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**Ancillary Studies Framework for Post-COVID Conditions Under Longitudinal Follow-Up in RECOVER (RECOVER 2.0)**

I. Background and Rationale

Recent experience from RECOVER-Adult 1.0 and biospecimen collection efforts has established a robust platform to observe post-acute sequelae of SARS-CoV-2 (PASC, commonly known as Long COVID). The natural history of Long COVID remains incompletely understood across symptom domains including neurocognitive changes, cardiovascular risk, immune dysregulation, and persistent inflammation. In response, a longitudinal follow-up protocol aims to extend clinical, biologic, and data collection activities over multiple years. Importantly, the platform is designed so that ancillary investigators can propose mechanistic ancillary studies, thereby taking advantage of the centralized repository of clinical data, imaging, and biospecimens.

II. Objectives

a. Primary Objectives

- To characterize the natural course and evolution of persistent symptoms following SARS-CoV-2 infection over time.
- To integrate objective clinical assessments (e.g., neurocognitive testing, cardiopulmonary evaluations, and infection-associated chronic condition symptomology) with serial biospecimen collections for biomarker analysis.

b. Secondary and Ancillary Objectives

- To build a flexible, cost-effective platform that permits ancillary studies on mechanistic pathways such as immune dysregulation, autoimmunity, endothelial dysfunction, neurological injury, cardiopulmonary complications, sleep disturbances and metabolic derangements.
- To allow external investigators to propose targeted studies by leveraging the infrastructure (e.g., advanced imaging, invasive phenotyping, deeper omics analyses), data, and biospecimen repository established through the longitudinal follow-up.

III. Study Design and Population Overall Design

a. This is a prospective, observational extension of the RECOVER-Adult Cohort study (RECOVER-Adult).

- The study will follow adults originally enrolled in RECOVER-Adult, using a case-cohort design. Participation in the extended follow-up is offered to eligible participants based on random sampling of the cohort supplemented with participants who experienced Long COVID as defined by the RECOVER Long COVID research index; the National Academies of Sciences, Engineering, and Medicine (NASEM) Long COVID criteria; and symptom severity.

- Data collection will include two in-person visits for comprehensive clinical and biospecimen evaluations and remote surveys every six months.

b. Population

- Adults previously enrolled in RECOVER-Adult 1.0 who had a confirmed or suspected SARS-CoV-2 infection. Given the high prevalence of prior infection, historical controls may be leveraged as needed.
- Subgroups (e.g., those developing new comorbidities or distinctive syndromic patterns) will be identified to support hypothesis-driven ancillary studies.

IV. Follow-Up Activities Scheduled Assessments

a. Remote Surveys (semiannually over three years)

- Collection of patient-reported outcomes including symptom burden (e.g., fatigue, pain, cognitive changes), mental health indices, functional status, quality of life, and socioeconomic metrics.
- Tracking of vaccination status, re-infections, and any therapeutic interventions.

b. In-Person Clinical Visits (annually; minimum two visits over three years)

- Detailed physical examinations; standardized neurocognitive testing (using updated National Institutes of Health [NIH] Toolbox assessments in a controlled setting); cardiopulmonary assessments (including six-minute walk test and measures of oxygenation); and infection-associated chronic condition (IACC) assessments (including orthostatic tolerance test, symptom diaries and surveys).
- Phlebotomy for comprehensive biospecimen banking (e.g., blood, plasma, serum) for downstream biomarker analyses.
- Ancillary studies may have the opportunity to add advanced phenotyping protocols (for example, tilt-table testing, cardiopulmonary exercise tests, invasive sampling such as muscle biopsies) for participants who meet pre-specified criteria.

c. Ancillary Enrollment Procedures

- A structured process for external investigators to apply for access to data and/or propose additional in-person assessments utilizing the follow-up visits.
- Ancillary studies might include specialized imaging (e.g., brain MRI, PET for neuroinflammation), detailed omics analysis (genomics, epigenomics, proteomics), assessments of immune dysfunction or interventions tailored to specific clinical phenotypes.

d. Data Collection and Biospecimen Management

- Clinical and survey data are integrated into a centralized RECOVER Data Gateway, updated every three months.

- Biospecimens are processed, cryopreserved, and linked to clinical data via de-identified codes.
- Ancillary investigators can request access to data and/or samples through an application process managed by the Data Resource Core.

V. Statistical and Analytic Overview

- Longitudinal models (including semi-parametric and causal inference methods) will be used to evaluate symptom trajectories in association with biologic markers.
- Advanced analytic techniques (e.g., machine learning, meta-learners for subgroup identification) will accommodate potential heterogeneity in outcomes and adjustments for time-varying confounders.
- The protocol incorporates plans to address missing data and potential informative loss to follow-up.
- Ancillary study proposals will be reviewed for integration with this dataset and can include additional statistical analyses specific to mechanistic questions.

VI. Timeline and Milestones

- Year 1 Q1–Q2 (6 months): Enrollment of prior RECOVER-Adult participants into the extension follow-up.
- Year 1 Q3–Year 3 Q2 (24 months): Annual in-person visits and biospecimen collection.
- Year 1 Q3–Year 3 Q4 (30 months): Semiannual remote surveys.
- Year 4: Study closeout, data cleaning, and analysis.
- Ongoing data curation and scheduled updates via the Data Gateway.
- Ancillary proposals will be solicited shortly after the initiation of the follow-up extension and will run concurrently.

VII. Regulatory Oversight and Safety

- The study will operate under IRB-approved protocols in line with NIH guidelines and utilize a single IRB.
- Data security and participant confidentiality are ensured by centralized data storage in a secure NIH STRIDES cloud environment.
- Adverse events will be monitored during in-person visits, and any incidental findings will be communicated to participants per protocol guidelines.
- The RECOVER Observational Safety and Monitoring Board will oversee activities semiannually.
- Data will be shared in accordance with NIH Scientific Data Sharing Policy and RECOVER Sharing Data for Broader Impact policies and processes.

## VIII. Summary and Opportunities for Collaboration

This protocol summary outlines a robust follow-up platform designed to rigorously study the long-term sequelae of COVID-19. Its flexible design permits not only detailed natural history analyses but also invites ancillary studies to address mechanistic hypotheses. External investigators will be encouraged to propose ancillary studies by leveraging this integrated infrastructure—thereby accelerating discovery on PASC pathogenesis and informing targeted interventions.

This high-level overview offers potential collaborators a clear introduction to the longitudinal follow-up activities and outlines how ancillary proposals can be seamlessly integrated with the current RECOVER extension protocol.