

A METHOD OF ASSESSING A DISEASE STATE IN A SUBJECT INFECTED WITH CORONAVIRUS

TECHNICAL FIELD

5

The present disclosure relates broadly to a method of assessing a disease state in a subject infected with coronavirus, such as identifying a risk of severe disease in a subject infected with SARS-CoV-2, and related methods and products thereof.

10

BACKGROUND

The Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Since its outbreak in 2019, COVID-19 has been declared a pandemic by the World Health Organisation (WHO).

The most common symptoms of COVID-19 are fever, cough, headaches, fatigue, muscle or body aches, loss of taste or smell, sore throat, nausea, and diarrhea. Whilst COVID-19 has some similar symptoms as Influenza (Flu) and both are considered contagious respiratory illness, the two diseases are caused by different viruses and COVID-19 has been considered to be more contagious and causing more severe disease in some patients. For example, COVID-19 has also been known to cause signs of severe disease such as trouble breathing, chest pain or pressure, and the like. As patients who develop severe disease can rapidly progress to acute respiratory distress syndrome that results in respiratory failure and/or death, there is a need to identify these subsets of patients to ensure appropriate treatment can be provided at an early stage of the disease.

In view of the ongoing SARS-CoV-2 pandemic, there is a need to provide a method of assessing a disease state in a subject infected with coronavirus.

30

SUMMARY

In one aspect, there is provided a method of identifying a risk of severe disease in a subject infected with SARS-CoV-2, the method comprising: determining a level of one or more immune mediators in the subject's sample; and identifying a risk of severe

35

disease based on the level of the one or more immune mediators, wherein the risk of severe disease is positively associated with the level of the one or more immune mediators and wherein the one or more immune mediators comprises at least one of cytokines, chemokines, growth factors or combinations thereof.

5 In one embodiment, the one or more immune mediators comprises at least one of interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), interferon gamma-induced protein 10 (IP-10), interleukin-18 (IL-18), interleukin-1 receptor antagonist (IL-1RA), platelet-derived growth factor-BB (PDGF-BB), hepatocyte growth factor (HGF), macrophage inflammatory protein-1 alpha (MIP-1 α), interleukin-12p70 (IL-12p70),
10 vascular endothelial growth factor A (VEGF-A) or combinations thereof.

In one embodiment, the sample comprises a sample collected from the subject during a virus-shedding phase.

In one embodiment, the one or more immune mediators comprises at least one of HGF, VEGF-A, MCP-1, IL-6 or combinations thereof.

15 In one embodiment, the one or more immune mediators comprises at least one of HGF, VEGF-A or combinations thereof.

In one embodiment, the sample comprises a sample collected from the subject during a convalescent phase.

In one embodiment, the sample comprises blood or a fraction thereof.

20 In one embodiment, determining a level of one or more immune mediators in a sample comprises contacting the sample with beads that are configured to bind to the one or more immune mediators.

In one aspect, there is provided a method of treating SARS-CoV-2 infection, the method comprising: administering to a subject in need thereof, an agent that is capable
25 of reducing a level of one or more immune mediators in the subject, wherein the one or more immune mediators comprises at least one of cytokines, chemokines, growth factors or combinations thereof.

In one embodiment, the one or more immune mediators comprises at least one of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70, VEGF-A or
30 combinations thereof.

In one embodiment, the agent comprises an IL-6 inhibitor.

In one embodiment, the IL-6 inhibitor comprises an antigen-binding protein comprising the following CDR sequences:

heavy-chain CDR1: GYSITSDHA (SEQ ID NO: 1);

35 heavy-chain CDR2: ISYSGIT (SEQ ID NO: 2);

heavy-chain CDR3: ARSLARTTAMDY (SEQ ID NO: 3);
light-chain CDR1: QDISSY (SEQ ID NO: 4);
light-chain CDR2: YTS (SEQ ID NO: 5);
light-chain CDR3: QQGNTLPYT (SEQ ID NO: 6);

5

heavy-chain CDR1: RFTFDDYA (SEQ ID NO: 9);
heavy-chain CDR2: ISWNSGRI (SEQ ID NO: 10);
heavy-chain CDR3: AKGRDSFDI (SEQ ID NO: 11);
light-chain CDR1: QGISSW (SEQ ID NO: 12);
light-chain CDR2: GAS (SEQ ID NO: 13);
light-chain CDR3: QQANSFPYT (SEQ ID NO: 14); or

10

conservative sequence variants thereof.

In one embodiment, the antigen-binding protein comprises at least one of
15 tocilizumab, sarilumab, antigen-binding fragments thereof or combinations thereof.

In one aspect, there is provided a method of evaluating the efficacy of a treatment
regimen in a subject infected with SARS-CoV-2, the method comprising: determining a
level of one or more immune mediators in a sample collected from the subject at a first
time point; determining a level of the one or more immune mediators in a sample
20 collected from the subject at a second time point following the administration of the
treatment regimen; comparing the level of the one or more immune mediators at the
second time point to the first time point; and identifying the treatment regimen as being
effective when there is a reduction in the level of the one or more immune mediators at
the second time point as compared to the first time point, wherein the one or more
25 immune mediators comprises at least one of cytokines, chemokines, growth factors or
combinations thereof.

In one embodiment, the one or more immune mediators comprises at least one of
IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70, VEGF-A or
combinations thereof, optionally wherein the one or more immune mediators comprises
30 at least one of HGF, VEGF-A, MCP-1, IL-6 or combinations thereof, further optionally
wherein the one or more immune mediators comprises at least one of HGF, VEGF-A or
combinations thereof.

In one embodiment, where the treatment regimen is not identified as being
effective, the method further comprises changing the treatment regimen administered to
35 the subject.

In one embodiment, wherein changing the treatment regimen comprises administering to the subject an agent that is capable of reducing a level of one or more immune mediators in the subject, optionally wherein the agent comprises an IL-6 inhibitor, further optionally wherein the IL-6 inhibitor comprises an antigen-binding

5 protein comprising the following CDR sequences:

heavy-chain CDR1: GYSITSDHA;
heavy-chain CDR2: ISYSGIT;
heavy-chain CDR3: ARSLARTTAMDY;
light-chain CDR1: QDISSY;
10 light-chain CDR2: YTS;
light-chain CDR3: QQGNTLPYT;

heavy-chain CDR1: RFTFDDYA;
heavy-chain CDR2: ISWNSGRI;
15 heavy-chain CDR3: AKGRDSFDI;
light-chain CDR1: QGISSW;
light-chain CDR2: GAS;
light-chain CDR3: QQANSFPYT; or

20 conservative sequence variants thereof,

further optionally wherein the antigen-binding protein comprises at least one of tocilizumab, sarilumab, antigen-binding fragments thereof or combinations thereof.

In one embodiment, the sample comprises blood or a fraction thereof.

25 In one embodiment, determining a level of one or more immune mediators in a sample comprises contacting the sample with beads that are configured to bind to the one or more immune mediators.

In one aspect, there is provided a multiplex array for identifying a risk of severe disease in a subject infected with SARS-CoV-2 or for evaluating the efficacy of a
30 treatment regimen in a subject infected with SARS-CoV-2, the array comprising: beads that are configured to bind to one or more immune mediators selected from the group consisting of: IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70, VEGF-A and combinations thereof.

DEFINITIONS

The term "treatment", "treat" and "therapy", and synonyms thereof as used herein refer to both therapeutic treatment and prophylactic or preventative measures, wherein
5 the object is to prevent or slow down (lessen) a medical condition, which includes but is not limited to diseases (such as a coronavirus infection), symptoms and disorders. A medical condition also includes a body's response to a disease or disorder, e.g. inflammation. Those in need of such treatment include those already with a medical condition as well as those prone to getting the medical condition or those in whom a
10 medical condition is to be prevented.

The term "subject" as used herein includes patients and non-patients. The term "patient" refers to individuals suffering or are likely to suffer from a medical condition such as coronavirus infection, while "non-patients" refer to individuals not suffering and are likely to not suffer from the medical condition. "Non-patients" include healthy individuals,
15 non-diseased individuals and/or an individual free from the medical condition. The term "subject" includes humans and animals. Animals include murine and the like. "Murine" refers to any mammal from the family Muridae, such as mouse, rat, and the like.

The term "micro" as used herein is to be interpreted broadly to include dimensions from about 1 micron to about 1000 microns.

20 The term "nano" as used herein is to be interpreted broadly to include dimensions less than about 1000 nm.

The term "particle" as used herein broadly refers to a discrete entity or a discrete body. The particle described herein can include an organic, an inorganic or a biological particle. The particle used described herein may also be a macro-particle that is formed
25 by an aggregate of a plurality of sub-particles or a fragment of a small object. The particle of the present disclosure may be spherical, substantially spherical, or non-spherical, such as irregularly shaped particles or ellipsoidally shaped particles. The term "size" when used to refer to the particle broadly refers to the largest dimension of the particle. For example, when the particle is substantially spherical, the term "size" can refer to the diameter of the particle; or when the particle is substantially non-spherical, the term "size"
30 can refer to the largest length of the particle.

The terms "coupled" or "connected" as used in this description are intended to cover both directly connected or connected through one or more intermediate means, unless otherwise stated.

The term "associated with", used herein when referring to two elements refers to a broad relationship between the two elements. The relationship includes, but is not limited to a physical, a chemical or a biological relationship. For example, when element A is associated with element B, elements A and B may be directly or indirectly attached to each other or element A may contain element B or vice versa.

The term "adjacent" used herein when referring to two elements refers to one element being in close proximity to another element and may be but is not limited to the elements contacting each other or may further include the elements being separated by one or more further elements disposed therebetween.

The term "and/or", e.g., "X and/or Y" is understood to mean either "X and Y" or "X or Y" and should be taken to provide explicit support for both meanings or for either meaning.

Further, in the description herein, the word "substantially" whenever used is understood to include, but not restricted to, "entirely" or "completely" and the like. In addition, terms such as "comprising", "comprise", and the like whenever used, are intended to be non-restricting descriptive language in that they broadly include elements/components recited after such terms, in addition to other components not explicitly recited. For example, when "comprising" is used, reference to a "one" feature is also intended to be a reference to "at least one" of that feature. Terms such as "consisting", "consist", and the like, may in the appropriate context, be considered as a subset of terms such as "comprising", "comprise", and the like. Therefore, in embodiments disclosed herein using the terms such as "comprising", "comprise", and the like, it will be appreciated that these embodiments provide teaching for corresponding embodiments using terms such as "consisting", "consist", and the like. Further, terms such as "about", "approximately" and the like whenever used, typically means a reasonable variation, for example a variation of +/- 5% of the disclosed value, or a variance of 4% of the disclosed value, or a variance of 3% of the disclosed value, a variance of 2% of the disclosed value or a variance of 1% of the disclosed value.

Furthermore, in the description herein, certain values may be disclosed in a range. The values showing the end points of a range are intended to illustrate a preferred range. Whenever a range has been described, it is intended that the range covers and teaches all possible sub-ranges as well as individual numerical values within that range. That is, the end points of a range should not be interpreted as inflexible limitations. For example, a description of a range of 1% to 5% is intended to have specifically disclosed sub-ranges 1% to 2%, 1% to 3%, 1% to 4%, 2% to 3% etc., as well as individually, values

within that range such as 1%, 2%, 3%, 4% and 5%. It is to be appreciated that the individual numerical values within the range also include integers, fractions and decimals. Furthermore, whenever a range has been described, it is also intended that the range covers and teaches values of up to 2 additional decimal places or significant figures (where appropriate) from the shown numerical end points. For example, a description of a range of 1% to 5% is intended to have specifically disclosed the ranges 1.00% to 5.00% and also 1.0% to 5.0% and all their intermediate values (such as 1.01%, 1.02% ... 4.98%, 4.99%, 5.00% and 1.1%, 1.2% ... 4.8%, 4.9%, 5.0% etc.,) spanning the ranges. The intention of the above specific disclosure is applicable to any depth/breadth of a range.

Additionally, when describing some embodiments, the disclosure may have disclosed a method and/or process as a particular sequence of steps. However, unless otherwise required, it will be appreciated that the method or process should not be limited to the particular sequence of steps disclosed. Other sequences of steps may be possible. The particular order of the steps disclosed herein should not be construed as undue limitations. Unless otherwise required, a method and/or process disclosed herein should not be limited to the steps being carried out in the order written. The sequence of steps may be varied and still remain within the scope of the disclosure.

Furthermore, it will be appreciated that while the present disclosure provides embodiments having one or more of the features/characteristics discussed herein, one or more of these features/characteristics may also be disclaimed in other alternative embodiments and the present disclosure provides support for such disclaimers and these associated alternative embodiments.

DESCRIPTION OF EMBODIMENTS

Exemplary, non-limiting embodiments of a method of assessing a disease state in a subject infected with coronavirus, such as identifying a risk of severe disease in a subject infected with SARS-CoV-2, and related methods and products are disclosed hereinafter.

Subjects/patients with coronavirus infection such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (or COVID-19 infection) may present with a range of clinical manifestations ranging from mild to severe. When severe disease occurs, it may rapidly progress to acute respiratory distress syndrome, resulting in respiratory failure and death. Prospective/early prediction/identification of

subjects/patients who have severe disease or are more likely to develop severe disease/ experience disease progression/ have an adverse clinical outcome/ have an unfavourable prognosis may therefore aid disease management and improve patient outcomes. For example, subjects/patients who are predicted/identified to have severe
5 disease or to be more likely to develop severe disease/ experience disease progression/ have an adverse clinical outcome/ have an unfavourable prognosis may be more closely monitored and/or given prophylactic treatment.

In various embodiments therefore, there is provided a method of stratifying subjects such as coronavirus patients, for example, into cohorts based on their severity
10 of disease, presence of severe disease, prognosis of disease and/or possibility/likelihood to develop severe disease /experience disease progression/have an adverse clinical (or disease) outcome/ have an unfavourable prognosis. In various embodiments, there is provided a method of assessing a disease state in a subject infected with coronavirus or a coronavirus patient. Assessing a disease state may include, for example, determining
15 a severity of the disease, determining a presence of severe disease, a severe disease status, determining a prognosis of the disease and/or determining a possibility/likelihood to develop severe disease/ experience disease progression/ have an adverse clinical outcome/ have an unfavourable prognosis. The method may be a diagnostic method or a prognostic method. In various embodiments, assessing a disease state comprises
20 determining a risk of severe disease/ severe disease development/ severe disease outcome.

In various embodiments, stratifying the subjects/patients or assessing a disease state of the subject/patient comprises measuring/determining an amount/level/proportion of one or more biomarkers in sample(s) from the subject(s)/patient(s). In various
25 embodiments, the amount/level/proportion of the one or more biomarkers in the sample is associated/correlated with severity of disease, presence of severe disease, prognosis of disease and/or possibility/likelihood to develop severe disease /experience disease progression/have an adverse clinical outcome/ have an unfavourable prognosis.

In various embodiments, where the amount/level/proportion of the one or more
30 biomarkers in the sample deviates from or exceeds a threshold amount/level/proportion e.g., a predetermined threshold amount/level/proportion, it is indicative that the subject/patient has a more severe disease, a presence of severe disease, an unfavourable or less favourable prognosis of disease and/or a high/higher possibility/likelihood to develop severe disease /experience disease progression/have
35 an adverse clinical outcome/ have an unfavourable prognosis. It will be appreciated that

it is within the purview of a person skilled in the art to determine the suitable threshold level. For example, the suitable threshold level may be determined by determining a mean/average amount/level/proportion of the one or more biomarkers of a healthy population e.g., a population that does not suffer from coronavirus infection.

5 In various embodiments, a higher amount/level/proportion of the one or more biomarkers in a subject's/patient's sample (e.g., a higher amount/level/proportion that exceeds a threshold amount/level/proportion e.g., a predetermined threshold amount/level/proportion) is indicative the subject/patient has a more severe disease, a presence of severe disease, an unfavourable or less favourable prognosis of disease
10 and/or a high/higher possibility/likelihood to develop severe disease /experience disease progression/have an adverse clinical outcome/ have an unfavourable prognosis. In various embodiments, a lower amount/level/proportion of the one or more biomarkers in a subject's/patient's (e.g., a lower amount/level/proportion that may or may not exceed a threshold amount/level/proportion e.g., a predetermined threshold
15 amount/level/proportion) is indicative the subject/patient has a less severe disease, an absence of severe disease, a favourable or more favourable prognosis of disease and/or a low/lower possibility/likelihood to develop severe disease /experience disease progression/have an adverse clinical outcome/ have an unfavourable prognosis.

Examples of coronaviruses include, but are not limited to, human coronavirus
20 229E (HCoV 229E), human coronavirus OC43 (HCoV OC43), human coronavirus NL63 (HCoV NL63), and human coronavirus HKU1 (HCoV HKU1), Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV-1) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In one embodiment, the coronavirus comprises at least one of MERS-CoV, SARS-CoV-1,
25 or SARS-CoV-2. In one embodiment, coronavirus comprises SARS-CoV-2 (or COVID-19). The term "SARS-CoV-2" includes a virus having the sequence set forth in SEQ ID NO: 17, as well as variants/mutants thereof that share at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least
30 about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.1%, at least about 99.2%, at least about 99.3%, at least about 99.4%, at least about 99.5%, at least about 99.6%, at least about 99.7%, at least about 99.8%, at least about 99.9%, at least about 99.91%, at least about 99.92%,
35 at least about 99.93%, at least about 99.94%, at least about 99.95%, at least about

99.96%, at least about 99.97%, at least about 99.98% or at least about 99.99% sequence identity with the sequence over its entire length. Examples of SARS-CoV-2 mutants include, but are not limited to, B.1.1.7, B.1.351, B.1.427, B.1.429, B.1.526, B.1.526, P.1, P.2. and $\Delta 382$ variants.

5 In various embodiments, the one or more biomarkers comprises one or more immune mediators. An immune mediator may be any substance that is capable of affecting/regulating an activity of the immune system or an immune response. In some embodiments, an immune mediator interacts with one or more components of the immune systems. Examples of immune mediators include, but are not limited to,
10 cytokines, chemokines, and growth factors.

In various embodiments therefore, there is provided a method of identifying a risk of severe disease in a subject infected with SARS-CoV-2, the method comprising: determining a level of one or more immune mediators in the subject's sample; and
15 identifying a risk of severe disease based on the level of the one or more immune mediators. Identifying a risk of severe disease in a subject infected with SARS-CoV-2 may include identifying a predisposal/possibility/likelihood of the subject to develop severe disease, a degree of severity of severe disease in the subject and/or a prognosis of severe disease in the subject. Subjects suffering from severe disease may have pneumonia with hypoxia, an oxygen saturation of less than about or equal to about 94%,
20 require supplemental oxygen and/or intensive-care unit (ICU) care.

The subject may be one who has been infected with SARS-CoV-2 but has not yet progressed to a severe disease state or has not yet shown symptoms of severe disease. The subject may be symptomatic or asymptomatic. The subject may also be one who has been infected with SARS-CoV-2 and is already in a severe disease state
25 or is showing symptoms of a severe disease state. In some embodiments, the subject comprises a subject who is susceptible to developing severe disease, e.g., an aged subject and/or a subject with comorbidity.

In various embodiments, the one or more immune mediators comprises at least one of cytokines, chemokines, growth factors or combinations thereof. In various
30 embodiments, the one or more immune mediators comprises at least one immune mediator selected from the group consisting of cytokines, chemokines, growth factors and combinations thereof. In various embodiments, the one or more immune mediators is selected from the group consisting of cytokines, chemokines, growth factors and combinations thereof. In some embodiments, the method comprises
35 determining/measuring an amount/level/proportion of a further immune mediator that is

not one of cytokines, chemokines or growth factors, and/or determining/measuring an amount/level/proportion of a further non-immune mediator, in addition to determining/measuring an amount/level/proportion of at least one of cytokines, chemokines, growth factors or combinations thereof. In some embodiments, the method
5 does not comprise determining/measuring an amount/level/proportion of a further immune mediator that is not one of cytokines, chemokines or growth factors. In some embodiments, the method does not comprise determining/measuring an amount/level/proportion of a non-immune mediator.

In various embodiments, the risk of severe disease is associated/correlated with
10 the level of the one or more immune mediators. The association/correlation may be positive or negative, linear (i.e., the ratio of change is constant) or non-linear (i.e., the ratio of change is not constant) and monotonic or non-monotonic. In one embodiment, the risk of severe disease is positively associated with the level of the one or more immune mediators. For example, the higher the level of the one or more immune
15 mediators in the subject's sample, the higher/greater the risk of severe disease in the subject (e.g., a greater predisposal/possibility/likelihood to develop severe disease, a higher degree of severity of severe disease and/or a worse prognosis of severe disease.) For example, the lower the level of the one or more immune mediators in the subject's sample, the lower/smaller the risk of severe disease in the subject (e.g., a smaller
20 predisposal/possibility/likelihood to develop severe disease, a lower degree of severity of severe disease and/or a better prognosis of severe disease.)

In various embodiments therefore, there is provided a method of identifying a risk of severe disease in subject infected with SARS-CoV-2, the method comprising: determining a level of one or more immune mediators in a sample from the subject; and
25 identifying a risk of severe disease based on the level of the one or more immune mediators, wherein the risk of severe disease is positively associated with the level of the one or more immune mediators and wherein the one or more immune mediators comprises at least one of cytokines, chemokines, growth factors or combinations thereof.

In various embodiments, the one or more immune mediators comprises
30 inflammatory, pro-inflammatory and/or anti-inflammatory immune mediators. In various embodiments, the one or more immune mediators comprises inflammatory, pro-inflammatory and/or anti-inflammatory cytokines, chemokines, growth factors or combinations thereof. In various embodiments, the immune mediators, the cytokines, the chemokines and the growth factors may be granulocyte-macrophage colony- stimulating
35 factor (GM-CSF), epidermal growth factor (EGF), brain-derived neurotrophic factor, beta-

nerve growth factor (bNGF), basic fibroblast growth factor (FGF-2), hepatocyte growth factor (HGF), monocyte chemoattractant protein (MCP) 1, macrophage inflammatory protein (MIP) 1 α , MIP-1 β , RANTES (regulated on activation, normal T cell expressed and secreted), chemokine (C-X-C motif) ligand (CXCL) 1 (GRO- α), stromal cell-derived factor 1 (SDF-1 α), interferon (IFN) gamma-induced protein 10 (IP-10), eotaxin, IFN- α , IFN- γ , interleukin (IL) IL-1 α , IL-1 β , IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-18, IL-21, IL-22, IL-23, IL-27, IL-31, leukemia inhibitory factor (LIF), stem cell factor (SCF), tumor necrosis factor (TNF- α), TNF- β , vascular endothelial growth factors A and D (VEGF-A, VEGF-D), platelet derived growth factor (PDGF-BB), placental growth factor (PLGF-1) or the like. In various embodiments, the immune mediators, the cytokines, the chemokines and the growth factors may be interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), interferon gamma-induced protein 10 (IP-10), interleukin-18 (IL-18), interleukin-1 receptor antagonist (IL-1RA), platelet-derived growth factor-BB (PDGF-BB), hepatocyte growth factor (HGF), macrophage inflammatory protein-1 alpha (MIP-1 α), interleukin-12p70 (IL-12p70), vascular endothelial growth factor A (VEGF-A) or the like. In various embodiments, the one or more immune mediators comprises at least one of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70, VEGF-A or combinations thereof. In various embodiments, the one or more immune mediators comprises at least one immune mediator selected from the group consisting of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70, VEGF-A and combinations thereof. In various embodiments, the one or more immune mediators is selected from the group consisting of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70, VEGF-A and combinations thereof. In some embodiments, the method comprises determining/measuring a level of a further immune mediator that is not one of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70 and VEGF-A, and/or determining a level of a further non-immune mediator, in addition to determining a level of at least one of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70, VEGF-A and combinations thereof. In some embodiments, the method does not comprise determining a level of a further immune mediator that is not one of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70 and VEGF-A. In some embodiments, the method does not comprise determining a level of a further non-immune mediator. In some embodiments, other than any of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70 and VEGF-A, no other biomarkers are detected/measured. Advantageously, embodiments of the immune mediators as

disclosed herein are useful markers for determining/predicting disease severity, disease outcome and/or prognosis in coronavirus infection such as SARS-CoV-2 infection. In various examples, embodiments of the immune mediators were shown to be positively associated with a severity of disease. In various examples, the level of the immune mediators was found to be the highest in the patient group having both pneumonia and desaturation, followed by the patient group having pneumonia and no desaturation, followed by the patient group having no pneumonia and finally, the level was found to be the lowest in healthy individuals.

Further, in various examples, lower concentrations of chemokines IP-10 (CXCL10), MCP-1 (CCL2), anti-inflammatory protein IL-1RA, growth factors associated with lung injury and regeneration, including HGF, and VEGF-A, were detected in patients infected with the $\Delta 382$ -variant associated with a milder disease outcome compared with patients with the wild-type virus. In various examples, infection with SARS-CoV-2 clades L/V was associated with increased severity and more systemic release of pro-inflammatory MCP-1, IP-10 and IL-6 and anti-inflammatory IL-1RA. In various examples, patients had significantly lower pro-inflammatory cytokine levels (IL-6, IP-10, MCP-1 and VEGF-A) during intermittent viral RNA shedding period, compared with those without intermittent viral RNA shedding after complete virus clearance. Without being bound by theory, it is believed that weaker inflammatory response or suppression of inflammatory responses could be triggering virus re-activation, resulting in intermittent detection in the patients. In various examples, patients with prolonged fever were found to have higher levels of anti-inflammatory IL-1RA, pro-inflammatory IL-6, and chemokine interferon- γ IP-10 compared with controls. Notably, patients with prolonged fever had higher IP-10 levels as compared with patients with saddleback fever. In various examples, hypertension patients on ARB (angiotensin receptor blockers) treatment had significantly higher MCP-1 and IP-10 concentrations compared to non-ARB users. In various examples, convalescents had elevated levels of circulating endothelial cells (CECs), a biomarker of vascular injury, than healthy participants. CECs attributes of the convalescents correlated with systemic levels of IP-10, IL-18, IL-1RA and PDGF-BB. In various examples, recovered patients had elevated levels of pro-inflammatory IL-12p70 and pro-angiogenic VEGF-A at day 180 post infection. Higher levels of MCP-1 and PDGF-BB were detected in patients with persistent symptoms, versus symptom-free patients.

In some embodiments, the one or more immune mediators comprises a direct or indirect interacting partner of IL-6. In some embodiments, the one or more immune mediators comprises an immune mediator that does not interact with IL-6.

In various embodiments, determining a level of one or more immune mediators
5 comprises determining a level of at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine or at least about ten immune mediators. In various
10 embodiments, determining a level of one or more immune mediators comprises determining a level of about one, about two, about three, about four, about five, about six, about seven, about eight, about nine or about ten immune mediators. In various
15 embodiments, determining a level of one or more immune mediators comprises determining a level of no more than about one, no more than about two, no more than about three, no more than about four, no more than about five, no more than about six, no more than about seven, no more than about eight, no more than about nine or no
more than about ten immune mediators. In various embodiments, the one or more
immune mediators comprises at least one of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-
BB, HGF, MIP-1 α , IL-12p70, VEGF-A or combinations thereof.

In various embodiments, the one or more immune mediators comprises at least
one of HGF, VEGF-A, MCP-1, IL-6 or combinations thereof. In various embodiments, the
20 one or more immune mediators comprises at least one immune mediator selected from the group consisting of HGF, VEGF-A, MCP-1, IL-6 or combinations thereof. In various
embodiments, the one or more immune mediators is selected from the group consisting
of HGF, VEGF-A, MCP-1, IL-6 or combinations thereof. In various embodiments, the one
or more immune mediators comprises at least one of HGF, VEGF-A or combinations
25 thereof. In various embodiments, the one or more immune mediators comprises at least
one immune mediator selected from the group consisting of HGF, VEGF-A and
combinations thereof. In various embodiments, the one or more immune mediators is
selected from the group consisting of HGF, VEGF-A and combinations thereof. In various
examples, patients who recovered more quickly following the onset of severe
30 disease/ICU admission were found to have distinctly lower levels of HGF, VEGF-A,
MCP-1 and IL-6 (especially HGF and VEGF-A) as compared to other patients.
Advantageously, HGF, VEGF-A, MCP-1 and IL-6 (especially HGF and VEGF-A) are
strong prognostic markers. In various examples, HGF, VEGF-A, MCP-1 and IL-6
(especially HGF and VEGF-A), independently or in combination, may accurately predict
35 at any early stage, e.g., at the onset of severe disease, a duration of the severe disease

state. For example, a lower level of one or more of HGF, VEGF-A, MCP-1 and IL-6 detected in a sample at the onset of severe disease may be predictive/indicative of a shorter duration of the severe disease state while a higher level of one or more of HGF, VEGF-A, MCP-1 and IL-6 detected in a sample at the onset of severe disease may be
5 predictive/indicative of a longer duration of the severe disease state.

The sample may be collected from the subject at different and/or multiple stages/phases/timepoints of the infection. In various embodiments, the sample comprises a sample collected from the subject during an acute phase of the infection or a convalescent phase of the infection. In various embodiments, the sample comprises a
10 sample collected from the subject during a virus-shedding phase of the infection. In various embodiments, a subject in a virus-shedding phase comprises a subject showing a positive reverse transcriptase- polymerase chain reaction (RT-PCR) test result for the infection. In various embodiments, virus in a subject at a virus-shedding phase is detectable by RT-PCR test. In various embodiments, the sample is collected from the
15 subject no more than about 30 days, no more than about 25 days, no more than about 20 days, no more than about 15 days, no more than about 10 days, no more than about 7 days, no more than about 5 days, no more than about 3 days or no more than about 1 day post-illness onset (pio)/after appearance of symptoms/after being confirmed to be infected. In various embodiments, the sample is collected from the subject from about 0
20 to about 30 days, from about 0 to about 20 days, from about 0 to about 10 days, from about 0 to about 7 days, from about 0 to about 5 days or from about 0 to about 3 days pio/after appearance of symptoms/after being confirmed to be infected. In various embodiments, the sample is collected from the subject on the day of illness onset/appearance of symptoms/being confirmed to be infected. In various embodiments, the
25 sample is collected from the subject no more than about 50 days, no more than about 45 days, no more than about 40 days, no more than about 35 days, no more than about 30 days, no more than about 25 days, no more than about 20 days, no more than about 15 days, no more than about 10 days, no more than about 7 days, no more than about 5 days, no more than about 3 days or no more than about 1 day after onset of severe
30 disease or after ICU admission. In various embodiments, the sample is collected from the subject from about 0 to about 50 days, from about 0 to about 40 days, from about 0 to about 30 days, from about 0 to about 20 days, from about 0 to about 10 days, from about 0 to about 7 days, from about 0 to about 5 days or from about 0 to about 3 days after onset of severe disease or after ICU admission. In various embodiments, the
35 sample is collected from the subject on the day of onset of severe disease or ICU

admission. In various embodiments, the sample is collected from the subject when the subject shows one or more symptoms associated with SARS-CoV-2 or severe disease in SARS-CoV-2, such as but not limited to fever, cough/dry cough, fatigue, myalgia, headache, dyspnea, sore throat, diarrhea, nausea/vomiting, loss of smell, loss of taste, abdominal pain, rhinorrhea, pneumonia, hypoxia, an oxygen saturation of less than about
5 or equal to about 94% and the like.

In various embodiments, wherein the one or more immune comprises at least one of HGF, VEGF-A, MCP-1, IL-6 or combinations thereof, or wherein the one or more immune comprises at least one of HGF, VEGF-A or combinations thereof, the sample
10 comprises a sample collected from the subject during a convalescent phase. In various examples, a level of the immune mediators HGF, VEGF-A, MCP-1, IL-6 in a sample collected from a subject during a convalescent phase was demonstrated to be of particularly high prognostic value.

In various embodiments, the sample comprises a biological sample. Examples of
15 biological samples include, but are not limited to blood, serum, plasma, sputum, saliva, lavage fluid (e.g. bronchial lavage fluid, alveolar lavage fluid and bronchoalveolar lavage fluid), sputum, nasal fluid/swab/wash/aspirate, anterior nares fluid/swab/wash/aspirate, nasal mid-turbinate fluid/swab/wash/aspirate, pharyngeal fluid/swab/wash/aspirate, nasopharyngeal fluid/swab/wash/aspirate, oropharyngeal fluid/swab/wash/aspirate,
20 tissue biopsy e.g. lung biopsy, cerebrospinal fluid, urine, faeces, stool, anal swab, semen, sweat, tears, processed fractions thereof and the like. In various embodiments, the sample comprises a fluid biological sample or a liquid biological sample. In various embodiments, the sample comprises blood or a fraction thereof, e.g., whole blood, blood serum, blood plasma or processed fractions thereof. In various embodiments, the sample
25 comprises blood serum or blood plasma. In one embodiment, the sample comprises blood plasma.

In various embodiments, determining a level of one or more immune mediators in a sample comprises contacting/incubating the sample with a molecule capable of binding to the one or more immune mediators or having affinity for the one or more
30 immune mediators. The assay may comprise a bead-based assay. For example, a molecule capable of binding to the one or more immune mediators or having affinity for the one or more immune mediators may be coupled/conjugated to beads to capture the one or more immune mediators. In various embodiments therefore, determining a level of one or more immune mediators in a sample comprises contacting the sample with
35 beads/affinity beads that are configured to bind to the one or more immune mediators.

In various embodiments, the beads/affinity beads are coupled or conjugated to one or more molecules (e.g., capture molecules) capable of binding to the one or more immune mediators or having affinity for the one or more immune mediators. Examples of molecules that may be employed include, but are not limited to, peptides, proteins, anti-binding proteins, antibodies, aptamers, fragments thereof and the like. In various
5 embodiments, the beads comprise microbeads. In various embodiments, the beads comprise magnetic beads. In various embodiments, the beads are labelled. In various embodiments, the beads are labelled with dye(s) such as fluorescent dye(s). In various embodiments, determining a level of one or more immune mediators in a sample
10 comprises performing a bead-based assay. In various embodiments, the bead-based assay comprises a microbead-based assay. The level of the one or more immune mediators in the sample may be also determined using other methods known in the art e.g., using enzyme-linked immunosorbent assay (ELISA).

 In various embodiments, determining a level of one or more immune mediators
15 in a sample further comprises washing the beads (e.g., to remove any unbound materials). In various embodiments, determining a level of one or more immune mediators in a sample further comprises contacting/incubating the beads with a detecting molecule. In various embodiments, the detecting molecule is labelled. In various
 embodiments, the detecting molecule is labelled with dye(s) such as fluorescent dye(s).

20 In various embodiments, determining a level of one or more immune mediators in a sample further comprises suspending the beads in a sheath fluid. In various embodiments, determining a level of one or more immune mediators in a sample comprises or further comprises performing flow cytometry.

 In various embodiments, determining a level of one or more immune mediators
25 in a sample comprises performing a bead-based flow cytometry assay.

 In various embodiments, determining a level of one or more immune mediators in a sample comprises performing a multiplex assay. In various embodiments, determining a level of one or more immune mediators in a sample comprises contacting/incubating the sample with at least about two, at least about three, at least
30 about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine or at least about ten molecules for binding to at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine or at least about ten immune
 mediators. In various embodiments, determining a level of one or more immune
35 mediators in a sample comprises contacting/incubating the sample with about two, about

three, about four, about five, about six, about seven, about eight, about nine or about ten molecules for binding to about two, about three, about four, about five, about six, about seven, about eight, about nine or about ten immune mediators. In various embodiments, determining a level of one or more immune mediators in a sample comprises
5 contacting/incubating the sample with no more than about two, no more than about three, no more than about four, no more than about five, no more than about six, no more than about seven, no more than about eight, no more than about nine or no more than about ten molecules for binding to no more than about two, no more than about three, no more than about four, no more than about five, no more than about six, no more than about
10 seven, no more than about eight, no more than about nine or no more than about ten immune mediators.

In various embodiments, there is provided an array/panel comprising beads/affinity beads that are configured to bind to the one or more immune mediators. In various embodiments, the array/panel comprises a bead-based array/panel. In various
15 embodiments, the array/panel comprises a microbead-based array/panel. In various embodiments, the beads/affinity beads are coupled or conjugated to one or more molecules capable of binding to one or more immune mediators or having affinity for one or more immune mediators. The one or more immune mediators may comprise one or more features as described herein. Examples of molecules that may be employed
20 include, but are not limited to, peptides, proteins, anti-binding proteins, antibodies, aptamers, fragments thereof and the like.

In various embodiments, the array/panel comprises a multiplex array/panel. In various embodiments, the array/panel is configured to bind/capture at least about two, at least about three, at least about four, at least about five, at least about six, at least
25 about seven, at least about eight, at least about nine or at least about ten immune mediators. In various embodiments, the array/panel is configured to bind/capture about two, about three, about four, about five, about six, about seven, about eight, about nine or about ten immune mediators. In various embodiments, the array/panel is configured to bind/capture no more than about two, no more than about three, no more than about
30 four, no more than about five, no more than about six, no more than about seven, no more than about eight, no more than about nine or no more than about ten immune mediators. For example, the beads may comprise multiple different molecules for binding to or capturing multiple different immune mediators. In various embodiments, the beads are configured to bind to one or more immune mediators comprising at least one of IL-6,
35 MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70, VEGF-A or

combinations thereof. In various embodiments, the beads are configured to bind to one or more immune mediators selected from the group consisting of: IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70, VEGF-A and combinations thereof. Advantageously, embodiments of array/panel are useful for stratifying subjects with coronavirus, for assessing a disease state of a subject with coronavirus, for identifying a risk of severe disease in a subject with coronavirus, for evaluating the efficacy of a treatment in a subject with coronavirus and the like.

In various embodiments therefore, there is provided a multiplex array for identifying a risk of severe disease in a subject infected with SARS-CoV-2 or for evaluating the efficacy of a treatment in a subject infected with SARS-CoV-2, the array comprising: beads that are configured to bind to one or more immune mediators selected from the group consisting of: IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70, VEGF-A and combinations thereof.

In various embodiments, there is provided a method of evaluating the efficacy of a treatment in a subject infected with coronavirus such as SARS-CoV-2, the method comprising: determining a level of one or more immune mediators in a sample collected from the subject at a first time point; determining a level of the one or more immune mediators in a sample collected from the subject at a second time point e.g., following the administration of a treatment regimen; comparing the level of the one or more immune mediators at the second time point to the first time point; and identifying the treatment regimen as being effective when there is a reduction in the level of the one or more immune mediators at the second time point as compared to the first time point. In various embodiments, the method comprises identifying the treatment regimen as being in effective when there is no substantial change or no reduction or an increase in the level of the one or more immune mediators at the second time point as compared to the first time point. In various embodiments, the subject is not yet administered the treatment regimen at the first time point. In various embodiments, the first time point is earlier than the second timepoint. In various embodiments, the sample comprises one or more features as described herein. In various embodiments, the sample comprises blood or a fraction thereof. In various embodiments, the determining step comprises one or more features as described herein. In various embodiments, determining a level of one or more immune mediators in a sample comprises contacting the sample with beads that are configured to bind to the one or more immune mediators. In various embodiments, the one or more immune mediators comprises one or more features as described herein. In various embodiments, the one or more immune mediators comprises at least one of

cytokines, chemokines, growth factors or combinations thereof. In various embodiments, the one or more immune mediators comprises at least one immune mediator selected from the group consisting of cytokines, chemokines, growth factors and combinations thereof. In various embodiments, the one or more immune mediators is selected from

5 the group consisting of cytokines, chemokines, growth factors and combinations thereof. In some embodiments, the method comprises determining/measuring an amount/level/proportion of a further immune mediator that is not one of cytokines, chemokines or growth factors, and/or determining/measuring an amount/level/proportion of a further non-immune mediator, in addition to determining/measuring an

10 amount/level/proportion of at least one of cytokines, chemokines, growth factors or combinations thereof. In some embodiments, the method does not comprise determining/measuring an amount/level/proportion of a further immune mediator that is not one of cytokines, chemokines or growth factors. In some embodiments, the method does not comprise determining/measuring an amount/level/proportion of a non-immune

15 mediator. In various embodiments, the one or more immune mediators comprises at least one of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70, VEGF-A or combinations thereof, optionally wherein the one or more immune mediators comprises at least one of HGF, VEGF-A, MCP-1, IL-6 or combinations thereof, further optionally wherein the one or more immune mediators comprises at least one of HGF,

20 VEGF-A or combinations thereof.

In various embodiments, where the treatment regimen is not identified as being effective or where the treatment regimen is identified as being ineffective, the method further comprises changing the treatment regimen administered to the subject. Changing the treatment regimen may involve subjecting/exposing the subject to a second therapy

25 that is different from the current or the first therapy. Changing the treatment regimen may involve replacing the current treatment regimen received by the subject with another treatment regimen, or it may involve administering to the subject additional therapies in addition to the current treatment regimen, or where combination therapies are used in the current treatment regimen, it may involve removing one or more therapies. Changing

30 the treatment regimen may also involve changing certain aspects of the current treatment regimen, e.g., changing a dosage and/or dose frequency of a therapy. In various embodiments, changing the treatment regimen comprises administering to the subject an agent that is capable of reducing a level of one or more immune mediators in the subject. The one or more immune mediators may comprise one or more features as

35 described herein. In various embodiments, the one or more immune mediators

comprises at least one of cytokines, chemokines, growth factors or combinations thereof. In various embodiments, the one or more immune mediators comprises at least one of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70, VEGF-A or combinations thereof. In various embodiments, the one or more immune mediators
5 comprises IL-6 or is IL-6.

In various embodiments, there is provided a method of treating coronavirus infection, such as SARS-CoV-2 infection, the method comprising, administering to a subject in need thereof, an agent that is capable of reducing a level of one or more immune mediators in the subject. The one or more immune mediators may comprise one
10 or more features as described herein. In various embodiments, the one or more immune mediators comprises at least one of cytokines, chemokines, growth factors or combinations thereof. In various embodiments, the one or more immune mediators comprises at least one of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70, VEGF-A or combinations thereof. In various embodiments, the one or more
15 immune mediators comprises IL-6 or is IL-6. In various embodiments, the method comprises a method of treating severe disease in SARS-CoV-2 infection. In various embodiments, the method comprises prophylactic treatment of severe disease in SARS-CoV-2 infection, for example, in subjects who are likely or susceptible to developing severe disease.

20 An "agent" may be anything (physical, chemical, biological etc.) that an immune mediator may be exposed to e.g., in order to reduce its levels. Non-limiting examples of an "agent" include chemicals, compounds, compositions, molecules, small molecules, nucleic acid sequences, nucleic acid analogues, proteins, peptides, aptamers, antigen-binding proteins, antibodies, or fragments or derivatives thereof. The term "agent" in no
25 way excludes the use of two or more such agents. Accordingly, the term "agent" also contemplates mixtures, fusions, combinations, compositions and conjugates, for example, mixtures, fusions, combinations, compositions and conjugates of any chemicals, compounds, compositions, molecules, small molecules, nucleic acid sequences, nucleic acid analogues, proteins, peptides, aptamers, antigen-binding
30 proteins, antibodies, or fragments or derivatives thereof etc. In various embodiments, a derivative of a molecule is structurally related to the molecule. For example, the derivative may share a common structural feature, fundamental structure and/or underlying chemical basis with the molecule. A derivative is not limited to one produced or obtained from the molecule although it may be one produced or obtained from the
35 molecule. In some embodiments, the derivative is derivable, at least theoretically, from

the molecule through modification of the molecule. In some embodiments, a derivative of a molecule shares or at least retains to a certain extent a function, chemical property, biological property, chemical activity and/or biological activity associated with the molecule. A skilled person will be able to identify, on a case-by-case basis and upon
5 reading of the disclosure, the common structural feature, fundamental structure and/or underlying chemical basis of the molecule that have to be maintained in the derivative to retain the function, chemical property, biological property, chemical activity, and/or biological activity. A skilled person will also be able to identify assays that can prove the retention of the function, chemical property, biological property, chemical activity, and/or
10 biological activity. For example, a binding assay such as ELISA may be carried out to determine a binding property of a derivative of a molecule.

In various embodiments, the agent comprises an agent that is capable of reducing a level of IL-6. The agent may reduce a level of IL-6 through direct or indirect interaction with IL-6. For example, the agent may target an interacting partner e.g., a direct
15 interacting partner, of IL-6. In various embodiments, the agent comprises an IL-6 inhibitor/antagonist. The IL-6 inhibitor/antagonist may reduce an expression and/or an activity of IL-6. In various examples, for elderly patients ≥ 50 years old, a higher percentage of the patients in the patient group treated with COX-2 inhibitors showed reduction of IL-6 levels as compared to the control patient group. A greater reduction of
20 absolute IL-6 levels was also observed in the treatment group as compared to control group.

In various embodiments, the IL-6 inhibitor/antagonist comprises an antigen-binding protein.

An antigen-binding protein may include any protein construct that is capable of
25 binding to IL-6. Examples include, but are not limited to, antibodies and fragments thereof such as antigen-binding fragments. Non-limiting examples of antigen-binding fragments include one or more fragments or portions of an antibody that retain the ability to specifically bind to an antigen (e.g., IL-6), or synthetic modifications of an antibody fragments that retain the desired binding ability to the antigen. In various embodiments,
30 antigen-binding fragments include single domain antibodies, further engineered molecules (such as, but is not limited to diabodies, triabodies, tetrabodies, minibodies, and the like), Fab fragments, Fab' fragments, F(ab')₂ fragments, Fd fragments, Fv fragments, single-chain Fv (scFv) molecules, seFv molecules, scFv dimer, BsFv molecules, dsFv molecules, (dsFv)₂ molecules, dsFv-dsFv' molecules, Fv fragments,
35 dAb fragments, bispecific antibodies, ds diabodies, nanobodies, domain antibodies,

bivalent domain antibodies, and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (e.g., an isolated complementarity determining region (CDR)). In some embodiments, antigen-binding fragments may retain at least about one, at least about two, at least about three, at least
 5 about four, at least about five or at least about six of the CDR regions/sequences of the antibody.

In various embodiments, the antigen-binding protein comprises at least about one, at least about two, at least about three, at least about four, at least about five or at least about six of the CDRs of tocilizumab or sarilumab, or sequence variants e.g.,
 10 conservative sequence variants of the CDRs. In various embodiments, one or more CDRs of the antigen-binding protein shares at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence similarity/identity with one or
 15 more CDRs of tocilizumab or sarilumab. In various embodiments, one or more CDRs of the antigen-binding protein comprises no more than about ten, no more than about nine, no more than about eight, no more than seven, no more than about six, no more than about five, no more than about four, no more than about three, no more than about two or no more than about one amino acid difference with one or more CDRs of tocilizumab
 20 or sarilumab.

In various embodiments, the antigen-binding protein comprises a sequence set forth in Table 1 below, or a conservative sequence variant thereof, or a sequence sharing at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about
 25 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.1%, at least about 99.2%, at least about 99.3%, at least about 99.4%, at least about 99.5%, at least about 99.6%, at least about 99.7%, at least about 99.8% or at least about 99.9% sequence similarity/identity thereto.

30 **Table 1: Sequences of antigen-binding proteins 1 and 2**

	Region	Sequence	SEQ ID NO:
Antigen-binding	heavy-chain CDR1 (positions 27-38)	GYSIT...SDHA	1
	heavy-chain CDR2	ISYS...GIT	2

protein 1	(positions 56-65)		
	heavy-chain CDR3 (positions 105-117)	ARSLAR.TTAMDY	3
	light-chain CDR1 (positions 27-38)	QDI.....SSY	4
	light-chain CDR2 (positions 56-65)	YT.....S	5
	light-chain CDR3 (positions 105-117)	QQGNT....LPYT	6
	heavy chain variable domain	QVQLQESGPGGLVVRPSQTLSTCTVSGYSIT SDHAWSWVRQPPGRGLEWIGYISYSGITTY NPSLKSRTVTLRDTSKNQFSLRLSSVTAAD TAVYYCARSLARTTAMDYWGQGSGLTVSS	7
	light chain variable domain	DIQMTQSPSSLSASVGDRVITICRASQDISS YLNWYQQKPGKAPKLLIYYTSRLHSGVPSR FSGSGSGTDFTFTISSLQPEDIATYYCQQG NTLPYTFGQGTKVEIK	8
Antigen-binding protein 2	heavy-chain CDR1 (positions 27-38)	RFTF....DDYA	9
	heavy-chain CDR2 (positions 56-65)	ISWN..SGRI	10
	heavy-chain CDR3 (positions 105-117)	AKGRD....SFDI	11
	light-chain CDR1 (positions 27-38)	QGI.....SSW	12
	light-chain CDR2 (positions 56-65)	GA.....S	13
	light-chain CDR3 (positions 105-117)	QQANS....FPYT	14
	heavy chain variable domain	EVQLVESGGGLVQPGRSLRLSCAASRFTF DDYAMHWVRQAPGKGLEWVSGISWNSGR IGYADSVKGRFTISRDNALSLFLQMNGLR AEDTALYYCAKGRDSFDIWGQGTMTVSS	15
	light chain variable domain	DIQMTQSPSSVSASVGDRVITICRASQGIS SWLAWYQQKPGKAPKLLIYGASSLESGVP SRFSGSGSGTDFTLTISLQPEDFASYCQ QANSFPYTFGQGTKEIK	16

The table above defines the CDRs by the standardized IMGT numbering system, namely: CDR1=IMGT positions 27-38; CDR2=IMGT 56-65; and CDR3=IMGT 105-117.

- In various embodiments, the antigen-binding protein comprises at least about one, at least about two, at least about three, at least about four, at least about five or at least about six CDRs having sequences corresponding to those of SEQ ID NO: 1-6 and/or 9-14, or sequence variants e.g., conservative sequence variants thereof. In various embodiments, one or more CDRs of the antigen-binding protein shares at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at

least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence similarity/identity with one or more sequence of SEQ ID NO: 1-6 and/or 9-14. In various embodiments, one or more CDRs of the antigen-binding protein comprises no more than
5 about ten, no more than about nine, no more than about eight, no more than seven, no more than about six, no more than about five, no more than about four, no more than about three, no more than about two or no more than about one amino acid difference with one or more sequence of SEQ ID NO: 1-6 and/or 9-14.

In various embodiments, the antigen-binding protein comprises at least about one,
10 at least about two, at least about three, at least about four, at least about five or at least about six of the following CDR sequences: heavy-chain CDR1: GYSITSDHA; heavy-chain CDR2: ISYSGIT; heavy-chain CDR3: ARSLARTTAMDY; light-chain CDR1: QDISSY; light-chain CDR2: YTS; light-chain CDR3: QQGNTLPYT; or sequence variants e.g., conservative sequence variants thereof. In various embodiments, the antigen-binding protein comprises at least about one, at least about two, at least about three, at
15 least about four, at least about five or at least about six CDRs that share at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence
20 similarity/identity with at least about one, at least about two, at least about three, at least about four, at least about five or at least about six of the following CDR sequences: heavy-chain CDR1: GYSITSDHA; heavy-chain CDR2: ISYSGIT; heavy-chain CDR3: ARSLARTTAMDY; light-chain CDR1: QDISSY; light-chain CDR2: YTS and light-chain CDR3: QQGNTLPYT. In various embodiments, the antigen-binding protein comprises
25 at least about one, at least about two, at least about three, at least about four, at least about five or at least about six CDR sequences comprising no more than about ten, no more than about nine, no more than about eight, no more than seven, no more than about six, no more than about five, no more than about four, no more than about three, no more than about two or no more than about one amino acid difference with the
30 following CDR sequences: heavy-chain CDR1: GYSITSDHA; heavy-chain CDR2: ISYSGIT; heavy-chain CDR3: ARSLARTTAMDY; light-chain CDR1: QDISSY; light-chain CDR2: YTS and light-chain CDR3: QQGNTLPYT.

In various embodiments, the antigen-binding protein comprises at least about one, at least about two, at least about three, at least about four, at least about five or at least
35 about six of the following CDR sequences: heavy-chain CDR1: RFTFDDYA; heavy-chain

CDR2: ISWNSGRI; heavy-chain CDR3: AKGRDSFDI; light-chain CDR1: QGISSW; light-chain CDR2: GAS; light-chain CDR3: QQANSFPYT; or sequence variants e.g., conservative sequence variants thereof. In various embodiments, the antigen-binding protein comprises at least about one, at least about two, at least about three, at least about four, at least about five or at least about six CDRs that share at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence similarity/identity with at least about one, at least about two, at least about three, at least about four, at least about five or at least about six of the following CDR sequences: heavy-chain CDR1: RFTFDDYA; heavy-chain CDR2: ISWNSGRI; heavy-chain CDR3: AKGRDSFDI; light-chain CDR1: QGISSW; light-chain CDR2: GAS and light-chain CDR3: QQANSFPYT. In various embodiments, the antigen-binding protein comprises at least about one, at least about two, at least about three, at least about four, at least about five or at least about six CDR sequences comprising no more than about ten, no more than about nine, no more than about eight, no more than seven, no more than about six, no more than about five, no more than about four, no more than about three, no more than about two or no more than about one amino acid difference with the following CDR sequences: heavy-chain CDR1: RFTFDDYA; heavy-chain CDR2: ISWNSGRI; heavy-chain CDR3: AKGRDSFDI; light-chain CDR1: QGISSW; light-chain CDR2: GAS and light-chain CDR3: QQANSFPYT.

In various embodiments, the antigen-binding protein comprises an amino acid region, a light chain variable domain sequence and/or a heavy chain variable domain sequence corresponding to an amino acid region, a light chain variable domain sequence and/or a heavy chain variable domain sequence of tocilizumab or sarilumab, or a conservative sequence variant of a light chain variable domain sequence and/or a heavy chain variable domain sequence of tocilizumab or sarilumab. In various embodiments, the antigen-binding protein comprises an amino acid region or a light chain variable domain sequence and/or a heavy chain variable domain sequence sharing at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.1%, at least about 99.2%, at least about 99.3%, at least about 99.4%, at least about 99.5%, at least about 99.6%, at least about 99.7%, at least about 99.8% or at least about 99.9% sequence

similarity/identity with an amino acid region, a light chain variable domain sequence and/or a heavy chain variable domain sequence of tocilizumab or sarilumab. In various embodiments, the antigen-binding protein comprises an amino acid region, a light chain variable domain sequence and/or a heavy chain variable domain sequence comprising

5 no more than about ten, no more than about nine, no more than about eight, no more than seven, no more than about six, no more than about five, no more than about four, no more than about three, no more than about two or no more than about one amino acid difference with an amino acid region, a light chain variable domain sequence and/or a heavy chain variable domain sequence of tocilizumab or sarilumab.

10 In various embodiments, the antigen-binding protein comprises an amino acid region, a light chain variable domain sequence and/or a heavy chain variable domain sequence corresponding to SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 15 and/or SEQ ID NO: 16, or sequence variants e.g. conservative sequence variants thereof. In various embodiments, the antigen-binding protein comprises an amino acid region or a light

15 chain variable domain sequence and/or a heavy chain variable domain sequence sharing at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.1%, at least

20 about 99.2%, at least about 99.3%, at least about 99.4%, at least about 99.5%, at least about 99.6%, at least about 99.7%, at least about 99.8% or at least about 99.9% with SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 15 and/or SEQ ID NO: 16. In various embodiments, the antigen-binding protein comprises an amino acid region, a light chain variable domain sequence and/or a heavy chain variable domain sequence comprising

25 no more than about ten, no more than about nine, no more than about eight, no more than seven, no more than about six, no more than about five, no more than about four, no more than about three, no more than about two or no more than about one amino acid difference with SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 15 and/or SEQ ID NO: 16.

30 In various embodiments, the IL-6 inhibitor comprises an antigen-binding protein comprising the following CDR sequences:

heavy-chain CDR1: GYSITSDHA (SEQ ID NO: 1);

heavy-chain CDR2: ISYSGIT (SEQ ID NO: 2);

35 heavy-chain CDR3: ARSLARTTAMDY (SEQ ID NO: 3);

- light-chain CDR1: QDISSY (SEQ ID NO: 4);
 light-chain CDR2: YTS (SEQ ID NO: 5);
 light-chain CDR3: QQGNTLPYT (SEQ ID NO: 6);
- 5 heavy-chain CDR1: RFTFDDYA (SEQ ID NO: 9);
 heavy-chain CDR2: ISWNSGRI (SEQ ID NO: 10);
 heavy-chain CDR3: AKGRDSFDI (SEQ ID NO: 11);
 light-chain CDR1: QGISSW (SEQ ID NO: 12);
 light-chain CDR2: GAS (SEQ ID NO: 13);
- 10 light-chain CDR3: QQANSFPYT (SEQ ID NO: 14); or

conservative sequence variants thereof (e.g., an antigen-binding protein comprising the following CDR sequences: heavy-chain CDR1: a conservative sequence variant of GYSITSDHA; heavy-chain CDR2: ISYSGIT (SEQ ID NO: 2); heavy-chain CDR3: ARSLARTTAMDY (SEQ ID NO: 3); light-chain CDR1: QDISSY (SEQ ID NO: 4); light-chain CDR2: YTS (SEQ ID NO: 5); light-chain CDR3: QQGNTLPYT (SEQ ID NO: 6), or an antigen-binding protein comprising the following CDR sequences: heavy-chain CDR1: a conservative sequence variant of RFTFDDYA; heavy-chain CDR2: a conservative sequence variant of ISWNSGRI; heavy-chain CDR3: AKGRDSFDI (SEQ ID NO: 11); light-chain CDR1: QGISSW (SEQ ID NO: 12); light-chain CDR2: GAS (SEQ ID NO: 13); light-chain CDR3: QQANSFPYT (SEQ ID NO: 14) etc.).

In various embodiments, the antigen-binding protein comprises at least one of tocilizumab, sarilumab, antigen-binding fragments thereof or combinations thereof.

In various embodiments, there is provided a method of screening for/identifying an agent for treating coronavirus infection such as SARS-CoV-2 infection, the method comprising: contacting the one or more immune mediators (or a cell comprising the one or more immune mediators) with a candidate agent; and determining whether candidate agent is capable of binding to and/or inhibiting the one or more immune mediators, wherein where the candidate agent is capable of binding to and/or inhibiting the one or more immune mediators, identifying the candidate agent as an agent for treating coronavirus infection such as SARS-CoV-2 infection and/or wherein where the candidate agent is incapable of binding to and/or inhibiting the one or more immune mediators, concluding that the candidate agent is not an agent for treating coronavirus infection such as SARS-CoV-2 infection. In various embodiments, the method is a method of screening for/identifying an agent for treating severe disease in SARS-CoV-2 infection.

An agent may inhibit an immune mediator by occupying, or otherwise reducing access to, a region of the immune mediator for binding to an interaction partner. A binding activity for an immune mediator and/or inhibitory activity against an immune mediator may be determined by methods known to those skilled in the art. Examples of suitable methods include ELISA and competitive ELISA.

In various embodiments, there is provided an agent as disclosed herein for use in the treatment of coronavirus infection such as SARS-CoV-2 infection. In various embodiments, there is provided the use of an agent as disclosed herein in the manufacture of a medicament for the treatment of coronavirus infection such as SARS-CoV-2 infection.

Embodiments of the methods disclosed herein may be *in vitro*, *ex vivo* or *in vivo* methods.

In various embodiments, there is provided a method or a product as described herein.

It will be appreciated by a person skilled in the art that other variations and/or modifications may be made to the embodiments disclosed herein without departing from the spirit or scope of the disclosure as broadly described. For example, in the description herein, features of different exemplary embodiments may be mixed, combined, interchanged, incorporated, adopted, modified, included etc. or the like across different exemplary embodiments. The present embodiments are, therefore, to be considered in all respects to be illustrative and not restrictive.

DETAILED DESCRIPTION OF FIGURES

Example embodiments of the disclosure will be better understood and readily apparent to one of ordinary skill in the art from the following discussions and if applicable, in conjunction with the figures. It should be appreciated that other modifications related to structural, electrical and optical changes may be made without deviating from the scope of the invention. Example embodiments are not necessarily mutually exclusive as some may be combined with one or more embodiments to form new exemplary embodiments. The example embodiments should not be construed as limiting the scope of the disclosure.

FIG. 1A-C Immune signature of COVID-19 patients reveal cytokines associated with disease severity. Plasma fractions were isolated from the blood of

COVID-19 patients (n = 81) at different time-points. Time-points closest to symptom onset, including mild (no pneumonia), pneumonia with no desaturation, or pneumonia with desaturation, were chosen to identify cytokines that were associated with disease severity. Concentrations of 45 immune mediators were quantified using a 45-plex microbead-based immunoassay.

FIG. 1A Longitudinal profile of detectable cytokines in COVID-19 patients during the acute phase of disease. Statistical analyses were performed using unpaired t-test against the healthy baseline (*P <0.05; **P <0.01, ***P <0.001). Cytokine level for healthy controls (n = 23) is indicated by the dotted line.

FIG. 1B Heatmap of severity-associated cytokine levels in patients with different disease outcome (healthy controls, n=23; no pneumonia, n=34; pneumonia and no desaturation, n =28; pneumonia and desaturation, n=19). Each color represents the relative concentration of a particular analyte. White and black indicates low and high concentration, respectively.

FIG. 1C Longitudinal comparison of HGF, VEGF-A, MCP-1 and IL-6 cytokine levels in twelve ICU patients. CT009 and CT032 recovered from COVID-19 at 16 and 25 days post-ICU admission, respectively, while the remaining patients remained in hospital during time of study. Cytokine level for healthy controls (n = 23) is indicated by the dotted line. Patient samples that are not detectable are assigned the value of logarithmic transformation of Limit of Quantification (LOQ).

FIG. 2 Profiles of immune mediators of COVID-19 patients during virus-shedding period. Plasma samples of patients with different disease severity (no pneumonia, n=34; pneumonia, but no desaturation, n =28; pneumonia and desaturation, n=19) were subjected to multiplex microbead-based immunoassay. Profiles of significant immune mediators are illustrated as scatter plots. One-way ANOVAs were conducted on the logarithmically transformed concentration with post hoc t tests corrected using the method of Bonferroni. ANOVA results were corrected for multiple testing using the method of Benjamini and Hochberg (*p < 0.05, **p <0 .01, and ***p <0 .001). Cytokine level for healthy controls (n = 23) is indicated by the dotted line. Patient samples that are not detectable are assigned the value of logarithmic transformation of Limit of Quantification (LOQ).

FIG. 3 Immune signature of COVID-19 patients in ICU. Plasma fractions were isolated from the blood of COVID-19 patients at different time-points. Time-points closest to ICU admission were chosen to characterize trends of cytokines that were associated with disease severity. Concentrations of 45 immune mediators were quantified using a 45-plex microbead-based immunoassay. Longitudinal comparisons of IP-10, IL-18, IL-12p70, PDGF-BB, IL-1RA and MIP-1 α in twelve ICU patients were performed. CT009 and CT032 recovered from COVID-19 at 16 and 25 days post-ICU admission, while the remaining patients remained in hospital during time of study. Cytokine level for healthy controls (n = 23) is indicated by the dotted line. Patient samples that are not detectable are assigned the value of logarithmic transformation of Limit of Quantification (LOQ).

FIG. 4A-D Network analysis of immune mediators associated with disease severity in COVID-19 patients.

FIG. 4A Interactomic analysis by Ingenuity Pathway Analysis (IPA) Software. The network is displayed graphically as nodes and edges. Each node represents either severity-associated cytokine or disease condition. Thickness of edges represent the magnitude of biological relationship between connected nodes. Edges are drawn thicker when the association between parameters is stronger.

FIG. 4B IPA analysis of the significant immune mediators associated with disease severity in COVID-19 patients. The chart represents the top ten significantly associated canonical pathways with the immune mediators.

FIG. 4C Venn diagrams show common immune mediators between influenza virus infection and SARS-CoV-2 virus infection.

FIG. 4D Interactive relationships between the immune mediators were determined by STRING analysis, with a confidence threshold of 0.8. IL-6, interleukin 6; IL-1RA, interleukin 1 receptor antagonist; MCP-1, monocyte chemoattractant protein-1; HGF, hepatocyte growth factor; VEGF-A, vascular endothelial growth factor A; PDGF-BB, two beta subunits of platelet-derived growth factor; IP-10, IFN- γ inducible protein, IL-8, interleukin 8; NRP-2, neuropilin 2.

FIG. 5 Correlation of immune mediators with age in COVID-19 patients. Spearman rank correlation analysis was conducted on the logarithmically transformed

concentration and age in years of 81 COVID-19 patients. Profiles of significant immune mediators (IL-6 and IP-10) are illustrated as scatter plots with the results of the correlation of immune mediators against age of the patients shown as asterisks (**p < 0.001).

5

EXPERIMENTAL SECTION

Materials and methods

Multiplex Microbead-Based immunoassay

10 Triton™ X-100 (1%) (Sigma Aldrich) inactivated plasma immune mediator levels in the acute and convalescence phase of COVID-19 patients were measured using Cytokine/Chemokine/Growth Factor 45-Plex Human ProcartaPlex™ Panel 1 (ThermoFisher Scientific). Cytokines included granulocyte-macrophage colony-stimulating factor (GM-CSF), epidermal growth factor (EGF), brain-derived neurotrophic factor, beta-nerve growth factor (bNGF), basic fibroblast growth factor (FGF-2),
15 hepatocyte growth factor (HGF), monocyte chemoattractant protein (MCP) 1, macrophage inflammatory protein (MIP) 1 α , MIP-1 β , RANTES (regulated on activation, normal T cell expressed and secreted), chemokine (C-X-C motif) ligand (CXCL) 1 (GRO- α), stromal cell-derived factor 1 (SDF-1 α), interferon (IFN) gamma-induced protein 10 (IP-10), eotaxin, IFN- α , IFN- γ , interleukin (IL) IL-1 α , IL-1 β , IL-1RA, IL-2, IL-4, IL-5, IL-6,
20 IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-18, IL-21, IL-22, IL-23, IL-27, IL-31, leukemia inhibitory factor (LIF), stem cell factor (SCF), tumor necrosis factor (TNF- α), TNF- β , vascular endothelial growth factors A and D (VEGF-A, VEGF-D), platelet derived growth factor (PDGF-BB), and placental growth factor (PLGF-1).

25 Standards and plasma from COVID-19 patients and healthy controls were incubated with fluorescent-coded magnetic beads pre-coated with respective capture antibodies in a 96 black clear-bottom plate. After an overnight incubation at 4°C, plates were washed five times with wash buffer (PBS with 1% BSA (Capricorn Scientific) and 0.01% Tween20 (Promega)). Biotinylated detection antibodies were incubated with the
30 complex for 1 hour and washed five times with wash buffer. Subsequently, Streptavidin-PE was added and incubated for another 30 mins. Plates were washed five times again, then beads were re-suspended with sheath fluid before acquiring on the FLEXMAP® 3D (Luminex) using xPONENT® 4.0 (Luminex) acquisition software. Data analysis was

done on Bio-Plex Manager™ 6.1.1 (Bio-Rad). Standard curves were generated with a 5-PL (5-parameter logistic) algorithm, reporting values for both MFI and concentration data.

Data processing and statistical analysis

5 Internal control samples were included in each Luminex assays to remove any potential plate effects. Readouts of these samples were then used to normalize the assayed plates. A correction factor was obtained from the differences observed across the multiple assays and this correction factor was then used to normalize all the samples. The concentrations were logarithmically transformed to ensure normality.

10 Data processing and analysis were done in the R statistical language (version 3.3.1). Unpaired t test was applied to ascertain significant difference in the immune mediator levels between the COVID-19 patients and healthy controls at different time points post illness onset. The time points closest to event onset, including mild symptoms with no pneumonia, pneumonia with no desaturation, or pneumonia with desaturation, 15 were chosen to identify cytokines that were associated with disease severity. One-way ANOVA analysis with post-hoc t test corrected using the method of Bonferroni was used to discern the differences in immune mediator levels between the various disease severity groups. One-way ANOVA results were corrected for multiple testing using the method of Benjamini and Hochberg. P values less than 0.05 were considered to be 20 statistically significant. Plots were generated using GraphPad Prism version 7.

TM4-MeV Suite (version 10.2) was used to compute hierarchical clustering and heat map on the immune mediators. In the heat map presentation, the average concentration was computed for each measured immune mediator in its respective 25 group, and the average values were then scaled between 0 and 1 for visualization. An association analysis was done by combining concentrations of immune mediators and clinical data on disease severity (pneumonia, desaturation and ICU admission) into a network using Fisher's Exact, ANOVA and Pearson correlation results. Biological processes and immune pathways were predicted from the disease severity-associated 30 immune mediators with Ingenuity Pathway Analysis (IPA; Qiagen). Interaction networks of these immune mediators were also predicted and illustrated with STRING (version 11.0; available at: <https://string-db.org>).

Results

35 ***Immune signatures of COVID-19 patients***

Of the 100 patients included in this study, immune mediators in plasma fractions collected from 81 patients were quantified by multiplex microbead-based immunoassay. Collectively, levels of IL-2, IL-18, IFN- γ , TNF- α , MCP-1, HGF, BDNF, LIF, PLGF-1 and bNGF were significantly produced in high levels when compared to the healthy control baseline at post-illness onset (PIO) (FIG. 1A and Table 2). Stratification of COVID-19 patients during virus-shedding phase based on disease severity (Scale 1: no pneumonia; Scale 2: pneumonia, but no desaturation; Scale 3: pneumonia and desaturation) revealed several immune mediators that were associated with severity. These include pro-inflammatory cytokines IP-10, HGF, IL-6, MCP-1, IL-18, IL-12p70 and MIP-1 α , growth factors VEGF-A and PDGF-BB, and anti-inflammatory cytokine IL-1RA (FIG. 1B and FIG. 2). Baseline levels of these cytokines are shown in Table 3.

Table 2. Mean concentrations of immune mediators post-illness onset (PIO)

No.	Analyte PIO(Day)	Log10 [Cytokine] pg/mL																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	BDNF	1.48	1.40	1.56	1.66	1.95	1.58	1.90	1.63	1.57	1.59	1.88	1.73	1.97	1.86	2.15	1.85	1.63	1.75	1.32	1.76
2	EGF	0.56	1.36	0.91	1.17	1.09	0.94	1.21	0.87	1.04	0.83	1.07	1.07	1.88	1.09	1.91	1.02	0.84	0.95	0.69	0.94
3	Eotaxin	1.42	1.59	1.33	1.34	1.35	1.29	1.33	1.30	1.30	1.27	1.33	1.29	1.43	1.39	1.42	1.14	1.31	1.29	1.15	1.18
4	GRO- α	0.13	0.60	0.72	0.77	0.66	0.82	0.85	1.09	0.54	0.72	0.83	1.02	0.90	0.76	1.09	0.62	0.38	0.73	0.67	0.51
5	HGF	2.19	2.18	2.16	2.16	2.10	2.10	2.20	2.14	2.19	2.17	2.41	2.16	2.46	2.51	2.27	1.82	2.53	2.48	2.36	2.39
6	IFN- γ	1.64	1.41	1.60	1.45	1.65	1.53	1.37	1.69	1.57	1.81	1.62	1.61	1.61	1.64	1.61	1.47	1.39	1.41	1.44	1.01
7	IL-1 β	0.39	0.76	0.43	0.51	0.38	0.44	0.49	0.52	0.44	0.45	0.68	0.52	1.25	0.59	1.41	0.47	0.40	0.47	0.04	0.30
8	IL-12p70	0.26	0.22	0.26	0.28	0.23	0.19	0.39	0.43	0.23	0.39	0.41	0.37	1.07	0.36	1.24	0.23	0.13	0.23	0.29	0.09
9	IL-18	1.65	1.70	1.77	1.69	1.78	1.85	1.67	1.93	1.86	2.01	2.05	1.80	1.93	1.83	1.91	1.60	1.70	1.70	1.82	1.55
10	IL-1RA	3.07	2.75	3.12	2.83	3.01	2.66	2.85	3.08	3.11	2.99	3.14	2.61	3.45	3.57	3.66	2.60	3.96	3.06	2.74	2.83
11	IL-2	1.48	1.77	1.50	1.44	1.42	1.34	1.49	1.37	1.38	1.33	1.45	1.38	1.72	1.43	1.78	1.42	1.38	1.42	1.06	1.28
12	IL-5	1.22	1.22	0.80	0.99	0.86	0.81	0.97	1.03	0.71	0.82	0.87	0.82	1.95	1.03	1.55	0.87	0.68	0.59	0.14	0.65
13	IL-6	0.18	1.35	1.66	1.51	1.41	1.18	1.29	1.74	1.42	1.54	1.67	1.21	1.72	1.77	2.07	1.11	2.62	1.34	0.93	1.09
14	IP-10	1.47	1.80	1.93	1.90	1.87	1.74	1.71	2.19	1.86	2.01	2.02	1.70	1.65	2.15	1.43	1.40	2.03	1.43	1.41	1.21
15	IL-7	0.14	0.27	0.06	0.12	0.33	0.08	0.41	0.31	0.03	0.06	0.27	0.03	0.56	0.15	0.66	0.22	0.13	0.27	0.30	0.44
16	LIF	0.73	0.79	0.77	0.93	0.75	0.77	0.90	0.70	0.79	0.70	1.01	0.73	1.50	0.88	1.51	0.83	0.89	0.95	0.57	0.91
17	MCP-1	1.92	1.81	2.06	1.98	2.05	1.93	2.00	2.16	1.97	2.16	2.12	1.81	1.89	2.37	1.89	1.64	2.60	1.77	1.71	1.52
18	MIP-1 α	0.83	0.53	0.69	0.80	0.71	0.84	0.79	0.86	0.72	0.75	0.86	0.73	1.00	1.05	1.13	0.80	0.84	0.67	0.39	0.73
19	MIP-1 β	1.56	1.33	1.44	1.41	1.53	1.40	1.55	1.81	1.62	1.82	1.53	1.61	2.09	1.74	2.26	1.28	1.76	1.59	1.29	1.79
20	PDGF-BB	1.80	2.42	2.42	2.51	2.94	2.12	2.69	2.67	2.02	2.26	2.50	2.25	2.21	2.35	2.32	2.55	1.86	2.18	1.92	1.89

21	PLGF-1	1.32	2.12	1.56	1.79	1.92	1.92	1.34	1.92	1.74	1.50	1.40	1.71	1.55	2.60	1.72	2.56	1.83	1.30	1.48	1.17	1.50
22	RANTES	1.65	1.68	1.96	2.17	1.95	1.89	1.87	1.89	2.01	1.91	2.24	2.18	2.16	2.07	2.03	2.89	2.15	1.87	1.95	1.78	1.83
23	SCF	0.95	0.91	0.72	0.73	0.71	0.78	0.83	0.83	0.67	0.69	0.75	0.75	0.78	1.01	0.81	1.07	0.77	0.80	0.85	0.63	0.68
24	SDF-1 α	2.76	2.73	2.80	2.72	2.81	2.73	2.74	2.74	2.73	2.81	2.81	2.76	2.82	3.61	2.83	2.76	2.79	2.89	2.80	2.77	2.78
25	TNF- α	1.03	1.31	1.16	1.11	1.14	1.02	1.14	1.14	1.13	0.98	1.12	1.15	1.11	1.55	1.11	1.70	1.07	0.76	0.94	0.92	0.82
26	VEGF-A	1.83	1.70	2.15	2.03	2.31	1.90	2.21	2.21	2.46	2.07	2.43	2.34	1.99	2.91	2.32	3.02	1.96	2.10	2.19	2.17	2.06
27	bNGF	1.15	1.73	1.28	1.52	1.36	1.17	1.38	1.38	1.29	1.28	1.25	1.47	1.25	1.75	1.41	1.82	1.50	1.36	1.28	1.15	1.37

WO 2021/206637

Table 3. Concentrations of significant immune mediators in healthy controls (n = 23)

No.	Immune mediator	Log ₁₀ [Cytokine] (pg/ml); detectable mean (range)
1	HGF	1.90 (1.09 - 2.36)
2	VEGF-A	1.65
3	MCP-1	1.65 (0.004 - 2.07)
4	IL-6	0.87 (0.46 - 1.15)
5	IP-10	1.26 (0.82 - 1.67)
6	IL-18	1.72 (0.62 - 2.41)
7	PDGF-BB	1.94 (1.30 - 2.75)
8	IL-1RA	2.23
9	MIP-1 α	0.74 (0.18 - 1.22)
10	IL-12p70	< LOQ

Further interactome analyses by Ingenuity Pathway Analysis (IPA) showed association between the aforementioned immune mediators associated with severity and clinical parameters (pneumonia, desaturation and ICU admission). The intertwined relationship of the cytokines is shown (FIG. 4). In addition, IPA revealed several canonical pathways associated with these immune mediators, with the top ten canonical pathways involved in inflammatory diseases and cell signaling (FIG. 4B). The top canonical pathway, influenza pathogenesis, highlights the common immune mediators between Influenza virus infection and SARS-CoV-2 virus infection, including MCP-1, IL-1RA, IP-10 and IL-6 (FIG. 4C).

In addition to IPA, STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) prediction of protein-protein interactions was also performed on the immune mediators associated with severity, with a confidence threshold of 0.8 (FIG. 4D). Predicted interactive networks highlight IL-6 as a direct interacting partner with other severity-associated immune mediators.

Longitudinal comparison of these immune mediators associated with severity in 12 patients admitted into ICU was also performed to establish their role as plausible prognostic markers for severe COVID-19 (FIG. 1C). Two patients, ICU CT009 and CT032, recovered from COVID-19 at 16 days and 25 days post-ICU admission, respectively. Interestingly, HGF and VEGF-A were distinctly separated into two levels, with CT009 and CT032 having lower HGF and VEGF-A levels, and HGF approaching healthy baseline levels during the convalescence phase. Similarly, CT009 and CT032 also had lower levels of MCP-1 and IL-6 compared to the other patients admitted into ICU, despite having a less distinct separation (FIG. 1C). The longitudinal comparison of the other immune mediators, including IP-10, IL-18, PDGF-BB, IL-1RA, IL-12p70, and MIP-1 α revealed decreasing levels with days post-ICU admission and approaching healthy baseline levels during the latter period of post-ICU admission (FIG. 3).

Collectively, the upregulation of these specific cytokines could be influencing disease severity in COVID-19 patients.

30

Measuring the levels of prognostic cytokine markers to study their associations with disease outcomes after infection with different variants of SARS-CoV-2.

The Δ 382 variant of SARS-CoV-2 seems to be associated with a milder disease outcome. Lower concentrations of the chemokines IP-10 (CXCL10), MCP-1 (CCL2), the anti-inflammatory protein IL-1RA, growth factors associated with lung injury and

35

regeneration, including HGF, and VEGF-A, were detected in patients with the $\Delta 382$ -variant compared with patients with the wild-type virus.

Infection with SARS-CoV-2 clades L/V was associated with increased severity and more systemic release of pro-inflammatory MCP-1, IP-10 and IL-6 and the anti-inflammatory IL-1RA.

Measuring the levels of prognostic cytokine markers to study their associations with viral shedding and fever patterns in COVID-19.

COVID-19 patients had significantly lower pro-inflammatory cytokine levels (IL-6, IP-10, MCP-1 and VEGF-A) during intermittent viral RNA shedding period, compared with those without intermittent viral RNA shedding after complete virus clearance. A weaker inflammatory response or suppression of inflammatory responses could be triggering virus re-activation, resulting in intermittent detection in the patients. Whether milder inflammation and virus re-activation are causally related remains to be explored.

COVID-19 cases with prolonged fever were found to have higher levels of anti-inflammatory IL-1RA, pro-inflammatory IL-6, and chemokine interferon- γ IP-10 compared with controls. Notably, patients with prolonged fever had higher IP-10 levels as compared with patients with saddleback fever.

Measuring the levels of prognostic cytokine markers to understand the underlying immune responses related to treatment during COVID-19.

In the hypertension COVID-19 patients, the use of angiotensin receptor blockers (ARBs) was associated with a higher risk of ICU admission (aRR 2.19, 95% CI 1.08-4.43). These patients on ARB treatment had significantly higher MCP-1 and IP-10 concentrations compared to non-ARB users.

Measuring the levels of prognostic cytokine markers to study their associations with long term impacts of COVID-19

COVID-19 convalescents had elevated levels of circulating endothelial cells (CECs), a biomarker of vascular injury, than healthy participants. CECs attributes of convalescent COVID-19 patients correlated with systemic levels of IP-10, IL-18, IL-1RA and PDGF-BB.

Recovered COVID-19 patients had elevated levels of pro-inflammatory IL-12p70 and pro-angiogenic VEGF-A at day 180 post infection. Higher levels of MCP-1 and

PDGF-BB were detected in patients with persistent symptoms, versus symptom-free patients.

Measuring the levels of systemic cytokine IL-6 to evaluate the therapeutic efficacy and effects of cyclooxygenase inhibitors in high-risk COVID-19 patients

Retrospective safety and efficacy study on elderly patients ≥ 50 years old showed reduction of IL-6 levels in 5 of 6 (83.3%) treated with COX-2 inhibitors, versus 15 of 28 (53.6%) in the control group. A greater reduction of absolute IL-6 levels was also observed in treatment compared to control group, respectively (0.06 (0.06–0.06) vs 2.11 (0.06–15.10) pg/mL).

Consistent with Severe Acute Respiratory Syndrome (SARS) (Wong CK, 2004) and Middle East Respiratory Syndrome (MERS) (Min CK, 2016), SARS-CoV-2 infections trigger a cytokine storm with markedly increased levels of pro-inflammatory cytokines, chemokines and growth factors in COVID-19 patients. Robust induction of various inflammatory cytokines and chemokines, such as IL-2, IL-18, TNF- α and IFN- γ , suggests that innate immune cell responses and anti-viral T cell responses are responsible for SARS-CoV-2 pathogenesis in COVID-19 patients (Faure E, 2014).

In addition, elevation of growth factors, including HGF (4), VEGF-A (5) and PDGF-BB (6), further indicate the repair mechanism following acute lung injury during SARS-CoV-2 infection. Notably, patients with higher levels of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF and VEGF-A exhibited worse disease outcomes after infection with SARS-CoV-2. This further highlights the dysregulation of immune responses that could contribute to the development of severe disease in COVID-19 patients. Host-directed therapies that target the pro-inflammatory cytokine storm in SARS-CoV-2 patients could reduce immunopathology and limit the progression to severe disease outcome. STRING analysis revealed potential protein-to-protein interactions in severe COVID-19 infection, in which IL-6 is the direct interacting partner of other cytokines that are associated with disease severity. Thus, several approved IL-6 blockers with excellent safety profiles such as Actemra (Roche) or Kevzara (Regeneron) could potentially be repurposed to treat severe SARS-CoV-2 infection.

In summary, in a cohort study of 100 COVID-19 patients, it has been observed that patients with higher levels of IL-6, MCP-1, IP-10, IL-18, IL-1RA, PDGF-BB, HGF, MIP-1 α , IL-12p70 and VEGF-A exhibited worse disease outcomes after infection with SARS-CoV-2. This further highlights the dysregulation of immune responses that could contribute to the development of severe disease in COVID-19 patients. Host-directed

therapies that target the pro-inflammatory cytokine storm in SARS-CoV-2 patients could reduce immunopathology and limit the progression to severe disease outcome.

STRING (Search Tool for the Retrieval of Interacting Genes/ Proteins) prediction of protein-protein interactions was also performed on the immune mediators associated with severity, with a confidence threshold of 0.8 (FIG. 4D). Predicted interactive networks highlight IL-6 as a direct interacting partner with other severity-associated immune mediators, thereby suggesting that IL-6 could be a potential therapeutic target for treatment of COVID-19 patients

Thus, several approved IL-6 blockers with excellent safety profiles such as Actemra (Roche) or Kevzara (Regeneron) could potentially be repurposed to treat severe SARS-CoV-2 infection.

References

1. Wong CK, Lam CW, Wu AK, Ip WK, Lee NL, Chan IH, Lit LC, Hui DS, Chan MH, Chung SS, Sung JJ. 2004. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin Exp Immunol 136:95-103.
2. Min CK, Cheon S, Ha NY, Sohn KM, Kim Y, Aigerim A, Shin HM, Choi JY, Inn KS, Kim JH, Moon JY, Choi MS, Cho NH, Kim YS. 2016. Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity. Sci Rep 6:25359.
3. Faure E, Poissy J, Goffard A, Fournier C, Kipnis E, Titecat M, Bortolotti P, Martinez L, Dubucquoi S, Dessein R, Gosset P, Mathieu D, Guery B. 2014. Distinct immune response in two MERS-CoV-infected patients: can we go from bench to bedside? PLoS One 9:e88716.
4. Zeng L, Yang XT, Li HS, Li Y, Yang C, Gu W, Zhou YH, Du J, Wang HY, Sun JH, Wen DL, Jiang JX. 2016. The cellular kinetics of lung alveolar epithelial cells and its relationship with lung tissue repair after acute lung injury. Respir Res 17:164.
5. Zhang L, Yuan LJ, Zhao S, Shan Y, Wu HM, Xue XD. 2015. The role of placenta growth factor in the hyperoxia-induced acute lung injury in an animal model. Cell Biochem Funct 33:44-9.
6. Quinton LJ, Mizgerd JP, Hilliard KL, Jones MR, Kwon CY, Allen E. 2012. Leukemia inhibitory factor signaling is required for lung protection during pneumonia. J Immunol 188:6300-8.

7. Chioh, F.W., Fong, S.W., Young, B.E., Wu, K.X., Siau, A., Krishnan, S., Chan, Y.H., Carissimo, G., Teo, L.L., Gao, F., Tan, R.S., Zhong, L., Koh, A.S., Tan, S.Y., Tambyah, P.A., Renia, L., Ng, L.F., Lye, D.C., and Cheung, C. (2021). Convalescent COVID-19 patients are susceptible to endothelial dysfunction due to persistent immune activation. *Elife* 10. doi: 10.7554/eLife.64909
5
8. Dalan, R., Ang, L.W., Tan, W.Y.T., Fong, S.W., Tay, W.C., Chan, Y.H., Renia, L., Ng, L.F.P., Lye, D.C., Chew, D.E.K., et al. (2020). The association of hypertension and diabetes pharmacotherapy with COVID-19 severity and immune signatures: an observational study. *Eur Heart J Cardiovasc Pharmacother*. Published online 2020/08/09 DOI: 10.1093/ehjcvp/pvaa098.
10
9. Lee, P.H., Tay, W.C., Sutjipto, S., Fong, S.W., Ong, S.W.X., Wei, W.E., Chan, Y.H., Ling, L.M., Young, B.E., Toh, M.P.H., et al. (2020). Associations of viral ribonucleic acid (RNA) shedding patterns with clinical illness and immune responses in Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection. *Clin Transl Immunology*. 9(7), e1160. Published online 2020/08/04 DOI: 10.1002/cti2.1160.
15
10. Ng, D.H.L., Choy, C.Y., Chan, Y.H., Young, B.E., Fong, S.W., Ng, L.F.P., Renia, L., Lye, D.C., Chia, P.Y., and National Centre for Infectious Diseases, C.-O.R.T. (2020). Fever Patterns, Cytokine Profiles, and Outcomes in COVID-19. *Open Forum Infect Dis*. 7(9), ofaa375. Published online 2020/10/02 DOI: 10.1093/ofid/ofaa375.
20
11. Ong, S.W.X., Fong, S.-W., Young, B.E., Chan, Y.-H., Lee, B., Amrun, S.N., Chee, R.S.-L., Yeo, N.K.-W., Tambyah, P., Pada, S., et al., 2021. Persistent symptoms and association with inflammatory cytokine signatures in recovered COVID-19 patients. *Open Forum Infect Dis*. (In press).
25
12. Ong, S.W.X., Tan, W.Y.T., Chan, Y.H., Fong, S.W., Renia, L., Ng, L.F., Leo, Y.S., Lye, D.C., and Young, B.E. (2020). Safety and potential efficacy of cyclooxygenase-2 inhibitors in coronavirus disease 2019. *Clin Transl Immunology*. 9(7), e1159. Published online 2020/07/31 DOI: 10.1002/cti2.1159.
30
13. Young, B.E., Fong, S.W., Chan, Y.H., Mak, T.M., Ang, L.W., Anderson, D.E., Lee, C.Y., Amrun, S.N., Lee, B., Goh, Y.S., et al. (2020). Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study. *Lancet*. Published online 2020/08/22 DOI: 10.1016/S0140-6736(20)31757-8.

14. Young, B.E., Wei, W.E., Fong, S.W., Mak, T., Anderson, D.E., Chan, Y.H., Pung, R., Heng, C.S., Ang, L.W., Zheng, K.E., et al., 2021. Association of SARS-CoV-2 Clades with Clinical, Inflammatory and Virologic Outcomes: An Observational Study. *ebiomedicine* (In press).