

PROTOCOL

Title: Autoimmunity and The Role of T Cells in Parkinson's Disease
(PPMI Whole Blood T cells)

Sponsor: Michael J. Fox Foundation

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**Autoimmunity and The Role of T Cells in Parkinson's Disease
(PPMI Whole Blood T cells)**

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1. PURPOSE OF STUDY

The purpose of this protocol is to study T cell reactivity to α -synuclein in prodromal and early Parkinson's Disease (PD) participants compared to Healthy Controls (HC). Autoimmunity plays a crucial role in PD through T cell responses toward α -syn^{1,2,8}. Analysis of longitudinal changes in peripheral blood mononuclear cells (PBMCs) to identify autoimmune antigens, epitopes, and T cell receptors (TCRs) in Parkinson's Disease (PD) to investigate the association of Parkinson's Disease (PD) with general inflammation.

2. BACKGROUND AND AIMS

Research has played critical roles in advancing understanding of the role of the adaptive immune system in PD. It has been reported the presence of α -syn-responsive T cells in human PD, particularly for epitopes containing residue Y39 or phosphorylated S129 (pS129)^{1,2}, a component of Lewy bodies. Y39 peptides are presented by specific HLA alleles associated with PD by GWAS studies³. Findings of α -syn-responsive T cells in PD has been independently replicated⁴. It has been reported recently that α -syn-reactive T cells are mostly present during prodromal and early PD¹ during the rapid loss of SN DA neurons⁵. While there are suggestions of additional PD-associated epitopes (e.g.,⁶), there has been one instance where these were assayed in human (see also⁷). An important question is whether PD patients exhibit a general change in T cell responses or if it is specific to disease-associated proteins. To address this, we will examine if T cells from PD patients exhibit differential response to common antigenic proteins, e.g., pollens, CMV, EBV, pertussis, and influenza, which may be associated with PD and to which there is nearly universal response due in part to vaccination.

Aim 1. To determine changes in epitope-specific T cell reactivity against proteins for which processing is altered in neurodegenerative diseases including PD such as α -syn (including fibrils, which activate T cells¹), tau, amyloid- β and amyloid precursor protein, optineurin, GBA, LRRK2, TDP-43, OGDH, dysbindin, and ataxins. Publications confirm autoimmune T cell responses in individuals with PD against α -syn, and tau^{1,2,7}, but other candidates exist, and no studies have been performed on longitudinal samples. PBMCs from PD patients with recently diagnosed (<5 years) and long-term PD (>10 years), at-risk patients with REM sleep behavior disorder (RBD), and age-matched HC in longitudinal analysis will be examined. As not all PD patients respond to α -syn^{1,2}, other candidates contribute to autoimmune response will be explored by studying multiple antigens side-by-side, to determine if individuals possess poly-specific responses, or respond to a unique set of antigens. Enrolled participants will provide one blood draw but may be asked to provide additional blood draw at their annual PPMI visit(s). Magnitude and response quality by measuring proinflammatory (IFN γ , IL-5, and TNF α) and regulatory (IL-10) cytokines will be examined. Also, the antigen-specific TCR-repertoire will be examined to determine if TCRs promise a PD biomarker. Longitudinal samples will test if antigen-specific reactivity in recently diagnosed PD decreases over time, as predicted by a recent study², if reactivity predicts PD onset in RBD participants, and if the proinflammatory / regulatory cytokine ratio changes with a higher proportion of regulatory cytokines over time. We will determine if antigen-specific T cells are present during the prodromal phase in individuals

at risk of developing PD and if detection of these cells may provide earlier diagnosis of PD.

Aim 2. It is unknown if T cell changes in PD are specific to α -syn or represent altered general immune function. Inflammatory features are described in the brain of PD patients⁹ and anti-TNF treatment reduces PD incidence¹⁰, suggesting a preclinical heightened TNF response. Preliminary results suggests that PD patients who do not respond to α -syn (which may correspond to later stages) may display a depressed overall immune response. Characterization of antigen-specific responses will be determined in the same participants as above for Aim 1. Vaccination history, including seasonal influenza vaccination, will be recorded to investigate if induced vaccine responses differ between the cohorts. T cell reactivity will also be measured against α -syn, fibrils and epitopes and common antigens due to exposure (pollens, CMV, EBV), and vaccination (pertussis, tetanus, influenza) by Fluorospot and by intracellular cytokine assays for proinflammatory and regulatory cytokines including TNF α . Basal levels of T cell activation and reactivity by measuring T cell activation markers (OX40, PD-L1, CD137, CD25 and CD69) on unstimulated cells and response magnitude against polyclonal stimulation (PHA, anti-CD3/CD28 and PMA/IO) will be investigated. Participants will be analyzed based on reactivity to PD-specific antigens (α -syn and others in Aim 1) levels. Participants associated with neurodegeneration-antigen-specific T cell reactivity will be classified as exhibiting ongoing autoimmunity and compared to non-responsive PD patients (presumably past autoimmune responders and/or patients for whom T cell inflammation does not play a key role). This Aim will determine if PD is associated with general inflammation and altered immune response against frequently encountered foreign antigens.

3. STUDY DESIGN

This study will take place at up to 40 PPMI sites and will aim to enroll about 180 participants. It will involve 1 standard venous blood draw of approximately 50ml at the participant's baseline or annual visit (depending on when they enroll). Participants may be asked for an additional blood draw at future visits.

4. STUDY POPULATION

Approximately 180 participants enrolled in the PPMI Clinical will be recruited, including the following:

4.1 Prodromal participants n=approximately 120

4.1.1 RBD (n= approximately 60)

4.1.2 Hyposmic (n= approximately 60)

4.2 Parkinson's Disease Participants n= approximately 30

4.3 Healthy Controls n= approximately 30

5. RECRUITMENT METHODS

A total of 180 participants will be recruited, all of whom are already enrolled in PPMI Clinical and may be at various stages of participation and follow up. PPMI Clinical participants who are potentially eligible will be provided information regarding this sub-study and invited to participate. The clinical site staff will be responsible for recruiting

participants into this sub-study.

6. PARTICIPANT ELIGIBILITY

Participants must meet the following criteria to enroll:

7.1 Inclusion Criteria

- Enrolled in PPMI Clinical.
- Willing and able to provide informed consent.

7.2 Exclusion Criteria

- Known autoimmune condition, including rheumatoid arthritis, psoriasis, inflammatory bowel disease, systemic lupus erythematosus, etc.
- Known immune deficiency disorder.
- History of solid or hematological malignancy other than in situ carcinoma of the cervix or basal cell carcinoma of the skin (solid-within 1 year since removal).
- Taking immunomodulatory medication, such as Humira (adalimumab), Enbrel (etanercept), Stelara (ustekinumab), Azathioprine, Cyclosporine, Cytosan, Methotrexate, Tysabri (natalizumab), Rituxan (rituximab), beta-interferons, etc.
- History of receiving chemotherapy agents with ability to affect the immune system, or radiation therapy (within 1 year after the last chemotherapy or radiation therapy).
- Taking antibiotics or over the counter (OTC) “probiotic” preparations within 3 months of enrollment.

7. OBTAINING INFORMED CONSENT

The procedures and requirements of the study, together with any potential hazards/risks, and the freedom to withdraw from participation in the study at any time, will be explained to each potential participant as part of the consent process. The consent process will take place in a space that allows for privacy and confidentiality and should allow for enough time for the individual to consider participation and ask any questions. Consent will be obtained by the study Investigator or delegated study staff, as applicable. Each participant will sign such an informed consent to document agreement to participate in the study, as well as to document HIPAA authorization. The signed informed consent might be uploaded to a secure portal for remote monitoring.

It is the responsibility of the Investigator (or as delegated to the person obtaining consent) to make sure that the participant understands what she/he is agreeing to and that written informed consent is obtained before the participant is involved in any protocol-defined procedures. Each participant will be provided a copy of the consent form.

8. PARTICIPANT ID ASSIGNMENT

All participants will use their assigned PPMI study ID. The PPMI Participant ID number will be used to identify a participant on all study related documentation.

9. STUDY PROCEDURES

Assessments for this study will be performed as described below and in the PPMI Whole Blood T-Cells Schedule of Activities.

Once consent is obtained, and eligibility is confirmed by the Site Investigator, the participant may be enrolled into the study. Participants will be asked to donate 50ml of blood via standard venous blood draw into five (5) 10ml EDTA tubes (purple top) at their visit of consent. Each tube will be labeled with a de-identified participant ID and visit number. Post collection blood samples will be stored at ambient temperature (room temperature) at all times. Samples will then be shipped via courier within **24 hours** from the time of collection as per the PPMI Biologics Manual.

10. BIOLOGICS RESEARCH SAMPLING

Refer to the PPMI Biologics Manual for a detailed description of blood sampling and shipping.

10.1 Blood Samples

Whole blood (about 50 ml) will be collected to conduct analyses as described above in Section 2. No more than 110 ml will be drawn at any visit, including this sub-study and the PPMI Clinical blood samples.

All research samples will be sent to La Jolla Institute for Immunology (LJI) research lab to be stored indefinitely for research purposes. Samples will be made available to researchers to conduct analyses related to PD and other disorders. Participants will not receive any individual results of research analysis or testing conducted on the biologic samples.

11. CONCOMITANT MEDICATIONS

Concomitant medications refer to all medications taken between the dates of signed consent and completion of study. All concomitant medications reported at the time of the PPMI Clinical Screening visit and for the duration of participation are recorded on the PPMI Clinical study medication logs. Concomitant medications, including over the counter (OTC), dietary supplements (e.g., herbal remedies) or prescriptions, are permitted during the study period, except for the following medications: Taking immunomodulatory medication, such as Humira (adalimumab), Enbrel (etanercept), Stelara (ustekinumab), Azathioprine, Cyclosporine, Cytoxan, Methotrexate, Tysabri (natalizumab), Rituxan (rituximab), beta-interferons, etc. Taking antibiotics or OTC “probiotic” preparations within 3 months of study enrollment are prohibited.

12. RISKS TO PARTICIPANTS

12.1 Blood Sampling

Risks associated with venous blood draw include pain and bruising at the site where the blood is taken. Sometimes people can feel lightheaded or even faint after having blood drawn.

12.2 Confidentiality

There is a risk of loss of confidentiality. This risk will be minimized by removing individually identifying information from data and samples and replacing with a study code before sharing information with the LJI lab or a data repository.

13. POTENTIAL BENEFITS TO PARTICIPANTS

There are no direct anticipated benefits to study participants in this study. However, new information may be generated by the study that will support development of better treatments for Parkinson's Disease.

14. COSTS FOR PARTICIPATION

There are no additional costs for individuals participating in this study.

15. PAYMENT AND REIMBURSEMENT FOR PARTICIPATION

Participants will not be paid for activities completed in this study.

16. PARTICIPANT WITHDRAWALS

Study participants will be informed during the consent process that they have the right to withdraw from the study at any time without prejudice, and may be withdrawn at the Investigator's or Sponsor's discretion at any time. Any information that has already been collected prior to the study participant's withdrawal will not be removed.

17. PRIVACY AND CONFIDENTIALITY

Privacy of participants will be protected in that each person will have the option to voluntarily choose whether to participate in this study. It is the responsibility of the site Investigator to consider the participant's privacy and confidentiality when completing study visits and related protocol activities.

The Site Investigator must assure that the confidentiality of participants, including their personal identity and personal medical information, will be maintained at all times. U.S. sites have additional confidentiality obligations to study participants under the Health Insurance Portability and Accountability Act (HIPAA). Participants will be identified by participant ID numbers on data forms and other study materials submitted to the La Jolla Institute for Immunology (LJI) research lab.

Only study staff requiring access to related study documentation will have permission to view identifiable information.

18. DATA AND SAMPLE SHARING AND STORAGE FOR FUTURE USE

Data collected for this study will be maintained and stored indefinitely at the study Cores on secure, password protected systems. All study information (data and samples) will be accessed only by those who require access as pertains to the individual's role on the study. All organizations responsible for data storage and review will observe the highest precautions to ensure data integrity and security.

Data collected for this study may be transferred and shared across participating PPMI Cores for conducting analyses as pertains to the study. All PPMI data will be incorporated into the PPMI database to create a fully harmonized PPMI database.

Data obtained during the conduct of this study will be sent to the Laboratory of Neuro Imaging (LONI) in Los Angeles, California to be stored indefinitely for research purposes. Research data will be made available to researchers to conduct analyses related to PD and other disorders. Researchers will be required to comply with the PPMI data agreement to receive data. All personally identifiable information will be removed before it is shared outside the study.

19. ANALYSIS PLAN

T cells will be assessed in RBD participants, as compared to PD and healthy control participants as described in Section 2 Aims. At the end of 3 years and following analysis, the RBD participants will be designated as a confirmed PD or non-PD diagnosis.

20. REFERENCES

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21. Appendix 1-Schedule of Activities

PPMI Whole Blood T Cells Schedule of Activities

Visit	Sample 1 (VS1)	Sample 2 (VS2)*	Sample 3 (VS3)*
Assessment			
<i>Consent Activities</i>			
Documentation of Informed Consent	X		
Informed Consent Tracking Log	X		
<i>General Activities</i>			
Review Inclusion/Exclusion Criteria	I		
Screen Fail	As Needed		
Conclusion Page	As Needed		
<i>Biological Samples</i>			
Whole blood sample	X	X	X
<i>General Health</i>			
Vaccination history	X	X	X

I = Investigator completed assessment

X = Investigator or Coordinator completed assessment (or as otherwise delegated)

*participants may be asked to return for additional blood sampling