out' catheterization

5.6.1.1 Procedure Visit

Thoracic duct cannulation procedure (described above) and procurement of up to 100 mL of lymph fluid (at two levels) and withdrawal of the catheter. Blood draw (up to 100 mL) by phlebotomy. Participants are monitoring in the IR recovery room until clinically released.

5.6.1.2 Post-Procedure Contact

Participants will be contacted on post-procedure Days 1 and 3. This can be done remotely as part of video-telemedicine visit if all is well, or in person if any concern at all. They will come to clinic for final site inspection on Day 7 to ensure wound healing and absence of infection.

21 "Indwelling" catheter and serial sampling

5.6.1.3 Visits 1-3

For participants undergoing indwelling catheter and serial sampling, thoracic duct cannulation procedure (described above) and procurement of up to 100 mL of lymph fluid (at two levels) will be done on Day 0 with conversion to an indwelling peripheral line. Blood draw (up to 100 mL) will be done by phlebotomy. Patients will return on days 1 and 2 for additional thoracic duct sampling. After the sampling on day 2, and when relevant, OMB treatment will be initiated.

5.6.1.4 Follow-up Visits 4-7

Patients will return to clinic on days 4, 9, 16, and 21 after the cannulation procedure and be assessed for any possible adverse events. Up to 50 mL of lymph fluid will be drawn from the peripheral line connected to the indwelling catheter in the thoracic duct. These visits will last approximately 1-2 hours. At the last visit, the interventional radiology team will remove the catheter after collecting up to 50mL of lymph fluid. Blood draw (50 mL) by phlebotomy may be obtained at each visit.

5.6.1.5 Follow-up visits 1-7 for participants receiving OMB

Participants receiving OMB will return to clinic 1, 3, 6, 12, 18, and 24 months after starting OMB for visits and clinical testing. Research blood work (up to 50 mL) by peripheral phlebotomy may be collected at each visit. Pre- and post-procedure MRIs should not be more than 2 months apart. Another research brain MRI will be obtained at 24 months +/- 2 months a

5.6.1.6 Study completion and discontinuation

A patient will be considered to have completed the study when the patient has completed the last visit planned in the protocol.

22 Unscheduled Visit

An unscheduled visit (UNS) is a spontaneous visit and may take place at any time during the course of the clinical trial if and when the Investigator deems it necessary. The data collected during the UNS assessments must be recorded in the CRF.

5.7 Withdrawal of informed consent

Any participant may voluntarily withdraw consent to participate in the study for any reason at any time. Withdrawal of consent from the study is defined as when a participant:

Does not want to participate in the study anymore

or

Does not want any further visits or assessments

or

Does not want any further study related contacts

or

Does not want any analyses performed on the biological material obtained

If a participant withdraws consent after partial or complete collection of lymph fluid, we will ask the participant to provide their withdrawal of consent in writing. Otherwise the material collected will be analyzed.

5.8 Loss to follow up

For participants whose status is unclear because they fail to appear for study visits without stating an intention to discontinue or withdraw, the investigators will show "due diligence" by documenting in the source documents steps taken to contact the participant, e.g. dates of telephone calls, emails, text messages, etc. A patient will be considered as lost to follow-up until the time point of his/her scheduled end of study visit has passed.

5.9 Data Collection and Follow-up for Withdrawn Participants

All participants who have undergone thoracic duct cannulation will be contacted 1, 3, and 7 days following their procedure. This will be performed on participants who withdraw their consent before or after completion of the procedure. For patients withdrawing early from the serial sampling study, they will be contacted by telephone 1, 3, and 7 days following removal of the catheter to assess for adverse events.

23 Early Termination Visits

Participants who elect not to undergo thoracic duct cannulation after undergoing screening evaluations will receive copies of any laboratory test results obtained during screening evaluations.

If a participant asks to stop their participation during the course of the thoracic duct cannulation, the procedure will be discontinued and catheters will be removed based upon procedures felt by the radiologist performing the procedure to ensure the safety of the participant. If the participant has received sedating medication the participant will be monitored in the post-procedure area until the clinical staff believe it is safe for the participant to return home.

5.10 Pharmacokinetic Evaluation

Sampling for OMB pharmacokinetics will be done in the serial time point thoracic duct cannulation procedure and phlebotomy in patients pre- and post- OMB therapy. Details are provided elsewhere in the protocol.

5.11 Safety Evaluation for Thoracic Duct Procedures

The safety endpoints of the study will be those that represent complications of the study procedures, including adverse events associated with the use of medications that are a component of the study procedure, sedating agents and antibiotics.

Complications of the thoracic duct cannulation procedure that will be queried include: failure to successfully cannulate the thoracic duct, pain/discomfort at the site of the insertion of cannulas (groin and abdomen), swelling, infection, and fevers. Adverse events associated with the use of sedating agents and antibiotics that will be queried include nausea, vomiting, diarrhea, dizziness, confusion, rash, and fever. Participants will be asked to provide open ended responses to the question of whether they believe they have experienced any adverse events associated with study participation.

Adverse events will be graded as mild, moderate, severe, and life threatening in severity.

Participants who report adverse events following the procedure will be asked to report the date of resolution of their symptoms.

5.12 Visit schedule and assessments

Table lists all the assessments and indices with an X when the Visits are performed. Visit schedule is listed for healthy, participants undergoing single time procedure, and participants undergoing indwelling catheter procedure for ease of reading.

24 Visit schedule and assessment for 'in-and-out' cannulation

Table 1 Assessment Schedule for "in-and-out" cannulation

Period	Screening		Procedure		Follow up	
Visit Name	Screening	Baseline	Visit 1	Visit 2 (telephone Visit)	Visit 3 (telephone visit)	Visit 4
Day	-120 to -8	-7 to -1	0	1	3	7
Informed consent	X					
Demographics	X					
Medical History	X					
Inclusion / Exclusion criteria	X	X				

Period	Screening		Procedure		Follow up	
Visit Name	**Screening**	**Baseline**	**Visit 1**	**Visit 2 (telephone Visit)**	**Visit 3 (telephone visit)**	**Visit 4**
Day	**-120 to -8**	**-7 to -1**	**0**	**1**	**3**	**7**
Review of System	X	X	X	X	X	X
Vital Signs	X	X	X			X
Physical Exam (include height, weight)	X					X
MS only and chooses ofatumumab treatment: EDSS		X				
Pregnancy Test (urine dip or serum)		X				
Safety labs: CBC, CHEM7, LFTs, PT/INR	X					
Peripheral phlebotomy for research studies	X (Screening and/or baseline)		X			
Lymph fluid collection for research studies			X			
Concomitant medications	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X
MS only: MRI brain	X (within 4 weeks of cannulation event)					
MS only - If consented to receive OMB through study: OMB safety labs (quant IgG, QuantiFERON gold, Hepatitis B, C testing, CD19 count, and JCV titer)	X					

25 Visit schedule and assessment for procedure for “indwelling” catheter and OMB treatment

Table 2 Assessment schedule for indwelling cannulation

Period	Screening		Procedure and Treatment with OMB						
Visit Name	**Screening**	**Baseline**	**Visit 1 (procedure)**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**
Day	**-120 to -8**	**-7 to -1**	**0**	**1**	**2**	**4**	**9**	**16**	**21**
Informed consent	X								
Demographics	X								
Medical History	X								
Inclusion / Exclusion criteria	X	X							
Review of System			X	X	X	X	X	X	X

Period	Screening		Procedure and Treatment with OMB						
Visit Name	**Screening**	**Baseline**	**Visit 1 (procedure)**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**
Day	**-120 to -8**	**-7 to -1**	**0**	**1**	**2**	**4**	**9**	**16**	**21**
Washout Period (if applicable)	X	X							
MS only: Training for OMB injection			X						
Drug dispensation				X			X	X	
MS Relapse assessment	X			X	X	X	X	X	
MS only: EDSS,	X	X							
MS only: MRI brain		X (Within 4 weeks of cannulation event)							X[a]
Vital Signs	X	X	X	X	X	X	X	X	X
Physical Exam (may include height and weight)	X		X	X	X	X	X	X	X
Pregnancy Test (urine dip or serum)		X							
Safety labs: CBC, CHEM7, LFTs, PT/INR		X				X			X
Lymph fluid collection for research studies			X	X	X	X	X	X	X
Peripheral phlebotomy for research studies (possible)	X	X	X	X	X	X	X	X	X
MS only: OMB safety labs (quant IgG, QuantiFERON gold, Hepatitis B, C testing, cd19 count, JCV titer)	X								
Concomitant medications	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X
Treatment with OMB					X		X	X	

a. **Pre- and post-procedure MRIs should not be more than 2 months apart.**

26 Post procedure Visit schedule and Assessments for MS patients who receive 2-year OMB treatment with follow-up

Table 3 Assessment schedule for post-procedure follow-up for MS patients on treatment

Period	**Post procedure Follow-Up for MS patients on treatment**						
Visit Name	**Follow-up Visit 1**	**Follow-up Visit 2**	**Follow-up Visit 3**	**Follow-up Visit 4**	**Follow up Visit 5**	**Follow up visit 6**	**End of Study visit**
Day	**Post procedural visits + 1 Month ±14d**	**Post procedural visit + 3 Months ±14d**	**Post procedural visit + 6 Months ±14d**	**Post procedural visit + 12 Months ±14d**	**Post procedural visit + 18 Months ±14d**	**Post procedural visit + 24 Months ±14d**	**30 days post last dose***
MS Relapse assessment	X	X	X	X	X	X	
EDSS,				X		X	
Research MRI brain						X[a]	
Physical/Neurological Exam	X	X	X	X	X	X	
OMB Safety labs (cbc, chem7, LFT, JCV, IgG, CD19)			X	X	X	X	
Peripheral phlebotomy for research studies (e.g. NfL, GFAP)	X	X	X	X	X	X	
Adverse Events & Concomitant medication	X	X	X	X	X	X	X

[a] The 'month 24' MRI can be obtained within a +/- 2 month window

* Participants who prematurely discontinue study treatment will have their EOS visit as soon as possible

6 Visit schedule and assessment

Assessment schedule lists all of the assessments and indicates with an "X" when they are performed. All data obtained from these assessments must be supported in the participant's source documentation. Participants should be seen for all visits/assessments as outlined in the assessment schedule or as close to the designated day/time as possible. Missed or rescheduled visits should not lead to automatic discontinuation. In case a visit is performed outside of the schedule, subsequent visits shall be performed in keeping with the original visit schedule. In addition to the scheduled visits, participants may have unscheduled visits due to an acute illness of undetermined cause, for other reasons, or at the discretion of the Investigator. Data collected during unscheduled visits will be recorded in the unscheduled visits CRF.

Participants who prematurely discontinue the study for any reason should be scheduled for the end of study (EOS) visit as soon as possible and then enter the Safety Follow-up period according to the schedule in below tables. At this final visit, all dispensed investigational product should be reconciled, and the adverse event and concomitant medications recorded on the CRF.

6.1 Information collected on screen failures

All participants who sign informed consent but discontinue prior to Baseline Visit are considered screen failures. The Screening Visit Date, Demography, Informed Consent, Inclusion/Exclusion Criteria, Participant Rescreening, and the Screening Phase Disposition eCRFs must be completed. The Adverse Event eCRF and a paper SAE form must be completed for any SAE that occurs during the screening period. Adverse events that are not SAEs will be followed by the Investigator and collected only in the source data. The Withdrawal of Informed consent must be documented if consent was withdrawn during the Screening period before the participant was enrolled.

6.2 Rescreening

If a patient fails on one or more laboratory (or other) assessment criteria, as part of the Screening process, the assessment(s) may be repeated at the discretion of the Investigator, and the patient may be included if criteria are then met, provided the assessments are completed within the Screening or Baseline time window.

6.3 Magnetic Resonance Imaging (MRI)

MS patients in the 'in and out catheter' sampling will undergo a single brain MRI (+/- Gd) within 4 weeks prior to the catheterization, to document presence or absence of subclinical disease activity, in order to better interpret immunological results. MS patients undergoing 'indwelling catheter' sampling will undergo two brain MRIs (+/- Gd). The first within 4 weeks prior to the catheterization, and the second no more than 2 months following the initial MRI. Patients treated with OMB will have an additional MRI at month-24 (+/- 2 months).

Restrictions to MRI schedule

To avoid interferences caused by steroids (in regards to Gadolinium (Gd)-enhancing lesions) for the treatment of MS relapse, the following restrictions apply for this study:

- In case of relapse, if an MRI has been scheduled within 14 days of the initiation of steroid treatment, MRI (with Gd enhancement) should be performed **before** steroid treatment is initiated.
- No MRI (with Gd-enhancement) should be performed while a participant is on steroid therapy for relapse and within the following 14 days upon termination of steroid treatment.

Because of these restrictions, MRI scheduling can be adjusted accordingly. In case a visit is performed outside the visit window, any subsequent visits should be performed according to the original visit schedule.

Scanning

MRI scan sequences such as but not limited to will include conventional MRI measures of T1 hypointense images (with and without contrast medium, i.e., gadolinium-DTPA), T2 weighted images.

The gadolinium contrast medium may occasionally cause nausea and vomiting. Allergic reactions may also occur very rarely and, in extremely rare instances, can be potentially serious and require immediate anti-anaphylactic treatment. Any AE experience due to the contrast medium should be recorded on the AE eCRF.

7 Statistical Analysis Plan

This is an exploratory study. There is no data describing the distribution of populations of immune cells in the thoracic duct in MS patients compared to their blood. Descriptive statistics will be used as well as nonparametric Mann Whitney U tests, the Wilcoxon matched pairs signed rank test or the Friedman test with Dunn's multiple comparison post-test, as appropriate, to compare immune cell subset frequencies and antigen specific responses between thoracic duct and blood, between patients and controls, and prior to and following aCD20 treatment in each compartment. Correlation analyses will we analyzed using the nonparametric Spearman test. Differentially expressed genes between groups will be ranked using Smyth's variance-moderated t-test. Statistical plan will be discussed further amongst collaborators.

Based on our Penn collaborator's prior work characterizing TD cells, we anticipate that our cohort size will generate a sufficiently powered dataset to successfully address our research questions including our primary outcome aiming to document anti-CD20 treatment effect on TD immune cell populations. As an example, in Vella et al (JCI, 2019) experiments with as few as n=4 and typically n=6-10 samples were sufficient to distinguish features of follicular T-helper (Tfh) cells and B cells in the TD from those in the blood in individuals not undergoing immune therapy. We expect a robust aCD20 treatment effect that we already know will impact the blood cells so we should be well powered to comment on aCD20 treatment effects on TD cells.

Power calculations were utilized to determine the effect sizes for secondary outcomes including comparison immune cell subset abundance differences between the compartments of MS patients and controls, with a goal of 80% power and a Type I error of 0.05. Our experimental design of 18 patients with MS and 6 healthy controls (and utilizing a Student's t-test), results in a Cohen's d effect size of 1.38. We next estimated our immune subset abundance standard deviation as 1.5% based on our existing immune phenotyping data. This results in our experimental design being powered to detect immune cell subset abundance differences of at least 1.95%.

Power calculations were also utilized to estimate effect sizes for gene expression changes within immune cell subsets, with a goal of 80% power and a False Discovery Rate of 0.05. We utilized a data-driven model specifically generated for power analysis in multiple-sample, single-cell transcriptomic studies (Schmid KT, et al. Nat Comm, 2021). With an experimental design of 18 patients with MS and 6 healthy controls, our experimental design is powered to detect gene expression changes with a magnitude log2-fold-change of at least 2.

7.1 Control of Bias and Confounding

There are several potential biases that may be introduced into the study based upon selection of participants. There may be differences in immunologic endpoints based upon participants' age, gender, race, and time from prior interferon/glatiramer acetate use, or use of other medication. Given the small sample size and pilot nature of the study we will not try to control or adjust analyses for these potential biases. In addition, given the small sample size and pilot nature of the research, we will not adjust our analyses for multiple comparisons.

7.2 Baseline Data

Baseline and demographic characteristics will be summarized by standard descriptive statistics (including mean and standard deviation for continuous variables such as age and standard percentages for categorical variables such as gender).

7.3 Analysis of Primary Outcome of Interest

This is an exploratory study. There is no data describing the distribution of populations of immune cells in the thoracic duct in MS patients compared to their blood. Descriptive statistics will be used as well as nonparametric Mann Whitney U tests, the Wilcoxon matched pairs signed rank test or the Friedman test with Dunn's multiple comparison post-test, as appropriate, to compare immune cell subset frequencies and antigen specific responses between thoracic duct and blood, between patients and controls, and prior to and following aCD20 treatment in each compartment. Correlation analyses will we analyzed using the nonparametric Spearman test. Differentially expressed genes between groups will be ranked using Smyth's variance-moderated t-test.

7.4 Pharmacokinetic Analysis

PK trough values at steady state for ofatumumab will be used to conduct a population pharmacokinetics analysis. Sampling time points are provided in the visit schedule. Samples for PK assessment should be taken prior to dosing at the Month 1 visit. For any later PK visits, if the visit coincides with the day the monthly injection is scheduled, the patient should not take the injection in the morning before coming to the site so that the PK sample can be drawn before the injection. The PK sample collection date and time must be entered on the appropriate CRF. A laboratory manual on sample collection, numbering, processing and shipment will be provided by the Research Collaborator.

8 Safety

Safety assessments are specified with the assessment schedule (Tables 1-3).

Safety assessments will include:

- Physical examination (including skin)
- Vital signs
- Height and Weight
- Laboratory evaluations

- Adverse events

Any new or worsening clinically relevant findings from such additional assessments meeting definition of an adverse event (AE) or serious AE should be recorded as AE/SAE. For details on AE collection and reporting, refer to Section 10.1.

8.1 Physical Examination (including skin)

A complete physical examination will be performed at the visits indicated and will include an assessment of skin, head and neck, lymph nodes, heart, lungs, abdomen, back, neurological function and comments on general appearance. A complete neurological examination will be part of the initial physical examination at Screening.

Information for all physical examinations (including skin examination) must be included in the source documentation at the study site. Clinically relevant findings that are present prior to signing informed consent must be recorded on the appropriate eCRF that captures medical history. Significant findings made after signing informed consent, which meet the definition of an Adverse Event must be recorded on the adverse events eCRF.

8.2 Vital Signs

Vital signs will include sitting pulse rate (measured as radial pulse for 60 seconds), sitting systolic and diastolic blood pressure and body temperature (oral, or per local practice and should be recorded in the relevant CRF page in Celsius) which will be assessed at the visits indicated.

After the participant has been sitting for five minutes, with back supported and both feet placed on the floor, systolic and diastolic blood pressure will be measured three times using an automated validated device, e.g. OMRON, with an appropriately sized cuff. The repeat sitting measurements will be made at 1 - 2 minute intervals and the mean of the three measurements will be used. In case the cuff sizes available are not large enough for the participant's arm circumference, a sphygmomanometer with an appropriately sized cuff may be used.

For drug treatment, vital signs should be obtained 30-60 minutes before the s.c. injection and again approximately 60 minutes post for the Day 2 administration. If premedication is administered, the vital signs should be taken prior to premedication administration.

8.3 Height and Weight

Height in centimeters (cm) and body weight (to the nearest 0.1 kilogram (kg) in indoor clothing, but without shoes) will be measured.

8.4 Laboratory evaluations

A local laboratory will be used for analysis of all specimens collected. Abnormal laboratory parameters, inconsistent with the clinical presentation of MS or which cause suspicion of an underlying medical condition, should be repeated for confirmation.

All abnormal lab results must be evaluated for criteria defining an adverse event and reported as such if the criteria are met. For those lab adverse events, repeated evaluations are mandatory until normalization of the result(s) or until the result is no longer considered clinically significant.

Table 3 Laboratory Assessments

Test Category	Test Name
Hematology	Blood samples will be collected according to the schedule the respective visit schedule. Hematocrit, Hemoglobin, Platelets, Red blood cell (RBC) count, Total WBC count and WBC differential counts (Basophils, Eosinophils, Lymphocytes, Monocytes, Neutrophils).
Chemistry	Blood samples will be collected according to the schedule as above. Electrolytes (Sodium, potassium, chlorine, bicarbonate, calcium, magnesium, phosphorus), random glucose, total protein, blood urea nitrogen, albumin, alkaline phosphatase, ALT, AST, y-GT, total bilirubin, conjugated bilirubin, creatinine, amylase, total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein, C-Reactive protein.
B-cell/T-cell sampling	$CD19^+$ B-cell counts and $CD3^+CD20^+$ T-cell counts. Samples will be collected according to the schedule in visit above.
Total IgG	Samples will be collected according to the schedule in visit above.
Biomarker sampling	Refer to visit above.
Additional tests	Testing of lab samples will be conducted at screening to determine the participant's eligibility for inclusion in the study with respect to hepatitis viruses. Testing for syphilis and tuberculosis at Screening is also needed. A positive result for any of the following serological markers for hepatitis B and C as below is an exclusion criterion: • HBs Ag and/or anti-HBc IgM and/or HB virus deoxyribonucleic acid (DNA) • anti-HBs negative and anti-HBc positive • anti-HC IgG (if positive IgG, HCV RNA PCR will be performed and if negative, participant can be enrolled)
Pregnancy Test	Serum or Urine pregnancy test

8.5 Pregnancy and assessments of fertility

Urine pregnancy tests (UPT) will be conducted for all female subjects of childbearing potential at Screening visit within 1 week of thoracic duct cannulation. If UPT cannot be collected for any reason, a serum pregnancy test will be collected instead.

In addition, the Investigator will review the contraception status with the participant at each visit to ascertain that the participant continues to comply with protocol requirements for highly effective contraception as shown in the below contraception guidance.

CONTRACEPTION GUIDANCE:

CONTRACEPTIVES ALLOWED DURING THE STUDY INCLUDE:

Highly Effective Methods[a] That Have Low User Dependency Failure rate of <1% per year when used consistently and correctly.

- Implantable progestogen-only hormone contraception associated with inhibition of ovulation[b]
  - Intrauterine device (IUD)
  - Intrauterine hormone-releasing system (IUS)[c]
- Bilateral tubal occlusion

Vasectomized partner

(Vasectomized partner is a highly effective contraceptive method provided that the partner is the sole sexual partner of the female subject of childbearing potential and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used. Spermatogenesis cycle is approximately 90 days.)

Highly Effective Methods[a] That Are User Dependent

Failure rate of <1% per year when used consistently and correctly.

- Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation[c]
  - oral
  - intravaginal
  - transdermal
  - injectable
- Progestogen-only hormone contraception associated with inhibition of ovulation[c]

- oral
  - injectable

Sexual abstinence

Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)

ACCEPTABLE METHODS[c]

1. Progestogen-only oral hormonal contraception where inhibition of ovulation is not the primary mode of action
2. Male or female condom with or without spermicide[e]
3. Cervical cap, diaphragm, or sponge with spermicide
4. A combination of male condom with either cervical cap, diaphragm, or sponge with spermicide (double-barrier methods)

a Failure rate of <1% per year when used consistently and correctly. Typical use failure rates differ from those when used consistently and correctly.

b If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable contraceptive methods are limited to those which inhibit ovulation as the primary mode of action.

c Considered effective, but not highly effective - failure rate of ≥1% per year. Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method (LAM) are not acceptable methods of contraception.

8.6 Appropriateness of safety measurements

The safety assessments included in this study are standard for the MS indication and study participant population and appropriate for people undergoing thoracic duct cannulation.

8.7 Additional assessments

27 Biomarkers

Blood samples will be drawn for analysis of exploratory biomarkers according to the schedule above. These specimens will be used to identify and/or verify potential markers that may be predictive of disease activity, disease course and/or clinical response to treatment. Analysis of these specimens will include NfL and GFAP. Samples for biomarker assessment should be taken prior to dosing at Visit 3. For any later biomarker visits, if the visit coincides with the s.c. injections, the participant should not take the injection in the morning before coming to the site so that the biomarker sample can be drawn prior to administration. The biomarker sample collection date and time must be entered on the appropriate eCRF.

The details describing the collection, handling, storage and shipment requirements of samples will be shared amongst the collaborators and the laboratory manual, whatever is applicable.

28 Immune cell analyses

Detailed analyses in terms of handling of specimens, sharing of specimens between the labs, the work performed through the Bar-Or Lab, the Prak Lab, and the the Research Collaborator lab of Traggiai (e.g. B cell functional and autoantibody testing) will be discussed amongst the collaborators. This will also include how specimens and the generated data are shared.

9 Study discontinuation and completion

9.1 Discontinuation

29 Discontinuation of study treatment

Discontinuation of study drug for a participant occurs when study drug is stopped earlier than scheduled in the protocol, and can be initiated by either the participant or the investigator.

The investigator must discontinue study treatment for a given participant if he/she believes that continuation would negatively affect the participant's well-being.

Study drug must be discontinued under the following circumstances:

- Participant/guardian decision
- Pregnancy
- Use of prohibited treatment
- Diagnosis of PML
- Participants with active serious infections or reactivation (e.g. tuberculosis, hepatitis B or C)
- Skin and/or mucosal reactions which raise the suspicion of severe generalized major skin reactions (Stevens-Johnson syndrome, or toxis epidermal necrolysis-Lyell's syndrome)
- Hypersensitivity to the study medication
- Any situation in which study participation might result in a safety risk to the participant
- Protocol violation that results in a significant risk to the participant's safety

- Emergence of certain adverse events, such as malignancy (except successfully treated basal cell carcinoma, *in situ* squamous cell carcinoma and *in situ* carcinoma of cervix or uterus), liver failure or serious chronic infection (such as human immunodeficiency virus (HIV))
- Laboratory abnormalities (e.g. liver functions tests (LFTs)) and abnormal test procedure defined in Appendix 1
- Severe hypoproteinemia
- Interstitial lung disease or new onset or worsening of pulmonary symptoms, such as persistent cough and dyspnea, with or without associated fever, suspicious of interstitial lung disease
- Non-compliance with OMB or study procedures
- No benefit of drug treatment

If discontinuation of OMB occurs, the investigator should make a reasonable effort to understand the primary reason for the participant's premature discontinuation of study drug and record this information.

Participants who discontinue study drug or who decide they do not wish to participate in the study further should NOT be considered withdrawn from the study UNLESS they withdraw their consent (see Section 6.4.3).

If they fail to return for these assessments for unknown reasons, every effort (e.g. telephone, e-mail, letter) should be made to contact the participant/pre-designated contact as specified in Section 6.4.4. This contact should preferably be done according to the study visit schedule.

If the participant cannot or is unwilling to attend any visit(s), the site staff should maintain regular contact with the participant, or with a person pre-designated by the participant. This contact should preferably be done according to the study visit schedule for up to 9 months post last OMB administration.

After OMB discontinuation, at a minimum, in abbreviated visits, the following data should be collected at clinic visits or via telephone/email contact:

- New / concomitant treatments
- Adverse Events / Serious Adverse Events

The investigator must record the participant's discontinuation from OMB.

9.2 Study completion and post-study treatment

Study completion is defined as when the last participant finishes their Study Completion visit and any repeat assessments associated with this visit have been documented and followed-up appropriately by the Investigator or, in the event of an early study termination decision, the date of that decision.

A participant is considered to have completed the study when they fulfill the following criteria:

- Participant has completed the study in its entirety according to the approved duration of the study
- The participant has **not** stopped the study due to one or more of the following reasons:
  - Discontinuation of study treatment (Section 10.1)

- Withdrawal of informed consent (Section 6.4.3)
- Lost to follow-up (Section 6.4.4)

End of Study (EOS) visit is mandatory for all participants. For participants that complete the study, the next (and last) scheduled visit is the EOS visit and should align with the overall visit schedule. Participants who prematurely discontinue study treatment will have their EOS visit as soon as possible. The Investigator provides follow-up medical care for all participants who are prematurely withdrawn from the study, or refers them for appropriate ongoing care.

10 Safety monitoring and reporting

10.1 Definition of adverse events and reporting requirements

30 Adverse events

An adverse event (AE) is any untoward medical occurrence (e.g. any unfavorable and unintended sign [including abnormal laboratory findings], symptom or disease) in a participant or clinical investigation participant after providing written informed consent for participation in the study.

An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Therefore, an AE may or may not be temporally or causally associated with the use of a medicinal (investigational) product.

Adverse events may be detected when they are volunteered by the participant during or between visits or through physical examination findings, laboratory test findings, or other assessments.

Adverse events must be recorded under the signs, symptoms, or diagnosis associated with them, and graded using the Common Toxicity Criteria (CTC) AE grade (1-4) (if the event is serious refer to Section 11.1.2).

All adverse events must be treated appropriately. Treatment may include one or more of the following:

- No action taken (e.g. further observation only)
- Drug interrupted/withdrawn
- Concomitant medication or non-drug therapy given
- Participant hospitalization/participant's hospitalization prolonged

- Its outcome (not recovered/not resolved; recovered/resolved; recovered/resolved with sequelae; fatal; or unknown).

Conditions that were already present at the time of informed consent should be recorded in medical history of the participant.

Adverse events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms.

Adverse event monitoring should be continued for at least 30 days following the last dose of study treatment.

Once an adverse event is detected, it must be followed until its resolution or until it is judged to be permanent (e.g. continuing at the end of the study), and assessment must be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the interventions required to treat it, and the outcome. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study intervention or study participation will be recorded and reported.

Research staff will elicit and record all adverse events on case report forms. Information on all adverse events will be recorded immediately in the CRF and an AE form. All clearly related signs and symptoms will be grouped under one diagnosis.

Information about adverse drug reactions for OMB can be found in the FDA prescribing guidelines. For information about risks and common side effects related to the AEP (cholestyramine or activated charcoal), please refer to the local product label. This information will be included in the participant informed consent and should be discussed with the participant during the study as needed. Any new information regarding the safety profile of ofatumumab that is identified will be communicated as appropriate. New information might require an update to the informed consent and has then to be discussed with the participant.

The Investigator must also instruct each participant to report any new adverse event (beyond the protocol observation period) that the participant, or the participant's personal physician, believes might reasonably be related to study treatment. This information must be recorded in the Investigator's source documents; however, if the AE meets the criteria of an SAE, it must be reported to the Research Collaborator.

31 Serious adverse events

Adverse events are classified as serious or non-serious. A serious adverse event is any AE that is:

- fatal
- life-threatening
- requires a hospital admission or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- constitutes a congenital anomaly/birth defect
- requires an intervention to prevent permanent impairment or damage

- an important medical event

All adverse events that do not meet any of the criteria for serious will be regarded as non-serious adverse events.

Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study intervention or study participation will be recorded and reported.

Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious reactions, such as important medical events that might not be immediately life threatening or result in death or hospitalization but might jeopardize the participant or might require intervention to prevent one of the other outcomes listed above. Such events should be considered as "medically significant." Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization or development of dependency or abuse (please refer to the ICH-E2D Guidelines).

All malignant neoplasms will be assessed as serious under "medically significant" if other seriousness criteria are not met.

Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse reaction.

All reports of intentional misuse and abuse of the product are also considered serious adverse event irrespective if a clinical event has occurred, and are to be reported as appropriate according to Section 10.1.3.. The Research Collaborator qualified medical personnel will be readily available to advise on trial related medical questions or problems.

32 SAE reporting

The Sponsor will be responsible for ensuring that all Adverse Events and Serious Adverse Events, including Suspected Unexpected Serious Adverse Reactions (SUSARs) and other relevant safety information are recorded and appropriately reported to the relevant health authorities, ethics committees and Study investigators according to Applicable Laws in the country where the Study is conducted.

To ensure participant safety, every SAE, regardless of causality, shall be reported to the Research Collaborator as outlined in the Collaborative Research Agreement. Information about all SAEs is collected and recorded on Penn's Serious Adverse Event Report Form. Each re-occurrence, complication, or progression of the original event must be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or withdrew from study participation.

Any SAEs experienced after the 30 day period after the final study visit or after the end of Safety Follow-up should only be reported to the Research Collaborator if the investigator suspects a causal relationship to study treatment.

33 Reporting MS relapse as a SAE

MS relapses are exempt from SAE reporting although they may meet the SAE definition on the basis that they are considered medically significant and are frequently associated with hospitalization. These events will therefore be reported on the MS relapse CRF instead of the SAE form. However, if, in the judgment of the Investigator, a MS relapse is unusually severe or medically unexpected and warrants specific notification, then an SAE form must be completed and submitted according to SAE reporting procedures outlined above.

34 Pregnancy reporting

To ensure participant safety, each pregnancy occurring after signing the informed consent and after receiving drug treatment must be recorded. Any reports of reports of drug exposure during pregnancy should be considered an SAE and be reported to Novartis as soon as it becomes available, but in any event within eight (8) calendar days of becoming aware of such information. For instances of drug exposure during pregnancy, the pregnancy should be followed up until after the Expected Delivery Date (EDD) to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

35 Reporting of study treatment errors including misuse/abuse

Medication errors are unintentional errors in the prescribing, dispensing, administration or monitoring of a medicine while under the control of a healthcare professional, participant or consumer (European Medicines Agency (EMA) definition).

Misuse refers to situations where the medicinal product is intentionally and inappropriately used not in accordance with the protocol.

Abuse corresponds to the persistent or sporadic, intentional excessive use of a medicinal product, which is accompanied by harmful physical or psychological effects.

Study treatment errors and uses outside of what is foreseen in the protocol will be recorded on the appropriate CRF irrespective of whether or not associated with an AE/SAE and reported to Safety only if associated with an SAE.

Table 4 Guidance for capturing the study treatment errors including misuse/abuse

Treatment error type	Document in Dosing CRF (Yes/No)	Document in AE eCRF	Complete SAE form
Unintentional study treatment error	Yes	Only if associated with an AE	Only if associated with an SAE
Misuse/Abuse	Yes	Yes	Only if associated with an SAE

For more information on AE and SAE definition and reporting requirements, please see the respective sections.

11 Data Collection and Database management

11.1 Data collection

Designated trained investigator staff will enter the data required by the protocol into the Penn Electronic Case Report Forms (eCRF) and database. The Investigator/designee is responsible for assuring that the data recorded on CRFs is complete, accurate, and that entry and updates are performed in a timely manner. The Investigator must certify that the data entered are complete and accurate.

All other data captured for this study will have either the CRF as source or an external originating source (either written or electronic) with the eCRF not being considered as source. All data will be recorded, handled, and stored in a way that allows its accurate reporting, interpretation, and verification.

11.2 Database management and quality control

Clinical data, imaging data, and laboratory outcomes will be kept in separate databases.
All clinical data relevant to the study outcomes will be maintained in PennChart. Any paper forms will be maintained in locked filing cabinets in the study PI's or research coordinator's office. The Division of Interventional Radiology will keep records of participants who undergo thoracic duct cannulation procedures to monitor safety outcomes of the procedures.

Laboratory outcomes of the study will be maintained in databases maintained by individual laboratories that will be supplied with clinical material for analysis labeled with coded participant identification numbers.

Image data acquired during research MRI studies are automatically deidentified of Private Health Information (PHI) and transferred to a secure cloud-based informatics platform.

11.3 Sample management and storage

Biological samples will be processed locally at Penn, under the biorepository of the CNET (director Bar-Or) will include sample allocation and packaging to ship to the Research Collaborator and/or other entities to run specialized tests as outlined in the protocol. Sample aliquots not immediately planned for testing will be stored in appropriate conditions in the laboratory of Dr. Amit Bar-Or. The Research Collaborator may request additional sample aliquots following discussions with the primary Penn research team. At the end of the collaborative research agreement, all unused aliquots will remain under the control of the primary Penn research team.

Consent will be obtained from the study participants to transfer samples and data outside of the USA to allow specialized analyses to be performed by the Research Collaborator and/or other entities.

12 Data analysis and statistical methods

Any data analysis carried out independently by the Investigator should be shared with the Research Collaborator according to the terms of the Collaborative Research Agreement (CRA).

Likewise, the data generated by the Research Collaborator and/or other entities should be shared with the Penn research team according to the terms of the CRA.

12.1 Analysis sets

The Full Analysis Set (FAS) comprises all participants in the study whether or not drug treatment was administered.

12.2 Participant demographics and other baseline characteristics

Demographics, MS disease history, MRI baseline characteristics and MS medication history will be summarized.

12.3 Treatments

Exposure to investigational study medication is defined as the number of days spent on study treatment divided by 365 days. Intermediate treatment interruptions will be subtracted from drug exposure.

Exposure to investigational study medication will be summarized with number and percentage of participants by time category, and with summary statistics of the number of participant years of exposure.

12.4 Analysis of the primary endpoint(s)

The primary objective of the study is exploratory. The study is designed to evaluate the safety and feasibility of collection of lymphoid fluid via thoracic duct cannulation in healthy people and people with MS.

36 Safety endpoints

Patients will be assessed for the following:

1. Safety profile of "in-and-out" thoracic duct cannulation in participants (healthy controls and MS) and single time-point sampling of lymphatic fluids and peripheral blood

2. Safety profile of "indwelling" thoracic duct cannulation in participants (healthy controls and MS) and serial sampling of lymphatic fluids and peripheral blood

A summary of AEs and SAEs will be provided to the Research Collaborator in final study report as outlined in the Collaborative Research Agreement.

12.5 Analysis of secondary endpoints

The secondary objective of the study is also exploratory. The study will assess the impact on anti-CD20 ofatumumab treatment in patients with MS on biological measures in the thoracic duct fluid and in the blood.

37 12.5.1 Definition of secondary endpoint(s)

Secondary endpoints are to compare immune cell profiles of blood and lymphatics between MS and healthy controls and in untreated MS patients before and following a B-cell depleting therapy (Ofatumumab), including:

- To evaluate the phenotype, function, and transcriptional profiles of immune cell sub-populations (including T cell, B cell and innate cell subsets)
- To identify CNS-antigen specific responses of immune cells and profile B cell repertoires
- To quantify soluble measures of inflammation and CNS injury in lymph fluid compared to serum
- To investigate the humoral autoantibody repertoire at serological and cellular level

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39 **12.5.2. Statistical model, hypothesis, and method of analysis**

Descriptive statistics will be used as well as nonparametric Mann Whitney U tests, the Wilcoxon matched pairs signed rank test or the Friedman test with Dunn's multiple comparison post-test, as appropriate, to compare immune cell subset frequencies and antigen specific responses between thoracic duct and blood, between patients and controls, and prior to and following aCD20 treatment in each compartment. Correlation analyses will we analyzed using the nonparametric Spearman test. Differentially expressed genes between groups will be ranked using Smyth's variance-moderated t-test. Statistical plan will be discussed further amongst collaborators.

12.6 Analysis of exploratory endpoints

The following exploratory endpoints will be evaluated during the 24-month follow-up period of patients that have been treated with OMB, in serum and compared to earlier results in serum and lymph fluid. Samples collected at Penn will be shipped to the Research Collaborator, or other collaborating laboratories for testing.

- Neurofilament light (NfL; SIMOA platform)
- Glial fibrillary acidic protein (GFAP; SIMOA platform)
- anti-NfL antibodies (ELISA)
- Somamers (>5000 aptamer panel; SOMALOGIC)
- Olink 21-panel MS (OCTAVE)

In parallel, the clonal diversity in the B-cell receptor (BCR) reportoire of the residual B-cells (under treatment with OMB) in samples from the thoracic duct and peripheral blood shall be evaluated (collaboration with NVS, Basel).

Table 6: Exploratory biomarkers to be tested in participants receiving OMB

No	Measures	Frequency (Months)	Matrix	Specifications
1	NfL	1M, 3M, 6M, 12M, 18M, 24M	Thoracic duct fluid & blood	SIMOA (Quanterix)
2	GFAP			SIMOA (Quanterix)
3	anti-NfL Abs			
4	SOMASCAN			SOMALOGIC (5000-aptamer panel)
5	O-LINK			OCTAVE (21-panel)
6	BCR clonal diversity	1M, 6M, 12M, 24M		NVS, Basel

13 Ethical Considerations and Risks

All protocol amendments will be submitted for review and approval by the University of Pennsylvania IRB prior to the start of any study procedures or changes in study procedures. Informed consent documents will be updated with any new safety information that might influence a participant's willingness to enroll in the study. An annual report will be submitted to the University of Pennsylvania IRB for continued renewal of the study.

13.1 Risks

Risks associated with participation in the study include those associated with the physical performance of the thoracic duct cannulation procedure, those associated with medications used during the performance of the thoracic duct cannulation procedure, those associated with phlebotomy, those associated with MRI (*MS subjects only*) and those associated with loss of confidentiality.

40 Risks of thoracic duct cannulation procedure

The members of the Interventional Radiology Division who perform this procedure keep careful records of adverse events associated with the procedure. The most common reported event is mild abdominal discomfort where the abdomen is punctured, and the cannula is inserted that may persist for a day or two post-procedure or minor infection at the site of injection of the lymph node in the groin or at the site of insertion of the catheter in the abdomen. The abdominal pain reported in association with the procedure often does not require treatment. Pain requiring treatment is typically controlled by acetaminophen or ibuprofen. The interventional radiologists participating in this study have performed over 250 thoracic duct cannulation procedures for the purposes of thoracic duct embolization. All participants have been monitored and 10 serious adverse events have been reported. Each of these events was associated with the embolization component of the procedure which will not be performed as part of this study. A list of these events is included in the table below. Because the procedure involves passing a catheter through the abdomen, it is theoretically possible that a participant could experience intraabdominal bleeding or infection. An intravenous line will be maintained during the procedure, with possible adverse events of bruising, bleeding, infection or a blood clot at the insertion site of the intravenous line.

41 Additional risks associated with indwelling catheter.

Indwelling catheterization with external drainage of the thoracic duct has been successfully accomplished in the treatment of congenital and acquired lymphatic diseases in the inpatient setting. Indwelling vascular lines are commonly used in the treatment of infection. The risks for this procedure include infection, clotting of the line preventing sampling, bleeding, injury to the lymphatic drainage system and potentially other unknown risks.

Overall, the potential risks of the proposed indwelling catheter study can be divided into three categories, including those associated with (i) the procedure itself including the cannulation of the thoracic duct and the 'externalization' (ie establishing indwelling antecubital access to

thoracic duct); (ii) maintaining the indwelling catheter for a period of 2-3 weeks; and (iii) any added risk of carrying out the procedure and maintaining the indwelling catheter in the face of anti-CD20 treatment with ofatumumab.

(i) The procedure: The risks associated with the cannulation component of the procedure are well established based on the substantial combined clinical and research experience with 'in and out' thoracic duct catheterization at Penn, and described in our previously approved research protocol (Penn IRB: 831994). As noted by our study's interventional radiology (IR) collaborator, Dr. Itkin, the additional step of externalization has not itself been viewed as adding significant risk and was previously approved as a 'modification' to the HIV research protocol (attached Penn IRB 823909; PIs Drs. Ian Frank and Max Itkin), to enable more efficient collection of thoracic duct fluid in the event that initial cannulation resulted in limited flow of lymph. Though externalization was approved in that protocol, the added procedure was never required.

(ii) Potential risks associated with maintaining the thoracic duct indwelling catheter: A published meta-analysis of studies of external drainage of the thoracic duct reported on a total of 71 studies involving a total of 1160 patients, where this approach was used in solid organ transplantation, acute pancreatitis, neoplastic disease, sepsis, and autoimmune disease, for periods of up to and over 4 weeks (Meta analysis: Wang et. al Journal of Surgical Research. Vol 204. 2016). The most common complication was wound infection at the site of cannulation which occurred in 5-10% of participants. Other rare complications were accumulation of lymph in the neck, back pain, and edema of the face or arm. There was no reported mortality related to thoracic duct drainage. We were surprised to find that the meta-analysis included one small study in patients with MS (Ring et al. Lancet, 1974) where thoracic duct drainage was assessed as a potential therapeutic intervention (the study reported on immunological and neurological outcomes but did not report on complications).

It is important to note that many of the prior studies took place between the 1960s - 1980s with techniques of thoracic duct cannulation and drainage that involved surgical incision of the neck and more invasive techniques to access the duct. The modernized IR techniques have become more refined with lower complication rates than past surgical approaches. Important differences are that the majority of the prior studies described in the meta-analysis employed larger catheters as well as continuous drainage, unlike our proposed approach which uses considerably finer catheters and only intermittent sampling of smaller lymph volumes. To date, our IR team at Penn has performed indwelling thoracic duct cannulation with drainage in 8 patients including 3 infants with anasarca and 5 adolescent patients with chylothorax. The duration of indwelling catheter and drainage averaged 9.9 days (with a range of 2 to 43 days). There were no intra-procedure complications, all 8 patients experienced symptomatic improvement and no line-infections developed this quite sick patient population. Our IR collaborators have a Penn IRB approved research protocol to perform thoracic duct cannulation with externalization and drainage in the context of sepsis (protocol :834599), though they have not yet initiated this trial.

Given our proposed use of the smaller bore catheters and intermittent sampling of much smaller volumes of lymph fluid (50mL per sample versus the liters that are drained in the clinical setting), we anticipate any added risks of indwelling catheter in the generally healthy MS patients who do not have abnormal thoracic duct anatomy will be minimal, particularly over a period of several weeks. According to our IR team, the closest approximation of risk associated with the indwelling thoracic duct catheter in this generally healthy population is the risk associated with a peripherally inserted central catheter (PICC) line. PICC line cannulation of 3 weeks or longer is routinely done in the appropriate clinical setting. We will use a similar 4-5 Fr line to access the thoracic duct through left arm vein. The greatest potential complication is infection. The risk of PICC line associated infections in the heme/onc population such as bone marrow transplantation patients who are much more profoundly immune compromised is low. In a study of oncology patients, 165 BMT patients had PICC lines for an average of 88.3 days and an infection rate of 0.96 infections per thousand line-days (Moturu, A et al. Infection rates for PICC lines in oncology patients. Journal of Vascular and Interventional Radiology, 2017). We anticipate an even lower risk of infection given the shorter duration of indwelling catheter and the healthier MS population.

(iii) Potential for added risk of carrying out the procedure, including maintaining the indwelling catheter, in the face of anti-CD20 treatment with ofatumumab: Anti-CD20 therapies have been used as front-line agents to treat B cell lymphomas for over 2 decades and in growing numbers of patients with a range of autoimmune conditions including MS. We found no studies that directly assess risks of catheterization or of indwelling catheters such as PICC lines in anti-CD20 treated patients. Several studies have attempted to isolate the added risk conferred by anti-CD20 therapy in patients undergoing surgical interventions and have reported relatively minor or no obvious added risks even to fairly involved surgery. For example, a study assessing the safety of surgery in 133 patients with rheumatoid arthritis on anti-CD20 (S. Godot et al. ACR, 2013) found that complications including infections did not differ in relation to anti-CD20 treatment including time from last infusion and duration on treatment. This may reflect the relatively limited degree of immune compromise conferred by anti-CD20 treatment. The role of B cells in host protection is largely attributed to their potential to become antibody secreting plasma cells and since plasma cells no longer express CD20, the anti-CD20 agents do not directly deplete plasma cells. It is well established that pre-existing protective antibodies are generally not impacted by anti-CD20 monotherapy for at least several years of ongoing therapy. Moreover, while the ability to mount novel antibody responses is clearly impaired in patients who are B cell depleted, their CD4 and CD8 T cell responses remain robust (Bar-Or et al Neurology 2020; Apsotolidis et al Nat Med 2021). Among anti-CD20 treated patients, those with MS are relatively unique in that they tend to be generally healthier, receive lower doses (than cancer patients) and invariably are on anti-CD20 as a monotherapy (unlike both cancer and autoimmune conditions like rheumatoid arthritis where patients are almost always on two or more therapies that impact the immune system). In particular, the approved MS dosing regimen of ofatumumab used in our study (30 mg sc monthly), is lower than the other approved anti-CD20, ocrelizumab (600 mg

IV every 6 months) and did not appear to increase infection rates in the pivotal phase III ASCLEPIOS I and ASCLEPIOS MS trials (Hauser et al, NEJM, 2020). These studies randomized over 1600 total MS patients 1:1 between ofatumumab and teriflunomide (itself not associated with more infections than placebo) and revealed no differences over two years of continuous treatment between ofatumumab and teriflunomide for either 'any infections' (approximately 50% for both) or 'serious infections' (approximately 2.3% for both). We do note that post-marketing studies of patients with MS on the anti-CD20 ocrelizumab (which has been approved for longer than ofatumumab) have recently shown that after several years of continuous treatment, patients may start dropping levels of circulating IgG which, in a small proportion of patients, was associated with slight increased risk of serious infections. Hence, while direct evidence is still lacking, and while continuous B cell depletion over a more extended timeframe will likely result in functional immune suppression in at least some patients, the observations described above are reassuring and would together suggest that the first few weeks of ofatumumab therapy would confer little to no added risk to the proposed procedure of thoracic duct cannulation, externalization, and maintenance as an indwelling catheter for a limited time period.

42 Risks associated with conscious sedation.

Conscious sedation will be achieved with intravenous midazolam and fentanyl, the standard drugs used in the clinical procedure.

43 Risks associated with fentanyl when given intravenously

Nausea (26.1%), vomiting (18.6%), muscle rigidity (10.4%), hypotension (8.8%), hypertension (8.8%), bradycardia (6.1%), sedation (5.3%), tachycardia (4.0%), dizziness (3.7%), apnea (3.5%), dyskinesia (3.2%), confusion (1.9%), visual disturbance (1.9%), arrhythmia (2.9%), vein pain (2.9%), bronchospasm (1.3%), laryngospasm (1.3%), rash (1.3%).

44 Risks associated with midazolam when given as a single agent for sedation in adults:

Hiccoughs (3.9%), nausea (2.8%), vomiting (2.6%), coughing (1.3%), over sedation (1.6%), headache (1.5%) and drowsiness (1.2%). Other possible events occurring at a frequency of <1.0% include dyspnea, tachypnea, bradycardia, tachycardia, amnesia, anxiety, insomnia, ataxia, and double vision.

45 Risks associated with antibiotics.

The antibiotics that will be used for prophylaxis against infection will be ampicillin/sulbactam, and for penicillin allergic patients the combination of levofloxacin and clindamycin. Single doses of antibiotics will be given, and therefore the risks are low.

Risks associated with ampicillin-sulbactam include diarrhea (3%), rash (2%), and in <1% of recipients: itching, nausea, vomiting, fatigue, headache, abdominal distention, swelling, chills,

tightness of the throat, elevated liver function tests, elevated renal function tests, anemia, low platelet counts, and low white blood cell counts.

Risks associated with levofloxacin (to be administered only to participants with penicillin allergies) include trouble sleeping (4%), headache (6%), dizziness (3%), nausea (7%), diarrhea (5%), constipation (3%), abdominal pain (2%), vomiting (2%), rash (2%), swelling (1%), and in rare cases rupture of the tendon.

Risks associated with clindamycin (to be administered only to participants with penicillin allergies) include: diarrhea, *Clostridia difficile* colitis, itching, skin rash, abnormal liver function tests, worsening renal functions, low white blood cell counts, low platelet counts (all less than 2%).

46 Risk of phlebotomy.

The most common risks associated with phlebotomy are bruising, bleeding, pain, and infection.

47 Risk of radiation.

Radiation is associated with an increased risk of cancer. The dose of radiation being administered in this study is not believed to result in a clinically significant increased risk of cancer.

48 Risks of MRI.

The known risks associated with MRI are minimal. Implanted medical devices and metallic foreign fragments inside the subject's body may pose a risk if s/he were to enter the MRI magnet room. Therefore, questions regarding medical and work history will be asked prior to the subject's exam. The greatest risk is a magnetic object flying through the air toward the magnet and hitting the subject. To reduce this risk, we require that all people involved with the study remove all magnetic metal from their clothing and all magnetic metal objects from their pockets. No magnetic metal objects are allowed to be brought into the magnet room at any time except by approved personnel. In addition, once the subject is in the magnet, the door to the room will be closed so that no one inadvertently walks into the room. There is no known health risk associated with exposure to magnetic fields during an MRI. There are minimal risks from the loud noise associated with the MRI scanner and from the discomfort of lying on a hard surface. We shall provide the subject with protective earplugs as necessary and make every attempt to ensure his/her comfort with blankets, etc. during his/her time in the scanner.

Gadolinium Based Contrast Agents - Retention: Traces of gadolinium may remain in the body long-term after contrast administration. This risk increases with the number of administrations, but reviews to date have not identified adverse health effects from gadolinium retained in the brain or bodily tissues after MRI.

Gadolinium Based Contrast Agents - IV Line Placement: Multiple needle-sticks may be necessary if a vein cannot be properly accessed and this will be carried out with the patient's permission.

IV Contrast Risks: There is a rare possibility that patients could have an adverse reaction to the contrast agent such as rash, hives, itching, mild headache and nausea. They may also experience some minor discomfort and low risk of bleeding, infection and bruising associated with Intravenous catheter placement.

Pregnancy: Gadolinium-based IV contrast agents are not approved in pregnant people and therefore those who are pregnant will be excluded from this trial.

Some of the pulse sequences associated with this MRI are not FDA approved but are considered to pose no more than minimal risk. Although there are no known risks related to MRI on pregnant people or a fetus, there is a possibility of yet undiscovered pregnancy related risks. Since there is no possible benefit from participating in this protocol for a pregnant patient, we will exclude pregnant subjects from this study.

The proposed MRIs are not clinical scans. It is possible that during the course of the research study, the research staff may notice an unexpected finding(s). Should this occur, the finding(s) will be considered by the appropriate personnel and the PI will inform the subject if necessary. These possible finding(s) may or may not be significant and may lead to the subject experiencing anxiety about his/her condition and they may lead to further work-up by his/her physician.

The subject might find parts of the testing difficult or tiring. If the subject finds that s/he is becoming tired or discouraged s/he can request a break and s/he may decline to participate in any aspect of the testing.

49 Loss of confidentiality.

Identities of participants will be known by research and clinical personnel performing participant recruitment and performance of the study procedures. Specimens obtained will be labeled with a participant number so that laboratory personnel handling the collected material will not know the identity of study participants. Clinical data abstracted from participants' medical records will be identified only with a participant number. The list linking the participant number and participant's identification will be kept in the research office of Dr. Bar-Or in a locked cabinet with access limited to the Clinical Coordinator and Principal Investigator (Dr. Bar-Or). A copy of this list linking participant's identification number and name will also be maintained in the research office of the Interventional Radiology Division so that safety data related to the study procedure can be effectively tracked.

Minimization of risks. Risks of study participation will be minimized as much as possible. The thoracic duct cannulation procedure will be performed only by interventional radiologists and support staff experienced in the performance of this procedure. The procedure will be halted if there is any indication that the participant is experiencing an adverse event prior to

the completion of the procedure. Participants will be contacted by telephone on the days following the procedure to whether adverse events have been encountered, and any adverse events can be quickly evaluated and treated.

Additional precautions in place for the indwelling group due to unknown risks of this procedure in the ambulatory setting. As described in the study protocol, subjects will be assessed at multiple time points during the study to evaluate for any adverse events. Each subject in the indwelling group will be assessed sequentially to identify any potential unknown risks and the IRB and study will be updated accordingly.

Alternatives to participation. This study is solely for research purposes. An individual may choose to not participate. A decision to not participate will have no influence on the care that individual receives.

50 Risks associated with Ofatumumab:

Ofatumumab (Kesimpta) has been approved by the FDA for patients with relapsing remitting MS and is commonly used in the Penn Neurology clinic. Ofatumumab has the potential for an increased risk of infections, including serious bacterial, fungal, and new or reactivated viral infections; some of these infections have been fatal in patients treated with other anti-CD20 antibodies. In the phase 3 clinical trials the overall rate of infections and serious infections in patients treated with Ofatumumab was similar to patients who were treated with teriflunomide (51.6% vs 52.7%, and 2.5% vs 1.8%, respectively). The most common infections reported by Ofatumumab -treated patients in the randomized clinical relapsing MS (RMS) trials included upper respiratory tract infection (39%) and urinary tract infection (10%).

Hepatitis B Virus

Reactivation

There were no reports of HBV reactivation in patients with MS treated with Ofatumumab. However, HBV reactivation, in some cases resulting in fulminant hepatitis, hepatic failure, and death, has occurred in patients being treated with ofatumumab for chronic lymphocytic leukemia (CLL) (at higher intravenous doses than the recommended dose in MS but for a shorter duration of treatment) and in patients treated with other anti-CD20 antibodies.

Infection

Ofatumumab is contraindicated in patients with active hepatitis B disease. Fatal infections caused by HBV in patients who have not been previously infected have occurred in patients being treated with ofatumumab for CLL (at higher intravenous doses than the recommended dose in MS but for a shorter duration of treatment). HBV screening should be performed in all patients before initiation of treatment with Ofatumumab. At a minimum, screening should include Hepatitis B surface antigen (HBsAg) and Hepatitis B Core Antibody (HBcAb) testing. These can be complemented with other appropriate markers as per local guidelines. For patients who are negative for HBsAg and positive for HB core antibody [HBcAb+] or are

carriers of HBV [HBsAg+], consult liver disease experts before starting and during treatment with Ofatumumab. These patients should be monitored and managed following local medical standards to prevent HBV infection or reactivation.

Progressive Multifocal Leukoencephalopathy

Progressive multifocal leukoencephalopathy (PML) is an opportunistic viral infection of the brain caused by the JC virus (JCV) that typically occurs in patients who are immunocompromised, and that usually leads to death or severe disability.

Although no cases of PML have been reported for Ofatumumab in the RMS clinical studies, PML resulting in death has occurred in patients being treated with ofatumumab for CLL (at substantially higher intravenous doses than the recommended dose in MS but for a shorter duration of treatment). In addition, JCV infection resulting in PML has also been observed in patients treated with other anti-CD20 antibodies and other MS therapies. At the first sign or symptom suggestive of PML, withhold Ofatumumab and perform an appropriate diagnostic evaluation. Magnetic resonance imaging (MRI) findings may be apparent before clinical signs or symptoms. Typical symptoms associated with PML are diverse, progress over days to weeks, and include progressive weakness on one side of the body or clumsiness of limbs, disturbance of vision, and changes in thinking, memory, and orientation leading to confusion and personality changes.

Vaccinations: Administer all immunizations according to immunization guidelines at least 4 weeks prior to initiation of Ofatumumab for live or live-attenuated vaccines, and whenever possible, at least 2 weeks prior to initiation of Ofatumumab for inactivated vaccines.

Ofatumumab may interfere with the effectiveness of inactivated vaccines.

The safety of immunization with live or live-attenuated vaccines following Ofatumumab therapy has not been studied. Vaccination with live or live-attenuated vaccines is not recommended during treatment and after discontinuation until B- cell repletion.

Injection-Related Reactions: In large phase 3 clinical trials, systemic and local injection reactions were reported in 21% and 11% of patients treated with Ofatumumab compared to 15% and 6% of patients treated with teriflunomide who received matching placebo injections, respectively.

Injection-related reactions with systemic symptoms observed in clinical studies occurred most commonly within 24 hours of the first injection but were also observed with later injections. Symptoms observed included fever, headache, myalgia, chills, and fatigue, and were predominantly (99.8%) mild to moderate in severity. There were no life-threatening injection reactions in the RMS clinical studies.

Local injection-site reaction symptoms observed in clinical studies included erythema, swelling, itching, and pain.

Only limited benefit of premedication with corticosteroids, antihistamines, or acetaminophen was observed in RMS clinical studies. The first injection of Ofatumumab should be performed under the guidance of an appropriately trained healthcare professional. If injection-related reactions occur, symptomatic treatment is recommended.

Reduction in Immunoglobulins: As expected with any B-cell depleting therapy, decreased immunoglobulin levels were observed. Decrease in immunoglobulin M (IgM) was reported in 7.7% of patients treated with Ofatumumab compared to 3.1% of patients treated with teriflunomide in RMS clinical trials. Treatment was discontinued because of decreased immunoglobulins in 3.4% of patients treated with Ofatumumab and in 0.8% of patients treated with teriflunomide. No decline in immunoglobulin G (IgG) was observed at the end of the study. Monitor the levels of quantitative serum immunoglobulins during treatment, especially in patients with opportunistic or recurrent infections, and after discontinuation of therapy until B-cell repletion. Consider discontinuing Ofatumumab therapy if a patient with low immunoglobulins develops a serious opportunistic infection or recurrent infections, or if prolonged hypogammaglobulinemia requires treatment with intravenous immunoglobulins.

Fetal Risk: Based on animal data, Ofatumumab can cause fetal harm due to B-cell lymphopenia and reduce antibody response in offspring exposed to Ofatumumab in utero. Transient peripheral B-cell depletion and lymphocytopenia have been reported in infants born to mothers exposed to other anti-CD20 B-cell depleting antibodies during pregnancy. Advise females of reproductive potential to use effective contraception while receiving Ofatumumab and for at least 6 months after the last dose

13.2 Benefits

This study endeavors to answer several important questions that may influence our understanding of immune mediated diseases including MS, its pathogenesis in such a manner as to influence how these diseases are treated, whether they can be cured, and whether a preventative vaccine or other preventative strategies can be developed. In this context, the research proposed may offer benefits to the community of people living with and at risk for MS.

13.3 Risk Benefit Assessment

Participation in this study involves risks that are more than minimal. However, the research staff has considerable experience with the conduct of the interventional procedures involved in this study for clinically indicated purposes, and the number of reported adverse events has been few and generally mild in severity. There are theoretical risks of participation that are potentially severe or life threatening, but these have not been encountered in clinical practice. There will no benefits that any individual participant will receive as a consequence of their participation. Despite advances in the treatment of MS, morbidity and mortality associated with the disease are considerable, and individuals almost always require continuous treatment for the remainder of their lives. This study will provide access to a unique body compartment that has never been evaluated in patients with MS that likely harbors a greater proportion of autoreactive cells than blood. In addition, the immune response directed against self-antigens

is likely to be more concentrated in this compartment than blood. Therefore, the general benefit related to an increase in our understanding of MS pathogenesis, with potential implications for new research findings that may be important in developing MS curative or preventative strategies outweighs the risks to individual participants.

14 Informed Consent Process / HIPAA Authorization

All subjects in this study will be provided a consent form describing this study and providing sufficient information for potential subjects to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, will be obtained before that subject undergoes any study procedure. The subject alone must sign the consent form, which will also be signed by the investigator-designated research professional obtaining the consent. No surrogates will be able to provide informed consent on behalf of the potential subject. Subjects will be consented by the study Principal Investigator, or appropriate designee, in a room located within a clinic providing care to patients. Healthy controls will be consented in research space used by the MS Clinical Research Team. Potential subjects will review the consent form in detail with the person designated to consent (either PI or a designee) and will have the ability to take the consent home for further review. The consent form will combine consent for trial participation with HIPAA Authorization in a combined document.

15 Study Finances

15.1 Funding Source

This study will be funded by Novartis through a research collaboration.

51 Conflict of Interest

All University of Pennsylvania Investigators will follow the University of Pennsylvania Policy on Conflicts of Interest Related to Research.

52 Subject Stipends or Payments

All subjects will be compensated by Greenphire ClinCard.

All subjects:
Screening visit: Transportation and parking costs (up to $50)

Thoracic duct cannulation procedure: ‘In-and-out’ catheterization (single time-point sampling): $500 in patient stipends for completion of the study, plus additional transportation and parking costs (up to $50). Patient stipends include:

- $50 after completion of the screening visit
- $100 after completion of the baseline visit
- $300 after the completion of the procedure
- $50 after the completion of visit 4

Thoracic duct cannulation procedure: “Indwelling” catheter with serial sampling: $3000 in patient stipends for completion of serial sampling study, plus additional parking/transportation costs (up to $50). Patient stipends include:

- $50 after completion of the screening visit
- $150 after completion of the baseline visit
- $1500 after the completion of the procedure
- $250 each for visits 3-6
- $300 after the completion of visit 7

16 Publication Plan

Results of the study will be published as a collaboration of University of Pennsylvania investigators and non-Penn investigators who participate in the analysis of specimens. The investigators will decide who will serve as the senior investigator for individual publications based upon the types of analyses that are the subject of publications. Criteria for authorship will follow the guidelines of the International Committee of Medical Journal Editors.

17 Ethical considerations and administrative procedures

17.1 Regulatory and ethical compliance

This clinical study was designed and shall be implemented, executed and reported in accordance with the protocol, with the International Conference on Harmonisation (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations (including European Directive 2001/20/EC, US CFR 21), and with the ethical principles laid down in the Declaration of Helsinki.

17.2 Responsibilities of the investigator and IRB/IEC

Before initiating a trial, the investigator/institution must obtain approval/favorable opinion from the Institutional Review Board/Independent Ethics Committee (IRB/IEC) for the trial protocol, written informed consent form, consent form updates, participant recruitment procedures (e.g. advertisements) and any other written information to be provided to participants.

18 Protocol adherence

This protocol defines the study objectives, the study procedures and the data to be collected on study participants. Additional assessments required to ensure safety of participants should be administered as deemed necessary on a case by case basis. Under no circumstances including incidental collection is an investigator allowed to collect additional data or conduct any additional procedures for any purpose involving any investigational drugs under the protocol, other than the purpose of the study. If despite this interdiction prohibition, data, information,

observation would be incidentally collected, the investigator shall immediately disclose it to the Sponsor and not use it for any purpose other than the study, except for the appropriate monitoring on study participants.

Investigators ascertain they will apply due diligence to avoid protocol deviations. If an investigator feels a protocol deviation would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by the Sponsor and approved by the IRB/IEC, where required, it cannot be implemented.

19 Thoracic Duct Complication Table

Table of Adverse Events Complicating the Thoracic Duct Cannulation Procedure at HUP

Event 1		
Sex: F	Age: 63	Year of service: 2015
History: Chylothorax and chylous ascites after surgery for gynecologic malignancy. Complication: Embolus of lipiodol/glue to left upper lobe. No symptoms. Hospitalized.		
Event 2		
Sex: M	Age: not documented	Year of service: 2009
History: Right chylous pleural effusion after esophagectomy. Complication: Fraction of Trufill glue traveled into left SCV and into lungs causing glue pulmonary embolism. Patient asymptomatic.		
Event 3		
Sex: not documented	Age: 58:	Year of service: 2009
History: S/p heart transplant with chyle leak and bilateral chylous pleural effusions. Complication: Patient removed sutures on feet with leakage of edema fluid.		
Event 4		
Sex: M	Age: 51	Year of service: 2009
History: Recurrent chylothorax Complication: Dislodgement of embolization coil into retroperitoneum. Patient asymptomatic.		
Event 5		
Sex: M	Age: not documented	Year of service: 2009
History: S/p esophagectomy with right chylous pleural effusion Complication: Decline in patient's oxygen saturation and blood pressure at the end of the procedure. Patient sent to emergency department.		
Event 6		
Sex: F	Age: not documented	Year of service: 2009
History: Recurrent chylothorax with failed embolization procedure on two occasions. Complication: Malpositioned embolization coil in retroperitoneum.		

Event 7		
Sex: M	Age: not documented	Year of service: 2012
History: Bilateral chylothorax s/p CABG. Complication: Pneumothorax s/p right thoracentesis.		
Event 8		
Sex: F	Age: not documented	Year of service: 2015
History: Bilateral chylothorax. Complication: Glue embolism into retroperitoneum and pulmonary circulation. Patient asymptomatic.		
Event 9		
Sex: F	Age: not documented	Year of service: 2013
History: Adenocarcinoma of lung, s/p resection with thoracic duct injury and chyle leak. Complications: Sheared tip of cannula in retroperitoneum after embolism coil failed to migrate. Patient asymptomatic.		
Event 10		
Sex: M	Age: not documented	Year of service: 2005
History: S/p gunshot wound to chest with chylothorax. Complication: Embolism of glue to left lower lobe. Patient asymptomatic.		

20 References

References are available upon request

Ahmed, R., Omidian, Z., Giwa, A., Cornwell, B., Majety, N., Bell, D.R., Lee, S., Zhang, H., Michels, A., Desiderio, S., Sadegh-Nasseri, S., Rabb, H., Gritsch, S., Suva, M.L., Cahan, P., Zhou, R., Jie, C., Donner, T., Hamad, A.R.A., 2019. A Public BCR Present in a Unique Dual-Receptor-Expressing Lymphocyte from Type 1 Diabetes Patients Encodes a Potent T Cell Autoantigen. Cell 177, 1583-1599.e16. https://doi.org/10.1016/j.cell.2019.05.007

Buggert, M., Nguyen, S., Salgado-Montes de Oca, G., Bengsch, B., Darko, S., Ransier, A., Roberts, E.R., Del Alcazar, D., Brody, I.B., Vella, L.A., Beura, L., Wijeyesinghe, S., Herati, R.S., Del Rio Estrada, P.M., Ablanedo-Terrazas, Y., Kuri-Cervantes, L., Sada Japp, A., Manne, S., Vartanian, S., Huffman, A., Sandberg, J.K., Gostick, E., Nadolski, G., Silvestri, G., Canaday, D.H., Price, D.A., Petrovas, C., Su, L.F., Vahedi, G., Dori, Y., Frank, I., Itkin, M.G., Wherry, E.J., Deeks, S.G., Naji, A., Reyes-Terán, G., Masopust, D., Douek, D.C., Betts, M.R., 2018. Identification and characterization of HIV-specific resident memory CD8+ T cells in human lymphoid tissue. Sci. Immunol. 3. https://doi.org/10.1126/sciimmunol.aar4526

Da Mesquita, S., Fu, Z., Kipnis, J., 2018. The Meningeal Lymphatic System: A New Player in Neurophysiology. Neuron 100, 375–388. https://doi.org/10.1016/j.neuron.2018.09.022

Itkin, M., Chen, E.H., 2011. Thoracic duct embolization. Semin. Interv. Radiol. 28, 261–266. https://doi.org/10.1055/s-0031-1280676

Li, R., Rezk, A., Miyazaki, Y., Hilgenberg, E., Touil, H., Shen, P., Moore, C.S., Michel, L., Althekair, F., Rajasekharan, S., Gommerman, J.L., Prat, A., Fillatreau, S., Bar-Or, A., Canadian B cells in MS Team, 2015. Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy. Sci. Transl. Med. 7, 310ra166. https://doi.org/10.1126/scitranslmed.aab4176

Lindner, J.M., Cornacchione, V., Sathe, A., Be, C., Srinivas, H., Riquet, E., Leber, X.-C., Hein, A., Wrobel, M.B., Scharenberg, M., Pietzonka, T., Wiesmann, C., Abend, J., Traggiai, E., 2019. Human Memory B Cells Harbor Diverse Cross-Neutralizing Antibodies against BK and JC Polyomaviruses. Immunity 50, 668-676.e5. https://doi.org/10.1016/j.immuni.2019.02.003

Nadolski, G.J., Itkin, M., 2012. Feasibility of ultrasound-guided intranodal lymphangiogram for thoracic duct embolization. J. Vasc. Interv. Radiol. JVIR 23, 613–616. https://doi.org/10.1016/j.jvir.2012.01.078

Polman, C.H., Reingold, S.C., Banwell, B., Clanet, M., Cohen, J.A., Filippi, M., Fujihara, K., Havrdova, E., Hutchinson, M., Kappos, L., Lublin, F.D., Montalban, X., O'Connor, P., Sandberg-Wollheim, M., Thompson, A.J., Waubant, E., Weinshenker, B., Wolinsky, J.S., 2011. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann. Neurol. 69, 292–302. https://doi.org/10.1002/ana.22366

Thompson, A.J., Banwell, B.L., Barkhof, F., Carroll, W.M., Coetzee, T., Comi, G., Correale, J., Fazekas, F., Filippi, M., Freedman, M.S., Fujihara, K., Galetta, S.L., Hartung, H.P., Kappos, L., Lublin, F.D., Marrie, R.A., Miller, A.E., Miller, D.H., Montalban, X., Mowry, E.M., Sorensen, P.S., Tintoré, M., Traboulsee, A.L., Trojano, M., Uitdehaag, B.M.J., Vukusic, S., Waubant, E., Weinshenker, B.G., Reingold, S.C., Cohen, J.A., 2018. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 17, 162–173. https://doi.org/10.1016/S1474-4422(17)30470-2

(1990) EuroQol--a new facility for the measurement of health-related quality of life. Health Policy p. 199-208.

(2010) United States Food and Drug Administration FDA Approves First Oral Drug to Reduce MS Relapses.

Bar-Or A, Grove RA, Austin DJ, et al (2018) Subcutaneous ofatumumab in patients with relapsing-remitting multiple sclerosis: The MIRROR study. Neurology p. e1805-e1814.

Barro C, Benkert P, Disanto G, et al (2018) Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain.

Buggert M, Vella LA, Nguyen S, Wu VH, Chen Z, Sekine T, Perez-Potti A, Maldini CR, Manne S, Darko S, Ransier A, Kuri-Cervantes L, Japp AS, Brody IB, Ivarsson MA, Gorin JB, Rivera-Ballesteros O, Hertwig L, Antel JP, Johnson ME, Okoye A, Picker L, Vahedi G, Sparrelid E, Llewellyn-Lacey S, Gostick E, Sandberg JK, Björkström N, Bar-Or A, Dori Y, Naji A, Canaday DH, Laufer TM, Wells AD, Price DA, Frank I, Douek DC, Wherry EJ, Itkin MG, Betts MR. The Identity of Human Tissue-Emigrant CD8⁺ T Cells. Cell. 2020 Dec 23;183(7):1946-1961.e15. PMID: 33306960.

Byrne E (2003) Randomized, comparative study of interferon beta-1a treatment regimens in MS: the EVIDENCE trial. Neurology p. 1872; author reply 1872-3.

Disanto G, Barro C, Benkert P, et al (2017) Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann. Neurol. p. 857-870.

Epstein LG, Prineas JW, Raine CS (1983) Attachment of myelin to coated pits on macrophages in experimental allergic encephalomyelitis. J. Neurol. Sci. p. 341-8.

Eriksson I, Cars T, Piehl F, et al (2018) Persistence with dimethyl fumarate in relapsing-remitting multiple sclerosis: a population-based cohort study. Eur. J. Clin. Pharmacol. p. 219-226.

Genain CP, Hauser SL (1996) Allergic Encephalomyelitis in Common Marmosets: Pathogenesis of a Multiple Sclerosis-like Lesion Methods p. 420-34.

Hauser SL, Bar-Or A, Comi G, et al (2017) Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis. N. Engl. J. Med. p. 221-234.

Hauser SL, Comi GC, Hartung HP et al. (2015) Efficacy and safety of ocrelizumab in relapsing multiple sclerosis - results of the interferon-beta-1a-controlled, double-blind, Phase III OPERA I and II studies.

Hobart J, Cano S (2009) Improving the evaluation of therapeutic interventions in multiple sclerosis: the role of new psychometric methods. Health Technol Assess p. iii, ix-x, 1-177.

Hobart J, Lamping D, Fitzpatrick R, et al (2001) The Multiple Sclerosis Impact Scale (MSIS-29): a new patient-based outcome measure. Brain p. 962-73.

Kappos L, De Stefano N, Freedman MS, et al (2016) Inclusion of brain volume loss in a revised measure of 'no evidence of disease activity' (NEDA-4) in relapsing-remitting multiple sclerosis. Mult. Scler. p. 1297-305.

Kappos L, Li D, Calabresi PA, et al (2011) Ocrelizumab in relapsing-remitting multiple sclerosis: a phase 2, randomised, placebo-controlled, multicentre trial. Lancet p. 1779-87.

Kuhle J, Barro C, Disanto G, et al (2016) Serum neurofilament light chain in early relapsing remitting MS is increased and correlates with CSF levels and with MRI measures of disease severity. Mult. Scler. p. 1550-1559.

Li R, Rezk A, Miyazaki Y, et al (2015) Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy. Sci Transl Med. 21;7(310):310

Lu G, Beadnall H, Wang C et al (2017) The prognostic utility of no evidence of disease activity (neda).

Lublin FD, Reingold SC, Cohen JA, et al (2014) Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology p. 278-86.

Lucchinetti CF, Popescu BF, Bunyan RF, et al (2011) Inflammatory cortical demyelination in early multiple sclerosis. N. Engl. J. Med. p. 2188-97.

Magliozzi R, Howell O, Vora A, et al (2007) Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain p. 1089-104.

McDonald WI, Compston A, Edan G, et al (2001) Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann. Neurol. p. 121-7.

Multiple sclerosis disease modifying drugs first line treatments.

NEDA-4 may predict MS outcomes better than NEDA-3.

Piehl F, Kockum I, Khademi M, et al (2018) Plasma neurofilament light chain levels in patients with MS switching from injectable therapies to fingolimod. Mult. Scler. p. 1046-1054.

Polman CH, Reingold SC, Banwell B, et al (2011) Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann. Neurol. p. 292-302.

Ray A, Mann MK, Basu S, et al (2011) A case for regulatory B cells in controlling the severity of autoimmune-mediated inflammation in experimental autoimmune encephalomyelitis and multiple sclerosis. J. Neuroimmunol. p. 1-9.

Reilly MC, Zbrozek AS, Dukes EM (1993) The validity and reproducibility of a work productivity and activity impairment instrument. Pharmacoeconomics p. 353-65.

Schmid, K.T., Höllbacher, B., Cruceanu, C. et al. scPower accelerates and optimizes the design of multi-sample single cell transcriptomic studies. Nat Commun 12, 6625 (2021). https://doi.org/10.1038/s41467-021-26779-7

Siller N, Kuhle J, Muthuraman M, et al (2018) Serum neurofilament light chain is a biomarker of acute and chronic neuronal damage in early multiple sclerosis. Mult. Scler. p. 1352458518765666.

Sorensen PS, Lisby S, Grove R, et al (2014) Safety and efficacy of ofatumumab in relapsing-remitting multiple sclerosis: a phase 2 study. Neurology p. 573-81.

Sormani MP, Bonzano L, Roccatagliata L, et al (2009) Magnetic resonance imaging as a potential surrogate for relapses in multiple sclerosis: a meta-analytic approach. Ann. Neurol. p. 268-75.

Sormani MP, Bruzzi P (2013) MRI lesions as a surrogate for relapses in multiple sclerosis: a meta-analysis of randomised trials. Lancet Neurol p. 669-76.

Teeling JL, Mackus WJ, Wiegman LJ, et al (2006) The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J. Immunol. p. 362-71.

Vella LA, Buggert M, Manne S, Herati RS, Sayin I, Kuri-Cervantes L, Bukh Brody I, O'Boyle KC, Kaprielian H, Giles JR, Nguyen S, Muselman A, Antel JP, Bar-Or A, Johnson ME, Canaday DH, Naji A, Ganusov VV, Laufer TM, Wells AD, Dori Y, Itkin MG, Betts MR, Wherry EJ. T follicular helper cells in human efferent lymph retain lymphoid characteristics. J Clin Invest. 2019 Jul 2;129(8):3185-3200. PMID: 31264971.

Voillet V, Buggert M, Slichter CK, Berkson JD, Mair F, Addison MM, Dori Y, Nadolski G, Itkin MG, Gottardo R, Betts MR, Prlic M. Human MAIT cells exit peripheral tissues and recirculate via lymph in steady state conditions. JCI Insight. 2018 Apr 5;3(7):e98487. PMID: 29618662.

Wingerchuk DM, Banwell B, Bennett JL, et al (2015) International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology p. 177-89. www.medscape.com/viewarticle/903910.