Protocol for "ImmuneRACE – Immune Response Action to COVID-19 Events"

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I. BACKGROUND

Adaptive Biotechnologies has developed a platform technology called T-cell receptor βeta-1 (TCRβ1) Assay for immune profiling, also referred to as immunoSEQ[§] in this proposal. The platform utilizes next-generation sequencing (NGS) to identify rearranged T-cell receptor beta (TRβ)gene sequences and the abundance of those sequences. This broad platform technology can be used to determine the diversity of the cellular adaptive immune system and track phenotype associated T-cell clones to query the current state and history of the adaptive immune system.

Detecting Specific TCRs for Diagnostics[1-3]

Despite the overwhelming number of possible T-cell receptors (TCRs), many TCRs are quite common and repeatedly recur within and between individuals, comprising the public T-cell repertoire[4]. These public TCRs are diagnostically useful because once a TCR has been associated with a particular antigen or disease state, the association is reliable even when that TCR is seen in a new individual. Using this technique, we have demonstrated that CMV infection can be assayed purely from the peripheral TCR repertoire[3].

When specific TCRs associated with particular antigens or disease states are known, scanning the T-cell repertoire for those specific sequences can be clinically useful. The purpose of this research study is to explore the immune system in individuals with coronavirus disease.

II. PROPOSED RESEARCH

The SARS-CoV-2 virus which causes coronavirus disease (COVID-19) is spreading rapidly throughout the world. Researchers, governments, and biotechnology companies are mobilizing to develop and disseminate diagnostic and therapeutic alternatives to try to curb this global pandemic. The vast majority of these R&D efforts are focused on the RNA of the virus itself rather than critical information held within the genetics of a patient's immune response to the virus and the disease patterns we can infer from studying the immune response at the population level.

First, we will identify the parts of the virus, called antigens, that induce a cellular immune response via T cells. We do this by rapidly determining the nearly full set of processed, presented, immunogenic, and immunodominant antigens from a disease. This has historically been very challenging for the immunology community and we are confident that our approach is a significant breakthrough in the field. Next, we use these antigens to identify tens of thousands of T-cell receptors from T cells that expand in response to the virus across the human population. This requires the scale of data generation and processing made possible by the Adaptive and Microsoft partnership.

In parallel, we will identify and confirm the TCR signature of the immune response to SARS-CoV-2 virus. To do this, we require DNA extracted from the peripheral blood or whole blood of

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approximately 1000 blood samples from patients with COVID-19. The immune signature we expect to identify of COVID-19 is a starting point to solve key public health challenges in the present. These samples can either be newly diagnosed patients or patients who have already recovered from the virus because the immune response will still be identifiable in the memory compartment.

III. PURPOSE OF THE STUDY

The primary aim is to increase the understanding of the immune system response in coronavirus diseases by assaying the peripheral immune repertoire for TCRs and a secondary aim of BCRs, specific to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus which causes COVID-19, with the potential to develop better diagnostic tests and treatments.

Adaptive and Microsoft are committed to making TCR repertoire data public to researchers around the world. We hypothesize that by studying the immune response in patients with COVID-19 and making these data public, we can help solve several key challenges plaguing this current pandemic.

Specific Aims

Aim 1. Comparison of disease-specific TCR signatures in patients and controls

We will run immunoSEQ on approximately 1000 patient samples with disease status unblinded. After sequencing these samples, we will quantitatively describe the compartment of the T-cell repertoire specific for the disease of interest. We will then use these data to construct a classifier that accurately distinguishes patients from controls.

Aim 2. Identify the immunodominant antigens that elicit a T-cell response to COVID-19

We developed technology to query the antigen specificity of the T-cell repertoire to hundreds of peptide epitopes in a single blood sample[5]. This technology not only allows us to parse out immunodominant epitopes of a given infection from irrelevant epitopes, but also allows us to determine the TCR sequences of the T cells responding to these immunodominant epitopes. We will use this technology to determine which peptide epitopes derived from the SARS-CoV-2 genome commonly elicit an immunodominant T-cell response in different samples. The TCR sequence data generated from these studies will be used to boost the diagnostic classifier obtained in Aim 1. In addition, the identification of immunodominant epitopes can be disseminated to fuel studies in vaccine design and antigen specific T-cell responses relating to clinical outcome in other labs.

Aim 3. Risk Stratification based on an individual's immune signature

There is a critical need for a reliable risk stratification test to enable treatment prioritization given the potentially massive number of symptomatic patients. Despite an emerging understanding of the diagnosis of COVID-19, how it spreads, and the death rate, there is no currently available way to predict who needs hospitalization beyond age associated risk and

limited epidemiologically linked comorbidities. We aim to utilize the immune signature as a way to predict disease severity in individuals by utilizing T cell sequencing capabilities and machine learning pipelines to identify T cells specific to the virus and assessing the strength of the immune response by the expansion of these T cells in a patient's blood. By understanding the strength and specificity of a patient's immune response, which may be temporarily bolstered by recent exposure to other coronaviruses (e.g., common cold) in some people, we may be able to significantly improve the diagnostic and risk stratification process.

Aim 4. Determine whether an immune signature can be detected in individuals exposed to SARS-CoV-2 earlier than currently available tests

Another critical need to contain the spread of SARS-CoV-2 is to determine the false negative rate of the RNA test in asymptomatic people and offer an alternative diagnostic that is more sensitive in this cohort. For example, it is possible that early stage disease is not picked up by RNA tests because the virus may be isolated to regions such as the lower respiratory cavity that are not assessed by standard testing methods. We aim to determine whether the immune response can be used to detect the virus from a simple blood test thereby providing a more sensitive test in asymptomatic people even if the virus itself is not directly detectable in the upper respiratory region.

Secondary Aim:
Aim 5. **Explore whether additional research assays could potential identify and/or confirm antigenic binding**

As a secondary aim, Adaptive will perform explorational research with additional sequencing-based research assays to profile the adaptive immune system, such as, but not limited to TCR pairSEQ[**6] and B-cell receptor (BCR) pairSEQ[††]. These assays use a combinatorial method for pairing TCR alpha and beta chain sequences and BCR heavy and light chain sequences. The output is a large set of full length paired BCR or TCR sequences, which allows reconstruction of a functional antibody or TCR. We regularly utilize the results of our pairSEQ assays to identify and/or confirm antigenic binding. For the case of BCR pairSEQ, the resulting antibody sequences could potentially have therapeutic value for imparting passive immunity.

IV. STUDY POPULATION

Participants

Prospective ascertainment of approximately 1000 individuals, between the ages of 18 – 89, who reside within the United States. Blood samples and nose or throat swabs will be collected at affiliated sites or with mobile phlebotomy. These samples will be shipped frozen or transported refrigerated or at room temperature to Adaptive Biotechnologies for processing, including, but not limited to DNA extraction and analysis. Minors, pregnant women, prisoners, mentally disabled persons, and wards-of-the-state will be excluded to prevent any risk to vulnerable populations. The selection of participants will be equitable per 45 CFR 111(a). The inclusion and exclusion criteria for the three cohorts are included below.

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Cohort 1. Exposed to coronavirus disease

Inclusion criteria

Participants must satisfy the following criteria to be enrolled in the study:

i. Individuals exposed to someone with a confirmed diagnosis of coronavirus disease within 2 weeks of exposure (or at the discretion of the investigator)
ii. Male and female participants of any race and ethnicity between 18 to 89 years of age (inclusive) at the time of enrolling in the study
iii. Must be able to communicate with the investigator, understand and comply with the requirements of the study

Exclusion Criteria

The presence of any of the following will exclude a participant from enrollment:

i. Individuals who have not been exposed to a person with a confirmed diagnosis of coronavirus disease within 2 weeks of exposure (or at the discretion of the investigator)
ii. Protected populations including minors, pregnant women, prisoners, mentally disabled persons, and wards-of-the state
iii. Any significant condition, laboratory abnormality, or psychiatric illness that would prevent the participant from safely participating in the study
iv. Donated more than 500cc or 1 pint of blood in the past 60 days prior to the blood draw (at the discretion of the investigator)

Cohort 2. Active coronavirus disease

Inclusion criteria

Participants must satisfy the following criteria to be enrolled in the study:

i. Individuals with a diagnosis of coronavirus disease:
    a. Either by clinical diagnosis made by a medical professional, or
    b. By positive laboratory test, including but not limited to naso- or oropharyngeal swab (or at the discretion of the investigator)
ii. Male and female participants of any race and ethnicity between 18 to 89 years of age (inclusive) at the time of enrolling in the study
iii. Must be able to communicate with the investigator, understand and comply with the requirements of the study

Exclusion Criteria

The presence of any of the following will exclude a participant from enrollment:

i. Individuals without a diagnosis of coronavirus disease
ii. Protected populations including minors, pregnant women, prisoners, mentally disabled persons, and wards-of-the state
iii. Any significant condition, laboratory abnormality, or psychiatric illness that would prevent the participant from safely participating in the study

iv. Donated more than 500cc or 1 pint of blood in the past 60 days prior to the blood draw (at the discretion of the investigator)

Cohort 3. Recovered from coronavirus disease

Inclusion criteria

Participants must satisfy the following criteria to be enrolled in the study:

i. Individuals previously diagnosed with coronavirus disease and cleared from active infection (within 2 weeks or at the discretion of the investigator) by:
   a. Testing negative on two consecutive naso- or oropharyngeal swab tests following initial diagnosis, or
   b. Cleared by a healthcare professional or public health authority, or
   c. Resolution of symptoms related to COVID-19 (or at the discretion of the investigator)
ii. Male and female participants of any race and ethnicity between 18 to 89 years of age (inclusive) at the time of enrolling in the study
iii. Must be able to communicate with the investigator, understand and comply with the requirements of the study

Exclusion Criteria

The presence of any of the following will exclude a participant from enrollment:

i. Individuals without a previous diagnosis of coronavirus disease at the discretion of the investigator
ii. Protected populations including minors, pregnant women, prisoners, mentally disabled persons, and wards-of-the state
iii. Any significant condition, laboratory abnormality, or psychiatric illness that would prevent the participant from safely participating in the study
iv. Donated more than 500cc or 1 pint of blood in the past 60 days prior to the blood draw (at the discretion of the investigator)

V. METHODS AND PROCEDURES

Sample and Data Collection Plan

Blood Draw and Sample Processing

Standard blood draws will be performed via routine venipuncture during either routine clinical care of the participants or scheduled remote phlebotomy appointments for the research study. Whole blood will be collected in volumes of approximately 10-60 mL (~1-6 X 10 mL tubes). All blood samples will be shipped frozen (at -20 to -80 °C) or transported refrigerated (at 2 to 8°C) or at room temperature within 72 hours of being drawn to Adaptive Biotechnologies for further testing. Blood will undergo DNA extraction, and the immunoSEQ Assay will be performed. This will quantitatively describe the compartment of the T-cell repertoire specific for the disease of interest compared to controls. In addition, additional research assays may be applied.

Depending on the needs of the study, Adaptive will coordinate with the phlebotomist blood draws that will be transported and processed same day at room temperature. These samples must be processed and cryopreserved within 6 hours of the sample blood draw. Both the PBMCs and serum will be processed and frozen. For samples undergoing this route, participants that will be prioritized include, but are not limited to individuals with a confirmed diagnosis of coronavirus disease and reporting symptoms for at least two weeks prior to blood draw.

Nose or Throat Swabs
A nasopharyngeal or oropharyngeal swab will be collected by inserting a swab into the nose or throat of the participant. Samples collected will be shipped frozen at -80 °C to Adaptive within 1 month of collection or stored at 4 °C and transported to Adaptive within 72 hours. All samples will be stored at -80°C until further testing is performed. Testing may include, but not limited to, clinically available tests for coronavirus disease or other respiratory illnesses.

Study Visit

During the study visit, the examiner will collect information including, but not limited to, participant's diagnosis of coronavirus disease and symptoms associated with coronavirus disease. This information will be used in the analysis to better understand and interpret the test results. This information will be stored with an alphanumeric code. All information will be de-identified in accordance with HIPAA standards.

Electronic Questionnaire
An electronic questionnaire will be administered in this study to collect information pertaining to the participant's medical history, symptoms, and diagnostic tests performed for coronavirus disease. This information will be used in the analysis to better understand and interpret the test results. This information will be stored with an alphanumeric code. All information will be de-identified in accordance with HIPAA standards.

As part of this study, medical records, with the participant's consent, may be accessed to confirm medical information pertaining the participant's medical history, symptoms, and diagnostic tests performed for coronavirus disease.

As part of this study, participants may be asked to provide diagnostic test results performed for coronavirus disease, if available. Diagnostic test results can be provided via secure email or secure fax.

Optional Participation
Participants will have the option to undergo up to four additional blood draws and four additional questionnaires about the participant's symptoms and medical information relating to coronavirus disease throughout a 4-month time period. Each additional blood draw will be approximately 10-60 mL (1-6 x 10mL tube(s)). Optional blood draws are at the discretion of the investigator and dependent on the needs of the study.

Additional tests may be run to diagnose or support the diagnosis may be run on the participant's sample from all cohorts. This is explicitly stated in the consenting information sheet.

Shipping

Batches of samples can be transported to Adaptive by courier. All sample shipments will follow regulations for UN 3373 Biological Substance, Category B. Shipment notification and the electronic manifest should be provided before sample receipt at Adaptive.

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Metadata Summary:

For each sample the following metadata will be included, but not limited to:

i. Basic demographics (age at collection, gender, ethnicity/race)
ii. Symptoms associated with coronavirus disease and at time of blood draw
iii. Required hospitalizations relating to coronavirus disease
iv. Date and city/state of diagnosis of coronavirus disease
v. Test results for coronavirus disease
vi. Other comorbid conditions that may impact results and interpretation (for example, asthma, chronic lung disease, autoimmune conditions)
vii. Medications that may impact results and interpretation

Data Analysis

Data analysis will be performed by Adaptive Biotechnologies scientists using customized molecular assays and software designed to capture detailed information about the immune repertoire. In collaboration with Microsoft, we will use these data to construct a classifier that accurately distinguishes cases from controls with the use of machine learning and artificial intelligence (AI) based on TCR repertoire analysis. A rigorous statistical analysis will be performed.

For the secondary aim, TCR pairSEQ and/or BCR pairSEQ assay analysis will be performed. This involves a combinatorial method for pairing TCR alpha and beta chain sequences and BCR heavy and light chain sequences will be performed, allowing reconstruction of a functional antibody or TCR.

This study is considered minimal risk and does not require additional data monitoring measures in place.

Immunodominant peptide analysis

Cryopreserved PBMC will be used to run the assay, which will link TCR sequences to T-cell specificity of peptides derived from the SARS-CoV-2 genome as described by Klinger et al.[5]. Briefly, memory T cells from SARS-CoV-2 exposed individuals will be incubated with epitopes derived from the entire genome of SARS-CoV-2. The SARS-CoV-2 specific memory T cells will be isolated and sequenced. The TCR sequences of the memory response will be used to broaden the diagnostic signal obtained by repertoire analysis alone. In addition, the SARS-CoV-2 antigenic epitopes that are found to be immunogenic in participants who have cleared the infection will be identified and shared with the community so that further studies can be targeted at these epitopes.

Data Storage and Confidentiality

DNA extracted from the samples will be stored at -20 to -80 °C until the sample is exhausted. Samples and associated clinical information will be de-identified prior to being transferred or upon arrival to Adaptive Biotechnologies. The samples will be stored and tracked by the research technicians using a laboratory information management system (LIMS). The samples may be used for research assays in areas beyond the disease of interest at the conclusion of this study.

To the extent, the results of this research study are presented at meetings or in publications, the identity of participants will not be included in the information that is disclosed. In addition, records related to this study may be reviewed during a regulatory inspection.

VI. RISK/BENEFIT ASSESSMENT

Risk Category: Minimal

Potential Risk - Physical

Physical risks associated with drawing blood, include soreness at the site of puncture, bruising, and in the rare case, infection of the blood draw site, fainting, nerve, or tendon damage.

Physical risks associated with a nose or throat swab include: a very small risk of bleeding when swabbing the inside of your nose or throat (mucosal membranes).

Mitigating strategies to minimize risk:

Standard procedures to minimize discomfort, and the chance of infection resulting from venipuncture, nasal or throat swabs will be utilized.

Potential Risk – Loss of Confidentiality

There may be a risk of loss of confidentiality of the protected health information of the participants.

Mitigating strategies to minimize risk:

All data, blood samples from participants will be de-identified at Adaptive in accordance with the HIPAA Safe Harbor Method. In addition, staff sign confidentiality agreements as conditions of their employment and are trained annually on the HIPAA Privacy and Security Rule. Delegated research staff will be able to view information about the participant, the code given their samples, and the resulting data derived from the analysis. Therefore, appropriate data security measures will be in place to prevent unauthorized access to individually identifiable data.

Benefits

No direct benefit will be seen by the study participants.

Participants are made aware that allowing the study to use their blood samples and analyze their DNA may help develop better lab tests and lead to a better scientific understanding of coronavirus disease.

In addition, de-identified data will be shared with the global research community with the potential of developing better tests and treatment for coronavirus disease.

The participants are informed that they will not receive individual results from this study. Moreover, we will not give the results to their doctor or put them in their medical records.

Alternatives to Participation

Options include not participating or participating in other research studies.

VII. PARTICIPANT IDENTIFICATION, RECRUITMENT AND CONSENT

Method of Participant Identification and Recruitment

Prospective participants will be identified and recruited by, but not limited to, clinicians and other health care personnel at participating sites, online and printed marketing material, and radio and television advertisements. The identification and recruitment of participants will protect privacy and be free of undue influence. All material will be submitted to the IRB for approval.

Process of Consent

Eligible individuals will be given an information sheet and will electronically consent to participate in the study. We are requesting a waiver of signed consent given:

- The research involves no more than minimal risk
- The waiver of informed consent will not adversely affect the rights and welfare of the participant
- It is not practicable to conduct the research without the waiver or alteration
- Whenever appropriate, participants will be provided with additional pertinent information after their participation.

Participants who wish to participate in this study will demonstrate consent by clicking "ACCEPT" to an information sheet that describes the study. They will not be able to participate if they do not click "ACCEPT". Additional information about the study will be available to participants as FAQ. Please reference the FAQ and eConsent information sheet for further details. There will be no elements of coercion or undue influence. Consent will be obtained via an eConsent process and/or through a research coordinator or other appropriate study staff when applicable. Documentation of consent and contact information for those who wish to be contacted in future studies will be obtained and stored on a secure, password-protected server. If eConsent is not available or handwritten signatures are required, a paper consent will be used, the participant will demonstrate consent by signing at the bottom of the information consent sheet that describes the study. For certain instances where a wet ink signature is required, a HIPAA form to be shared with the provider network will be issued for authorization of release of medical records.

Costs to the Participant

Adaptive Biotechnologies, the Sponsor, or study partners (Microsoft, LabCorp/Covance and Illumina), will pay for procedures associated with the study and necessary follow-up. Participants will not incur any costs nor will insurance be billed for research procedures in this study.

Payment for Participation

Participants will be given a $50 gift card for their participation in the study. Participants must complete the blood draw, nasal or oral swab, and study questionnaire prior to receiving the gift card.

Participants will be provided an additional $50 gift card after completing each additional blood draw and questionnaire about their symptoms and medical information relating to COVID-19.

VIII. SPONSOR

Adaptive, or study partners (Microsoft, LabCorp/Covance, and Illumina), will sponsor the entirety of this study and all related costs including resource needs to curate metadata.

REFERENCES

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