NCT05482867

Exploratory Open-Label Study for the Development of a Method to Detect Dendritic Cell Recruitment in Alzheimer's Disease (DC RAD)

EXPLORATORY OPEN-LABEL STUDY FOR THE DEVELOPMENT OF A METHOD TO DETECT DENDRITIC CELL RECRUITMENT IN ALZHEIMER'S DISEASE (DC RAD)

PROTOCOL NUMBER:	ICG-001
STUDY PHASE:	Phase 0 Proof of Concept
PROTOCOL VERSION:	6.0
ORIGINAL PROTOCOL DATE:	13 June 2023
SPONSORED BY:	MindImmune Therapeutics, Inc. 130 Flagg Rd Kingston, RI 02881 Phone: (860) 271-9706

PROTOCOL APPROVAL

PROTOCOL TITLE:

EXPLORATORY OPEN-LABEL STUDY FOR THE DEVELOPMENT OF A METHOD TO DETECT DENDRITIC CELL RECRUITMENT IN ALZHEIMER'S DISEASE (DC RAD)

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PROTOCOL DATE: 13 June 2023

SPONSOR: MindImmune Therapeutics, Inc.
130 Flagg Rd
Kingston, RI 02881
Phone: (860)271-9706

STUDY PRODUCT: Indocyanine Green (ICG)

Sponsor Approval:

Date: 02 April 2024

Signature: Frank S. Menniti, Ph.D. *Electronically signed by: Frank S. Menniti, Ph.D. Reason: I approve this document. Date: Apr 2, 2024 16:00 EDT*

Frank S. Menniti, Ph.D.
Chief Science Officer

1 PROCEDURES IN CASE OF EMERGENCY

Table 1 Sponsor/CRO Contact Information

Role in Study	Name	Address and Telephone Number
Clinical Study Leader	Thomas J. Hochadel, Pharm.D. President	Cognitive Research Corporation (CRC) 200 Central Ave, Suite 1200 Saint Petersburg, FL 33701 Telephone: 727-897-9000 Cell: 727-515-2147 tomhochadel@cogres.com
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2 INVESTIGATOR AGREEMENT

EXPLORATORY OPEN-LABEL STUDY FOR THE DEVELOPMENT OF A METHOD TO DETECT DENDRITIC CELL RECRUITMENT IN ALZHEIMER'S DISEASE (DC RAD)

PROTOCOL NUMBER: ICG-001

I have read the protocol and agree that it, along with the related Clinical Trial Agreement, contains all the details necessary to carry out the study. I will conduct this study according to the protocol and will complete the study in the time agreed. Potential additions or modifications to the study will be by mutual written agreement between MindImmune Therapeutics, Inc. and me and will be documented and filed, if required, with the Institutional Review Board and the United States Food and Drug Administration.

I will provide copies of the protocol and other pertinent information to all individuals responsible for assisting me in the study.

MindImmune Therapeutics, Inc., Cognitive Research Corporation, and their designees will have access to source documentation from which case reports have been generated.

Investigator

Signature: _____ Date: _____

Investigator

Name (print): _____

3 SYNOPSIS

Name of sponsor/company: MindImmune Therapeutics, Inc.

Name of investigational product: indocyanine green (ICG)

Protocol number: ICG-001

Title of study: EXPLORATORY OPEN-LABEL STUDY FOR THE DEVELOPMENT OF A METHOD TO DETECT DENDRITIC CELL RECRUITMENT IN ALZHEIMER'S DISEASE (DC RAD)

Estimated number of study center(s): The study will be conducted at one site.

Phase of development: P0

Objectives:

Biomarker Proof of Concept Part 1

- Characterize indocyanine green (ICG) labeling of peripheral immune phagocytic cells in healthy adult subjects.

Biomarker Proof of Concept Part 2

- Determine the presence of ICG in the brain of adult subjects diagnosed with Alzheimer's disease (AD).

Safety and Tolerability

- Examine the safety and tolerability of ICG infusion in normal healthy adult volunteers, normal healthy elderly volunteers, and in AD subjects.

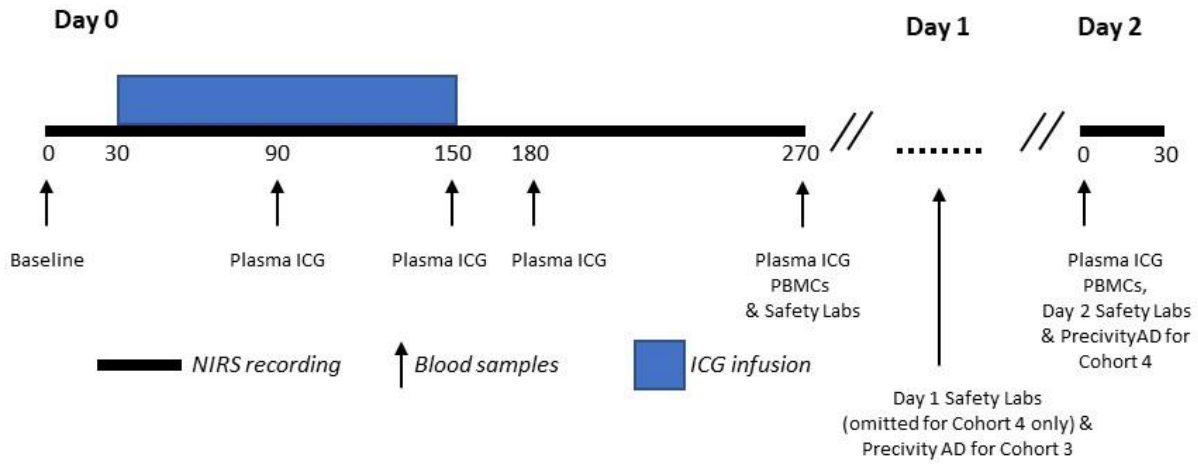
Study Design:

This is a proof-of-concept study designed to confirm that human phagocytic cells can be labeled with the near-infrared dye indocyanine green (ICG) and the presence of the labeled cells 48 hours later in cerebral cortex can be inferred using near infrared spectroscopy (NIRS).

To develop a method to detect dendritic cell recruitment in Alzheimer's disease (DC RAD), this study is designed in 2 parts. The first part assesses the safety and efficacy of indocyanine green (ICG) in labeling peripheral immune phagocytic cells in healthy adult subjects. The second part is designed to determine the presence of ICG in the brain of adult subjects diagnosed with Alzheimer's disease (AD).

In the first part of the study, ICG will be delivered by intravenous infusion to healthy subjects to verify that peripheral immune phagocytic cells, of which DCs are a subset, can be labeled with ICG. If ICG labeling of phagocytic cells is confirmed, then in the second part of the study the presence of ICG in brain of AD patients, putatively carried in by ICG-labeled cells, will be investigated by NIRS using the INVOS 7100 cerebral oximeter.

For all subjects, the study will follow timeline shown:



On Day 0, NIRS recording will commence and continue throughout a 270-minute session. Following baseline NIRS recording from 30 minutes pre-infusion to minute 0, an intravenous infusion of ICG will commence and be maintained until minute 120 post-infusion (120-minute infusion duration). After cessation of the infusion, NIRS recording will continue until minute 240 post-infusion. Blood samples will be drawn at 30 minutes pre-infusion and at minutes 60, 120, 150, and 240 (relative to the start of the ICG infusion). All blood samples will be assayed for plasma ICG concentration.

Peripheral blood mononuclear cells (PBMCs) will be isolated from the blood sample drawn on Day 0 at minute 240 (relative to the start of the ICG infusion) and on Day 2 (48 hours after the start of the ICG infusion). The percentage of cells labeled with ICG determined by flow cytometry or other appropriate method.

For healthy elderly (Cohort 3) a plasma sample obtained on Day 1 and for AD patients (Cohort 4) a plasma sample obtained on Day 2 will be assayed for biomarkers predictive of brain amyloid deposition using the PrecivityAD™ test.

Approximately 48 hours after ICG infusion (Day 2); a blood sample will be drawn at minute 0 (48 hours from start of ICG infusion) and an NIRS recording will be made for 30 minutes. Subjects will be discharged at the end of the 30-minute recording. The Day 1 blood and urine samples collected for all subjects except Cohort 4 will be used for safety labs. The Day 2 blood and urine samples collected for all subjects will be used for safety labs, for assay for plasma ICG level, and for isolation of PBMC for determination of the percentage of cells labeled with ICG.

Cohort 1, healthy adults (n = 5), will receive an ICG infusion of 1 mg/min using sterile water as the infusion vehicle. If no dose limiting adverse effects are observed in Cohort 1, then Cohort 2, healthy adults (n = 10), will receive an ICG infusion of 2 mg/min using sterile water as the infusion vehicle. If there are no dose limiting adverse events and there is evidence of ICG-labeling of PBMCs, the 2 mg/min infusion rate will be used for the remainder of the study.

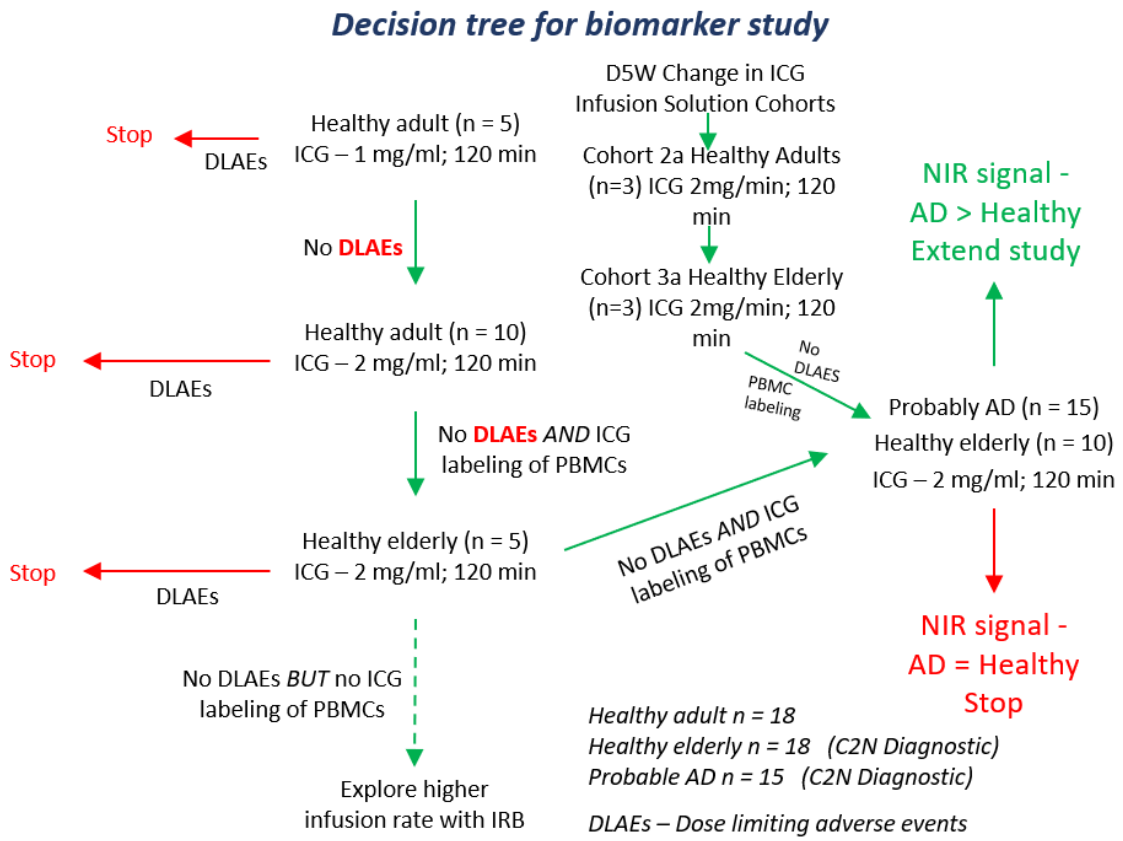
Cohort 3, healthy elderly adults, will receive an ICG infusion of 2 mg/min using sterile water as the infusion vehicle, and a sample will be taken on Day 1 for the PrecivityAD™ biomarker test. The first 5 subjects will serve as a satellite group. If no dose limiting adverse effects are observed in the satellite group, then a further 10 healthy elderly adults will receive a 2 mg/min ICG infusion using sterile water as the infusion vehicle (n = 15 total for Cohort 3).

Two additional Cohorts (Cohorts 2a and 3a) will receive IV infusions of ICG using dextrose 5% in water (D5W) as the infusion vehicle to evaluate the safety of D5W as an infusion vehicle and assess

the comparability of results on ICG exposure, NIRS signals and % labeling of PBMC using sterile water vs. D5W as the vehicle. Three (3) additional healthy adults (Cohort 2a) and three (3) additional healthy elderly adults (Cohort 3a) will be infused with ICG in D5W at the rate of 2 mg/min for 120 minutes. If no dose limiting adverse events are observed in Cohorts 2a or 3a and data on all measures is comparable to that collected in previous cohorts, D5W will be adopted as the IV infusion vehicle for Cohort 4 dosing.

For Cohort 4, in recognition of the unique limitations and needs of the AD subjects and their caregivers, the requirement for remaining in the clinic throughout the entire protocol will be eliminated. Cohort 4 subjects will receive an ICG infusion of 2 mg/min (using D5W as the infusion vehicle if the criteria described above are met); the Day 0 safety assessments, NIRS recording, and blood sampling will be identical to that used with prior cohorts. At the end of Day 0 testing, Cohort 4 subjects will be discharged and return after 48 hours for completion of the Day 2 assessments. The blood collection and sample processing for the PrecivityAD test for Cohort 4 will be conducted on Day 2. Waiving the Day 1 safety assessments for Cohort 4 is supported by the fact that, per the study decision tree (see below), Cohort 4 will only proceed if no dose limiting adverse effects are observed in previous cohorts. The first 5 subjects will serve as a satellite group. If no dose limiting adverse effects are observed, then a further 10 AD patients will be enrolled (n = 15 total for Cohort 4).

The study decision tree is shown.



Baseline data will be collected to include urine analysis, urine drug screen, urine pregnancy, clinical laboratory tests, vital signs, physical examination finding, and ECG. Any abnormal vital sign measurement, clinical laboratory test, physical examination finding, or ECG parameter deemed clinically significant by the investigator will be repeated, including test results obtained on the final study day or upon early termination. For any test abnormality deemed clinically significant, repeat analysis will be performed during the follow-up period and until the value returns to baseline (or within normal limits) or the investigator deems the abnormality to be stable and no longer of clinical concern.

Number of Subjects Planned: 51 total subjects

Diagnosis and Main Criteria for Eligibility:
Subjects must fulfill all inclusion criteria and meet none of the exclusion criteria to be eligible for participation in the study:

Inclusion Criteria

1.All Healthy Subjects

- Subjects are determined by the investigator to be medically stable and expected to complete the trial as designed
- Subjects have adequate hearing, vision, and language skills to perform neuropsychiatric testing and interviews as specified in the protocol
- Subjects are able to understand and agree to comply with the study procedures and report for scheduled office visits

- Subjects are able to reliably communicate with study personnel about adverse events (AEs) and concomitant medications
- Signed written informed consent according to institutional guidelines

2. Healthy Adult subjects
   - Male or female and between the ages of 21 to 55 inclusive

3 Healthy Elderly subjects
   - Male or female and between the ages of 65 to 90 inclusive
   - MMSE score > 26

4. Alzheimer's patients
   - Male or female and between the ages of 50 to 90 inclusive
   - Patients satisfying the criteria for the clinical diagnosis of probable AD based on National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) OR 6 months documented cognitive decline by physician OR Amyloid or Tau pathology confirmed by PET scan with ligand.
   - MMSE score between 16 and 25
   - Modified Hachinski Ischemia Scale (MHIS) score of ≤ 4

Exclusion Criteria

1. Sex and Reproductive Status
   - Women who test positive for pregnancy
   - Pre-menopausal women who are not practicing two methods of birth control for 3 months prior and a week after the ICG infusion

2. Medical History
   - Subjects with a history of any anaphylactic reactions
   - Subjects with a history of allergic reaction to ICG
   - Subjects with a history of iodine sensitivity and/or allergic reaction to iodine
   - Subjects with a history of a clinically significant hepatic disease

3. Target Disease Exceptions
   - Any subject diagnosed to have an autoimmune disorder, including
     - Psoriasis
     - Lupus
     - Rheumatoid arthritis
     - Crohn's disease
     - multiple sclerosis
     - alopecia areata
   - Any subject who has any unstable cardiovascular (included uncontrolled hypertension), pulmonary, or GI disease
   - For Alzheimer's patients, a medical condition other than AD that could explain or contribute significantly to the patient's dementia: e.g., Down Syndrome, abnormal thyroid function, posttraumatic physical conditions, syphilis, Parkinson's disease, multi-infarct dementia, Huntington's disease, normal pressure hydrocephalus, central nervous system (CNS) tumor, frontotemporal dementia, seizure disorder (other than childhood febrile seizures), and subdural hematoma

4. Concurrent Medications

- Any subject who is immunocompromised at screening including taking medications that are systemic immunosuppressives including corticosteroids but not NSAIDS
- Any subject currently prescribed a biologic immunosuppressive therapy or having taken such therapy in the prior 3 months.

5. Physical and Laboratory Test Findings at Screening

- Any subject with uncontrolled hypertension at screening (e.g., repeated diastolic measurements ≥ 96 mmHg).
- Any subject with either of the following hepatic test abnormalities at screening:
  - Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) > 2.0 times the institutional ULN
  - Total Bilirubin > 2 times the institutional ULN
- Any subject with P-Amylase or Lipase values > 2 times the ULN at screening
- Any subject at screening with insulin-dependent diabetes mellitus or HbA1C > 7.5%
- Any subject at screening with pathologic renal findings as defined by the presence of Calculated glomerular filtration rate (GFR) (creatinine clearance) < 30 ml/min/1.73m2 (Cockcroft-Gault formula for GFR estimate)
- Any subject at screening with any of the following hematologic abnormalities:
  - Hemoglobin < 10g/dL
  - WBC < 3.0 x 103/mm3
  - Platelet count < 100,000/mm3
- Any subject who has a known infection with a human immunodeficiency virus.
- For Cohort 4: Any subject who has abnormal results for thyroid-stimulating hormone, folate, or Vitamin B12 at Screening that indicate a possible alternative diagnosis that may cause cognitive decline.

Test Product, Dose, and Mode of Administration:

This study is designed to evaluate the use of a specific near infrared dye (ICG) to label peripheral immune phagocytic cells. ICG, as a solution in sterile water or D5W, will be administered by IV infusion at 1 mg or 2 mg per minute using an infusion pump.

Reference therapy, dosage and mode of Administration:

Not applicable

Duration of Treatment:

Infusion will commence over 120 minutes on Day 0 with a +/- 5 minute window.

Criteria for Evaluation:

Pharmacodynamic assessment:

1) Determine the preliminary pharmacodynamic assessment of intravenous infusion of ICG to ICG-label peripheral immune phagocytic cells as measured ex vivo in PBMCs isolated from blood samples. Peripheral blood mononuclear cells (PBMCs) will be isolated from the minute 240 blood sample and the percentage of cells labeled with ICG determined by flow cytometry or other appropriate method.

2) Determine the persistence of ICG-labeling of peripheral immune cells after 48 hours. On Day 2 at time 0 (48 h after the start of ICG infusion on Day 0), blood will be drawn and an NIRS recording will be made for 30 minutes. The blood sample will be assayed for plasma ICG, and peripheral blood mononuclear cells (PBMCs) will be isolated from the sample and the percentage of cells labeled with ICG determined by appropriate analytical methods.

Safety and tolerability assessments:

All observed and volunteered AEs will be recorded. Vital signs including systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, will be measured at screening, Day 0 immediately before the start of the NIRs recording, immediately after the start of the ICG infusion and at 30min, 90min, 120min (relative to the start of the ICG infusion), during the NIRs recording after the stop of the ICG infusion at 150min, 210min, and 240min (relative to the start of the ICG infusion), and on Day 2 before discharge. ECG will be collected within 30 minutes pre-infusion and within 30 minutes of the end of the ICG infusion, each within 30 minutes allowing a +5 minute window.

On Day 1 (omitted for Cohort 4 only) Safety Assessments to include:

- Urine analysis, Hematology and Serum Chemistry

On Day 2 (all cohorts) Safety Assessments to include:

- ECG obtained before discharge
- Urine analysis, Hematology and Serum Chemistry

Additional Assessments:

- Demographic Data (Screening)
- Medical History (Screening)
- Prior and Concomitant Medication (Screening; concomitant medication only additionally reviewed/recorded on Days 0-2 and during Day 7-16 telephone visit)
- Physical Examination (Screening and Day 2 prior to discharge)
- Suicidality (Screening and Day 2 prior to discharge)
- Pregnancy (Screening)

Statistical Analysis

General

Safety and tolerability of ICG will be determined by AEs. Data will be summarized by cohort using descriptive statistics (number of subjects, mean, median, standard deviation, minimum, and maximum) for continuous variables and summarized by cohort using frequencies and percentages for categorical variables.

Pharmacodynamic Assessments

Determination of the percent of PBMCs labeled with ICG as measured by appropriate analytical methods.

Determination if there is a NIRS signal in cortex that is higher than the baseline signal recorded on Day 0. The distribution of those elevated signals will be examined across the study population.

Safety Analyses

Safety data analyses will be conducted on all subjects who have started at least 1 infusion of ICG. The number and percentage of subjects experiencing 1 or more AEs will be summarized by infusion rate, relationship to ICG, and severity. AEs will be coded using Medical Dictionary for Regulatory Activities (MedDRA) terminology. Listings of subjects who withdraw from the study due to an AE, serious AEs and/or death or lack of treatment effect will be presented. Laboratory parameters will be summarized for each cohort using descriptive statistics and data listings of clinically significant abnormalities. Vital signs and ECG data will be summarized by changes from baseline values using descriptive statistics.

Sample Size Determination

Due to the exploratory nature of this study, no formal sample size can be determined.

Table 2 Schedule of Visits and Assessments

Visit	Screening[1]	Dosing and Measurements[2]	24 hours[2]	48 hours and Early Termination[2]	Telephone Follow-up
Day	-28 to -1	0	1	2 and ET	Day 7-16
Informed Consent[1]	X				
Inclusion/Exclusion Criteria[3]	X	X			
Demographics	X				
Medical and Psychiatric History	X				
Concomitant Medications	X	X	X	X	X
Suicide Screening	X			X	
12 – Lead ECG[4]	X	X		X	
Physical Exam[5,6]	X			X	
Safety labs[7]	X	X	X	X	
Urine Pregnancy testing	X				
Vital Signs Measurements[8, 9]	X	X		X	
HIV Testing	X				
AE Monitoring	X	X	X	X	X
Urine Toxicology/BAL[10]	X	X			
Urinalysis[11]	X	X	X	X	
PrecivityAD™ test [12]			X [19]	X [20]	
MMSE [13]	X				
MHIS [14]	X				
Administration of ICG by Infusion[15]		X			
Scan by near infrared spectroscopy (NIRS) [16]		X		X	
Analytical measurement of labeled cells (PBMC) [17]		X		X	
ICG Concentration Levels [18]		X		X	

Notes:

1. All procedures must be completed after signing informed consent.
2. All timed measurements and procedures on Day 0 and Day 1 (if applicable) are to be completed within a ± 5-minute window; vital signs will be completed within a +/- 7 minute window. Day 2 windows will be +/- 2hrs.
   Overnight stays for Cohort 4 AD subjects will not be required; Day 1 procedures will not be performed for Cohort 4.
3. Inclusion/Exclusion criteria evaluated at Screening and Day 0.
4. ECG will be performed at Screening, Day 0 within 30 minutes pre-infusion and within 30 minutes of the end of the ICG infusion allowing a +5 minute window and on Day 2 after completion of the 30 minute NIRs recording prior to discharge.
5. The Physical Exam will be performed at Screening and on Day 2 prior to discharge.
6. Height to be collected at Screening only. Weight to be collected at Screening only.
7. On Day 0, after dosing and completion of NIRs, on Day 1 (except for Cohort 4), and on Day 2 Hematology, and Serum Chemistry including fractionated bilirubin are to be collected.
8. Vital sign measurements will include measurement of oxygen saturation. Oxygen saturation will be collected at screening and prior to the NIRs recording Day 0 only. Resting vital signs (SBP, DBP and HR) will be taken upon having the subject sitting/semi-recumbent or supine/recumbent position for 5 min at Screening, on Day 0 immediately prior to the start of the NIRs recording, and immediately after the start of the ICG infusion, and at 30min, 90min, 120min, 150min, 210min, and 240min after the start of infusion, and prior to discharge on Day 2.
9. Orthostatic measurement of systolic, diastolic blood pressure and heart rate will be measured after the subject has been standing for a total of 5 minutes. On Day 0, the orthostatic measurements will be taken prior to the start of the NIRs recording, at 30 minutes after the start of the ICG infusion and 240 minutes after the start of ICG infusion. If the first measurement of any vital signs (SBP, DBP and pulse) shows the following, vital signs will be measured again in triplicate (same arm, separated by at least 1 minute) for SBP <90 mmHg, DBP <60 mmHg, and pulse <60 bpm. No upper limit will be set as the investigator or designee may repeat any vital sign at their discretion.

10. Urine drug testing will include opioids (fentanyl), buprenorphine, methadone, benzodiazepines, cocaine (benzoylecgonine), amphetamines, and other drugs. Breath alcohol levels (BAL) will be assessed at Screening and Day 0 prior to start of NIRs recording.
11. At Screening, Day 0, after dosing and completion of NIRs, Day 1 (if applicable) and on Day 2 a Urinalysis is to be collected.
12. PrecivityAD™ test to be drawn from Cohorts 3 and 4 only. Precivity AD™ for Cohort 3 will be drawn anytime on Day 1; Precivity AD™ for Cohort 4 will be drawn on Day 2 and will be given priority over the other assessments on Day 2.
13. Mini Mental State Examination (MMSE) to be completed only for Cohorts 3 and 4.
14. Modified Hachinski Ischemia Scale (MHIS) to be completed only for Cohort 4
15. Administration of ICG will be done on Day 0 after all pre-dose procedures, 30mins relative to the start of the NIRs recording.
16. Day 0 all timed measurements and procedures are relative to the start of the ICG infusion. On Day 2, the 30min NIRs recording begins 48hrs after the start of the ICG infusion on Day 0.
17. Analytical measurement of labeled cells (PBMC) are measured on Day 0 at 240 minutes (relative to the start of ICG infusion)) and on Day 2 (48 hrs. after the start of the ICG infusion).
18. ICG levels will be drawn on Day 0 (relative to the start of ICG infusion) at 30 minutes pre-infusion and at minutes 60, 120, 150, and 240 post-infusion and on Day 2 (48hrs after the start of the ICG infusion).
19. Cohort 3 only.
20. Cohort 4 only.

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5 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AD	Alzheimer's Disease
AE	Adverse event
BAL	Beath alcohol level
CR3	Complement receptor 3
CRF	Case Report Form
CRO	Contract Research Organization
DBP	Diastolic blood pressure
DC	Innate immune dendritic cells
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	Electrocardiogram
GCP	Good Clinical Practices
ICH	International Conference on Harmonization
ICF	Informed Consent Form
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Regulatory Activities
MHIS	Modified Hachinski Ischemia Scale
µg	microgram
mg	milligram
ml	milliliter
mm	millimeters
mmHg	millimeters of mercury
MMSE	Mini Mental State Examination
NIRS	near infrared spectroscopy
PBMC	peripheral blood mononuclear cells
PP	per protocol
SAE	serious adverse event
SAP	Statistical Analysis Plan
SBP	systolic blood pressure
TEAE	treatment-emergent adverse event
TSH	thyroid-stimulating hormone

6 INTRODUCTION

6.1 Background and Rationale

Data indicate that neuroinflammation is a principal effector of neurodegeneration in Alzheimer's disease (AD) [1, 2]. MindImmune has identified a key neuroinflammatory mechanism that is a possible target for therapeutic intervention. In AD, neuroinflammation is damaging and appears to be driven by chronic activation of elements of the complement system [3-6]. Stevens and colleagues [7, 8] have proposed that the synaptic attack at the core of AD pathology is signaled by complement receptor 3 (CR3), which results in an aberrant reactivation of a developmental synaptic pruning mechanism [9]. It has been known for decades that a unique innate immune cell is closely associated with the dense core plaques seen in post-mortem cortical samples from AD patients [10, 11]. Recently, expression profiling of these cells indicate that they have a phagocytic phenotype [12]. The localization to areas of synaptic damage, expression of CR3, and the phagocytic profile recommend these cells as prime candidates as the effectors of the complement-signaled destruction of synapses in AD [7, 13]. Thus, these cells are the focus of intensive investigation as potential points of intervention in the development of novel AD therapeutics [14].

MindImmune has new evidence indicating the pathology-associated cells in the AD brain are recruited from the periphery and are of a phenotype consistent with innate immune dendritic cells (DCs). Blocking recruitment of DCs into brain eliminates the CR3-expressing innate immune cells from the vicinity of plaques. Eliminating the presence of these deleterious effector cells lessens the synaptic pathology. Given that this synaptic pathology is at the core of AD symptoms and disease progression, MindImmune is pursuing DC recruitment blockade as a novel therapeutic approach to AD.

6.2 Translational biomarker assay to track DC recruitment.

One of the techniques adopted by MindImmune to measure DC recruitment into brain in an AD mouse model involves labeling DCs in the periphery by systemic administration of the near infrared dye indocyanine green (ICG) and then detecting the presence of the labeled cells in brain 24 to 72 h later by near infrared spectroscopy (NIRS). ICG is approved by regulatory agencies and used extensively in humans for over 60 years and is considered very safe. In this study it is proposed to translate this preclinical methodology to humans. ICG will be delivered using precedented intravenous infusion protocols [39-42] to label DCs in the periphery. Recruitment of ICG-labeled DCs into brain will be measured using the portable NIRS technology standardly used clinically to measure cerebral oxygenation with the INVOS 7100 device.

Development of this assay of DC recruitment is preliminary to its use in early clinical studies to demonstrate proof-of-mechanism for a MindImmune DC recruitment inhibitor. DC recruitment measured by this assay could also be used as a more general biomarker of ongoing neuroinflammation in AD and in other neuroinflammatory conditions.

7 OBJECTIVES

The primary objectives of the current study are:

Biomarker Proof of Concept Part 1

- Characterize indocyanine green (ICG) labeling of peripheral immune phagocytic cells in healthy adult subjects.

Biomarker Proof of Concept Part 2

- Determine the presence of ICG-labeled cells in the brain of adult subjects diagnosed with Alzheimer's disease (AD).

Safety and Tolerability

- Examine the safety and tolerability of ICG infusion in normal healthy adult volunteers, normal healthy elderly volunteers, and in AD subjects.

8 STUDY DESIGN

8.1 Overall Study Design and Plan

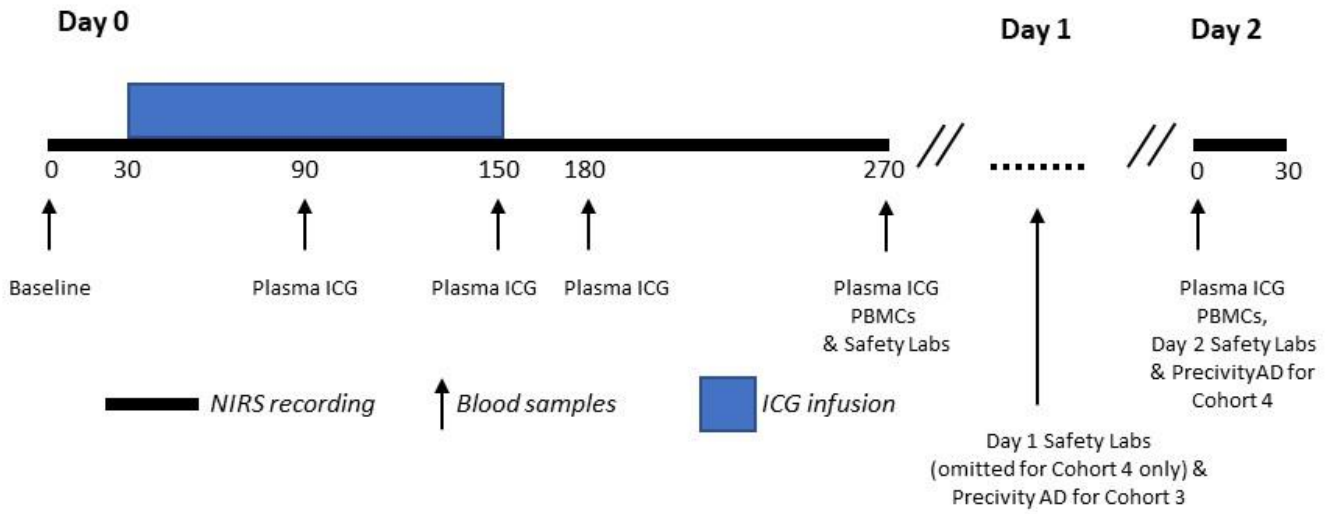
This proof-of-concept study is designed to determine if human phagocytic cells can be labeled with the near-infrared dye indocyanine green (ICG) in the periphery and the presence of ICG-labeled cells can then be detected 48 h later in the cerebral cortex by near infrared spectroscopy (NIRS).

To develop a method to detect dendritic cell recruitment in Alzheimer's disease (DC RAD), this study is designed in 2 parts. The first part is to characterize the efficacy of intravenous infusion of indocyanine green (ICG) for labeling of peripheral immune phagocytic cells in healthy adult subjects. The second part is designed to determine the presence of ICG-labeled cells in the brain of adult subjects diagnosed with Alzheimer's disease (AD).

In the first part of the study, ICG will be delivered by intravenous infusion to healthy subjects to verify that peripheral immune phagocytic cells, of which DCs are a subset, can be labeled with ICG. Labeling will be confirmed by analysis of the PBMC fraction. If ICG labeling of phagocytic cells is confirmed, then in the second part of the study the presence of ICG in brain of AD patients, putatively carried in by ICG-labeled cells, will be investigated by NIRS using the INVOS 7100 cerebral oximeter.

For all subjects, the study will follow the timeline shown in Figure 1

Figure 1 Study Timeline



On Day 0, NIRS recording will commence and continue throughout a 270-minute session. All timed measures are relative to the initiation of the ICG infusion. All timed measures on Day 0 and Day 1 are to be completed within a ± 5-minute window with the exception of vital signs, which will be completed within a +/- 7 minute window. Following baseline recording from 30 minutes pre-infusion to minute 0, an intravenous infusion of ICG will commence and be maintained from minute 0 to minute 120 (120 minutes duration). After cessation of the infusion, NIRS recording will continue until minute 240. Blood samples will be drawn relative to the start of infusion at 30 minutes pre-infusion, and at minutes 60, 120, 150, and 240 post-infusion. All blood samples will be assayed for plasma ICG concentration. For Cohorts 3 and 4 (healthy elderly and AD patients), a separate blood sample collected on Day 1 for

Cohort 3 and on Day 2 for Cohort 4 will be used for assay for biomarkers predictive of brain amyloid deposition-relevant pathologies using the PrecivityAD™ test [15]. An additional blood sample will be collected at the 240-minute timepoint, coinciding with the end of the NIRs recording. This sample will be used to determine the presence of ICG-labeled cells. Procedure for the preparation of the PBMC Fraction is included in Appendix 18.2. Subjects (except for Cohort 4) will remain in the clinic for approximately 48 hours; Cohort 4 subjects will return to the clinic for Day 2 procedures.

Approximately 48 hours after ICG infusion, blood samples will be drawn and an NIRS recording will be made for 30 minutes. Subjects will be discharged at the end of the 30-minute recording. One of the Day 2 blood sample will be used for assay of plasma ICG level and a separate blood sample will be used for isolation of PBMCs for determination of the percentage of cells labeled with ICG. The procedure for PBMC Isolation is in Appendix 18.2.

Cohort 1, healthy adults (n = 5), will receive an ICG infusion of 1 mg/min using sterile water as the infusion vehicle. If no dose limiting adverse effects are observed in Cohort 1, then Cohort 2, healthy adults (n = 10), will receive an ICG infusion of 2 mg/min using sterile water as the infusion vehicle. If there are no dose limiting adverse events and there is evidence of ICG-labeling of PBMCs, the 2 mg/min infusion rate will be used for the remainder of the study.

Cohort 3, healthy elderly adults, will receive an ICG infusion of 2 mg/min using sterile water as the infusion vehicle, and a sample will be taken on Day 1 for the PrecivityAD™ biomarker test. The first 5 subjects will serve as a satellite group. If no dose limiting adverse effects are observed in the satellite group, then a further 10 healthy elderly will receive a 2 mg/min ICG infusion using sterile water as the infusion vehicle (n = 15 total for Cohort 3).

Two additional Cohorts (Cohorts 2a and 3a) will receive IV infusions of ICG using dextrose 5% in water (D5W) as the infusion vehicle to evaluate the safety of D5W as an fusion vehicle and assess the comparability of results on ICG exposure, NIRS signals, and % labeling of PBMC using sterile water vs.D5W as the vehicle. Three (3) additional healthy adults (Cohort 2a) and three (3) additional healthy elderly adults (Cohort 3a) will be infused with ICG in D5W at the rate of 2 mg/min for 120 minutes. If no dose limiting adverse events are reported and data on all measures is comparable to that collected in previous cohorts (including evidence of ICG-labeling of PBMCs), D5W will be adopted as the IV infusion vehicle for Cohort 4 dosing.

For Cohort 4, in recognition of the unique limitations and needs of the AD patients and their caregivers, the requirement for remaining in the clinic throughout the entire protocol will be eliminated. Cohort 4 subjects will receive an ICG infusion of 2 mg/min (using D5W as the infusion vehicle if the criteria described above are met); the Day 0 safety assessments, NIRS recording, and blood sampling will be identical to that used with prior cohorts. At the end of Day 0 testing, Cohort 4 subjects will be discharged. All Cohort 4 subjects will be required to return on Day 2 for completion of the Day 2 assessments. The blood collection and sample processing for PrecivityAD™ biomarker testing for Cohort 4 will be conducted on Day 2. Waiving the Day 1 safety assessments for Cohort 4 is supported by the fact that, per the study decision tree (Figure 3), Cohort 4 will only proceed if no dose limiting adverse effects are observed in previous cohorts. The first 5 subjects will serve as a satellite group. If no dose limiting adverse effects are observed, then a further 10 AD patients will receive a 2 mg/min ICG infusion (n = 15 total for Cohort 4). Recruitment of AD patients will begin after three (3) subjects are dosed in Cohort 2a and three (3) subjects are dosed in Cohort 3a without any dose limiting adverse events and evidence of ICG-labeling of PBMCs is confirmed.

Table 3 Study Cohorts and Dosing Scheme

Cohort	Demographic	Number of subjects	ICG infusion rate	Infusion Vehicle
1	**Healthy Adults**	5	1 mg/min	Sterile Water
2	**Healthy Adults**	10	2 mg/min	Sterile Water
3	**Healthy Elderly**	15	2 mg/min	Sterile Water
2a	**Healthy Adults**	3	2 mg/min	D5W
3a	**Healthy Elderly**	3	2 mg/min	D5W
4	**AD patients**	15	2 mg/min	D5W[1]

1. If no dose limiting adverse effects are observed in Cohorts 2a and 3a and ICG exposure, NIRS signals, and % labeling of PBMC are comparable for Cohorts 2a and 3a compared to Cohorts 2 and 3, respectively.

Baseline data will be collected to include urine analysis, urine drug screen, urine pregnancy, clinical laboratory tests, vital signs, physical examination finding, and ECG. Any abnormal vital sign measurement, clinical laboratory test, physical examination finding, or ECG parameter deemed clinically significant by the investigator will be repeated, including test results obtained on Day 0, Day 1 (except Cohort 4), and the final study day (Day 2) or upon early termination.

For any test abnormality deemed clinically significant, repeat analysis will be performed during the follow-up period and until the value returns to baseline (or within normal limits) or the investigator deems the abnormality to be stable and no longer of clinical concern**.**

8.2 ICG pharmaceutical properties

ICG is a tricarbocyanine dye that is approved to determine cardiac output, hepatic function, liver blood flow and for ophthalmic angiography and visualization of vascular beds during surgery [18, 19]. ICG absorbs light in the near-infrared range (peak absorbance of 805 nm) and is also a fluorescence emitter (peak emission at 830nm). Because light in this near-infrared range is not absorbed by bone and tissue, ICG can be detected as it transits through the vasculature using different types of near-infrared light detection technologies [20-26]. Of note, oxygen-saturated hemoglobin also absorbs 805 nm light, which is the basis for the development and use of NIRS instruments such as the INVOS 7100 to monitor cerebral oxygenation [45].

ICG was FDA-approved in 1959 for use in hepatic function diagnostics. Shortly thereafter, ICG began to be used in as a tool to measure cardiac function. ICG was first used in 1969 for retinal diagnostic procedures. ICG is now routinely used to image vascular beds and monitoring tissue perfusion before, during, and after surgery or transplantation. The range of medical uses for ICG continues to expand. In a highly cited review of ICG imaging in surgery by Alander et al published in 2012, the authors noted over 6000 publications in PubMed [19]. A current PubMed search on the term ‘indocyanine green’ returns over 14,000 publications, with the rate of new publications increasing year over year.

8.3 Adverse reactions to ICG

Preclinical assessments. The Akorn product insert for ICG notes an absence of animal toxicological data describing the signs, symptoms, or laboratory findings accompanying ICG overdosage other than determinations of LD50s in mouse, rat, and rabbit. The LD50 doses after intravenous bolus administration ranged from 50 to 80 mg/kg). Safety margins for humans were extrapolated from the preclinical species LD50s based on body surface area extrapolation and the LD50 values are reported as

2.4 to 13-fold higher than the maximum recommended human dose of 2 mg/kg. However, ICG has a very low volume of distribution and is essentially confined to the plasma compartment in all species examined. For this type of drug, safety margin based on body surface extrapolation is not appropriate for estimating human safety margins. Instead, safety margins are more simply estimated based on direct comparison of the mg/kg of the drug dose administered [46]. Using this more appropriate method to estimate safety margins, the LD50 values in the non-human studies are 25 to 40-fold higher than the maximum recommended human dose of 2 mg/kg. These estimated safety margins are more in line with the very low incidence of adverse reactions to ICG in humans in clinical practice (see below). In MindImmune studies, ICG has been administered to over 200 mice by intraperitoneal bolus injection at 20-40 mg/kg; in these studies, no adverse reactions to ICG administration were observed.

Safety profile based on human clinical experience. The safety profile of ICG in humans has been reviewed in multiple publications [27-36]. For intravenous bolus administration at recommended doses as described in the package insert (Appendix 18.1), the incidence of adverse reaction is rare and commonly stated to be between 1:10,000 to 1:40,000. Reactions are typically mild and transient. As summarized by Hope-Ross et al [27], AEs associated with ICG administration appear to be 'pseudo-allergic' (see also [32, 33, 36]). This type of AE is managed by administration of antihistamines and support protocols used to manage allergic drug reactions. Such adverse reactions typically occur shortly after injection, suggesting that they are C_{max} driven (see further below).

More severe allergic reactions may be attributable to reaction to sodium iodide in the ICG formulation. Currently, sodium iodide is restricted to less than 5% of drug product. Nonetheless, ICG administration is contraindicated or used cautiously in individuals with known iodide or other drug allergies and such individuals will be excluded from the proposed trial.

ICG administration has also been linked to rare episodes of hypotension following ICG administration during cardiac catheterization [30]. At the time of the Hope-Ross et al. review in 1994, there were 3 deaths in ICG-administered subjects for an estimated number of ICG dosings exceeding 1,000,000. Causes of death were uncertain.

There is no evidence of differential safety of ICG in the elderly. According to the product insert, animal reproduction studies have not been conducted with ICG and it is not known whether this drug is excreted in human milk. Thus, pregnant women and pre-menopausal women not using birth control methods will be excluded from the proposed study and women of childbearing age will be screened for pregnancy prior to enrollment.

8.4 Dose Justification

For approved uses and imaging vascular beds, ICG is administered as an intravenous bolus delivered as rapidly as possible. ICG is administered at fixed doses up to 25 mg/individual, with routine doses of 5 or 12.5 mg/individual or on a mg/kg body weight basis at typical doses of 0.25 or 0.5 mg/kg. The total ICG dose is recommended to be no higher than 2 mg/kg. The dye is confined to the plasma compartment (Vd ~ 0.05 L/kg) where it is highly plasma protein bound ($\geq$98%). Given this biodistribution, plasma C_{max} levels may be estimated to range from 5 to 40 μg/ml for 0.25 to 2 mg/kg doses, respectively. These estimates are closely in line with direct measurement of ICG plasma concentrations shortly after bolus injection (e.g. [25]).

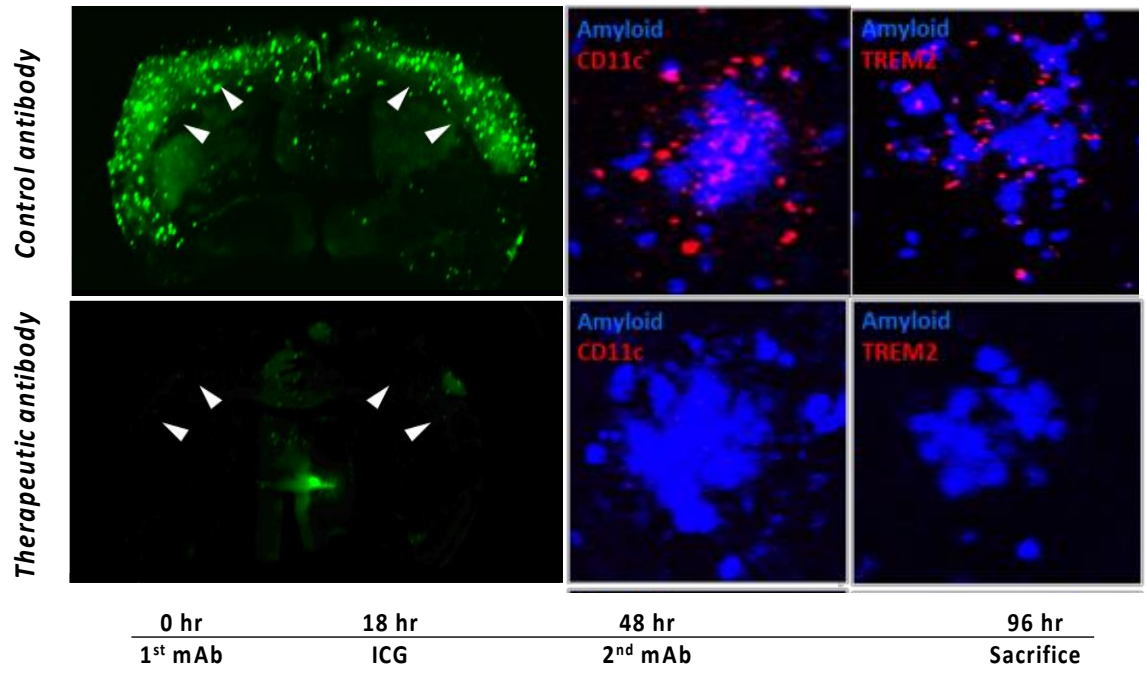
ICG is eliminated exclusively by liver extraction and excreted in bile. The plasma half-life of ICG is approximately 4 minutes and in healthy individuals less than 4% of the drug remains in the plasma compartment 20 minutes after bolus administration.

8.4.1 ICG to label and track DCs

The use of ICG to label immune cells was first described by Sim et al to track leukocytes into retina in a mouse model of uveitis [37]. Using mouse and human isolated peripheral blood mononuclear cells (PBMCs), it was found that both the ICG concentration and length of incubation influenced the degree of ICG labeling, with optimal labeling among the conditions explored achieved at an ICG concentration of 6 µg/ml incubated for 30 minutes at 37°C. However, Sim et al observed poor labeling of retinal leukocytes in mice in vivo when the dye was delivered by intravenous bolus. In contrast, labeling was robust if the dye was administered by intraperitoneal injection. Sim et al hypothesized that the prolonged ICG exposure after intraperitoneal administration provided a more adequate time window for phagocytic leukocytes to sequester the dye. Bell et al subsequently replicated the finding that human blood-derived macrophages in culture sequester ICG when incubated with the dye [38]. However, mirroring the results in mice, Bell et al saw no evidence of robust ICG-labeling of cells in retina in patients administered a standard intravenous bolus dose of 25 mg ICG for retinal angiography. Bell et al conjectured that "… (ICG) administration by slow intravenous infusion … may be preferable and our in vitro modelling suggests a sustained blood level of even 6 µg/ml of ICG for two hours might enable strong cell marking to be reliably detectable…" but did not test this hypothesis.

MindImmune adapted the paradigm of Sim et al to use ICG to label and track DCs into the brain of mice with AD-relevant pathology. As illustrated in Figure 1Figure 2, amyloid depositing APP/PS1 mice administered 1 mg ICG by intraperitoneal injection evidenced a robust accumulation of ICG-labeled cells in the vicinity of amyloid plaques. These cells bear markers of DCs, including CD11c and TREM2. MindImmune has identified a receptor on DCs that when neutralized in the periphery blocks recruitment into brain. Significantly, when DC recruitment is blocked in this way, evidence of active synaptic attack by these cells is rapidly attenuated. These data support MindImmune's pursuit of DC recruitment blockade as a novel therapeutic approach to AD. The human biomarker assay to ICG-label and track DCs into the brains of AD patients may provide early support for this therapeutic hypothesis and serve as a proof-of-mechanism biomarker for DC recruitment inhibitors.

Figure 2 ICG labeled DCs in cortex of APP/PS1 amyloid mouse model.



APP/PS1 mice were treated with a control (upper panels) or therapeutic (lower panels) antibody and then administered ICG on the timeline depicted below the figure. In the control antibody treated mice, ICG-labeled cells (upper left panel, green) are abundantly clustered around amyloid plaques. At higher magnification (upper right panels) these cells are observed to express the DC markers CD11c and TREM2 (red, amyloid plaque in blue). Treatment with the therapeutic antibody largely eliminates ICG-labeled, CD11c+, TREM2+ cells from vicinity of plaques (bottom panels).

MindImmune has determined the plasma ICG concentrations that yield the observed robust ICG-labeling of cells in brain of APP/PS1 mice. Intraperitoneal administration of 1 mg ICG results in a large and variable peak in plasma ICG concentration that plateaus to 2-4 μg/ml sustained for 2-3 hours. This ICG plasma exposure time course is in the concentration range and of a duration in line with that predicted by Sim et al [37] and Bell et al [38] to yield robust immune cells ICG labeling. Based on our data and that from these publications, MindImmune proposes to administer ICG to humans by intravenous infusion to enable ICG-labeling and tracking of DCs into the brains of AD patients.

8.4.2 ICG intravenous infusion

To achieve sustained ICG exposures in humans, MindImmune proposes an intravenous infusion paradigm. There are several published studies that have reported on ICG intravenous infusion in humans [39-42]. Although the number of subjects exposed to ICG by infusion is small relative to the very large number that have received ICG by intravenous bolus, there is no indication of new or unanticipated serious adverse reaction. There is some indication of injection site irritation. Data from these infusion studies were informative in choosing the specified parameters for the proposed study and these results are summarized in the Table 4. Two key issues were considered in choosing infusion parameters, 1) an ICG infusion rate and duration that would achieve target plasma exposures, while 2) minimizing safety concerns.

Table 4 ICG Infusion Studies Summary

	Infusion rate, mg/min Duration, min	Total dose	Approximate steady state plasma levels	Number of subjects, adverse events
Skak & Keiding (1987) *Liver* **7:**155	0.05 to 0.16 Up to 320 min	Up to 50 mg	0.3 ∝g/ml at 0.16 mg/ml	52 - liver disease 61 - abdominal angina 17 – hospitalized, no liver disease 8 - healthy volunteers No AEs reported
Clements et al (1987) *J Hepatol* **5:**282	0.25 Up to 60	25 mg	Not specified	13 with and 12 without liver cirrhosis No AEs reported
Soons et al (1991) *Br J Clin Pharm* **32**:697	0.5 to 2 150	150 mg	1 to 3 ∝g/ml	6 ~20% incidence of pain, irritation at infusion site
Schoemaker et al (1997) *Br J Clin Pharm* **45**:463	0.75 200	150 mg	1.25 ∝g/ml	8 1 AE - phlebitis at injection site

In the published studies noted in the table, plasma ICG levels were found to reach a stable steady state within minutes after the start of infusion, consistent with the short half-life of the dye and a distribution limited to the plasma compartment. In the article by Soons et al., steady state plasma levels were found to be dose proportional over infusion rates from 0.5 to 2 mg/min [40] with proportional steady-state plasma concentrations of approximately 0.7 to 3.0 μg/ml. Thus, a 2 mg/min infusion rate is predicted to yield a sustained ICG plasma level (~3.0 μg/ml) in the range predicted to yield robust labeling based on the studies of Sim et al [37] and Bell et al [38] and in the range that has resulted in robust ICG labeling of DCs to an extent that enabled ready detection of these cells in the brain in APP/PS1 mice.

The MindImmune mouse data and the published studies further suggest that an infusion duration of 120 min would favor robust DC labeling. An infusion of 2 mg/min for 120 minutes results in a total ICG dose of 240 mg. For perspective, the highest recommended dose of ICG, 2 mg/kg, results in an approximate total dose of 140 mg for a 70 kg individual. Furthermore, in the infusion studies noted in the table, highest total doses were 150 mg. Thus, the total ICG dose for the proposed infusion protocol is only ~60% higher than the highest recommended dose and is considered to remain in a range associated with infrequent and mild adverse reactions in general clinical use. Supporting this conclusion 2 recent publications report the administration of ICG as an intravenous bolus at a dose of 5 mg/kg, for total dose of ~350 mg, to visualize glioblastoma prior to surgery (the SWIG' technique; [43, 44]). No adverse reactions to this higher dose of ICG were reported, albeit only approximately 25 patients have been treated to date.

It is also noteworthy that the most common adverse reactions to intravenous bolus ICG administration occur shortly after drug administration. This suggests that the adverse reactions are C_{max} related. Plasma C_{max} concentrations of ICG after bolus administration are calculated to range from ~ 5 μg/ml for the lowest routinely used dose of 0.25 mg/kg to ~40 μg/ml for the highest recommended dose of 2 mg/kg. For the SWIG technique, the C_{max} ICG concentrations after bolus administration of 5 mg/kg is calculated to exceed 100 μg/ml. In contrast, intravenous infusion of ICG at 2 mg/min is predicted to result in a C_{max} exposure of ~3 μg/ml, based on calculation and clinical exposure data published in the literature (Table 3). Thus, in so far as adverse reaction to ICG is C_{max} driven, the proposed intravenous infusion rate of 2 mg/kg will result in C_{max} ICG plasma exposures that are well within the margins produced by routinely used dosing regimens that are considered safe and well tolerated. Subjects will be closely monitored for adverse reactions during the infusion. The infusion will be stopped if moderate to severe reactions emerge and given the short half-life (~4 minutes), the plasma concentration will rapidly abate.

8.5 Study Sites

The study will be conducted at one site.

9 SUBJECT POPULATION

9.1 Selection of Study Population

Subjects must fulfill all the inclusion criteria and meet none of the exclusion criteria to be eligible for participation in the study. The goal is to include at least 15 subjects in each target population. Subjects will be enrolled until at least this target goal is reached.

9.1.1 Inclusion Criteria

1.All Healthy Subjects

- Subjects are determined by the investigator to be medically stable and expected to complete the trial as designed
- Subjects have adequate hearing, vision, and language skills to perform neuropsychiatric testing and interviews as specified in the protocol
- Subjects are able to understand and agree to comply with the study procedures and report for scheduled office visits
- Subjects are able to reliably communicate with study personnel about adverse events (AEs) and concomitant medications
- Signed written informed consent according to institutional guidelines

2. Healthy Adult subjects

- Male or female and between the ages of 21 to 55 inclusive

3 Healthy Elderly subjects

- Male or female and between the ages of 65 to 90 inclusive
- MMSE score > 26

4. Alzheimer’s patients

- Male or female and between the ages of 50 to 90 inclusive
- Patients satisfying the criteria for the clinical diagnosis of probable AD based on National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) OR 6 months documented cognitive decline by physician OR Amyloid or Tau pathology confirmed by PET scan with ligand.
- MMSE score between 16 and 25
- Modified Hachinski Ischemia Scale (MHIS) score of ≤ 4

9.1.2 Exclusion Criteria

1. Sex and Reproductive Status

- Women who test positive for pregnancy
- Pre-menopausal women who are not practicing two methods of birth control for 3 months prior and a week after the ICG infusion

2. Medical History

- Subjects with a history of any anaphylactic reactions
- Subjects with a history of allergic reaction to ICG
- Subjects with a history of iodine sensitivity and/or allergic reaction to iodine
- Subjects with a history of a clinically significant hepatic disease

3. Target Disease Exceptions

- Any subject diagnosed to have an autoimmune disorder, including
  - Psoriasis
  - Lupus
  - Rheumatoid arthritis
  - Crohn's disease
  - multiple sclerosis
  - alopecia areata
- Any subject who has any unstable cardiovascular (included uncontrolled hypertension), pulmonary, or GI disease
- For Alzheimer's patients, a medical condition other than AD that could explain or contribute significantly to the patient's dementia: e.g., Down Syndrome, abnormal thyroid function, posttraumatic physical conditions, syphilis, Parkinson's disease, multi-infarct dementia, Huntington's disease, normal pressure hydrocephalus, central nervous system (CNS) tumor, frontotemporal dementia, seizure disorder (other than childhood febrile seizures), and subdural hematoma

4. Concurrent Medications

- Any subject who is immunocompromised at screening including taking medications that are systemic immunosuppressives including corticosteroids but excluding NSAIDS.
- Any subject prescribed a biologic immunosuppressive therapy or having taken such therapy in the prior 3 months, including
  - abatacept (Orencia)
  - adalimumab (Humira)
  - anakinra (Kineret)
  - certolizumab (Cimzia)
  - etanercept (Enbrel)
  - golimumab (Simponi)
  - infliximab (Remicade)
  - ixekizumab (Taltz)
  - natalizumab (Tysabri)
  - rituximab (Rituxan)
  - secukinumab (Cosentyx)
  - tocilizumab (Actemra)
  - ustekinumab (Stelara)
  - vedolizumab (Entyvio)
  - Monoclonal antibodies
  - basiliximab (Simulect)
  - daclizumab (Zinbryta)

5. Physical and Laboratory Test Findings at Screening

- Any subject with uncontrolled hypertension at screening (e.g., repeated diastolic measurements ≥ 96 mmHg).
- Any subject with either of the following hepatic test abnormalities at screening:
  - Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) > 2.0 times the institutional ULN
  - Total Bilirubin > 2 times the institutional ULN

- Any subject with P-Amylase or Lipase values > 2 times the ULN at screening
- Any subject at screening with insulin-dependent diabetes mellitus or HbA1C > 7.5%
- Any subject at screening with pathologic renal findings as defined by the presence of Calculated glomerular filtration rate (GFR) (creatinine clearance) < 30 ml/min/1.73m2 (Cockcroft-Gault formula for GFR estimate)
- Any subject at screening with any of the following hematologic abnormalities:
  - Hemoglobin < 10g/dL
  - WBC < 3.0 x 103/mm3
  - Platelet count < 100,000/mm3
- Any subject who has a known infection with a human immunodeficiency virus.
- For Cohort 4: Any subject who has abnormal results for thyroid-stimulating hormone (TSH), folate, or Vitamin B12 at Screening that indicate a possible alternative diagnosis that may cause cognitive decline.

9.2 Removal of Subjects from Therapy or Assessment

All subjects are free to withdraw from participation in this study at any time for any reason and without prejudice.

The investigator may terminate a subject at any time for any effect that is intolerable to the subject or otherwise considered unacceptable, for intolerable or unacceptable AEs, intercurrent illness, noncompliance with study procedures, administrative reasons, or unsuitability for the study in the investigator's opinion to protect the subject's best interest. Moderate to severe AEs occurring during the infusion will result in termination of the infusion.

If a subject is withdrawn from dosing before completing the study, the reason for withdrawal will be entered on the appropriate Case Report Form (CRF). Whenever possible and reasonable, Early Termination evaluations should be performed at the time of premature discontinuation of dosing.

10 STUDY TREATMENTS

10.1 Identification of Investigational Product

ICG is manufactured and sold by Akorn, Inc., Lake Forest, IL, and Diagnostic Green, LLC, Farmington Hills, MI.

The product is identified as IC-Green® (indocyanine green for injection, USP) (Akorn) or Indocyanine Green for Injection USP (Diagnostic Green). It is supplied as single-dose vials containing varying amounts of lyophilized ICG, together with vials containing sterile water for injection to be used for reconstitution. For this study, ICG products containing 25 mg ICG per single-dose vial will be used.

For some cohorts, the protocol will be utilizing D5W for infusion rather than Sterile Water (see Section 8.1 and Table 3 for details). See Pharmacy Manual for detailed dosing instructions.

Either IC-Green® (Akorn) or Indocyanine Green for Injection USP (Diagnostic Green) may be used in this study. Both products are listed as therapeutic equivalents in the FDA's Orange Book, *Approved Drug Products with Therapeutic Equivalence Evaluations*: "Approved drug products are considered to be therapeutic equivalents if they are pharmaceutical equivalents for which bioequivalence has been demonstrated, and they can be expected to have the same clinical effect and safety profile when administered to patients under the conditions specified in the labeling" [47].

The Akorn product label was approved in 2006 under Supplemental NDA 11-525-S-017. The Diagnostic Green product label was approved in 2007 under Abbreviated NDA 40-811. The Akorn product insert and the Diagnostic Green product insert are included as Appendix 18.1.

10.2 Treatment administration

Study site personnel will administer the treatments.

10.3 Storage

No special transport or storage conditions are required. Infusion solutions will be stored at room temperature, protected from light (i.e., wrapped in aluminum foil) and infusion initiated within 6 hours of preparation.

10.4 Drug Accountability

The Investigator must maintain adequate records showing the receipt, dispensing, return, or other disposition of investigational product, including the date, quantity, batch or code number, and identification of subjects (subject number and initials) who received investigational product. The Investigator will not supply study drug to any person except those named as sub-investigators on the United States Food and Drug Administration Form 1572, designated staff, and subjects in this study. Investigational product may not be relabeled or reassigned for use by other subjects.

10.5 Blinding and Unblinding Treatment Assignment

This study will be conducted under open-label conditions.

10.6 Selection of Dose in the Study

MindImmune has determined target plasma ICG concentrations expected to yield robust ICG-labeling of peripheral phagocytic immune cells, including DCs, based on published and in-house preclinical studies in mouse models and published ex vivo studies using human PBMCs [37] [38]. Based on MindImmune data

and information from these publications, MindImmune intends to administer ICG to humans by intravenous infusion to enable ICG-labeling and tracking of DCs into the brains of AD patients.

10.7 Treatment Compliance

All unused study product will be disposed by investigator. Drug accountability will be performed by study site personnel.

Since treatments will be administered by site staff in this inpatient study, treatment compliance is expected to be 100%.

10.7.1 Concomitant Medications

All concomitant medications used to include over-the-counter medications and herbal and nutritional supplements will be recorded in the source document and on the appropriate CRF. The medication name, dose, frequency, date, and indication for use must be recorded on the CRF. Medications and therapies that are considered necessary for the subject's welfare and will not interfere with the response to the investigational product may be given at the discretion of the investigator.

10.7.2 Permitted Therapies

Concomitant medications are allowed unless specifically prohibited (see Section Prohibited Therapies) but should be limited to only those medications considered necessary and if the dose is stable at the time of the screening visit and stable throughout the study. Guaifenesin for cough, antacids for indigestion and nausea, Ondansetron (Zofran) for nausea/vomiting, medications for diarrhea, constipation, acetaminophen or ibuprofen for aches and pains.

10.7.3 Prohibited Therapies

Prohibited therapies are medications that are systemic immunosuppressives including corticosteroids taken in the prior three months to the study. Systemic immunosuppressives are:

- abatacept (Orencia)
- adalimumab (Humira)
- anakinra (Kineret)
- certolizumab (Cimzia)
- etanercept (Enbrel)
- golimumab (Simponi)
- infliximab (Remicade)
- ixekizumab (Taltz)
- natalizumab (Tysabri)
- rituximab (Rituxan)
- secukinumab (Cosentyx)
- tocilizumab (Actemra)
- ustekinumab (Stelara)
- vedolizumab (Entyvio)

- Monoclonal antibodies
- basiliximab (Simulect)
- daclizumab (Zinbryta)

11 STUDY PROCEDURES

Subjects will provide written informed consent before any study-related procedures are initiated, including the cessation of prohibited concomitant therapy.

11.1 Screening Days -28 to -1 (within 28 days prior to inpatient admission)

The following procedures and assessments will be performed at Screening:

- Obtain written informed consent. No study procedures may be performed prior to completion of the ICF process.
- Review inclusion and exclusion criteria.
- Collect demographic information.
- Record medical and psychiatric histories
- Mini Mental State Examination (MMSE) to be completed only for Cohorts 3 and 4.
- Modified Hachinski Ischemia Scale (MHIS) to be completed only for Cohort 4
- Record prior and concomitant medications.
- Perform physical examination, including weight and height.
- Record vital signs including systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate and pulse oximetry measurement.
- Record a 12-lead ECG.
- Perform rapid urine pregnancy test for all females of childbearing potential
- Collect blood and urine samples for clinical laboratory tests (hematology, Serum chemistry, HIV, urinalysis, urine drug screen [for opioids, buprenorphine, benzodiazepines, cocaine, amphetamines, and other drugs]), and breath alcohol level (BAL).
- Record AEs (after signing the ICF).

All screening procedures must be completed after signing informed consent and prior to subject randomization.

11.2 Treatment Phase and Study Days 0, 1, and 2

On Study Days 0 - 2 subjects in each cohort will receive the treatments appropriate to each cohort. Cohort 4 will not receive any assessments on Day 1. The following assessments (within a ± 5-minute window, unless otherwise noted) and data collected will include:

- Vital signs including systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, will be measured on Day 0 (immediately prior to the start of the NIRs recording) then immediately after the start of infusion and at 30 min, 90 min, 120 min, 150 min, 210 min, and 240 mins (+/- 7 min window) after the start of infusion and on Day 2 before discharge.
- Pulse oximetry will be measured on Day 0 prior to start of the NIRs recording.
- Urine toxicity and Breath Alcohol levels on Day 0 test prior to start of NIRs monitoring

- 12-Lead ECG on Day 0 will be collected within 30 minutes pre-infusion and within 30 minutes (with a +5 min window) of the end of the ICG infusion and on Day 2 after the 30-minute NIRs recording before discharge.
- On Day 0, after dosing and completion of NIRs, on Day 1 (except for Cohort 4), and on Day 2, samples for Hematology, Serum Chemistry including fractionated bilirubin, and Urinalysis are to be collected.
- Physical Examination will be conducted on Day 2 before discharge.
- Suicidality screening on Day 2 before discharge
- NIRs recording on Day 0 for 270-minutes and on Day 2 for 30-minutes
- ICG concentrations on Day 0 and Day 2
- PBMC measurements on Day 0 and Day 2
- Record concomitant medication use.
- Assess and record AEs.
- Administration of ICG Infusion on Day 0 only
- Precivity AD test on Day 1 only for Cohort 3
- Precivity AD test on Day 2 only for Cohort 4

11.3 Telephone Visit Day 7-16 *(*Approximately 1 week after the end of the study*)*

The following assessments and procedures will be conducted approximately 1 week after the end of the study:

- Record concomitant medication use
- Assess and record AEs

12 STUDY ASSESSMENTS

We predict that in all subjects during the ICG infusion the NIRS signal recorded with the INVOS 7100 will be elevated above baseline [16, 17]. We predict that in the healthy adult and elderly subjects the NIRS signal will return to baseline rapidly after cessation of the ICG infusion with a time course reflecting the rapid elimination of ICG and that the NIRS signal will remain at baseline level on Day 2. We hypothesize that in the AD patients, the NIRS signal may not return to baseline on Day 0 and will be elevated over baseline on Day 2. This will be inferred to reflect the accumulation of ICG-labeled immune cells in the brain.

Proof-of-concept will be achieved if the NIRS signal detected in frontal cortex of patients with AD is greater than in healthy elderly controls. In addition, we will examine in post hoc analyses whether there is any relationship between the NIRS signal after cessation of ICG infusion and the results of the PrecivityAD™ screen.

12.1 Efficacy

Biomarker Proof of Concept Part 1

- Establish efficacy of an intravenous infusion of indocyanine green (ICG) to label peripheral immune phagocytic cells with the dye in healthy adult subjects.

Biomarker Proof of Concept Part 2

- Determine if the presence of ICG-labeled cells trafficked into the brain of adult subjects diagnosed with Alzheimer's disease (AD) can be determined by inference of the detection of an increase in absorbance of near infrared light (805 nm) in the cerebral cortex of Alzheimer's patients compared to healthy elderly control subjects measured using the INVOS 7100 NIRs cerebral oximeter.

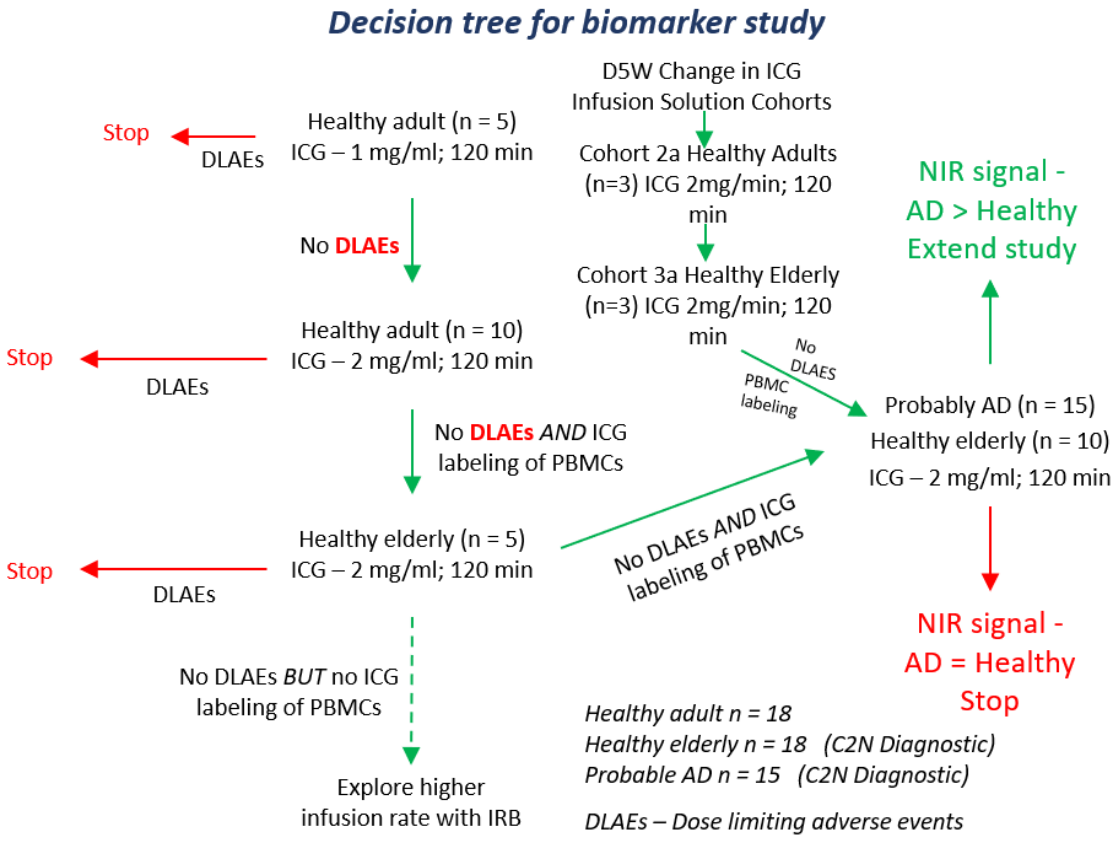
The efficacy of ICG to label peripheral immune phagocytic cells will be determined in blood samples collect at the end of the Day 0 and the beginning of Day 2 (see Figure 1). Peripheral blood mononuclear cells (PBMCs) will be isolated from the blood samples and the percentage of cells labeled with ICG determined by appropriate analytical methods.

The plasma ICG concentrations during the ICG intravenous infusion and the degree to which ICG has been eliminated from plasma after the infusion will be determined from blood samples collected on Day 0 30 minutes before start of the infusion (background), 60 and 120 minutes after the start of the infusion, and 30 and 120 minutes after the end of the infusion (see Figure 1). An addition blood sample will be collected on Day 2. Plasma will be separated and the ICG concentration measured by NIR spectroscopy. Concentration will be calculated based on comparison to an ICG standard curve of known ICG concentration.

The presence of ICG labeled cells in brain will be inferred from the detection of an increase in absorbance of near infrared light at 805 nm measured using the INVOS 7100 cerebral oximeter. This device is used routinely to determine changes in cerebral blood oxygenation by measuring changes in near infrared light absorbance at 805 nm that results from changes in the oxygenation level of blood hemoglobin. The INVOS 7100 detector has been calibrated to measure changes in this near infrared signal precisely in cortex. The presence of ICG-labeled cells in cortex will be inferred from an increase in the near infrared light absorbance over the background signal from hemoglobin. Data from the literature and analyses by MindImmune indicated that the instrument is well suited to detecting such an increase. At the start of session on Day 0, a light source/detector array will be placed over the temple as per the protocol specified in the INVOS manual. Baseline signal will be collected for 30 minutes prior to the initiation of the ICG intravenous infusion. Based on data from the literature and MindImmune calculations, it is anticipated that the signal will increase quickly to a plateau level and remain elevated

throughout the ICG infusion. In healthy subjects it is anticipated that the signal will return to baseline level within 20-30 min after cessation of the infusion. In AD subjects, the signal may not return to baseline or begin a second phase of increase due to the influx of ICG-labeled cells into the cortex. On Day 2, subjects will again have a light source detector attached to the temple and signal will be recorded for 30 minutes. It is anticipated that in the healthy subjects, the signal on Day 2 will be essentially equivalent to that during the baseline recording on Day 0. A signal higher than Day 0 baseline in AD subjects will be inferred to reflect the presence of ICG-labeled cells in cortex.

Figure 3 Decision Tree for Study



12.1.1 Analytical Procedures

12.1.1.1 Bioanalytical Sample Analyses and Methodology

- Plasma samples to measure ICG concentration after collection will be frozen and shipped to MindImmune for analysis. ICG levels in plasma will be determined by NIRS spectroscopy. Concentration will be estimated by extrapolation from a standard curve.
- Plasma samples for analysis of biomarkers in the PrecivityAD battery will be prepared according to detailed instructions provided by to vendor, C2N Diagnostics (Protocol for Sample Collection for Biomarker Studies (Appendix 18.3). Data from these analyses will be provided to MindImmune by C2N Diagnostics.
- PBMCs for analysis will be prepared by site adapted from the detailed protocol provided in Appendix 18.2. Samples will be shipped to shipped to MindImmune for analysis.
- Data from these analyses will be made available to the site as needed for preparation of a final report.

12.2 SAFETY

Safety and Tolerability of ICG will be assessed by adverse events, laboratory safety parameters, and vital signs.

12.2.1 Adverse Events

12.2.1.1 Adverse Event Definitions

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

Preexisting diseases or conditions will not be considered AEs unless there is an increase in the frequency or severity, or a change in the quality, of the disease or condition. Worsening of a preexisting condition is considered an AE.

An expected AE is one for which the nature or severity is consistent with the known AE profile of the product. The investigational product in this study is a marketed drug; the known information is in the current package insert. Appendix 18.1

An unexpected AE is one for which the specificity or severity is not consistent with the current package insert. For example, hepatic necrosis would be unexpected (greater severity) if the package insert listed only elevated hepatic enzymes or hepatitis. Likewise, cerebral thromboembolism and cerebral vasculitis would be unexpected (greater specificity) if the package insert only listed cerebral vascular accidents.

Furthermore, reports that add significant information on specificity or severity of a known, already documented adverse reaction constitute unexpected AEs. Examples include acute renal failure as an expected adverse reaction with a subsequent new occurrence of interstitial nephritis and hepatitis with a first occurrence of fulminate hepatitis.

A serious AE (SAE) is any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly
- Is an important medical event

Medical and scientific judgment should be used in deciding whether it is appropriate to consider other situations serious, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent another of the outcomes listed in the definition previously. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization.

An elective hospital admission to treat a condition present before exposure to the investigational product or a hospital admission for a diagnostic evaluation of an AE does not qualify the condition or event as an

SAE. A newly diagnosed pregnancy in a subject who has received a study drug is not considered an SAE; however, the medical monitor should be made aware of a newly diagnosed pregnancy as soon as possible after site notification. A congenital anomaly in an infant born to a mother who was exposed to the study drug during pregnancy is an SAE.

12.2.1.2 Eliciting and Documenting Adverse Events

The investigator is responsible for ensuring that all AEs and SAEs are recorded in the CRF and reported to the medical monitor. Adverse events will be collected from the signing of the informed consent through Day 2 or Early Termination visit and at the Telephone follow up visit.

AEs will be documented from any data collected on the AE page of the CRF (e.g., clinical laboratory values, physical examination findings, and ECG changes) or other documents that are relevant to subject safety.

12.2.1.3 Reporting Adverse Events

All AEs reported or observed during the study will be recorded on the AE page of the CRF. Information to be collected includes drug treatment, type of event, time of onset, dose, investigator-specified assessment of severity and relationship to investigational product, time of resolution of the event, seriousness, as well as any required treatments or evaluations, and outcome. Adverse events resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. All AEs will be followed to adequate resolution. The latest version of the MedDRA will be used to code all AEs.

Any medical condition that is present at the time that the subject is screened but does not deteriorate should not be reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

The investigator or designee must report any AE that meets the criteria for an SAE (Section 12.2.1.1) to the medical monitor within 24 hours of first becoming aware of the event by telephone. At the time of first notification, the investigator or designee should provide at a minimum the following information if available:

- Investigator information (name, phone, fax, e-mail).
- Protocol number.
- Subject's study identification and initials.
- Subject's date of birth.
- Date of dose of study drug.
- Time and date of occurrence of the event.
- A brief description of the event, outcome to date, and any actions taken.

Within 24 hours of the initial notification, the investigator must e-mail a written SAE report form to the medical monitor/Safety team. Any missing or additional relevant information about the SAE should be provided in a written follow-up SAE report form. The investigator should also ensure that any additional information requested about the event (e.g., hospital reports, autopsy reports) is provided as soon as it is available.

The investigator is required to comply with applicable regulations (including local laws and guidance) regarding the notification of the Institutional Review Board (IRB).

The following contact information is to be used for SAE reporting:

Cognitive Research Corporation: safety@cogres.com

12.2.1.3.1 Assessment of Severity

The severity or intensity of an AE refers to the extent to which it affects the subject's daily activities. Severity will be rated as mild, moderate, or severe using the following criteria:

Mild:	Is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
Moderate:	Is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
Severe:	Interrupts usual activities of daily living, significantly affects clinical status, or may require intensive therapeutic intervention.

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

12.2.1.3.2 Assessment of Relationship

The investigator's assessment of an AE's relationship to investigational product is part of the documentation process but is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

The relationship or association of the study drug in causing or contributing to the AE will be characterized using the following classification and criteria:

Not related:	An AE with sufficient evidence to accept that there is no causal relationship to administration of study drug (e.g., no temporal relationship because the study drug was administered after the onset of the event, an investigation shows that study drug was not administered, and another cause was proven.)
Unlikely/Remotely related:	An AE, including a clinical laboratory test abnormality, with a temporal relationship to administration of study drug that makes a causal relationship improbable and in which other drugs, events, or underlying disease provide plausible explanations.
Possibly related:	An AE with a reasonable time sequence to administration of study drug but that could also be explained by concurrent disease or other drugs or events. Information on drug withdrawal may be lacking or unclear.

Probably related:	An AE with a reasonable time temporal sequence from administration of the study drug; or the AE follows a known pattern of or response to the study drug; or an alternative explanation (e.g. concomitant disease, environment factors, and/or concomitant medications) is less likely than attribution to the study drug; or the AE diminishes or disappears upon cessation of study drug.
Definitely Related:	An AE occurring in a plausible time relationship to administration of study drug and that cannot be explained by a concurrent disease or other drugs or events. The response to withdrawal of the drug (de-challenge) is clinically reasonable.

12.2.1.3.3 Definition of Adverse Event Outcome at the Time of Last Observation

The AE outcome at the time of last observation will be classified as "resolved," "resolved with sequelae," "ongoing," "death," "other," or "unknown."

"Death" should only be selected as an outcome when the AE resulted in death. If more than 1 AE is possibly related to the subject's death, the outcome of death should be indicated for each such AE. Although "death" is usually an event outcome, events such as sudden death or unexplained death should be reported as SAEs.

12.2.1.4 Follow-up of Adverse Events

Any AE will be followed (up to approximately 1 week after dosing) to a satisfactory resolution or until the investigator deems the event to be chronic or not clinically significant or the subject to be stable. All findings relevant to the final outcome of an AE must be reported in the subject's medical record and recorded on the appropriate CRF.

12.2.2 Laboratory Safety Assessments

Samples for the following laboratory tests will be collected at the time points specified in the Schedule of Visits and Assessments (Table 2).

Hematology:	Consists of complete blood count (hemoglobin, hematocrit, white blood cell count with differential, red blood cell count, and platelet count)
Serology:	HIV
Serum chemistry:	Includes blood urea nitrogen, creatinine, total bilirubin (screening), fractionated bilirubin (Days 0, 1, & 2), alkaline phosphatase, aspartate aminotransferase (serum glutamic-oxaloacetic transaminase), alanine aminotransferase (serum glutamic pyruvic transaminase), glucose, albumin, total protein, HbA1C, lipase, amylase, and eGFR. Conducted at screening only: TSH, folate, and Vitamin B12.
Urinalysis:	Includes pH, specific gravity, protein, glucose, ketones, bilirubin, blood, nitrites, leukocytes, urobilinogen, microscopic urine analysis if dipstick positive
Urine pregnancy test	Conducted for females of childbearing potential only
Urine drug screen	Opioids, (fentanyl), buprenorphine, methadone, benzodiazepines, cocaine (benzoylecgonine), amphetamines, and other drugs. Results of the UDS for Inclusion/Exclusion is for the PI to determine if the subject if eligible.
Breath alcohol level	Conducted at screening and prior to the start of NIRs monitoring on Day 0.

12.2.3 Vital Signs

Resting vital signs, including systolic, diastolic blood pressure and heart rate (measured as pulse) will be measured after the subject has been in a sitting/semi-recumbent or supine/recumbent position for at least 5 minutes at the time points specified in the Schedule of Assessments Table 2.

Orthostatic measurement of systolic, diastolic blood pressure and heart rate will be measured after the subject has been standing for a total of 5 minutes. On Day 0, the orthostatic measurements will be taken prior to the start of the NIRs recording, at 30 min after the start of infusion, and 240 min after the start of the ICG infusion.

If the first measurement of any vital signs (SBP, DBP and pulse) shows the following, vital signs will be measured again in triplicate (same arm, separated by at least 1 minute) for SBP <90 mmHg, DBP <60 mmHg, and pulse <60 bpm. No upper limit will be set as the investigator or designee may repeat any vital sign at their discretion.

12.2.4 Electrocardiogram

A 12-lead ECG with rhythm strip will be performed at screening, Day 0 within 30 minutes pre-infusion and within 30 minutes of the end of the ICG infusion; on Day 2 after 30 mins of NIRs recording before discharge.

12.2.5 Physical Examination

A standard physical examination will be performed at screening and study Day 2 or Early Termination Visit. The examination will include assessment of skin, head, ears, eyes, nose, throat, neck, thyroid, lungs, heart, cardiovascular, abdomen, lymph nodes, and musculoskeletal system/extremities. Interim physical examinations will be performed at the investigator's discretion, if necessary, to evaluate AEs or clinical laboratory abnormalities.

Height and weight will be measured at Screening only.

12.2.6 PrecivityAD™ test

A plasma sample will be taken from each subject in Cohorts 3 and 4 and assayed for biomarkers predictive of brain amyloid deposition using the PrecivityAD™ test

12.2.7 Concomitant Medications

Concomitant medications will be reviewed and documented during screening, Day 0 through Day 2, and at the Telephone Follow-up visit.

13 STATISTICAL METHODS

13.1 General Considerations

A Statistical Analysis Plan (SAP) that describes the details of the analyses to be conducted will be finalized and provided in the Clinical Study Report.

13.2 Analysis Populations

The following analysis populations are planned:

- Safety Population: All subjects who receive study drug.
- Full Analysis Set (FAS): All subjects in the Safety Population who have at least one plasma ICG level or PBMCs.

13.3 Subject Disposition and Demographic Characteristics

Subject disposition will include the number of subjects who enroll in the study and the number and percentage of subjects included in each analysis population by treatment. The frequency and percentage of subjects who withdraw or discontinue from the study, along with the reason for withdrawal or discontinuation, will be summarized by cohort.

Demographics and baseline characteristics, including age, sex, race, weight, height and body mass index, will be summarized by cohort for the Safety Population.

13.4 Pharmacodynamic Assessment

13.4.1 Primary Endpoint

The FAS will be the primary analysis population for efficacy. Efficacy objects will be assessed using descriptive statistics.

13.4.2 Safety Analyses

All safety analyses will be performed using the Safety Population. All subjects who received at least one dose of study drug will be included in the population for safety analysis.

The number and percentage of subjects experiencing 1 or more AEs will be summarized by cohort, relationship to study drug, and severity. AEs will be coded using Medical Dictionary for Regulatory Activities (MedDRA) terminology. Listings of subjects who withdraw from the study due to an AE, serious AEs and/or death will be presented. Laboratory parameters will be summarized by cohort using descriptive statistics and data listings of clinically significant abnormalities. Vital signs and ECG data will be summarized by changes from baseline values using descriptive statistics.

13.5 Sample Size Determination

Due to the exploratory nature of this study, no formal sample size calculations were performed.

14 STUDY CONDUCT

Steps to ensure the accuracy and reliability of data include review of protocol procedures with the investigator and associated personnel prior to the study, and strict data management procedures.

14.1 Sponsor and Investigator Responsibilities

14.1.1 Sponsor Responsibilities

The sponsor is obligated to conduct the study in accordance with strict ethical principles. The sponsor reserves the right to withdraw a subject from the study or to discontinue the study.

The sponsor agrees to provide the investigator with sufficient material and support to permit the investigator to conduct the study according to the study protocol.

14.1.2 Investigator Responsibilities

By signing the Investigator's Agreement, the investigator indicates that he or she has carefully read the protocol, fully understands the requirements, and agrees to conduct the study in accordance with the procedures and requirements described in this protocol.

The investigator also agrees to conduct this study in accordance with all laws, regulations, and guidelines of the pertinent regulatory authorities, including and in accordance with the April 1996 International Conference on Harmonisation (ICH) Guidance for Industry E6 Good Clinical Practice (GCP) and in agreement with the 1996 Version of the Declaration of Helsinki. While delegation of certain aspects of the study to sub-investigators and study coordinators is appropriate, the investigator will remain personally accountable for closely overseeing the study and for ensuring compliance with the protocol and all applicable regulations and guidelines. The investigator is responsible for maintaining a list of all persons that have been delegated study-related responsibilities (e.g., sub-investigators and study coordinators) and his or her specific study-related duties.

Investigators should ensure that all persons who have been delegated study-related responsibilities are adequately qualified and informed about the protocol, study drugs, and their specific duties within the context of the study. Investigators are responsible for providing the sponsor with documentation of the qualifications, GCP training, and research experience for themselves and their staff as required by the sponsor and the relevant governing authorities.

To ensure compliance with the guidelines, the study will be audited by an independent person. The investigator agrees, by written consent to this protocol, to cooperate fully with compliance checks by allowing access to all study documentation by authorized individuals.

14.2 Site Initiation

Study personnel may not screen or enroll subjects into the study until after receiving notification from the sponsor or its designee that the study can be initiated at the study site. The study site will not be authorized for study initiation until:

1. The study site has received the appropriate IRB approval for the protocol and the appropriate informed consent.
2. All GCP documents have been submitted to and approved by the sponsor or its designee.
3. The study site has a Clinical Trial Agreement in place.
4. Study site personnel, including the investigator, have participated in a study initiation meeting.

14.3 Study Documents

All documentation and material provided by the sponsor for this study are to be retained in a secure location and treated as confidential material.

14.3.1 Good Clinical Practice Documents

The GCP documents are listed below.

- Signed original protocol (i.e., Investigator's Agreement).
- Curricula vitae of all investigators and sub-investigators.
- Name and address of the laboratories.
- List of laboratory reference ranges, and if available, a quality certificate.
- Signature Log/Delegation of Study-related Duties.
- United States Food and Drug Administration Form 1572.
- Any other relevant GCP documents.

The GCP documents must be received from the investigator and reviewed and approved by the sponsor or designee before the study site can initiate the study and before the sponsor will authorize shipment of study drug to the study site. Copies of the investigator's GCP documents must be retained at the study site in a secure location. Additional documents, including a copy of the protocol and applicable amendment(s), the study drug, CRF completion guidelines, copies of regulatory references, copies of IRB correspondence, and study drug accountability records should also be retained as part of the investigator's GCP documents. It is the investigator's responsibility to ensure that copies of all required GCP documents are organized, current, and available for inspection.

14.3.2 Case Report Forms

By signing the Investigator's Agreement, the investigator agrees to maintain accurate CRFs and source documentation as part of the case histories for all subjects who sign an informed consent form.

Case report forms are considered confidential documents and should be handled and stored accordingly. The sponsor or its designee will provide the necessary training on the use of the specific CRF system used during the study to ensure that the study information is captured accurately and appropriately.

To ensure data accuracy, CRF data for individual subject visits should be completed as soon as possible after the visit. All requested information must be entered in the CRF according to the completion guidelines provided by the sponsor or its designee.

The CRFs may be signed by the investigator or a sub-investigator. These signatures serve to attest that the information contained in the CRF is accurate and true.

14.3.3 Source Documents

All information recorded in the CRF must be supported by corresponding source documentation. Examples of acceptable source documentation include, but are not limited to, hospital records, clinic and office charts, laboratory notes, and recorded data from automated instruments, memoranda, and pharmacy dispensing records.

During the study, select CRF data may be used as original data collection tools as long as a description of this documentation process is maintained in the investigator's study files. Before the study starts, a list

identifying any data to be recorded directly on the CRFs (i.e., no prior written or electronic record of data) and considered to be source data will be provided.

Clinical laboratory data required by the protocol will be obtained by local labs. A paper copy of the laboratory results will be provided to the study site and should be retained with each subject's source data.

14.4 Data Quality Control

The sponsor and its designees will perform quality control checks on this clinical study.

14.4.1 Monitoring Procedures

The sponsor or designee will conduct site visits to monitor the study and ensure compliance with the protocol, GCP, and applicable regulations and guidelines. The assigned clinical research associate (CRA) will visit the investigator and study site at periodic intervals and maintain periodic communication. The investigator agrees to allow the CRA and other authorized sponsor personnel access. The CRA will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the investigator and staff. While on site, the CRA will review:

- Regulatory documents, directly comparing entries in the CRF with the source documents.
- Consenting procedures.
- AE procedures.
- Storage and accountability of study drug and study materials.

The CRA will ask for clarification or correction of any noted inconsistencies. Procedures for correcting CRFs are described in the study manual. As representatives of the sponsor, CRAs are responsible for notifying project management of any noted protocol deviations.

By signing the Investigator's Agreement, the investigator agrees to meet with the CRA during study site visits; to ensure that study staff is available to the CRA as needed; to provide the CRA access to all study documentation, to the clinical supplies dispensing and storage area; and to assist the monitors in their activities, if requested. Further, the investigator agrees to allow the sponsor or designee auditors or inspectors from regulatory agencies to review records and to assist the inspectors in their duties, if requested.

14.4.2 Data Management

The sponsor or designee will be responsible for activities associated with the data management of this study. The standard procedures for handling and processing records will be followed per GCP and the sponsor's or Contract Research Organization's (CRO) standard operating procedures. A comprehensive data management plan will be developed including a data management plan, database contents, annotated CRF, self-evident correction conventions, query contacts, and consistency checks.

Study site personnel will be responsible for providing resolutions to all data queries. The investigator will be required to document data review to ensure the accuracy of the corrected and/or clarified data. Procedures for soliciting and documenting resolution to data queries are described in the Data Management Plan.

14.4.3 Quality Assurance/Audit

This study may be subject to audit by the sponsor or designee. The audits may be undertaken to check compliance with GCP guidelines and may include:

- In-house study file audit.
- Audit of computer database quality control.
- Audit of clinical report quality control.

The sponsor or designee may conduct additional audits on a selection of study sites, requiring access to subject notes, study documentation, and facilities or laboratories used for the study.

The study site, facilities, all data (including source data), and documentation will be made available for audit by quality assurance auditors and for IRB or regulatory authorities according to GCP guidelines. The investigator agrees to cooperate with the auditor during the visit and will be available to supply the auditor with CRFs or other files necessary to conduct that audit. Any findings will be strictly confidential.

If a regulatory authority informs the investigator that it intends to conduct an inspection, the investigator shall notify the sponsor immediately.

14.5 Study Termination

The study may be terminated at the sponsor's discretion at any time and for any reason.

14.5.1 Regular Study Termination

The end of this study is defined as the date of the last visit of the last subject (last subject out or last subject last visit) participating in the study. Within 90 days of the end of the clinical study, the sponsor or designee will notify the IRB and regulatory authorities about the regular termination of the study as required.

14.5.2 Premature Study Termination

The study may be terminated prematurely for any reason and at any time by the sponsor, IRB, regulatory authorities, or the investigator. A decision to prematurely terminate the study is binding to all investigators of all study sites.

Within 15 days of premature termination of a clinical study, the sponsor or designee will notify the IRB and regulatory authorities as required. The sponsor or designee must clearly explain the reasons for premature termination.

If the study is terminated prematurely, the investigator must inform their subjects and take care of appropriate follow-up and further treatment of subjects to ensure protection of the subjects' interests. Study sites may be asked to have all subjects currently participating in the study complete all of the assessments for the Early Termination visit.

14.6 Study Site Closure

At the end of the study, study sites will be closed for participation. The sponsor may terminate participation of a study site at any time. Examples of conditions that may require premature termination of a study site include, but are not limited to, the following:

- Noncompliance with the protocol, applicable regulations, and guidelines, or both.
- Inadequate subject enrollment.

14.6.1 Record Retention

The investigator shall retain and preserve one copy of all data generated in the course of the study, specifically including, but not limited to, those defined by GCP as essential until at least 2 years after the notification of submission of the final study report to regulatory authorities by the sponsor.

These documents should be retained for a longer period, however, if required by the applicable regulatory requirement(s) or if needed by the sponsor.

At the end of such period, the investigator shall notify the sponsor in writing of his or her intent to destroy all such material. The sponsor shall have 30 days to respond to the investigator's notice, and the sponsor shall have a further opportunity to retain such materials at the sponsor's expense.

After completing the study, the sponsor will be provided with the original CRFs or at least a legible copy and retain the documents at least 5 years after the completion of the study.

One copy will remain with the investigator. The investigator shall arrange for the retention of the subject identification codes, subject files, and other source data until at least 5 years after notification of submission of the final study report to the regulatory authorities by the sponsor. These documents need to be retained for a longer period of time if required by applicable regulatory authorities or by agreement with the sponsor.

At the end of such period, the investigator shall notify the sponsor in writing of his or her intent to destroy all such material. The sponsor shall have 30 days to respond to the investigator's notice, and the sponsor shall have a further opportunity to retain such materials at the sponsor's expense.

Copies of these study records (and all study-related documents, including source data) shall be kept by the investigator for the maximum period of time permitted by the hospital, institution, or private practice.

14.6.2 Sample Retention

Samples may be used for purposes related to this research. The samples will be stored until the sponsor has determined that specimens are no longer needed and the decision has been made that none of the samples needs to be reanalyzed. In addition, identifiable samples can be destroyed at any time at the request of the subject.

14.7 Changes to the Protocol

This protocol cannot be altered or changed except through a formal protocol amendment, which requires the written approval by the sponsor. The protocol amendment must be signed by the investigator and approved by the IRB before it may be implemented. Protocol amendments will be filed with the appropriate regulatory agency.

14.8 Use of Information

All information about the study, the sponsor's operations, patent applications, formulas, manufacturing processes, basic scientific data, and formulation information supplied by the sponsor or designee to the investigator and not previously published, is considered confidential and remains the sole property of the sponsor. Case report forms also remain the property of the sponsor. The investigator agrees to use this information for purposes of study execution through finalization and will not use it for other purposes without the written consent of the sponsor.

The information developed in this study will be used by the sponsor in connection with the continued development of the study biomarker and thus may be disclosed as required to other clinical investigators or government regulatory agencies.

15 FINAL CLINICAL STUDY REPORT

The final study report will be written according to the "Guideline for Industry (Structure and Content of Clinical Study Reports)" from the International Conference on Harmonisation (ICH) E3. The final study report will present a narrative description of the clinical, analytical, pharmacokinetic, and statistical results. Tables and figures will be "integrated" into the main text, with appendices at the end of the report (e.g., the protocol, sample CRFs, investigator-related information, test/reference product information, subject data listings).

The final clinical study report will be submitted to the sponsor.

16 ETHICAL AND LEGAL CONSIDERATIONS

16.1 Declaration of Helsinki and Good Clinical Practice

This study will be conducted in compliance with the November 2016 ICH Guidance for Industry E6(R2) GCP and the 1996 Version of the Declaration of Helsinki.

16.2 Subject Information and Informed Consent

A properly constituted, valid IRB must review and approve the protocol, the investigator's informed consent document, and related subject information and recruitment materials before the start of the study.

It is the responsibility of the investigator to ensure that informed consent has been obtained from the subject before any activity or procedure is undertaken that is not part of routine care.

16.3 Approval by Institutional Review Board

A valid IRB must review and approve this protocol before study initiation. Written notification of approval is to be submitted by the investigator to the sponsor monitor before shipment of investigational drug supplies and will include the date of the committee's approval and the chairperson's signature. This written approval must consist of a completed sponsor IRB Approval Form or written documentation from the IRB containing the same information.

Until written approval by the IRB has been received by the investigator, no subject may undergo any procedure solely for determining eligibility for this study.

Protocol amendments must also be reviewed and approved by the IRB. Written approval from the IRB, or a designee, must be received by the sponsor before implementation. This written approval will consist of a completed IRB Approval form or written documentation from the IRB containing the same information.

17 REFERENCES

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18 APPENDICES

- 18.1 Akorn package insert
- 18.2 PBMC preparation
- 18.3 Instructions for sample preparation and shipment of samples to C2N Diagnostics for analysis of biomarkers in the PrecivityAD battery.

18.1 ICG Package Insert

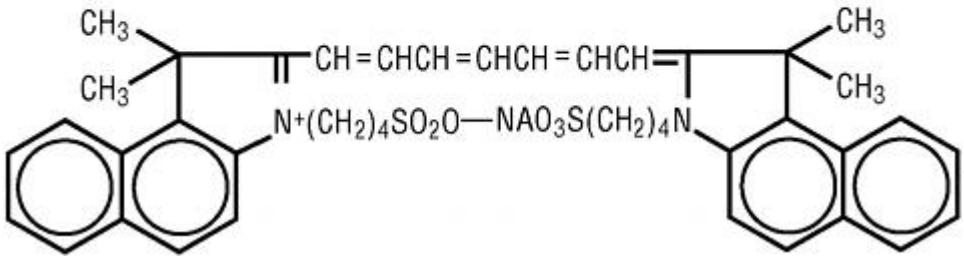
IC-GREEN™

(Indocyanine Green for Injection, USP)
Sterile

Description: IC-GREEN™ is a sterile, lyophilized green powder containing 25 mg of indocyanine green with no more than 5 % sodium iodide. It is packaged with an Aqueous Solvent consisting of Sterile Water for Injection used to dissolve the indocyanine green. IC-GREEN™ is to be administered intravenously.

Idocyanine green is a water soluble, tricarbocyanine dye with peak spectral absorption at 800 nm. The chemical name for Indocyanine Green is 1 H-Benz[e]indolium,2-[7[1,3-dihydro-1,1-dimethyl-3-(-4-sulfobutyl)-2H-benz[e]indo-2-ylidene]-1,3,5-heptatrienyl]-1,1-dimethyl-3-(4-sulfobutyl)-,hydroxide, innersalt, sodium. 2-[7-[1,1-Dimethyl-3-(4-sulfobuttyl)benz[e]indolin-2-ylidene]-1,3,5-heptatrienyl]-1,1-dimethyl-3-(4-sulfobutyl)-1Hbenz[e]indolium hydroxide , inner salt, sodium salt. IC-GREEN™ has a pH pf approximately 6.5 when reconstituted. Each vial of IC-GREEN™ contains 25 mg of indocyanine green as a sterile lyophilized powder.

$C_{43}H_{47}N_2NaO_6S_2$ Molecular Weight 774.96



Clinical Pharmacology: Following intravenous injection, IC-GREEN™ is rapidly bound to plasma protein, of which albumin is the principle carrier (95%). IC-GREEN™ undergoes no significant extrahepatic or enterohepatic circulation; simultaneous arterial and venous blood estimations have shown negligible renal, peripheral, lung or cerebro-spinal uptake of the dye. IC-GREEN™ is taken up from the plasma almost exclusively by the hepatic parenchymal cells and is secreted entirely into the bile. After biliary obstruction, the dye appears in the hepatic lymph, independently of the bile, suggesting that the biliary mucosa is sufficiently intact to prevent diffusion of the dye, though allowing diffusion of bilirubin. These characteristics make IC-GREEN™ a helpful index of hepatic function.

The plasma fractional disappearance rate at the recommended 0.5 mg/kg dose has been reported to be significantly greater in women than in men, although there was no significant difference in the calculated value for clearance.

Indications and Usage: For determining cardiac output, hepatic function and liver blood flow, and for ophthalmic angiography.

Contraindications: IC-GREEN™ contains sodium iodide and should be used with caution in patients who have a history of allergy to iodides.

Warnings: Anaphylactic deaths have been reported following IC-GREEN™ administration during cardiac catheterization.

Precautions: General: IC-GREEN™ Powder and Solution: IC-GREEN™ is unstable in aqueous solution and must be used within 6 hours. However, the dye is stable in plasma and whole blood so that samples obtained in discontinuous sampling techniques may be read hours later. Sterile techniques should be used in handling the dye solution as well as in the performance of the dilution curves.

IC-GREEN™ (indocyanine green for injection) powder may cling to the vial or lump together because it is freeze-dried in the vials.

Drug Interactions: Heparin preparations containing sodium bisulfate reduce the absorption peak of IC-GREEN™ in blood and, therefore, should not be used as an anticoagulant for the collection of samples for analysis.

Drug/Laboratory Test Interactions: Radioactive iodine uptake studies should not be performed for at least a week following the use of IC-GREEN™.

Carcinogenesis, Mutagenesis, Impairment of Fertility: No studies have been performed to evaluate the carcinogenicity, mutagenicity, or impairment of fertility.

Pregnancy: Teratogenic Effects: Pregnancy Category C: Animal Reproduction studies have not been conducted with IC-GREEN™. It is also not known whether IC-GREEN™ can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. IC-GREEN™ should be given to a pregnant woman only if clearly indicated.

Nursing Mothers: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when IC-GREEN™ is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in pediatric patients have been established.

Adverse Reactions: Anaphylactic or urticarial reactions have been reported in patients with or without history of allergy to iodides. If such reactions occur, treatment with the appropriate agents, e.g., epinephrine, antihistamines, and corticosteroids should be administered.

Overdosage: There are no data available describing the signs, symptoms, or laboratory findings accompanying overdosage. The LD_{50} after I.V. administration ranges between 60 and 80 mg/kg in mice, 50 and 70 mg/kg in rats and 50 and 80 mg/kg in rabbits.

Dosage and Administration: *INDICATOR-DILUTION STUDIES:* IC-GREEN™ permits recording of the indicator-dilution curves for both diagnostic and research purpose independently of fluctuations in oxygen saturation. In the performance of dye dilution curves, a known amount of dye is usually injected as a single bolus as rapidly as possible via a cardiac catheter into selected sites in the vascular system. A recording instrument (oximeter or densitometer) is attached to a needle or catheter for sampling of the dye-blood mixture from a systemic arterial sampling site.

Under sterile conditions, the IC-GREEN™ powder should be dissolved with the Aqueous Solvent provided for this product, and the solution used within 6 hours after it is prepared. If a precipitate is

present, discard the solution. The amount of solvent to be used can be calculated from the dosage form which follows. It is recommended that the syringe used for injection of the dye be rinsed with this diluent. Saline is used in all other parts of the catheterization procedure.

This matter of rinsing the dye syringe with distilled water may not be critical, since it is known that an amount of sodium chloride sufficient to make an isotonic solution may be added to dye *that has first been dissolved in distilled water.* This procedure has been used for constant-rate injection techniques without precipitation of the dye.

The usual doses of IC-GREEN™ which have been used for dilution curves are as follows:

Adults -5.0 mg

Children -2.5 mg

Infants -1.25 mg

These doses of the dye are usually injected in a mL volume. An average of five dilution curves are required in the performance of a diagnostic cardiac catheterization. The total dose of dye injected should be kept below 2 mg/kg.

Calibrating Dye Curves: To quantitate the dilution curves, standard dilutions of IC-GREEN™ in whole blood are made as follows. It is strongly recommended that the same dye that was used for the injections be used in the preparation of these standard dilutions. The most concentrated dye solution is made by accurately diluting 1 mL of the 5 mg/ mL dye with 7 mL of distilled water. This concentration is then successively halved by diluting 4 mL of the previous concentration with 4 mL of distilled water. (If a 2.5 mg/ mL concentration was used for the dilution curves, 1 mL of the 2.5 mg/ mL dye is added to 3 mL of distilled water to make the most concentrated "standard" solution. This concentration is then successively halved by diluting 2 mL of the previous concentration with 2 mL of distilled water.) Then 0.2 mL portions (accurately measured from a calibrated syringe) of these dye solutions are added to 5 mL aliquots of the subject's blood, giving final concentrations of the dye in blood beginning with 24.0 mg/liter, approximately (actual concentration depends on the exact volume of dye added). This concentration is, of course, successively halved in the succeeding aliquots of the subject's blood. These aliquots of blood containing known amounts of dye, as well as a blank sample of which

0.2 mL of saline containing no dye has been added, are then passed through the detecting instrument and a calibration curve is constructed from the deflections recorded.

HEPATIC FUNCTION STUDIES: Due to its absorption spectrum, changing concentrations of IC-GREEN™ (indocyanine green for injection) in the blood can be monitored by ear densitometry or by obtaining blood specimens at timed intervals. The technique for both methods is as follows.

The patient should be studied in a fasting, basal state. The patient should be weighed and the dosage calculated on the basis of 0.5 mg/kg of body weight.

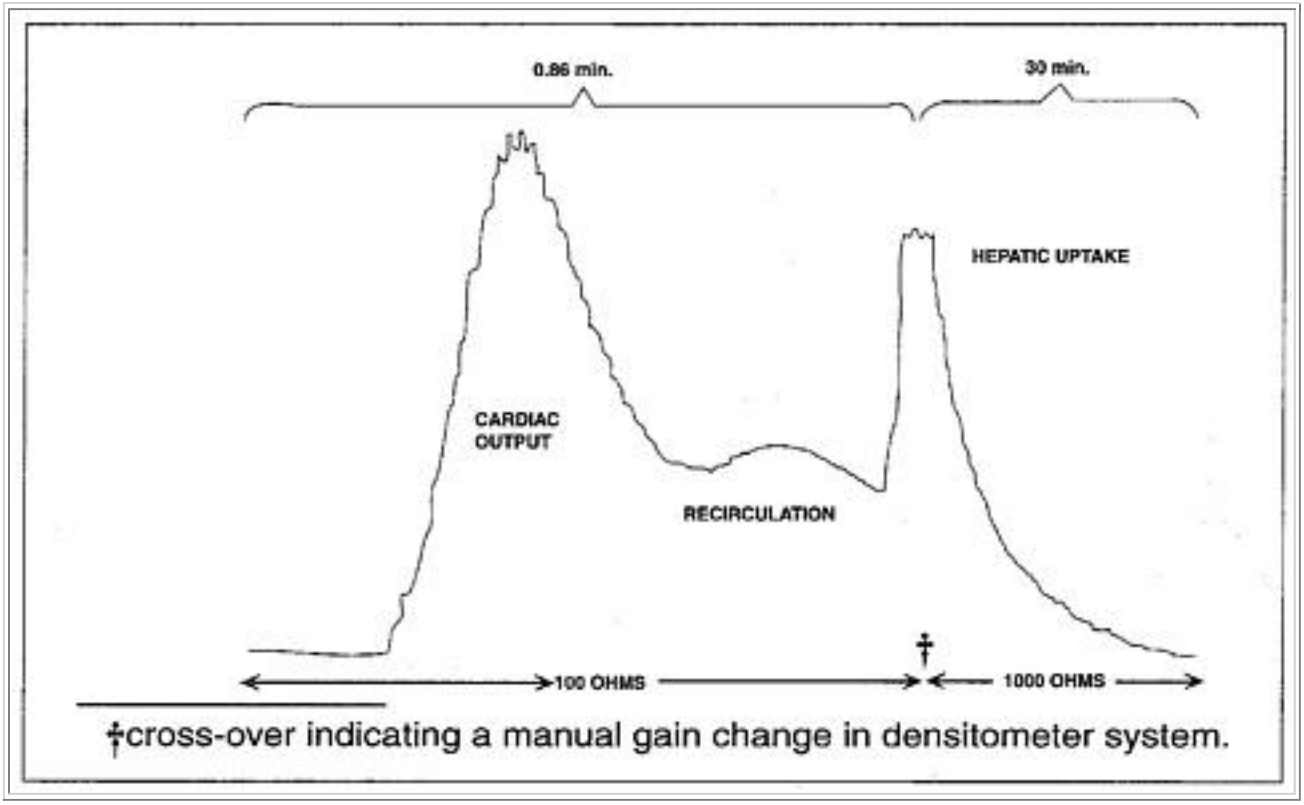
Under sterile conditions, the IC-GREEN™ powder should be dissolved with the Aqueous Solvent provided. Exactly 5 mL of aqueous solvent should be added to the 25 mg vial giving 5 mg of dye per mL of solution.

Inject the correct amount of dye into the lumen of an arm vein as rapidly as possible, without allowing the dye to escape outside the vein. (If *the photometric method is used, prior to injecting IC-GREEN™,*

withdraw 6 mL of venous blood from the patient's arm for serum blank and standard curve construction, and through the same needle, inject the correct amount of dye)

Ear Densitometry: Ear oximetry has also been used and makes it possible to monitor the appearance and disappearance of IC-GREEN™ without the necessity of withdrawal and spectrophotometric analysis of blood samples for calibration. An ear densitometer which has a compensatory photo-electric cell to correct for changes in blood volume and hematocrit, and a detection photocell which registers levels has been described. This device permits simultaneous measurement of cardiac output, blood volume and hepatic clearance of IC-GREEN™* and was found to provide a reliable index of plasma removal kinetics after single injections or continuous intrusions of IC-GREEN™. This technique was employed in newborn infants, healthy adults and in children and adults with liver disease. The normal subject has a removal rate of 18-24% per minute. Due to the absence of extra-hepatic removal, IC-GREEN™ was found to be ideally suited for serial study of severe chronic liver disease and to provide a stable measurement of hepatic blood flow. In larger doses, IC-GREEN™ has proven to be particularly valuable in detecting drug-induced alterations of hepatic function and in the detection of mild liver injury.

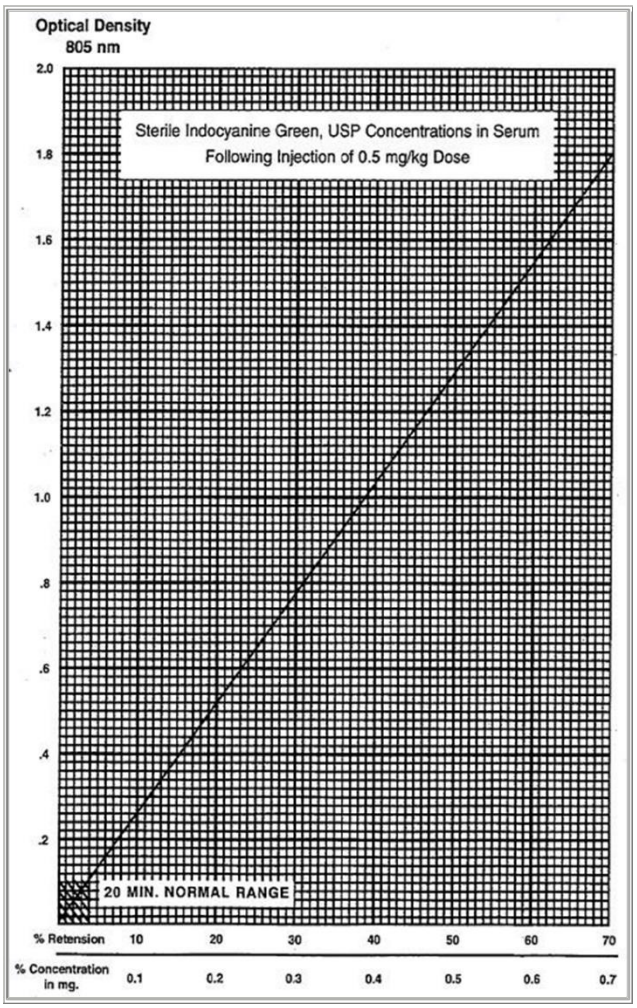
Using the ear densitometer, a dosage of 0.5 mg/kg in normal subjects gives the following clearance pattern.



Photometric Method-

Determination Using Percentage Retention of Dye:

A typical curve obtained by plotting dye concentration versus optical density is shown below. Percent retention can be read from this plot.



∗ Dichromatic earpiece densitometer supplied by The Waters Company, Rochester, Minnesota.

If more accurate results are desired, a curve using the patient's blood and the vial of IC-GREEN™ being used in the determination can be constructed as follows:

1. Take 6 mL of non-dye-containing venous blood from the patient's arm. Place in a test tube and allow the blood to clot. The serum is separated by centrifugation.
2. Pipette 1 mLof the serum into a microcuvette.
3. Add 1 lambda ((lambda)) of the 5 mg/mL aqueous IC-GREEN™ (sterile indocyanine green) solution to the serum, giving a dilution of 5 mg/liter, the standard for 50% retention. (The addition of 2 lambda (lambda) of the 5 mg/mL IC-GREEN™ solution would give 100% retention; however, this concentration cannot be read on the spectrophotometer.)
4. The optical density of this solution is read at 805 nm, using normal serum as the blank.
5. Plot the 50% figure obtained in Step 4, and draw a line connecting this point with the zero coordinates.

Percentage Retention: A single 20-minute sample (withdrawn from a vein in the opposite arm to that injected) is allowed to clot, centrifuged and its optical density is determined at 805 nm using the patient's

normal serum as the blank. Dye concentration is read from the curve above. A single 20- minute sample of serum in healthy subjects should contain no more than 4% of the initial concentration of the dye. The use of percentage retention is less accurate than percentage disappearance rate, but provides reproducible results. Hemolysis does not interfere with a reading.

Determination Using Disappearance Rate of Dye: To calculate the percentage disappearance rate, obtain samples at 5, 10, 15 and 20 minutes after injecting the dye. Prepare the sample as in the previous section and measure the optical densities at 805 nm, using the patient's normal serum as the blank. The IC-GREEN™ concentration in each timed specimen can be determined by using the concentration curve illustrated. Plot values on semilogarithmic paper.

Specimens containing IC-GREEN™ should be read at the same temperature since its optical density is influenced by temperature variations.

Normal Values: Percentage disappearance rate in healthy subjects is 18-24% per minute. Normal biological half-time is 2.5-3.0 minutes.

OPHTHALMIC ANGIOGRAPHY STUDIES: The excitation and emission spectra (Figure 1) and the absorption spectra (Figure 2) of IC-GREEN™ make it useful in ophthalmic angiography. The peak absorption and emission of IC-GREEN™ lie in a region (800-850 nm) where transmission of energy by the pigment epithelium is more efficient than in the region of visible light energy. IC-GREEN™ also has the property of being nearly 98% bound to blood protein, and therefore, excessive dye extravasation does not take place in the highly fenestrated choroidal vasculature It is, therefore, useful in both absorption and fluorescence infrared angiography of the choroidal vasculature when using appropriate filters and film in a fundus camera.

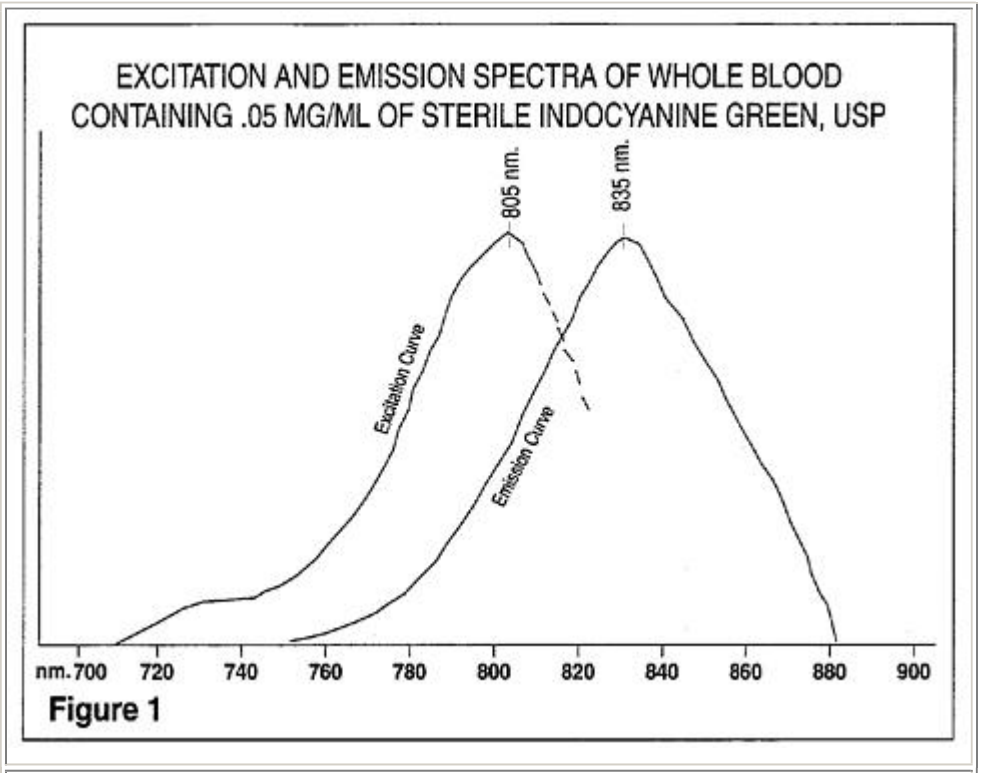


Figure 1

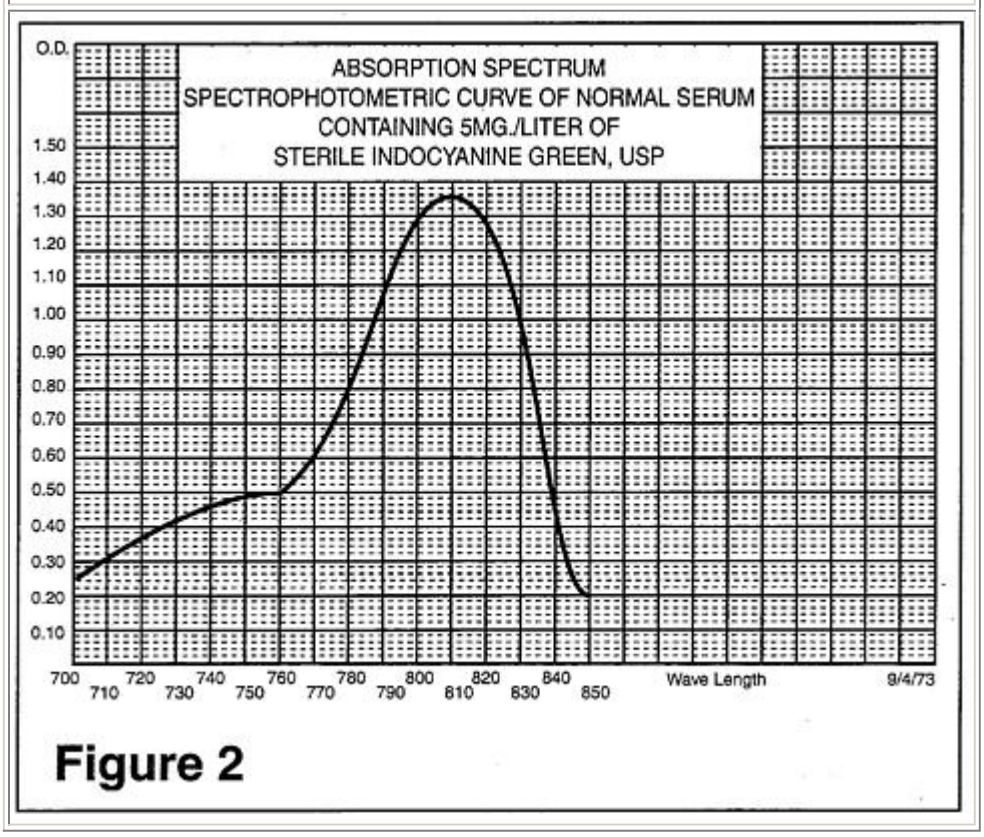


Figure 2

Dosages up to 40 mg IC-GREEN™ dye in 2 mL of aqueous solvent have been found to give optimal angiograms, depending on the imaging equipment and technique used. The antecubital vein injected IC-GREEN™ dye bolus should immediately be followed by a 5 mL bolus of normal saline.

Clinically, angiograms of uniformly good quality can be assured only after taking care to optimize the contributions of all possible factors such as, patient cooperation and dye injection. The foregoing injection regimen is designed to provide delivery of a spatially limited dye bolus of optimal concentration to the choroidal vasculature following intravenous injection.

How Supplied: IC-GREEN™ is supplied in a kit (NDC 17478-701-02) containing six 25 mg IC- GREEN™ vials and six 10mL Aqueous Solvent ampules:

NDC 17478-701-25 IC-Green vial. 25 mg fill in 50 mL vial.

NDC 17478-701-10 Aqueous Solvent ampule. 10 mL fill in 10 mL ampule.

Storage: Store at 15° to 25° C (59° to 77°). Rx Only

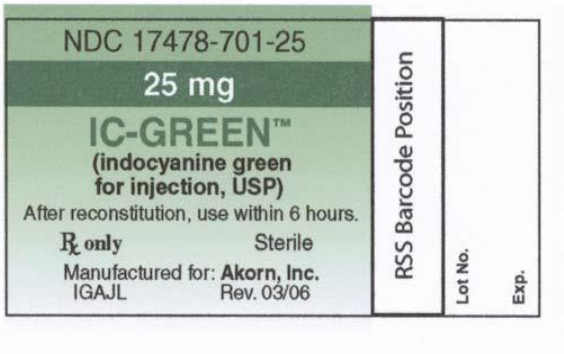
Manufactured for:

Akorn, Inc Buffalo Grove, IL 60089

IGGON Rev 03/06

PROPOSED CONTAINER LABEL FOR IC-GREEN

(not actual size)



PROPOSED KIT CARTON LABEL FOR IC-GREEN

(not actual size)

Indocyanine Green for Injection USP

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use INDOCYANINE GREEN for injection safely and effectively. See full prescribing information for INDOCYANINE GREEN for injection.

INDOCYANINE GREEN for injection, for intravenous injection

Initial U.S. Approval: 1959

INDICATIONS AND USAGE

Indocyanine Green for injection a tricarbocyanine dye, is indicated:

- For determining cardiac output, hepatic function and liver blood flow. (1.1)
- For ophthalmic angiography. (1.2)

DOSAGE AND ADMINISTRATION

Indicator-Dilution Studies. (2.1)
Under sterile conditions, the Indocyanine Green for injection powder should be dissolved with the Sterile Water for injection provided and the solution used within 6 hours after it is prepared. The usual doses of Indocyanine Green for injection for dilution curves are: Adults 5.0 mg, Children - 2.5 mg, and Infants - 1.25 mg.

Hepatic Function Studies. (2.2)
Under sterile conditions, the Indocyanine Green for injection powder should be dissolved with the Sterile Water for injection provided. The patient should be weighed and the dosage calculated on the basis of 0.5 mg/kg of body weight. Exactly 5 mL of Sterile Water for injection should be added to the 25 mg vial giving 5 mg of dye per mL of solution.

Ophthalmic Angiography Studies. (2.3)
Dosages up to 40 mg Indocyanine Green for injection dye in 2 mL of Sterile Water for injection should be administered. A 5 mL bolus of normal saline should immediately follow the injection of the dye.

DOSAGE FORMS AND STRENGTHS

Indocyanine Green for injection is a sterile, lyophilized green powder containing 25 mg of indocyanine green with no more than 5% sodium iodide. (3)

CONTRAINDICATIONS

Indocyanine Green for injection contains sodium iodide and should be used with caution in patients who have a history of allergy to iodides because of the risk of anaphylaxis. (4)

WARNINGS AND PRECAUTIONS

- Deaths due to anaphylaxis have been reported following Indocyanine Green for injection administration during cardiac catheterization. (5.1)
- Indocyanine Green for injection is unstable in aqueous solution and must be used within 6 hours. (5.2)
- Radioactive iodine uptake studies should not be performed for at least a week following the use of Indocyanine Green for injection. (5.3)

ADVERSE REACTIONS

Most common adverse reactions are anaphylactic or urticarial reactions. These have been reported in patients with and without a history of allergy to iodides. (6)

To report SUSPECTED ADVERSE REACTIONS, contact Diagnostic Green LLC at 1-844-424-3784 (1-844-ICG-DRUG) or e-mail: drugsafety@diagnosticgreen.com; or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

DRUG INTERACTIONS

Products containing sodium bisulfite reduce the absorption peak of Indocyanine Green for injection in blood. (7)

Revised: 04/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

Indocyanine Green for injection is indicated:

1.1 For Determining Cardiac Output, Hepatic Function and Liver Blood Flow

1.2 For ophthalmic angiography

2 DOSAGE AND ADMINISTRATION

2.1 Indicator-Dilution Studies

In the performance of dye dilution curves, a known amount of dye is injected as a single bolus as rapidly as possible via a cardiac catheter into selected sites in the vascular system. A recording instrument (oximeter or densitometer) is attached to a needle or catheter for sampling of the dye-blood mixture from a systemic arterial sampling site.

Under sterile conditions, the Indocyanine Green for injection powder should be dissolved with the Sterile Water for injection provided for this product, and the solution used within 6 hours after it is prepared. If a precipitate is present, discard the solution.

The usual doses of Indocyanine Green for injection for dilution curves are as follows:

Adults - 5.0 mg
Children - 2.5 mg
Infants - 1.25 mg

These doses of the dye are usually injected in 1 mL volume. An average of five dilution curves are recommended in the performance of a diagnostic cardiac catheterization. The total dose of dye injected should be kept below 2 mg/kg.

While sterile water for injection may be used to rinse the syringe, isotonic saline should be used to flush the residual dye from the cardiac catheter into the circulation so as to avoid hemolysis. With the exception of the rinsing of the dye injection syringe, saline should be used in all other parts of the catheterization procedure.

Calibrating Dye Curves: To quantitate the dilution curves, standard dilutions of Indocyanine Green for injection in whole blood are made as follows. It is strongly recommended that the same dye that was used for the injections be used in the preparation of these standard dilutions. The most concentrated dye solution is made by accurately diluting 1 mL of the 5 mg/mL dye with 7 mL of distilled water. This concentration is then successively halved by diluting 4 mL of the previous concentration with 4 mL of distilled water.

If a 2.5 mg/mL concentration was used for the dilution curves, 1 mL of the 2.5 mg/mL dye is added to 3 mL of distilled water to make the most concentrated "standard" solution. This concentration is then successively halved by diluting 2 mL of the previous concentration with 2 mL of distilled water.

Then 0.2 mL portions (accurately measured from a calibrated syringe) of these dye solutions are added to 5 mL aliquots of the subject's blood, giving final concentrations of the dye in blood beginning with 24.0 mg/liter, approximately (actual concentration depends on the exact volume of dye added). This concentration is, of course, successively halved in the succeeding aliquots of the subject's blood. These aliquots of blood containing known amounts of dye, as well as a blank sample to which 0.2 mL of saline containing no dye has been added, are then passed through the detecting instrument and a calibration curve is constructed from the deflections recorded.

2.2 Hepatic Function Studies

Due to its absorption spectrum, changing concentrations of Indocyanine Green for injection in the blood can be monitored by ear densitometry or by obtaining blood specimens at timed intervals. The technique for both methods is as follows.

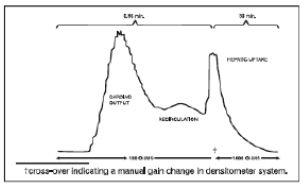
The patient should be studied in a fasting, basal state. The patient should be weighed and the dosage calculated on the basis of 0.5 mg/kg of body weight.

Under sterile conditions, the Indocyanine Green for injection powder should be dissolved with the Sterile Water for injection provided. Exactly 5 mL of Sterile Water for injection should be added to the 25 mg vial giving 5 mg of dye per mL of solution.

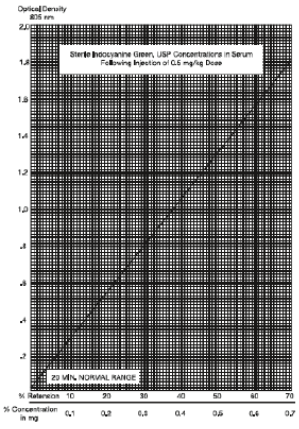
Inject the calculated amount of dye (0.5 mg/kg of body weight) into the lumen of an arm vein as rapidly as possible, without allowing the dye to escape outside the vein. *(If the photometric method is used, prior to injecting Indocyanine Green for injection, withdraw 6 mL of venous blood from the patient's arm for serum blank and standard curve construction, and through the same needle, inject the correct amount of dye.)*

Ear Densitometry: Ear oximetry has also been used and makes it possible to monitor the appearance and disappearance of Indocyanine Green for injection without the necessity of withdrawal and spectrophotometric analysis of blood samples for calibration. An ear densitometer which has a compensatory photo-electric cell to correct for changes in blood volume and hematocrit, and a detection photo cell which registers levels should be used. This device permits simultaneous measurement of cardiac output, blood volume and hepatic clearance of Indocyanine Green for injection*. This technique has been employed in newborn infants, healthy adults and in children and adults with liver disease. The normal subject has a removal rate of 18 to 24% per minute. Due to the absence of extra-hepatic removal, Indocyanine Green for injection was found to be suited for serial study of severe chronic liver disease and to provide a stable measurement of hepatic blood flow. In larger doses, Indocyanine Green for injection can be used in detecting drug-induced alterations of hepatic function and in the detection of mild liver injury.

Using the ear densitometer, a dosage of 0.5 mg/kg in normal subjects gives the following clearance pattern.



Cross-over indicating a manual gain change in densitometer system.



*Dichromatic earpiece densitometer supplied by The Waters Company, Rochester, Minnesota.

Photometric Method -

Determination Using Percentage Retention of Dye:

A typical curve obtained by plotting dye concentration versus optical density is shown. The percent retention can be read from this plot. If more accurate results are desired, a curve using the patient's blood and the vial of Indocyanine Green for injection being used in the determination can be constructed as follows:

1. Take 6 mL of non-dye-containing venous blood from the patient's arm. Place in a test tube and allow the blood to clot. The serum should be separated by centrifugation.
2. Pipette 1 mL of the serum into a microcuvette.

3. Add 1 lambda (λ) of the 5 mg/mL aqueous Indocyanine Green for injection (sterile indocyanine green) solution to the serum, giving a dilution of 5 mg/liter, the standard for 50% retention. (The addition of 2 lambda (λ) of the 5 mg/mL Indocyanine Green for injection solution would give 100% retention; however, this concentration cannot be read on the spectrophotometer.)
4. The optical density of this solution should be read at 805 nm, using normal serum as the blank.
5. Using graph paper similar to that used in the illustration, plot the 50% figure obtained in Step 4, and draw a line connecting this point with the zero coordinates.

Percentage Retention: A single 20-minute sample (withdrawn from a vein in the opposite arm to that injected) should be collected and allowed to clot, centrifuged and its optical density determined at 805 nm using the patient's normal serum as the blank. The dye concentration can be read from the curve above. A single 20-minute sample of serum in healthy subjects should contain no more than 4% of the initial concentration of the dye. The use of percentage retention is less accurate than percentage disappearance rate. Hemolysis is not expected to interfere with a reading.

Determination Using Disappearance Rate of Dye: To calculate the percentage disappearance rate, obtain samples at 5, 10, 15 and 20 minutes after injecting the dye. Prepare the sample as in the previous section and measure the optical densities at 805 nm, using the patient's normal serum as the blank. The Indocyanine Green for injection concentration in each timed specimen should be determined by using the concentration curve illustrated. Values should be plotted on semilogarithmic paper.

Specimens containing Indocyanine Green for injection should be read at the same temperature since its optical density is influenced by temperature variations.

Normal Values: Percentage disappearance rate in healthy subjects is 18 to 24% per minute. Normal biological half-time is 2.5 to 3.0 minutes.

2.3 Ophthalmic Angiography Studies

The excitation and emission spectra (Figure 1) and the absorption spectra (Figure 2) of Indocyanine Green for injection make it useful in ophthalmic angiography.

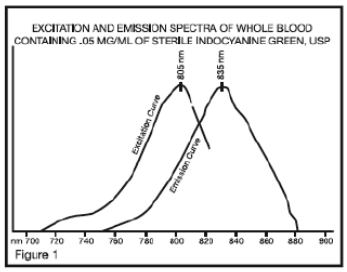


Figure 1

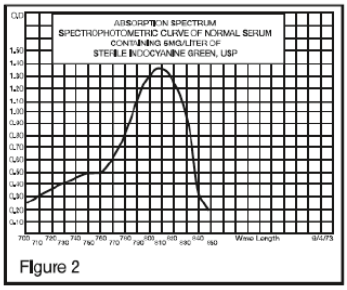


Figure 2

8.3 Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Indocyanine Green for injection is administered to a nursing woman.

8.4 Pediatric Use

Safety and effectiveness in pediatric patients have been established. See DOSAGE AND ADMINISTRATION (2) for specific dosing information in pediatric patients.

8.5 Geriatric Use

No overall differences in safety or effectiveness have been observed between elderly and younger patients.

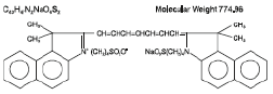
10 OVERDOSAGE

There are no data available describing the signs, symptoms, or laboratory findings accompanying overdosage. The LD50 after intravenous administration ranges between 60 and 80 mg/kg in mice, 50 and 70 mg/kg in rats and 50 and 80 mg/kg in rabbits. Based on body surface area, these doses are 2.4 to 13-fold the maximum recommended human (MRHD) dose of 2 mg/kg for indicator-dilution studies, 10 to 52-fold the MRHD of 0.5 mg/kg for hepatic-function studies, and 7 to 39-fold the MRHD of 0.67 mg/kg for ophthalmic angiography studies.

11 DESCRIPTION

Indocyanine Green for injection USP is a sterile, lyophilized green powder containing 25 mg of indocyanine green with no more than 5% sodium iodide. It is packaged with Sterile Water for injection, USP used to dissolve the indocyanine green. Indocyanine Green for injection USP is to be administered intravenously.

Indocyanine green is a water soluble, tricarbocyanine dye with a peak spectral absorption at 800 nm. The chemical name for Indocyanine Green is 1 *H*-Benz[*e*]indolium, 2-[7-[1,3-dihydro-1,1-dimethyl-3-(4-sulfobutyl)-2*H*-benz[*e*] indol-2-ylidene]-1,3,5-heptatrienyl]-1,1-dimethyl-3-(4-sulfobutyl)-,hydroxide, inner salt, sodium salt. Indocyanine Green for injection USP has a pH of approximately 6.5 when reconstituted. Each vial of Indocyanine Green for injection USP contains 25 mg of indocyanine green as a sterile lyophilized powder.



12 CLINICAL PHARMACOLOGY

Indocyanine Green for injection permits recording of the indicator-dilution curves for both diagnostic and research purposes independently of fluctuations in oxygen saturation. Following intravenous injection, Indocyanine Green for injection is rapidly bound to plasma protein, of which albumin is the principle carrier (95%). Indocyanine Green for injection undergoes no significant extrahepatic or enterohepatic circulation; simultaneous arterial and venous blood estimations have shown negligible renal, peripheral, lung or cerebro-spinal uptake of the dye. Indocyanine Green for injection is taken up from the plasma almost exclusively by the hepatic parenchymal cells and is secreted entirely into the bile. After biliary obstruction, the dye appears in the hepatic lymph, independently of the bile, suggesting that the biliary mucosa is sufficiently intact to prevent diffusion of the dye, though allowing diffusion of bilirubin. These characteristics make Indocyanine Green for injection a helpful index of hepatic function.

Dosages up to 40 mg Indocyanine Green for injection dye in 2 mL of Sterile Water for injection should be used, depending on the imaging equipment and technique used. The antecubital vein can be injected with an Indocyanine Green for injection dye bolus and should immediately be followed by a 5 mL bolus of normal saline.

3 DOSAGE FORMS AND STRENGTHS

Indocyanine Green for injection, USP is a sterile, lyophilized green powder containing 25 mg of indocyanine green with no more than 5% sodium iodide.

4 CONTRAINDICATIONS

Indocyanine Green for injection contains sodium iodide and should be used with caution in patients who have a history of allergy to iodides because of the risk of anaphylaxis.

5 WARNINGS AND PRECAUTIONS

5.1 Anaphylaxis

Deaths from anaphylaxis have been reported following Indocyanine Green for injection administration during cardiac catheterization.

5.2 Drug Instability

Indocyanine Green for injection is unstable in aqueous solution and must be used within 6 hours. However, the dye is stable in plasma and whole blood so that samples obtained in discontinuous sampling techniques may be read hours later. Sterile techniques should be used in handling the dye solution as well as in the performance of the dilution curves. If a precipitate is present, discard the solution.

5.3 Drug/Laboratory Test Interactions

Radioactive iodine uptake studies should not be performed for at least a week following the use of Indocyanine Green for injection.

6 ADVERSE REACTIONS

Anaphylactic or urticarial reactions have been reported in patients with or without history of allergy to iodides. If such reactions occur, treat with the appropriate agents, e.g., epinephrine, antihistamines, and corticosteroids.

7 DRUG INTERACTIONS

Preparations containing sodium bisulfite, including some heparin products reduce the absorption peak of Indocyanine Green for injection in blood and, therefore, should not be used as an anticoagulant for the collection of samples for analysis.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Animal reproduction studies have not been conducted with Indocyanine Green for injection. It is also not known whether Indocyanine Green for injection can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Indocyanine Green for injection should be given to a pregnant woman only if clearly indicated.

The peak absorption and emission of Indocyanine Green for injection lie in a region (800 to 850 nm) where transmission of energy by the pigment epithelium is more efficient than in the region of visible light energy. Indocyanine Green for injection also has the property of being nearly 98% bound to blood protein, and therefore, excessive dye extravasation does not take place in the highly fenestrated choroidal vasculature. It is, therefore, useful in both absorption and fluorescence infrared angiography of the choroidal vasculature when using appropriate filters and film in a fundus camera.

The plasma fractional disappearance rate at the recommended 0.5 mg/kg dose has been reported to be significantly greater in women than in men, although there was no significant difference in the calculated value for clearance.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No studies have been performed to evaluate the carcinogenicity, mutagenicity, or impairment of fertility.

16 HOW SUPPLIED/STORAGE AND HANDLING

Indocyanine Green for injection USP is supplied in a kit (NDC 70100-424-02) containing six 25 mg Indocyanine Green for injection USP vials and six 10 mL Sterile Water for injection, USP plastic vials:

NDC 70100-424-01 Indocyanine Green for injection USP vial. 25 mg fill in 25 mL vial.

NDC 63323-185-10 (or NDC 0409-4887-17) Sterile Water for injection, USP, 10 mL fill in 10 mL plastic vials.

Each combination of Indocyanine Green for injection USP vial and Sterile Water for injection, USP plastic vial is intended for single-patient-use.

STORAGE: Store at 20° to 25°C (68° to 77°F).

Manufactured by:
Patheon Italia S.p.A.
20900 Monza (MB), ITALY

Distributed by:
Diagnostic Green LLC
Farmington Hills, MI 48331 USA

Sterile Water for injection, USP is manufactured by:
Fresenius Kabi USA, LLC
Grand Island, NY 14072 USA
or
Hospira, Inc.
Rocky Mount, NC 27804 USA

50426

18.2 Recommended Procedure for isolation and preservation of human PBMCs for determination of ICG uptake

Background. Indocyanine green (ICG) is a near infrared dye commonly used for imaging vascular beds in various clinical settings. It has been demonstrated in mice in vivo and in isolated human cells in vitro that with prolonged exposure, phagocytic cells take up ICG and sequester the dye for long periods. This allows for tracking of these ICG-labeled cells into different compartments including brain by near infrared spectroscopy (NIRS). The goal of the present clinical trial is to determine 1) if a prolonged intravenous infusion of ICG in humans is sufficient to label phagocytic cells and then 2) to determine if these labeled cells can be tracked into the brain of Alzheimer's disease patients using NIRS. PBMCs will be the used to measure ICG labeling of cells. The PBMC cell fraction contains a population of phagocytic cells, and these are readily detected using the protocol described here developed by MindImmune in studies using mice. PBMCs are also advantageous because they are readily and simply isolated from blood samples and are abundant.

Technical considerations. In the course of preclinical proof of concept studies, MindImmune has found that it is critical to completely eliminate any plasma contamination from the isolated PBMC preparation because residual plasma ICG can skew cellular ICG quantitation. To minimize plasma contamination, freshly isolated, well-washed PBMCs are plated in 24-well tissue culture plates and allowed to adhere before final washing and fixation.

Briefly:

1. PBMCs are isolated from blood samples using SepMate-50 tubes and Lymphoprep CellMat density gradient medium according to standard protocols as recommended by the manufacturer
2. Isolated PBMCs are washed by repeated dilution in Phosphate Buffered Saline with 2% Fetal Bovine Serum (PBS – 2%FBS) and recollection by centrifugation
3. Washed PBMCs are counted, and a fixed number of cells are distributed into a 24-well tissue culture plate and incubated for 1.5 hours to allow the cells to adhere to the plate.
4. Adhered cells are gently washed by media exchange and then fixed with paraformaldehyde.
5. A reagent is added to stain all cells for exact quantitation of cells/well.
6. Cells are covered with a slide mounting fixative, which is left to harden.
7. Fixed cells are then to be shipped to MindImmune for quantitation of ICG level.

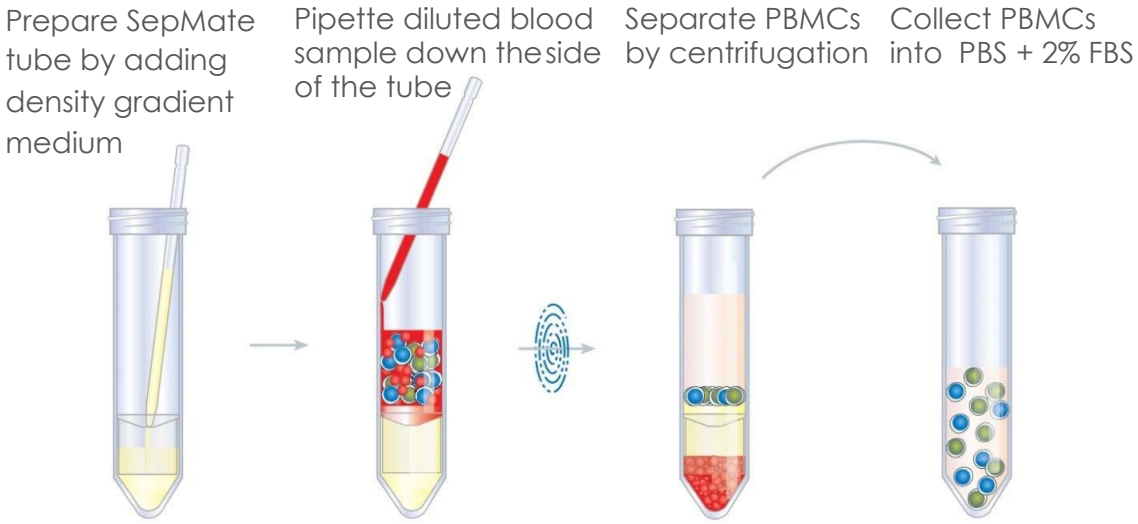
Protocol details. The procedure below is modified from that recommended by the manufacturer of the SepMate system (STEMCELL Technologies Inc. 2323 - 222 Third Street, Cambridge, MA 02142. https://www.stemcell.com/products/brands/sepmate-pbmc-isolation.html).

Preparation – PBMCs should be isolated no longer than 4 hours after blood samples have been obtained. Supplies and reagents (PBS + 2% FBS, density gradient medium, and centrifuge) should be at room temperature ready for use. For centrifugations, the centrifuge brake is on.

See YouTube video for a short review of the SepMate procedure (https://youtu.be/8bL_59lC7Nw)

1. Add 15 ml of Lymphoprep CellMat density gradient medium to the SepMate50 tube by carefully pipetting it through the central hole of the SepMate insert.
2. Dilute 7.5 ml blood sample with an equal volume of PBS + 2% FBS. Mix gently.
3. Keeping the SepMate tube vertical, add the diluted blood sample (15ml) by pipetting it down the side of the tube. The sample will mix with the density gradient medium above the insert.
4. Centrifuge at 1200 x g for 10 minutes at room temperature.
5. Carefully pipette off most of the supernatant above the PBMC layer and retain in a separate tube for plasma analyses.
6. Carefully pour off the PBMCs and retain in a separate tube. Bring volume up to 15 ml by addition of PBS + 2%FBS. Discard SepMate tube.
7. Centifuge PBMCs at 300 x g for 10 minutes. Discard supernatant. Wash cell 2 more times by resuspending cells in 15 ml PBS + 2% FBS and centrifuge at 300 xg for 9 minutes with centrifuge brake on.
8. Remove supernatant from final wash and resuspend PBMC pellet in 3.0 ml of PBS + 2% FBS.
9. Perform cell count
10. Dilute cells with PBS + 2% FBS to a density of 4 million cells/ml. Plate cells in triplicate wells at densities of 4 million cells per well. Incubate for 1.5 hours at room temperature to allow the cells to firmly stick to the plate.
11. Gently aspirate most of plating media and add 1.0 ml of PBS containing 4 % paraformaldehyde. Incubate for 10 minutes at room temperature.
12. Gently aspirate and add 0.25 ml cell mount. Let harden at room temperature. Seal plate with parafilm and ship to MindImmune.

Schematic of PBMC isolation workflow from STEMCELL Technologies



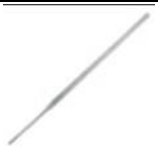
18.3 Protocol for Sample Collection for Biomarker Studies

Plasma Collection Manual for C_2N Biomarker Studies

Platform: PrecivityAD™ (Plasma Aß42/Aß40 & ApoE proteotyping for reporting the Amyloid Probability Score)

Materials Needed

Table 1. Blood collection supplies required

Product	# Units	Manufacturer	Catalog #	Image
13 x 75 mm 4.0 mL K_2 EDTA Vacutainer® Plus, plastic whole blood tube. Lavender BD Hemogard™ closure. See thru label.	1	Becton Dickinson	367862	
Vacutainer® Eclipse™ Blood Collection Needle (22 Gauge, 1.25 in. (32 mm) Needle) with Pre-Attached Holder **OR** Vacutainer® blood collection set with pre-attached holder; 21- or 23-Gauge needle with 12-inch tubing	1	Becton Dickinson	368651	
	1	Becton Dickinson	367352 368656 368689 368688	
Sarstedt 2.0 mL SC (screw-cap) Micro Tube protein LB (Low Bind)	1	Sarstedt	72.694.600	
2.0 mL Sarstedt Micro Tube label with unique sample ID#, bar code or QR code	1			
Calibrated Variable- (0.1 – 5.0 mL) air-displacement hand-held pipette with corresponding non- sterile polypropylene pipette tips **OR** 9 Inch Polypropylene Plasteur® Pipet, Individually Wrapped, Sterile with Small Latex Aspirating Bulbs (2 mL, 57 mm)	1	e.g., Gilson PIPETMAN, Globe Scientific	e.g., Gilson Pipette SKU F123790 & tips D1000; Gilson pipette SKU: FA10007 & tips D5000; Globe Scientific Pipette 3302-500 and tips C151153	
	1	Celltreat Scientific	229281	
	1	VWR	82024-554	

Store plasma sample tubes at -80°C in cryoboxes (e.g., SKU SB2CC-100) with 100-cell divider [5.25" (L) x 5.25” (W) x 2” (H)] until shipped to C_2N Diagnostics in batches of n=100 samples (desirable).

Sample collection procedures

Goal: Collect venous blood into one (1) four (4.0) mL K_2 EDTA Vacutainer®, centrifuge to separate plasma from cells, pipette 1.0 mL plasma aliquot into one labeled Sarstedt 2.0 mL screw-cap protein Low Bind Micro Tube, cap, freeze immediately on dry ice or -80ºC freezer. Plasma sample processing, aliquoting and freezing MUST be completed within 90 minutes of phlebotomy.

1. Label the lavender top K_2 EDTA Vacutainer® (4.0 mL) and one (1) Sarstedt 2.0 mL screw-cap Micro Tubes each with a unique sample identification number (ID#) or bar code, or QR code. No other private or personally identifiable information should be included on the labels affixed to the Sarstedt Micro Tube. Private information should be kept by the Site in a database/case report form along with the corresponding unique sample ID#/bar code/QR code that is affixed to the Sarstedt Micro Tube.
2. Apply a tourniquet, identify and prepare a suitable antecubital or arm vein for phlebotomy. Use either the Vacutainer® Eclipse™ Blood Collection Needle with Pre-Attached Holder OR the Vacutainer® blood collection set with pre-attached holder (butterfly set) to collect venous blood into the 4.0 mL K_2 EDTA Vacutainer® until the tube is full; gently mix by inversion 10-12 times. Store tube on ice until centrifuged.
3. Remove the blood collection needle or butterfly from the participant's arm vein. Apply sterile gauze and instruct participant to hold pressure on the needle insertion site. Apply bandage when insertion site has clotted.
4. Within 30 minutes of blood collection, centrifuge the 4.0 mL Vacutainer® tube (remember to balance the rotor). Centrifuge at 4ºC (room temperature is acceptable) for 15 minutes using a swinging bucket rotor at 500-700 x g with the brake on, or in another centrifuge and rotor at a comparable g-force.
5. Immediately after centrifugation, carefully transfer 1.0 mL plasma aliquot into one (1) Sarstedt 2.0 mL Micro Tube. DO NOT disrupt the plasma/cell interface when transferring plasma. For this transfer, use either the calibrated air-displacement hand-held pipette (calibrated to deliver 1.0 mL plasma) with the corresponding non-sterile polypropylene pipette tip, OR the polypropylene transfer pipet fitted with the latex aspirating bulb. This polypropylene transfer pipet does not have graduations – so you must use the graduations on the Sarstedt Micro Tube in order to ensure that you aliquot 1.0 mL plasma into the Sarstedt tube. After aliquoting plasma into the Sarstedt Micro Tube, immediately cap and freeze the plasma sample tube on dry ice or at -80ºC. If a -70º to -80ºC freezer is not available, sample tubes may be stored in a -20ºC freezer, but only for LESS THAN 6 MONTHS before they are shipped to C_2N for analysis. Discard the 4.0 mL Vacutainer® tube.
    i) Sarstedt Micro Tubes must contain 1.0 mL plasma, but do not completely fill the 2.0 mL tube with plasma (Plasma will expand when frozen and break open the tube).
    ii) If the red cells are disrupted while transferring plasma from a Vacutainer® into the Sarstedt Micro Tube, then the Vacutainer® MUST be re-centrifuged (Step 4) immediately to reestablish a distinct interface between the yellow-gold plasma layer and the white/red blood cells, and to complete the plasma aliquoting and freezing processes.
6. All plasma sample tubes must be stored frozen until shipped with plenty of dry ice by express courier service to C_2N Diagnostics. Place an absorbent towel and each cryobox with frozen samples into a plastic zip-lock bag. Add dry ice and the sealed zip-lock bag with cryobox to a styrofoam container. Enclose the styrofoam container in a properly labeled cardboard shipping container. Affix the sample manifest (log) and express courier waybill to the shipper, ship Priority Overnight to arrive by 10 am next day (<u>not</u> First Delivery next day), note the tracking number, contact C_2N with the tracking # and courier name before you ship, and then ship to C_2N Diagnostics.

The sample manifest can be a printed or digital excel file, but please use this format:

Unique Sample ID#	Location in Sample Box	Participant Age (yr)	Comments (e.g., baseline, visit #, visit date, early discontinuation, deviation, etc.)
11XXYYZZ99	Column A Row 1, A2… Column B Row 1, B2… …etc.	XX	

7. Shipping information: C_2N Diagnostics Attn: Scott Harpstrite - Research Samples, 4340 Duncan Avenue, Saint Louis, MO 63110 (314) 464-0008 sharpstrite@c2n.com

- Please notify Scott with the courier's name and the tracking number on the day you ship.
- Please ship on Sun-Mon-Tues-Weds or Thurs so the shipment arrives at C_2N on Mon-Tues- Weds-Thurs-Fri. In general, C_2N staff are NOT available to receive shipments that arrive on weekends or US Federal Holidays (below). Rare exceptions to C_2N's availability to receive shipments MUST be approved by Scott Harpstrite AT LEAST 3 WORKING DAYS in advance of shipping to C_2N Diagnostics.
- If you have any questions about sample shipping to C_2N, especially shipments scheduled around the time of US Federal Holidays (below), please contact Scott Harpstrite AT LEAST 3 WORKING DAYS in advance of shipping to confirm our availability to receive shipments.

  Birthday of Martin Luther King, Jr. - Third Monday in January Washington's Birthday - Third Monday in February

  Memorial Day - Last Monday in May
  Juneteenth Day – June 19th
  Independence Day - July 4th

  Labor Day - First Monday in September

  Indigenous People's/Columbus Day - Second Monday in October Veterans Day - November 11th

  Thanksgiving Day - Fourth Thursday-Friday in November Christmas & New Year's Day - December 24th through January 2nd