A guide to immunotherapy for COVID-19

Editor summary:

This review aims to support clinical decision-making by providing an overview of the evidence for immunotherapy strategies in patients with COVID-19.

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A guide to immunotherapy for COVID-19

Frank L. van de Veerdonk[1,*], Evangelos Giamarellos-Bourboulis[2], Peter Pickkers[3], Lennie Derde[4], Helen Leavis[5], Reinout van Crevel[1], Job Engel[1], W. Joost Wiersinga[6], Alexander P.J. Vlaar[7], Manu Shankar-Hari[8], Tom van der Poll[6], Marc Bonten[9], Derek C. Angus[10], Jos W. M. van der Meer[1], Mihai G. Netea[1,11*]

[1] Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands.

[2] Department of Infectious Diseases, Attikon Hospital, University of Athens, Greece

[3] Department of Intensive Care Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands

[4] Department of Intensive Care, University Medical Center Utrecht, the Netherlands

[5] Department of Internal Medicine, University Medical Center Utrecht, the Netherlands

[6] Division of Infectious Diseases, Center for Experimental Molecular Medicine (CEMM), Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands

[7] Department of Intensive Care Medicine and Laboratory of Experimental Intensive Care Medicine and Anesthesiology, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands
[8] School of Immunobiology and Microbial Sciences, King's College London, UK
[9] Julius Center for Health Sciences and Primary Care, Utrecht, The Netherlands
[10] UPMC and University of Pittsburgh, Pittsburgh, USA
[11] Department of Immunology and Metabolism, Life & Medical Sciences Institute, University of Bonn, Germany

*Correspondence:
Frank L van de Veerdonk (frank.vandeveerdonk@radboudumc.nl)
Mihai G Netea (mihai.netea@radboudumc.nl)

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Abstract

Immune dysregulation is an important component of the pathophysiology of Covid-19. A large body of literature has reported the effect of immune-based therapies in patients with Covid-19, with some remarkable successes such as the use of steroids or anti-cytokine therapies. However, challenges in clinical decision-making arise from the complexity of the disease phenotypes and patient heterogeneity, as well as the variable quality of evidence from immunotherapy studies. The present review aims to support clinical decision-making by providing an overview of the evidence generated by major clinical trials of host-directed therapy. We discuss patient stratification and propose an algorithm to guide the use of immunotherapy strategies in the clinic. This will not only help to guide treatment decisions, but may also help us design future trials investigating immunotherapy in other severe infections.

Introduction

The Covid-19 pandemic forced the world to accelerate vaccine and drug development and evaluation at an unparalleled pace. Currently, the Covid-19 treatment armamentarium is largely represented by antiviral agents (often administered in early stages of disease) and immunotherapeutic agents that modulate the host immune response (often administered in more advanced stages of disease)—with the rationale for immunotherapy being that dysregulation of host responses feature prominently in COVID-19 pathophysiology. Host-directed therapy is however a relatively complex approach, and several important aspects need to be considered.

First, apparently obvious choices based on knowledge extrapolated from analogous conditions may be inappropriate in the face of novel diseases with complex immunopathology. Indeed, the initial expert opinion to avoid corticosteroids as immunomodulatory treatment for Covid-19, while later on they became standard-of-care (SoC), underscores the importance of obtaining solid evidence based on robust clinical trials. Second, the host-pathogen response and resulting immunologic milieu is very heterogenous, implying that not every patient will benefit from the same immunomodulatory treatment strategy. Furthermore, this heterogeneity may not be clinically evident at the bedside, potentially necessitating the evaluation and deployment of biomarkers to guide patient-specific immune therapy. Third, all of this complexity needs to be dissected, understood, and then re-packaged in updated treatment algorithms in a setting of constant change in available evidence.

Here, we attempt to provide guidance for immunotherapy of patients with Covid-19, based on consideration of these three major points. We will provide an overview of evidence from the major clinical trials of host-directed therapy, discuss the patient stratification, and propose an algorithm to guide the use of immunotherapy strategies.

Immune pathophysiology of COVID-19

Covid-19 is a complex disease in which respiratory manifestations associated with viral replication are accompanied by systemic effects, implying that SARS-CoV-2 infection is likely to generate a broadly dysregulated immune response. In the pathophysiology of COVID-19, we can identify disease triggers, mediators, and effector pathways (**Figure 1**), which can be targeted by immunotherapy.

While the disease trigger is infection with SARS-CoV-2 and the first steps of the infection are relatively similar in most patients, the heterogeneity of Covid-19 increases with severity of disease and is largely determined by variability of the host immune response at the level of mediators and effectors. Infection is initiated when the spike (S) glycoprotein of SARS-CoV-2 binds to the human

angiotensin-converting enzyme (ACE)2 receptor on the epithelial cell surface, with the host transmembrane protease serine 2 (TMPRSS2) promoting the entry of the virus into the cell[1,2]. ACE2 is highly expressed in the epithelial cells of the nasal cavity, providing a point of entry for SARS-CoV-2[3]. The virus is also recognized by pattern recognition receptors on immune cells, which are responsible for the initiation of the host defense mechanisms. The subsequent production of immune mediators such as cytokines and complement – produced locally in moderate amounts – is essential to fight the infection; however, these can be deleterious when produced in excess[4].

Several studies have shown that the IL-1–IL-6 axis is likely to represent one of the most biologically relevant signaling pathways in the SARS-CoV-2-induced hyperinflammatory reaction[5–7]. Interestingly, in patients with severe Covid-19, low HLA-DR expression on circulating monocytes (a marker of immunosuppression) was clearly evident, but the monocytes retained normal or high cytokine production capacity (in contrast to bacterial sepsis)[5,8]. At the cellular level, Covid-19 is associated with a marked decrease in circulating $CD4^+$ and $CD8^+$ T lymphocytes[9], reminiscent of sepsis-associated lymphopenia[10], and this is associated with disease severity and poor outcome[11]. In addition to this reduction in lymphocyte numbers, their function and capacity to release type II interferons is also severely affected in patients with severe COVID-19[12–15].

Additional important pathophysiological processes in COVID-19 are induced at the level of effector pathways, such as the coagulation system. Thrombi occur when hypercoagulability, endothelial injury and blood stasis converge, and these conditions are frequently encountered in severe COVID-19. Subsequently, arterial and venous thromboembolism have been frequently reported: studies show that between 21-69% of patients with severe COVID-19 develop thromboembolic complications[16]. It is believed that inflammatory processes play an important role in the induction of thromboembolic processes, leading to severe complications[17–19]. In later phases, patients may develop pulmonary fibrosis or may enter a more chronic phase called ‘long covid’[20].

All in all, the pathophysiology of Covid-19 is complex, comprising an interaction between hyperinflammation, defective lymphocyte function, endothelial dysfunction, thromboembolic complications, and fibrotic processes in the lung. These processes are not only complex, but also highly variable between patients, likely related to the heterogeneity of the host immune response. This warrants a stratified immunotherapy approach in clinical trials for Covid-19.

Immunotherapy for COVID-19

From the start of the COVID-19 pandemic it became clear that dysregulation of immune responses against SARS-CoV-2 is one of the main features of disease pathogenesis, especially in patients with severe disease, and studies aimed at rebalancing this using modulators of immune responses were initiated early on. Our aim is to provide an overview of the immunotherapies targeting different components of COVID-19 pathophysiology, and to propose a practical approach for the use of host-directed strategies in clinical practice. **Table 1** provides an overview of the most important clinical trials of immunotherapy in COVID-19.

Anti-virus immunotherapies (anti-trigger)

Eliminating the virus as early as possible is likely to prevent or limit the cascade of immune dysregulation and therefore severity of disease. One aspect important to mention is that new studies provided important information on antiviral therapy, such as remdesivir and molnupiravir in COVID-19. However, since these are not considered immunomodulatory drugs, we will not focus on their use, but on the studies using immunotherapeutic drugs. Immune-based virus elimination with either polyclonal convalescent plasma (CP) or human monoclonal antibodies to SARS–CoV-2 spike protein might prevent infection in susceptible individuals at risk, or might improve outcomes in those who have established COVID-19. The underpinning biology with immunoglobulin therapies is the provision of immediate antiviral humoral immunity that on the one hand reduces the viral load, and on the other hand may induce immunomodulation through Fc gamma receptors[21,22], with both mechanisms contributing to reduction of illness severity and improved outcomes. It must be noted, however, that the role of Fc gamma receptors remains controversial in COVID-19 pathogenesis, with some literature referring to its role as a disease-enhancing factor [23,24].

There is relatively solid data for efficacy of CP when high titer plasma is used early in severe infection, with early data going back to the 1930s, and this treatment has been explored in COVID-19 from the very beginning of the pandemic[25–27]. A living systematic review by the Cochrane Collaboration on SARS-CoV-2 convalescent plasma analyzed data from randomized clinical trials, as of 20-May-2021[28]. There was no difference in all-cause 28-day mortality (risk ratio (RR) 0.98, 95% confidence interval (CI) 0.92 to 1.05; 7 RCTs, 12,646 participants; high-certainty evidence). Similarly, neither the United Kingdom RECOVERY[29] trial that enrolled mainly ward patients, nor the global REMAP-CAP Trial[30,31] in which most patients were mechanically ventilated, showed any benefit for treatment with convalescent plasma. However, in immunocompromised patients and older patients who may be immunosenescent[32], early administration of convalescent plasma seems potentially beneficial although this is based on smaller trials with fewer patients included[33].

Another strategy is the use of monoclonal antibodies which differ from convalescent plasma, since they act against one predefined target, such as the spike protein, with high neutralizing activity. In high-risk ambulatory patients a combination of bamlanivimab and etesevimab reduced COVID-19-related hospitalizations, reduced viral load, illness duration and decreased mortality[34]. Another antibody preparation, a combination of the monoclonal antibodies casirivimab and imdevimab (REGEN-COV), reduced 28-day mortality among hospitalized patients who were seronegative at baseline[35]. Anti-viral immunotherapy is likely to exert therapeutic potential when given early, especially before the endogenous development of antibodies. While this treatment may not be of benefit when endogenous antibody production is mounted in later stages of disease, it theoretically may benefit some patients, such as those who are immunocompromised patients and remain seronegative with persistent detectable viral loads[36,37].

Immunotherapies targeting immune mediators of host defense

The immune response can also be modulated by targeting the mediators that are triggered by the virus and which drive several effector mechanisms (Figure 1). These can be non-specific and broad, such as corticosteroids or very targeted, for example inhibiting one specific cytokine.

Corticosteroids

In a retrospective cohort study of 201 patients admitted with confirmed Covid-19 pneumonia in Wuhan, China in early 2020, treatment with methylprednisolone was associated with reduced risk of death (HR, 0.38; 95% confidence interval [CI], 0.20-0.72) among patients with Acute Respiratory Distress Syndrome (ARDS)[38]. Yet, effectiveness of untargeted immune suppression needed to be demonstrated with high-quality evidence, ideally from randomized studies, to be accepted by the scientific community. To this end, the RECOVERY RCT (an adaptive platform design) was the first to report that dexamethasone (6 mg/kg for 10 days) reduced 28-day mortality in patients hospitalized with Covid-19[39]. In that study, 2104 patients were assigned to receive dexamethasone and 4321 to receive usual care. Overall, 28-day mortality was 22.9% in the dexamethasone group and 25.7% in the control group (age-adjusted rate ratio 0.83; 95% CI, 0.75-0.93). However, reduced incidence of death in the dexamethasone arm was found for those receiving invasive mechanical ventilation (rate ratio, 0.64; 95% CI, 0.51-0.81) and those receiving oxygen without invasive mechanical ventilation (rate ratio, 0.82; 95% CI, 0.72-0.94) – in other words, the patients who were more sick at the time of treatment who seemed to benefit from corticosteroids . Similar protective effects of steroids in patients with severe COVID-19 were reported in REMAP-CAP, another adaptive platform study, in which 403 patients

were included in a corticosteroid evaluation domain[40]. The median adjusted odds ratio and Bayesian probability of superiority for the primary end point (combined organ support-free days at 21 days and mortality) were 1.43 (95% credible interval, 0.91-2.27) and 93% for fixed-dose hydrocortisone, and 1.22 (95% credible interval, 0.76-1.94) and 80% for shock-dependent hydrocortisone, compared with control. Two other large studies from Brazil and France also supported benefit from corticosteroids in patients with severe COVID-19[41,42]. After release of these results, similar corticosteroid trials terminated enrollment and combined their data in a prospective meta-analysis led by WHO[43], which provides a high level of evidence for the effectiveness of corticosteroids in hospitalized patients with Covid-19 who need respiratory support.

The observation that the beneficial effects of steroids are significant in sicker patients could be explained by the pleotropic effects of steroids that target different pathophysiological components of COVID-19 present in severe disease. Although this might explain why so many patients benefit, it also makes it challenging to define who needs to be treated with corticosteroids when progressing towards severe disease (Figure 2). Another important consideration is the possible over-use of corticosteroids, especially in the early phase of disease when such treatment might lead to detrimental effects, further supporting the need for guidance of immunotherapy.

Kinase inhibitors

Tyrosine kinases also have pleotropic effects and are seen as attractive targets in Covid-19, given their established druggability and the fact that most tyrosine kinase inhibitors (TKI) have a well-known clinical safety profile[44,45]. TKIs can block cytokine signaling pathways and many immune effector pathways.

A double-blind, randomized, placebo-controlled trial of 1033 adults hospitalized with Covid-19 who were randomly assigned to receive oral baricitinib (a Janus tyrosine kinase (JAK) inhibitor), or placebo for up to 14 days demonstrated that patients receiving baricitinib had a shorter time to recovery than patients in the placebo group (median 7 vs 8 days)[46]. Importantly, the effect was more pronounced in the subgroup requiring high-flow oxygen or noninvasive ventilation when compared to placebo (10 vs 18 days). In a phase 3, double-blind, randomised, placebo-controlled trial with 1525 participants, 764 received baricitinib and 76 placebo[47]. There was a 38.2% relative reduction in mortality, with the 28-day all-cause mortality being 8% for baricitinib and 13% for placebo with a hazard ratio [HR] 0.57 [95% CI 0.41-0.78]. This was an additional effect to standard treatment including corticosteroids, since 79.3% of participants with available data received systemic corticosteroids at baseline. The FDA has recently authorized baracitinib for emergency use in Covid-19. A Dutch clinical trial of 400 hospitalized patients with Covid-19 found a beneficial effect of oral

imatinib (a cytosolic multi-tyrosine kinase inhibitor) compared to placebo on duration of mechanical ventilation (7 days vs 12 days) and 28-day mortality (8% vs 14%)[48]. It should be noted that the primary endpoint was not met, which was time to discontinuation of mechanical ventilation and supplemental oxygen for more than 48 consecutive hours while being alive during a 28-day period. However, the beneficial findings warrant follow-up trials to validate these outcomes and select which patients might benefit from imatinib care. Other kinase inhibitors under investigation in RCTs in hospitalized Covid-19 patients include those targeting Bruton's tyrosine kinases (e.g. ibrutinib, acalabrutinib and zanubrutinib), phosphatidylinositol 3-kinase (PI3K)/ mammalian target of rapamycin (mTOR) inhibitors (duvelisib and temsirolimus) and JAK inhibitors such as ruxolitinib and tofacitinib[45]. Very recently, in a trial in Brazil, 289 patients hospitalized for Covid-19 were randomized to receive tofacitinib or placebo. They showed a cumulative incidence of death or respiratory failure of 18.1% in the tofacitinib group and 29.0% in the placebo group (risk ratio, 0.63; 95% confidence interval [CI], 0.41 to 0.97; P=0.04) at day 28[49]. Therefore, TKIs have good rationale to be explored in COVID-19 and the results reported thus far encourage further exploration in larger trials.

Targeted strategies: anti-cytokine treatment

Both IL-1 and IL-6 induce local effects such as macrophage activation, endothelial leakage, liquid extravasation as well as systemic effects including fever, somnolence, and synthesis of acute phase proteins. While moderate induction of inflammation is necessary for host defense, overabundant release of these mediators is deleterious. The CORIMUNO-ANA study randomized 116 patients with mild-to-moderate Covid-19 pneumonia to placebo or the IL-1 inhibitor anakinra, the only immunological criterium being a plasma C-reactive protein (CRP) level higher than 25 mg/L. No significant effect of blocking IL-1 with anakinra was observed on the proportion of patients who died or needed non-invasive or mechanical ventilation at day 4, or on survival without need for mechanical or non-invasive ventilation at day 14[50]. In line with this, anakinra had no effect on survival or release from organ support in the REMAP-CAP trial, in which 378 patients with Covid-19 needing organ support (without further immunological stratification) in the ICU were treated with anakinra and compared to 418 controls[51].

In contrast, patient stratification based on immunological profiles did identify patients likely to benefit from IL-1 blockade. The soluble urokinase plasminogen receptor (suPAR) was found to be associated with the risk for progression into severe respiratory failure and this formed the basis of a biomarker-driven immunotherapy trial[52,53] (**BOX1**). In the open-label single-arm phase 2 SAVE study, 130 patients with COVID-19 pneumonia and plasma suPAR of 6 ng/ml or more received SoC treatment and anakinra

(which blocks both Il-1a ansd Il-1b) 100mg subcutaneously daily for 10 days. The incidence of severe respiratory failure and/or death after 14 days was 22.3% compared to 59.2% of matched patients receiving SoC alone[54].

These results provided the rationale for the double-blind randomized phase 3 SAVE-MORE trial, in which 594 patients with moderate to severe COVID-19 pneumonia (WHO scale 3-5) and suPAR of 6 ng/ml or more were randomized to treatment with SoC and placebo (n= 189) or SoC and anakinra (n=405). Anakinra treatment provided 2.78 times higher odds for clinical improvement based on the 11-point WHO Clinical Progression Scale towards both full resolution and critical illness or death after 28 days[55]. 28-day mortality was lower among patients allocated to anakinra treatment - 6.9% in the control group versus 3.2% treated with anakinra. Overall, 85.9% of patients were co-administered dexamethasone, but anakinra still improved outcomes in this context. The results of the SAVE-MORE trial suggest that anakinra treatment guided by suPAR is a therapeutic strategy before progression into critical illness.

A trial including 454 patients randomized 1:1 to placebo or canakinumab, which blocks only IL-1b, did not reach significance for its primary outcome which was survival without invasive mechanical ventilation at day 29[56]. Patients enrolled were hypoxic and hospitalized without the need for invasive mechanical ventilation. COVID-19-related mortality occurred in 11 of 223 patients (4.9%) in the canakinumab group vs 16 of 222 (7.2%) in the placebo group, with a rate difference of −2.3% (95%CI, −6.7%to 2.2%) and an odds ratio of 0.67 (95%CI, 0.30 to 1.50).

For patients with hypoxemia and in need of oxygen therapy, anti-IL-6 strategies have been shown to be beneficial by the large-scale platforms RECOVERY[57] and REMAP-CAP[58]. In the open-label, randomized RECOVERY trial, which predominantly included patients that were not critically-ill, 2094 patients received usual care and 2022 patients received the IL-6 inhibitor tocilizumab. Mortality was decreased from 35% in the usual care arm to 31% in the tocilizumab arm (p: 0.0028)[57]. The REMAP-CAP trial included 2274 critically ill participants, with 972 participants receiving tocilizumab, 485 randomized to sarilumab, 378 to anakinra and 418 to control. Tocilizumab and sarilumab were both effective, when compared with control, and likely to be equivalent in terms of improving survival and release from organ support. However, anakinra was not effective in this population. Median organ support-free days were 7 (interquartile range [IQR] –1, 16), 9 (IQR –1, 17), 0 (IQR –1, 15) and 0 (IQR –1, 15) for tocilizumab, sarilumab, anakinra and control, respectively. Median adjusted odds ratios for hospital survival were 1.42 (95% credible interval (CrI) 1.05, 1.93), 1.51 (95%CrI 1.06, 2.20) and 0.97 (95%CrI 0.66, 1.40) for tocilizumab, sarilumab and anakinra respectively, compared to control[58]. The WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group published a prospective meta-analysis of clinical trials of patients hospitalized for COVID-19 that showed an

association with lower 28-day all-cause mortality in patients treated with an IL-6 antagonists compared with patients that received usual care or placebo[59]. Collectively, these data support the use of blocking IL-6 in patients with Covid-19 that are hospitalized and in need of oxygen supplementation.

Other proinflammatory cytokines besides those in the IL-1–IL-6 axis are also involved in Covid-19-mediated inflammation; one attractive approach is to inhibit neutrophil recruitment in the lung through inhibition of GM-CSF. In the double-blind randomized trial OSCAR, patients with respiratory distress were randomized to receive one infusion of the monoclocal anti-GM-CSF otilimab (n=395) or placebo (n=398). The primary study endpoint was the rate of patients being alive and free of respiratory failure by day 28: this was 71% in the placebo group and 67% in the otilimab group (p: 0.09). However, in the group of patients aged 70 years or more there was a significant effect of otilimab on the primary endpoint, namely 66% in the placebo compared to 46% in the group that received otilimab (p: 0.009)[60], which provides the rationale to further explore otilimab in patients aged 70 years or more. Nontheless, one should be cautious with such age-dependent interpretations, as this may imply opposite negative effects in the younger patients. Other cytokine-targeted therapies, such as anti-TNF, are currently being studied (NCT04705844). Cytokine-targeted treatment strategies in COVID-19 seem to be an attractive approach and might benefit from biomarker-based precision RCTs that help identify which patients are likely to benefit most.

Anti-complement therapies: anti-C5a

Complement activation seems to contribute to the pathophysiology of severe COVID-19. Autopsies of patients with severe COVID-19 showed widespread complement activation in the lung and kidney[61,62]. The potent anaphylatoxin C5a increases adherence and migration of neutrophils and monocytes to blood vessel walls; this causes tissue damage by oxidative radical formation and enzyme release, but also induces release of tissue factor from endothelial cells and neutrophils, thereby activating the coagulation system[63–65]. In patients with severe COVID-19, high concentrations of C5a are associated with poor outcome[66]. Based on these observations, anti-complement therapies have been investigated in severe COVID-19. One randomized phase 2 open label trial (n=30) investigated blockade of C5a using a chimeric monoclonal IgG4 antibody (vilobelimab) that specifically binds with high affinity to the soluble form of human C5a, and was shown to be safe in severe COVID-19 patients. In this study, infections considered as serious adverse events were reported in three (20%) patients receiving direct C5a inhibition, versus five (33%) patients in the control group[67]. The secondary outcomes including severe pulmonary embolism and mortality, were in favor of anti-C5a treatment. Currently, a phase 3 trial (NCT04333420) targeting to enroll 360 severe COVID-19 patients and using 28-day mortality as the primary endpoint is ongoing.

Stimulators of anti-viral defense: interferons

Type I IFNs are crucial for antiviral host responses and they have been previously used with partial success against SARS[68]. Daily inhalations with IFNβ-1a for 14 days versus placebo was investigated in a double-blind RCT in 101 patients with COVID-19 in the UK. Patients receiving inhaled IFNβ-1a had greater odds of improvement (odds ratio 2·32 [95% CI 1·07-5·04]; p=0·033) on day 15 or 16, and were more likely to recover during treatment (hazard ratio 2·19 [95% CI 1·03-4·69]; p=0·043)[69]. In a multicentre, prospective, open-label, randomised, phase 2 trial in China, 127 patients received either triple antiviral therapy (lopinavir, ritonavir and ribavirin) and three doses of 8 million international units of IFNβ-1b on alternate days (n=86) or lopinavir and ritonavir (n=41). Again, triple antiviral therapy plus IFNβ-1b resulted in shorter viral shedding and faster clinical improvement compared to lopinavir-ritonavir alone in patients with mild to moderate COVID-19[70]. In contrast, in the WHO Solidarity trial in which IFNβ-1a was given s.c and i.v for 6 days, death occurred in 243 of 2050 patients receiving IFNβ-1a and in 216 of 2050 receiving its control (rate ratio, 1.16; 95% CI, 0.96 to 1.39; P=0.11)[71]. An important note is that half of the patients in the Solidarity trial received corticosteroids that might affect interferon signaling, but the clinical relevance of this is uncertain.

IFN gamma (IFNγ) is a type II interferon that has an important role in boosting the innate host defense and might therefore act as an immunostimulatory agent. In a case series of five patients with persistent high viral loads and poor clinical condition with secondary infectious complications, recombinant IFNγ showed viral culture conversion from positive to negative and rapid decrease in viral load by PCR without subsequent signs of hyperinflammation [72]. In another report with 6 non-immunocompromised patients with ventilator associated pneumonia (VAP), IFNγ treatment led to a fast increase in HLA-DR^{high} monocytes in all but one patient, and was well tolerated[73]. IFNγ might represent an immunostimulatory agent that could help clear viral infection and be beneficial in the setting of secondary infectons in critically ill patients with Covid-19. Other strategies to boost the immune system are checkpoint inhibitors or recombinant IL-7 and these are currently under investigation (NCT04335305, NCT04379076)[74].

Immunotherapies targeting effector pathways

Inhibitors of local pulmonary oedema: kallikrein-kinin system (KKS)

Timely inhibition of the KKS in Covid-19 patients is proposed to counteract pulmonary edema and suppress thromboinflammation[75,76], thereby limiting disease severity. In a case-control study of nine Covid-19 patients treated with icatibant (bradykinin B2 receptor antagonist) and 18 matched controls,

icatibant showed promising results compared to SoC treatment[77]. Directly after treatment with three doses of 30 mg of icatibant, a reduction in oxygen supplementation of three L/min or greater was observed in 89% of patients in the intervention group compared to 17% of patients in the control group. Another case-control study investigating the effects of icatibant and an inhibitor of C1-esterase/kallikrein in 30 patients found no significant effect on clinical outcome, but found that both drugs were safe and had beneficial effects on lung CT severity scores and blood eosinophil counts[78]. Disease severity and timing of treatment may be important factors determining the efficacy of icatibant treatment in Covid-19. Several other drugs that modulate the KKS are currently under investigation.

Modulation of immune-thrombotic complications

Damage of the vascular endothelium induced by the inflammatory reaction, together with activation of platelets and the coagulation system, are key pathophysiological features of COVID-19[79,80]. These host response aberrations have been implicated in the high occurrence of venous thromboembolic (VTE) disease or arterial thrombosis in COVID-19 despite conventional thromboprophylaxis[81]. Consequently, many clinicians and scientific societies proposed the use of thromboprophylaxis medication at higher doses than usual in clinical practice, and over 75 RCTs related to antithrombotic therapy in hospitalized COVID-19 patients have been initiated[80].

In a multicenter RCT conducted in Iran encompassing 562 ICU patients with COVID-19, intermediate-dose prophylactic anticoagulation (enoxaparin 1 mg/kg) compared with standard-dose prophylactic anticoagulation (enoxaparin 40 mg) did not impact the primary outcome, which was a composite of venous or arterial thrombosis, treatment with extracorporeal membrane oxygenation or mortality[82]. In a multicenter RCT done in Brazil in 615 hospitalized patients, of whom 94% were considered clinically stable, anticoagulation with rivaroxaban or enoxaparin followed by rivaroxaban to day 30 did not improve clinical outcomes and increased bleeding compared to prophylactic anticoagulation[83].

On the other hand, two open-label adaptive multiplatform RCTs evaluating the use of therapeutic-dose anticoagulation with heparin in hospitalized non-critically ill[84] and critically ill COVID-19 patients[85] respectively were performed. The primary outcome of these RCTs was organ support-free days, an ordinal scale composed of survival to hospital discharge and – in survivors – the number of days free of organ support to day 21. Among the 2219 non-critically ill patients, the probability that therapeutic anticoagulation increased organ support-free days compared to standard thromboprophylaxis was 98.6% (adjusted odds ratio 1.27, 95% credible interval 1.03-1.58)[84]. Major bleeding occurred in 1.9% of patients with therapeutic heparin and 0.9% of patients with standard thromboprophylaxis[84]. In contrast, in critically ill patients (n = 1098) therapeutic anticoagulation with heparin did not improve survival or days free of organ support[85]. Major bleeding occurred in 3.8% of patients assigned to therapeutic anticoagulation versus 2.3% of patients on standard thromboprophylaxis[85].

Collectively, the results of these first RCTs suggest that therapeutic dose heparin might be beneficial in hospitalized non-ICU COVID-19 patients, whereas therapeutic dose oral anticoagulants are not. In addition, therapeutic dose heparin does not improve the outcome of critically ill COVID-19 patients and likely is associated with harm. A mechanistic explanation for these observations is currently not known and the results are counterintuitive from the coagulation point of view; this is most likely due to the use of a pleiotropic drug (heparin) in a heterogeneous disease (COVID-19), underscoring the importance of patient stratification – in other words, precision medicine. It is tempting to speculate that these differences are explained by heterologous effects on immune-effector pathways, but this remains to be demonstrated. Other antithrombic drugs under investigation in RCTs in hospitalized COVID-19 patients include tissue type plasminogen activator (a profibrinolytic agent), several antiplatelet drugs (dipyramidole, aspirin, clopidogrel) and nafamostat (a serine protease inhibitor and a short-acting anticoagulant). Moreover, several trials have been initiated to evaluate the effect of thromboprophylaxis in post-discharge COVID-19 patients[80].

Anti-fibrotic therapies in COVID-19

Development of fibrosis may be related to organizing pneumonia following acute lung injury or the abnormal immune response in the lung, as pulmonary compartmentalization of hyperinflammation is present in COVID-19 patients[4,86]. It is currently unknown why some may recover from this insult, while others respond with an unchecked cellular proliferation, including accumulation of fibroblasts and myofibroblasts and deposition of collagen to result in pulmonary fibrosis. For these latter patients with COVID-19, available anti-fibrotic therapies may be beneficial. Apart from steroids, new compounds, mainly tyrosine kinase inhibitors, have demonstrated efficacy in patients suffering from idiopathic pulmonary fibrosis[87,88]. In addition, preclinical data suggests beneficial effects of Janus Kinase-signal transducer and activator of transcription (JAK-STAT) inhibitors in preventing pulmonary fibrosis[89]. However, to date there are no data on antifibrotic treatment in COVID-19 and multiple clinical trials are currently ongoing.

The immunotherapeutic approach in the clinic

The large number of trials performed since the beginning of the pandemic have provided an unprecedented amount of knowledge for a disease which is known for such a short time, but this also raises the challenge of discerning the best path for a systematic and rational treatment of the patient with COVID-19. The first important step in approaching the patient with COVID-19 is to determine

the severity of the disease, which is one of the most important criteria for patient stratification. Many clinical trials have used the criterium of severity when investigating different approaches of immunotherapy in hospitalized patients outside of the intensive care unit (moderate-to-severe patients), or in the intensive care unit (severe-to-critically ill patients). It is important to note that immunotherapy in COVID-19 is dynamic and in constant development. Therefore, we aim to provide guidance on immunotherapeutic strategies that are supported in expert guidelines, such as anti-IL-6R blockade and corticosteroids; however, we will augment this guidance with possible treatment options for when patients fail to respond and there is a clear clinical rationale for an alternative therapy, even if not yet formally tested in large RCTs.

The patient with moderate disease at high risk for worsening

From the perspective of patient stratification based on severity, the first major group of COVID-19 patients are those with moderate disease hospitalized on the medical wards. The aim of immunotherapy in these patients would be to prevent worsening of the disease, and potentially reducing the duration of hospitalization. The patients with mild disease that do not need hospitalization are believed to be able to recover without the need of immunotherapy, and no studies have been conducted on host-directed therapy in this subgroup.

For patients in the medical wards, a number of immunotherapeutic approaches have been proposed (**Figure 2**). First, the data available on anti-coagulant therapy suggest that therapeutic-dose heparin might be beneficial in these non-ICU COVID-19 patients (but not patients on the ICU). Second, the serological status of the patient should be assessed; if the patient is seronegative, passive immunization with antibody cocktails should be considered. Third, if the patients are seropositive and addition of antibody cocktails is not expected to be useful, additional steps need to be taken if the patient displays signs of worsening. If the patient needs oxygen supplementation, treatment with dexamethasone should be initiated. Moreover, initiation of anti-IL-6 therapy (tocilizumab, sarilumab) is advised if the patient needs oxygen and CRP is higher than 50 (this limit differs in the guidelines of various countries). Furthermore, treatment with the kinase inhibitor baricitinib has been shown to improve outcome in patients with high-flow oxygen therapy and noninvasive ventilation[46]. If the patient does not need oxygen therapy but biomarkers indicate worsening inflammation, for example suPAR higher than 6 ng/ml, or the surrogate markers CRP (more than 50 mg/L) and ferritin (higher than 700 mg/L) are present, then administration of the IL-1 receptor blocker anakinra should be considered[55].

The ICU patient

Monoclonal antibodies against COVID-19 are a possible option in patients that have no seroconversion during infection or after vaccination. Treatment with corticosteroids and anti-IL-6 should be initiated within 48 hours of admission to the ICU. When a patient is transferred from the ward and has not yet received dexamethasone or tocilizumab, it is still an option to start corticosteroids and anti-IL-6 treatment.

Difficult therapeutic decisions on patients with severe COVID-19 may need to be taken if severe complications develop during the ICU stay. When signs of immunoparalysis are present, reflected by lymphopenia, low HLA-DR expression on monocytes, opportunistic infections (e.g. aspergillosis, herpes infections), or a persistent high SARS-CoV-2 load, then stimulatory immunotherapy would be a rational step – but this has not been formally tested in RCTs. From a pathophysiological point of view, and based on small case-series, one might consider immunostimulatory treatments such as recombinant IFNg. Similar approaches boosting adaptive immune responses are currently under investigation in clinical trials, such as with recombinant IL-7 and checkpoint inhibitors. Targeting pulmonary fibrosis is another challenge and might benefit from biomarker-directed therapy, although there is no current data on this in COVID-19 yet. High-dose steroids have been proposed with evidence coming from trials in the ICU before the pandemic. An overview of the potential approach to immunotherapy in the ICU patient with COVID-19 is presented in **Figure 3**.

The patient with multi-system inflammatory syndrome: MIS-C and MIS-A

Early in the pandemic, children were seen to present with diverse COVID-19 symptoms, such as persistent fever, headache, fatigue, abdominal pain, vomiting, conjunctival injection, myocarditis and rash, usually 2-6 weeks after mild; this condition was named multisystem inflammatory syndromes in children (MIS-C). A similar syndrome has been described in adults (MIS-A). Some of the children with MIS-C developed multi-organ failure and shock or coronary aneurysms.

The American College of Rheumatology treatment guideline recommends intravenous immunoglobulin (IVIG) and/or high-dose glucocorticoids as first-line therapy in MIS-C[90]. Approximately 30–80% of patients do not respond to IVIG and may require adjunctive immunomodulatory therapy[91–96]. Pulse methylprednisolone, additional dosing of IVIG, anakinra, tocilizumab and infliximab have all been used as escalation therapy[93,97–100] in MIS-C. Far fewer cases of MIS-A have been reported in the literature[101–103]. These adult patients were treated with glucocorticoids, with or without IVIG, and anticoagulants with mostly favorable outcomes.

In two large observational cohort studies, the effects of different treatment strategies on short term outcome were compared, with propensity score adjustments for confounding. The Overcoming COVID

consortium reported a lower risk of cardiovascular dysfunction and a lower need for vasopressors and adjunctive therapy in initial treatment with IVIG plus glucocorticoids compared to IVIG monotherapy[104]. Yet, in the Best Available Treatment Study (BATS), treatment with IVIG, IVIG plus glucocorticoids, or glucocorticoids monotherapy did not yield statistically significant differences for end points of ventilation, inotropic support, or death, or for improvement on an ordinal clinical-severity scale[105]. Both studies reported reduced risks for escalation therapy in patients treated with IVIG plus glucocorticoids compared to IVIG monotherapy, which corroborates the findings from a smaller French study[106]. Yet, glucocorticoid monotherapy and IVIG monotherapy was equally effective.

Differences in study results could result from genetic differences between study populations, differences in viral strain-dependent hyperimmune responses, and, of course, suboptimal adjustments for all potential confounders, in particular confounding by indication. Therefore, randomized controlled trials are needed to determine the optimal therapy for MIS-C and MIS-A. Currently, there is one recruiting RCT comparing infliximab, glucocorticoids or anakinra as escalation therapy after IVIG monotherapy (NCT04898231). In addition, treatment with mesenchymal stromal cells is currently being evaluated in open label studies (NCT04549285, NCT04456439).

Future outlook and conclusions

The immunotherapy of COVID-19 has booked important successes, being the first severe acute infectious disease in which a strong level of evidence permits recommendation of immunotherapy, as detailed above. However, major quandaries remain in the day-to-day clinical practice, and they should be addressed as a matter of urgency.

One major quandary with which we are confronted is the treatment of the COVID-19 patient who does not improve, despite treatment with immunotherapy such as dexamethasone and anti-IL-6 therapy. Some of these patients remain strongly hyperinflammatory, and no formal RCTs of follow-up immunotherapy have been performed to help guide our decision; this is a substantial unmet need. However, such studies will be more challenging to perform than earlier trials and until such data are available, one can rationally argue that the addition of an alternative immunotherapy should be considered (**Table 1**).

It will be thus very important to further pursue clinical studies to identify novel immunotherapies that could further improve the outcome of severe cases. If the patient is still not improving despite the available combinations, other immunomodulatory drugs could be an option to further dampen the hyperactive immune status, such as blocking C5a, anti-GM-CSF, or anti-TNF. However, the level of evidence for anti-cytokine therapies in the ICU patients beyond anti-IL-6 is very low. Furthermore, an

increased risk of secondary infections can be anticipated when blocking more components of the immune system. Therefore, escalation of immunosuppressive treatment is currently not advocated outside of clinical trials. An overview of novel potential therapies that need to be formally tested in future clinical trials is presented in **Figure 4**.

A second quandary that has been only superficially addressed until now is represented by the pathophysiological heterogeneity of COVID-19. Several interventions proven to be effective work by modulating the host's immune response, or cascades downstream of the immune response. However, the host response to SARS-CoV2 is complex, characterized by a plethora of pathways that can be both beneficial and deleterious. Not surprisingly, agents that modify these pathways can be beneficial for some patients and ineffective or even harmful in others. Further complexity arises when one considers that the agents themselves can have additive, multiplying or negative effects when used in combination. These variable treatment effects, dependent on a patient's particular immune state, the disease course and on the use of co-interventions, likely explain some of the disparate findings from some clinical trials. The weaving together of findings from these experiments into an overarching conceptual model is a largely theoretical exercise at this point. Consequently, the current evidence-based guidelines appear somewhat simplistic and lacking in nuance for the individualized treatment many clinicians likely wish to prescribe. Nevertheless, there preliminary data suggest that defined subgroups of patients (based on their inflammatory response) may benefit more, or less, from immunomodulatory therapy. The way forward is to perform trials based on robust biomarkers, so that patients that are more likely to benefit from a given treatment, will receive it.

There are two broad barriers to the generation of robust experimental evidence supporting individualized treatment algorithms. First, the underlying heterogeneity in pathophysiology that likely drives differential treatment response may often be clinically invisible: two clinically similar patients may have diverse immune states. Second, traditional trial designs are not well-suited for efficient evaluation of differential treatment effects in different patient groups. The good news is that much of the evidence supporting best treatment has come from adaptive platform trials, like RECOVERY or REMAP-CAP. These designs are more flexible for the evaluation of combinations of therapies and evaluation of effects across different subgroups. And, indeed, one can argue that adaptive platform trials have been the dominant source of robust clinical evidence for COVID-19, perhaps ushering in a new paradigm for clinical research. Nonetheless, these trials have thus far still used relatively simple approaches for the assessment of subgroup effects and heterogeneity of treatment effect. Smaller trials, although they can provide clinical rationale and explore more personalized options when common approaches are not working, they often lack statistical power to confirm clinical efficacy. Therefore, immunotherapy in COVID-19 needs to be further explored through RCTs in order to consolidate knowledge and experience and to reveal the optimal biomarker-driven host-directed strategies.

One final quandary is that must be addressed in the future is availability of immunotherapy. While the approaches described here can be incorporated in standard-of-care protocols of high-income countries, these treatments are often not available in many low or middle income countries. Efforts should be made to increase availability of the current medications on the one hand, and also to explore cheaper but equally effective alternatives. Only by ensuring equal therapeutic opportunities for all our patients can we fulfil our mission for optimal treatment of COVID-19.

Table 1. Overview of the relevant immunotherapeutic targets and respective trials discussed in this paper.

Type of immunotherapy	Intervention	Trial / paper	Study population	Primary endpoint(s) / outcome measures	Overall conclusion	Ref
ANTI-TRIGGER						
Polyclonal convalescent plasma		AAAS9924 (NCT04359810)	Hospitalized patients with PCR- and radiographically confirmed COVID-19 and hypoxia requiring supplemental oxygen	Clinical status according to the WHO Clinical Progression Scale at day 28	No significant effect	25
		RECOVERY (NCT04381936)	Hospitalized patients with suspected or confirmed COVID-19	28-day mortality	No significant effect	29
		REMAP-CAP (NCT02735707)	Hospitalized patients with COVID-19 admitted to the ICU for organ support	Organ support-free days up to day 21 and in-hospital mortality	No significant effect	30
		INFANT-COVID19 (NCT04479163)	Older ambulatory patients with mild COVID-19 symptoms	Progression to severe respiratory disease	Beneficial	33
Anti-spike protein monoclonal antibodies	Bamlanivimab - Etesivimab	BLAZE-1 (NCT04427501)	Ambulatory patients with mild to moderate COVID-19	Change in SARS-CoV2 log viral load at day 11	Beneficial[1]	34
	Casirivimab - Imdevimab	RECOVERY (NCT04381936)	Hospitalized patients with suspected or confirmed COVID-19	28-day mortality	Beneficial in certain subgroups[2]	35
MEDIATOR-TARGETED						
Corticosteroids	Dexamethasone	RECOVERY (NCT04381936)	Hospitalized patients with suspected or confirmed COVID-19	28-day mortality	Beneficial in certain subgroups[3]	39
		CoDEX (NCT04327401)	Hospitalized patients with moderate to severe COVID-19-associated ARDS	Ventilator-free days up to day 28	Beneficial	41
	Hydrocortisone	REMAP-CAP (NCT02735707)	Hospitalized patients with COVID-19 admitted to the ICU for organ support	Organ support-free days up to day 21 and in-hospital mortality	Potentially beneficial[4]	40
Kinase inhibitors	Baricitinib	ACTT-2 (NCT04401579)	Hospitalized patients with COVID-19	Time to recovery	Beneficial	46

[1] Only for the bamlanivimab – etesivimab cocktail; not for bamlanivimab monotherapy.
[2] Only in patients who were seronegative at baseline.
[3] Only in patients receiving supplemental oxygen or invasive mechanical ventilation; not in those without respiratory support.
[4] Trial stopped early; no treatment strategy met criteria for statistical superiority.

			COV-BARRIER (NCT04421027)	Hospitalized patients with COVID-19	Progress to high-flow oxygen, (non-) invasive invasive ventilation, or death by day 28	Partially beneficial[5]	47
		Imatinib	COUNTER-COVID (NCT04394416)	Hospitalized patients with COVID-19 and hypoxia requiring supplemental oxygen	Time to discontinuation of respiratory support for >48 consecutive hours	Potentially beneficial[6]	48
		Tofacitinib	STOP-COVID (NCT04469114)	Hospitalized patients with PCR- and radiographically confirmed COVID-19	Death or respiratory failure by day 28	Beneficial	49
	Anti-cytokine treatment	Anti-IL-1					
		Anakinra	CORIMUNO-ANA-1 (NCT04341584)	Hospitalized patients with mild-to-moderate COVID-19	Death or need for (non-) invasive ventilation by day 4	No significant effect	50
			REMAP-CAP (NCT02735707)	Hospitalized patients with COVID-19 admitted to the ICU for organ support	Organ support-free days and in-hospital mortality up to day 21	No significant effect	51
			SAVE (NCT04357366)	Hospitalized patients with PCR- and radiographically confirmed COVID-19 and blood suPAR concentrations of ≥ 6ng/mL	Progression to severe respiratory failure by day 14	Beneficial	54
			SAVE-MORE (NCT04680949)	Hospitalized patients with PCR- and radiographically confirmed COVID-19 and blood suPAR concentrations of ≥ 6ng/mL	Clinical status according to the WHO Clinical Progression Scale at day 28	Beneficial	55
		Canakinumab	CAN-COVID (NCT04362813)	Hospitalized patients with PCR- and radiographically confirmed COVID-19, hypoxia, and systemic inflammation	Survival without progression to invasive mechanical ventilation from day 3-29	No significant effect	56
		Anti-IL-6					
		Tocilizumab	RECOVERY (NCT04381936)	Hospitalized patients with COVID-19, hypoxia, and systemic inflammation	28-day mortality	Beneficial	57
			REMAP-CAP (NCT02735707)	Hospitalized patients with COVID-19 admitted to the ICU for organ support	Organ support-free days and in-hospital mortality up to day 21	Beneficial	58
		Sarilumab	REMAP-CAP (NCT02735707)	Hospitalized patients with COVID-19 admitted to the ICU for organ support	Organ support-free days and in-hospital mortality up to day 21	Beneficial	58
		Anti-GM-CSF					
		Otilimab	OSCAR (NCT04376684)	Hospitalized patients with severe COVID-19, hypoxia, and systemic inflammation	Survival without progression to respiratory failure at day 28	Beneficial in certain subgroups[7]	60

[5] No significant effect on composite primary endpoint, but a significant effect on 28- and 60-day all-cause mortality was observed.
[6] Although the primary endpoint was not met, findings on mortality and duration of mechanical ventilation suggest a possible beneficial effect and warrant further trials.
[7] Only in patients of age 70 and above.

	Anti-complement treatment	Anti-C5a					
			PANAMO (NCT04333420)	Hospitalized patients with severe PCR- and radiographically confirmed COVID-19	Percentage change in PaO_2/FiO_2 in supine position between baseline and day 5	No significant effect	67
	Interferons	Type I interferons					
		IFNβ-1a	SG016 (NCT04385095)	Hospitalized patients with COVID-19	Clinical status according to the WHO Clinical Progression Scale at day 14	Beneficial	69
		IFNβ-1b	UW-20-074 (NCT04276688)	Hospitalized patients with COVID-19	Time to providing a nasopharyngeal swab negative for SARS-CoV2 RT-PCR	Beneficial[8]	70
			DisCoVeRy (NCT04315948)	Hospitalized patients with COVID-19	In-hospital mortality	No significant effect	71
		Type II interferons					
		IFNγ	Case series	Five critically ill COVID-19 patients	Change in SARS-CoV2 viral load, positive-to-negative viral culture conversion	Potentially beneficial	72
			Case series	Six COVID-19 patients with ventilator-associated pneumonia	Monocyte HLA-DR expression, total lymphocyte counts	Potentially beneficial	73
EFFECTOR-TARGETED							
	Kallikrein-kinin inhibitors	Icatibant	Case-control study	Nine patients with mild-to-moderate COVID-19 and hypoxia compared to 18 matched controls	Change in supplemental oxygen need expressed in liters per minute	Potentially beneficial	77
		Icatibant + C1-esterase inhibitor	Case-control study	30 patients with PCR- and radiographically confirmed COVID-19 and hypoxia	Time to clinical improvement as defined by the Cap-China Network	Partially beneficial[9]	78
	Thromboprophylaxis	Low molecular weight heparins					
		Enoxaparin (therapeutic dose)	INSPIRATION (NCT04486508)	Hospitalized patients with COVID-19 admitted to the ICU	Venous or arterial thrombosis, ECMO treatment, or mortality within 30 days	No significant effect	82
			ACTION (NCT04394377)	Hospitalized patients with COVID-19 and elevated D-dimer levels	Time to death, discharge, or duration of supplemental oxygen up to day 30	No significant effect	83
		Direct anticoagulants					
		Rivaroxaban (therapeutic dose)	ACTION (NCT04394377)	Hospitalized patients with COVID-19 and elevated D-dimer levels	Time to death, discharge, or duration of supplemental oxygen up to day 30	No significant effect	83
		Unfractioned heparin (therapeutic dose)	ATTACC, ACTIV-4a and REMAP-CAP	Hospitalized patients with mild-to-moderate COVID-19	Organ support-free days and in-hospital mortality up to day 21	Beneficial	84
			ATTACC, ACTIV-4a and REMAP-CAP	Hospitalized patients with severe COVID-19	Organ support-free days and in-hospital mortality up to day 21	No significant effect	85

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[8] Observed for triple antiviral therapy combined with IFNβ-1b compared to lopinavir-ritonavir alone.

[9] Although the primary endpoint was not met, there was evidence for safety and a significant improvement in lung computed tomography scores and blood eosinophil counts.

BOX 1. The role of biomarker-driven immunotherapy in COVID-19.

The emergence of SARS-CoV-2 infection has been associated with a flurry of studies investigating biomarkers associated with disease severity and outcome. Many inflammatory biomarkers, from the number of subpopulations of immune cells (e.g. lymphopenia, neutrophil-to-lymphocyte ratio), to circulating cytokines (e.g. IL-6, chemokines) or acute phase proteins (e.g. CRP, ferritin), to biomarkers of endothelial cell activation (e.g. suPAR) or complement, are associated with development of a severe COVID-19. Unfortunately, a large gap persists between the use of these biomarkers for predicting disease severity, and for patient stratification to improve host-directed (immune-based) therapies. In addition, more work needs to be done to understand the variability of various immunological biomarkers in time, which may also influence treatment approaches. While the low-hanging fruits of anti-COVID19 immunotherapy (e.g. steroids, anti-IL-6 therapies) have been already achieved, the next steps for optimising immunotherapy will require identification of patient sub-groups that would benefit from specific approaches: e.g. immune-modulating approaches in patients with hyperinflammation vs. Immune-stimulatory therpaies in those with immune paralysis. A blueprint for biomarker-guided therapies is provided by the use of suPAR to guide anakinra treatment in the sub-group of COVID19 patients with lung hyperinflammation[55], or the use of HLA-DR expression to guide IFNγ therapy in sepsis[107]. Intense Biomarker research focusing on patient stratification is warranted; in addition, biomarkers to enable the monitoring of the effects of immune-based therapies are also needed.

Figure legends

Figure 1. A summary of the pathophysiological factors targeted by immune-based therapies in COVID19, which can be categorized as triggers of the infection (eg, SARS-CoV-2 virus, recognition receptors), mediators of the immune response (cytokines, complement, etc), and immune-effector mechanisms (kallikrein system, thromboinflammation).

Figure 2. An algorithm for immunotherapy of the patient with moderate-to-severe COVID-19, without critical illness.

Figure 3. An algorithm for immunotherapy of the patient with critical illness due to COVID-19.

Figure 4. An overview of the options for immunotherapy in patients with COVID-19, depending on the stage of the disease (based on the WHO Clinical Progression Score). The treatment based on high quality randomized trials are presented in dark blue, while the more speculative treatment based on observational or small case series studies are presented in light blue.

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