Interleukin-6, a major player of cytokine storm in COVID-19 and its alleviation by therapeutic antibodies- (A review)

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Declaration by authors

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Both the authors have written and read the manuscript and provided their consent to submit the manuscript for publication in “Current Science”. In addition, we also declare that there is no conflict of interest in the publication of this manuscript.

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Abstract

Interleukin-6 (IL-6) is an important cytokine; plays a vital role in immune responses and inflammation. Here, the signalling functions of IL-6 through its receptors; physiological and pathological roles especially, its contribution to various auto-immune diseases, cancers and severe COVID-19/SARS-CoV2 infections are described. It is reported that in severe COVID-19 and auto-immune patients experience cytokine storms due to the hyper-activation of the IL-6 receptor pathway leading to detrimental effects. Blocking IL-6 receptor action by therapeutic antibodies has been considered an attractive strategy of treatment. The latest findings on the application of the anti-IL-6 therapeutic antibodies in COVID-19 patients are discussed.

Keywords: COVID-19, SARS-CoV2, Interleukin-6, cytokine storm, monoclonal antibodies, therapeutic antibodies
1 Introduction

1.1 Cytokines

Cytokine is a generic term; other terms include lymphokines (lymphocyte-made cytokines), monokine (monocyte-made cytokines), chemokine (chemotactic-activated cytokines), and interleukin (leukocyte-made cytokines that work on further cells). Cytokines are low molecular weight (<30 kDa), a soluble glycoprotein which are non-immunoglobulin in nature acting non-enzymatically through specific receptors. Additionally, cytokines bring out effects essentially in four different ways which include, pleiotropy, redundancy, synergy, and antagonism\(^1\).

Based on their presumed function or target of the action, cytokines are classified into six categories that includes, hematopoitins, interleukins (IL), interferons (IFN), chemokines, Tumour Necrosis Factor (TNF) and myokine.

1.2 SARS-CoV2 and cytokine storm

The SARS-CoV2, widely known as COVID-19, has resulted in the global pandemic that originated in December 2019. At the commencement of writing this paper, it has affected countries all over the globe, and about 530,896,347 cases have been validated, of which 6,301,020 people have lost their lives\(^2\). The major cause for this colossal loss is assumed to be the ‘cytokine storm’ also known as cytokine storm/release syndrome (CSS/CRS)\(^3\).

As made evident by various investigations, "Cytokine Release Syndrome" (CRS), is a phenomenon that is allegedly involved in COVID-19. Additionally, there is an uncontrolled release of cytokines like IL-1, IL-6, IL-12, and IL-18, TNF, IFN, and other inflammatory cytokines. This may raise alveolar-capillary gaseous exchange, lowering oxygen saturation in the pulmonary tissue as shown in Figure 1\(^4\).
In earlier days, an influenza-like symptoms aroused after a systemic infection like sepsis and also by immune therapies. This syndrome is currently known as cytokine storm. This terminology used when immune dysregulation is characterized by the activation of macrophages and lymphocytes. This condition instigates an enormous amount of cytokine secretions resulting in systemic inflammation and organ failures\(^5\).

Cells such as neutrophils, macrophages and natural killer (NK) cells are most often responsible for cytokine storm. In animal studies and for anticancer therapies in humans, the infusion of recombinant cytokines like IL-1, IL-6, IL-12, IL-18 and TNF-\(\alpha\) causes terrible effects or mortality aligned with the pivotal role of cytokines as mediators of hyper inflammation. Several key cytokines which play crucial role in cytokine storm are IFN-\(\gamma\), IL-1, IL-6, TNF-\(\alpha\) and IL-18. The levels of IL-6 are highly elevated in various immunopathology disorders and cytokine storm\(^5\).

It has been determined that the normal range of IL-6 in a healthy human is less than 7 pg/ml. Several patients who experience cytokine storm due to various reasons have shown that the IL-6 shoots up to 100 – 10000 pg/ml\(^6\).

### 1.3 Interleukin – 6 (IL-6)

IL-6 is a cytokine that is not only a pro-inflammatory molecule but also has anti-inflammatory properties. The formation of this cytokine is associated with several types of cells, including fibroblasts, keratinocytes, mesangial cells, vascular endothelial cells, mast cells, macrophages, dendritic cells and T and B cells\(^7\).

The IL-6 receptor consists of two subtypes namely, gp80 and CD126. Two forms of receptors are present for IL-6, such as the transmembrane IL-6 receptor (mIL-6R) located on the cell membrane and the circulating soluble IL-6 receptor (sIL-6R). These are formed by the means of alternative splicing or by cleaving of ‘A Disintegrin and A Metalloproteinase 17/TNF-\(\alpha\)
converting enzyme (ADAM17/TACE)’ by metalloprotease. Other names for mIL-6R include IL-6R-α, gp80 or CD126. IL-6 stimulates osteoclast formation when secreted by osteoblast. Smooth muscle cells synthesize the IL-6 as a pro-inflammatory reaction in blood vessels. IL-6 also controls the development of proteins that are involved in the regulation of gene expression. The pleotropic character of this interleukin can be clarified by the number of genes activated by IL-6 action. Hyper production of IL-6 has been linked with numerous chronic diseases like rheumatoid arthritis (RA) and Castleman’s disease. Thus, IL-6 and IL-6 receptors are the utmost analysed parameters for the possible clinical cure of such deep-rooted illness. It can also act as an inflammatory marker in severe COVID-19 infections with poor prognosis.

As per the recent data, seven members are known of the IL-6 family. They include IL-6, Ciliary neurotrophic factor (CNTF), Leukaemia inhibitory factor (LIF), Oncostatin M (OSM), cardiotrophin (CT-1), interleukin 11 (IL-11) and cardiotrophin like cytokine (CLCF1). All these members interact with gp130 in the existence of their receptor and comprise of a four-helix packet assembly. Recently, IL-27, IL-35 and IL-39 have been included additionally as new members of this group. These novel members are heterodimers and Epstein-Barr virus-induced gene 3 (EBI3) codes for their common protein subunit. EBI3 belongs to the cytokine receptor family. Hence, the structure of these three new members is similar to IL-6/sIL-6R and IL-12.

It has been noted that all the members have gp130 as signal transducer and use Janus Kinase – signal transducer and activator of transcription (JAK–STAT) pathway for signal transduction in quick response. The STAT is transcription factors which are activated by JAK that are present in the cytosol and then moved to the nucleus of the cell.
1.4 Physiological and pathological roles of IL-6

A homodimer of gp130 is assembled when the IL-6 binds with the mIL-6R or sIL-6R. After binding, a hexamer network is racked-up, comprising two units of IL-6, IL-6R, and gp130. This complex then further activates JAK which phosphorylates tyrosine remnant in the cytoplasmic domain of gp130. This further phosphorylates the STAT-3 which enters the nucleus and transmits IL-6 signal\textsuperscript{15}.

This whole process finally activates two signalling pathways as shown in Figure 2.

a) Gp130 Tyr759 derived SHP-2/ERK MAPK
b) Gp130 YXXQ mediated JAK/STAT pathway

Acute-phase response, angiogenesis, neutrophil trafficking, immune responses, bone metabolism, cartilage metabolism, anaemia of chronic diseases, cancer and lipid metabolism are some physiological and pathological roles of IL-6\textsuperscript{16}.

1.4.1 Acute-phase response:

During this response, the pathogens are neutralized and their further invasion is restricted, thereby minimizing tissue damage. As noticed in the inflammatory conditions of humans, a wide spectrum of acute-phase proteins such as synthesis and secretion of C-reactive protein (CRP), fibrinogen, serum amyloid A (SAA), α 1-antitrypsin, α 1-antichymotrypsin and haptoglobin are enhanced. Interestingly, fibronectin, albumin and transferrin are diminished as shown in Figure 3 while the entire process is promoted by IL-6\textsuperscript{17}. Injection of IL-6 may lead to various effects on an individual depending upon the type of injection. For example, the intracerebroventricular injection may raise body temperature, whereas, intravenous or intraperitoneal injection will have no consequence on temperature.

SAA is an acute-phase protein which is produced with IL-6 stimulation in the liver. In the event of inflammation, SAA replaces apo-lipoprotein and circulates with high-density lipoprotein.
Once the process is finished, SAA detaches from HDL and undergoes degradation. Due to this, the SAA gets deposited in the extracellular region of critical tissues as amino acid fibrils which results in renal breakdown and gastrointestinal tract malfunction. This cytokine plays an important role in the synthesis of hepcidin in the liver and is involved in regulating the iron recycling in the spleen and absorption in the intestine, leading to iron deficiency anaemia\textsuperscript{15}.

1.4.2 Angiogenesis:
The production of blood vessels is known as angiogenesis and it acts as an essential component of inflammation. IL-6 has been indicated to play a part in pathogenic angiogenesis in disorders like stroke, RA and various cancers. It has been noted that vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), transforming growth factor-\(\beta\) (TGF), IL-6, IL-8, IL-1 and TNF-\(\alpha\) show angiogenic activity. Also, a significant increase in VEGF may lead to RA pathogenesis\textsuperscript{18}. Elevated concentrations of IL-6 and CRP have been linked with age-related macular degeneration (AMD). Blocking IL-6 also suppresses choroidal neovascularization (CNV), the formation of new blood vessels originating from the choroid, resulting in vision loss.

1.4.3 Neutrophil trafficking:
IL-6, which signals through the typical gp130 receptor subunit, is a key control point for neutrophil trafficking along the inflammatory response\textsuperscript{19}. Also, IL-6 deals as a stimulus for myelopoiesis i.e. formation of tissue in bone marrow. Neutrophils drift towards the inflated location leading to the robust synthesis of inflammatory moderators like prostaglandins, complements, reactive oxygen species (ROS), proteases and cytokines. Additionally, IL-6 augments the expression of the adhesive molecule like Vascular Cell Adhesion Molecule (VCAM1) and Intercellular Adhesion Molecule (ICAM1). This in turn induces chemokines
such as CXCL-8 (CX chemokine ligand)/IL-8, CCL2/MCP-1 (Monocyte chemoattractant protein), CCLS/MCP-3 and CCL20 leading to a reduction in moving neutrophils in RA.

1.4.4 Immune response:

IL-6, which is produced quickly and temporarily in response to infections and tissue injury, commits to host defence by activating acute process responses, haematopoiesis, and immunological reactions\(^2\). As known, IL-6 plays a crucial role in B-cell differentiation. This compliments the synthesis of IgM, IgG and IgA in B-cell triggered with *Staphylococcus aureus* Cowan I or pokeweed mitogen. IL-6 also encourages antibody synthesis by developing the B-cell helper competence of CD\(^+\) T-cells i.e. enhances humoral immunity. IL-6 also causes B-cell irregularities linked with the inflammatory procedure. IL-6 trans-signalling was also found responsible for the induction of pre B-cell enhancing factor (PBEF) in synovial fibroblast cells. Blockage of IL-6 supresses anti–CD28 antibody motivated CD\(^+\) T-cell procreation which is due to the introduction of regulatory T-cells (T\(_{\text{reg}}\)) and suppression of IL-2. On the grounds of cytokine production, CD\(^+\) helper T-cells can be divided into T\(_{\text{H}1}\) and T\(_{\text{H}2}\). IL-6 increases T\(_{\text{H}2}\) development caused by IL-4 while inhibiting T\(_{\text{H}1}\) differentiation induced by IL-12.

1.4.5 Bone metabolism:

In RA and other bone disorders, IL-6 plays a key role in aberrant bone resorption. Synovial fluid from RA patients has elevated levels of IL-6, sIL-6R, and the IL-6–sIL-6R network, that trigger the development of osteoclasts. IL-6 suppresses the development of osteoclast precursor cells via regulating transcription linked with MAPK phosphatases and the ubiquitin pathway. This IL-6 induced suppression can be reversed by the addition of sICAM-I in a mono-culture system. At the cellular plane, bone estrogen reduces osteoclastic differentiation, thereby decreasing the number of osteoclasts and the number of functioning remodelling factors. This influence is presumably moderated by certain cytokines, with the most potent candidates being
IL-1 and IL-6 via a yet unexplained mechanism. IL-6 presence in bone marrow cells is controlled by estrogen\textsuperscript{21}.

It was observed that a regular number of osteoclast and normal active bone reabsorption was present in IL-6 deficient mice. According to the findings, IL-6 is not required for normal bone growth and osteoclastogenesis or osteoclastic bone-resorbing activity. The relevance of IL-6 in postmenopausal osteoporosis is debatable because bone deterioration has been shown in individuals with estrogen shortage postmenopausal osteoporosis, and anti-IL-6 antibody remedy is futile to prohibit bone destruction\textsuperscript{22}.

1.4.6 Cartilage metabolism:
An increased amount of IL-6 and a decrease in Insulin-like growth factor (IGF-1) leads to growth impairment including juvenile RA, Crohn’s disease, cystic fibrosis and immunodeficiency. The endochondral ossification induces lengthwise bone growth in the growth plate, which is monitored by aggregation of generic and hormonal dynamics, growth factors, surroundings and diet. Studies conveyed that IL-6 suppresses insulin-induced differentiation of chondrogenic pioneer cells. These cartilage cells are eventually transformed as benign tumours\textsuperscript{23}. This can be inhibited by reduction in the levels of IGF-1. IL-6 encourages the synthesis of matrix metalloproteinase (MMPs) such as MMP1, MMP3 & MMP13, and a disintegrin and metalloproteinase with thrombospondin motif – 4 (ADAMTS) from chondrocytes and synovial cells. In RA, IL-6 has been linked to cartilage degradation that was found to be based on IL-6 in murine models of RA\textsuperscript{24}.

1.5 Contribution of IL-6 in auto-immune diseases and the role of therapeutic antibodies
Autoimmune diseases include a group of illnesses that are poorly acknowledged, involving genetic and environmental aspects implicated in pathogenesis. Autoimmune disorders are
classified into tissue specific and systemic disorders. Cytokines, such as ILs and IFNs, are produced by a variety of immune cells which are involved in the regulation of physiological and pathophysiological processes. Few investigations demonstrated the critical roles of pro-inflammatory cytokines including TNF, IL-1, and IL-6 in the development of RA. In certain cases, IL-6 has been persistent in people with immune-inflammatory illnesses including Castleman's disease and RA. Administration of anti-IL-6 receptor antibodies are effective in a few patients with RA. This corroborates the findings in the animal models which suggested anti-IL6R antibody treatment is quite rewarding in IL-6 related immune diseases.

Concerning the pathological function of IL-6 in different inflammatory autoimmune disorders. The complementarity-determining regions (CDR) of the mouse anti-human IL-6R Ab were transplanted onto human IgG1 to develop tocilizumab, an IgG1 class humanised anti-IL-6R monoclonal Ab. By suppressing IL-6 attachment to both mIL-6R and sIL-6R, this Ab inhibited IL-6-mediated signal transduction.

1.5.1 Tocilizumab in RA

It was observed in clinical studies that Tocilizumab successfully suppresses the activity of the disease and defends against the worsening of joint damage, and increases the everyday activity and quality of life. While other biologics, including TNF inhibitors, a T cell stimulus inhibitor, and a B cell diminishing mediator, are also used to treat RA patients, tocilizumab proves to become the most potent in controlling the activity of the disease. Tocilizumab monotherapy seems to be the only biologic known to be more promising than the conventional drug methotrexate (MTX).

1.5.2 Tocilizumab in case of systemic juvenile idiopathic arthritis (SJIA)

SJIA is a long-lasting infant arthritis subset that contributes to systemic inflammation, joint damage, physical weakness, and development deficiency. IL-6 in the blood of patients was
significantly raised and it is well-linked with the activity of the disease\textsuperscript{27}. A total of 112 kids with dynamic SJIA were arbitrarily injected with placebo or tocilizumab in a worldwide stage III clinical trial. After the treatment, tocilizumab expressed promising results in the beginning of a novel phase in the cure of untreated juvenile diseases\textsuperscript{28}.

1.5.3 IL-6 in systemic lupus erythematosus (SLE)

IL-6 may play a direct role in causing tissue injury, according to data from many investigations. SLE patients had higher blood IL-6 levels in some, but not all investigations. Patients also showed a greater frequency of IL-6 secreting peripheral blood mononuclear cells (PBMCs) than healthy controls. Lymphoblastoid cells collected from SLE patients generated great amounts of IL-6, and inhibiting IL-6 \textit{in vitro} decreased the anti-dsDNA autoantibody synthesis\textsuperscript{29}. Similar observations were noted when autoreactive T-cells and autologous B-cells were given an anti-IL-6R monoclonal antibody.

1.5.4 IL-6 in multiple sclerosis (MS)

MS has been linked to an increased levels of IL-6 in the bloodstream. In the plasma of individuals with acute meningoencephalitis, elevated levels of IL-6 can also be seen. In the majority (91\%) of MS patients, IL-6 becomes detectable in their plasma with a mean value of 54 U/ml\textsuperscript{30}. The interplay of T and B cells has been suggested as a role in the formation of MS. In the early stages of MS, Th17 cells are thought to have a significant impact\textsuperscript{31}. Elevated differentiation of plasma cells of activated B-cell replicas is implicated in the formation of tissue destructing autoantibodies.

1.6 IL-6 in case of cancer

IL-6, one of the key cytokines in the tumour microenvironment, is a crucial component present at elevated levels and is thought to be unregulated in cancer. In almost all forms of tumours, the overexpression has been identified. Chronic inflammation in the tumour microenvironment
has been found to promote tumour development and cause resistance to chemotherapy and radiotherapy. Numerous cancers, like breast cancer, human prostate cancer, ovarian cancer and lung cancer have been found to over-express IL-6 cytokine in the tumour microenvironment. A major part is contributed by IL-6 in the maintenance and development of tumour cells. IL-6 is classified as a multipurpose cytokine that is chief signalling pathway stimulator of the JAK/STAT3. STAT3 has been used as an oncogene in many cancers and has been shown in several models to induce malignant cell transformation. This issue has a tendency to escalate in prostate cancers. IL-6 has been shown to specifically induce increased cellular invasions over basement membrane degeneration induced by the gene expression of matrix metalloproteinase. This indicates a vital role of IL-6 in the occurrence of various cancers. In particular, IL-6 has been proposed as a potential therapeutic target for the management and cure of these cancers.

1.7 Role of IL-6 in COVID-19 patients

IL-6 has gained a prominent recognition in recent years as a result of the emergence of the virus and its impact on the pathogenesis of the COVID-19. In patients with COVID-19, cytokine storm is one of the most important pathological conditions. The pathogenesis of COVID-19 disease remains uncertain and data for potential therapeutics have just started emerging. The COVID-19 pathogenesis for severe acute respiratory syndrome includes, TNF-α, IFN-γ, IL-1 and IL-12 serum cytokines and chemokine IL-6. The IL-6 is one of the main cytokines contributing to an inflammatory storm, which may result in an increased instability of the alveolar-capillary and blood gas exchange. In this inflammatory process, IL-6 tends to be the dominant cytokine. Flushing of virus from the body, perseverance of virus and spread of prolonged viral infection are some major impacts led by the imbalance of IL-6. High levels of pro-inflammatory cytokines such as IL-1, IL-6, IL-18, and TNF-α may be seen in severe cases. When a patient experiences cytokine storm, CRP was produced at high levels (>100
mg/l) by hepatocytes in response to IL-6. The peak serum level of ferritin reaches more than 1000 µg/l, an indicator of macrophage activeness. A German study has shown that IL-6 levels >80 pg/ml together with CRP levels >97 mg/l can lead to respiratory failure and death.

### 1.7.1 IL-6 in COVID-19 pathogenesis

In viral infections, the conflicting functions of IL-6 may be challenging, mainly in choosing potential therapeutic options. The laboratory experiments have shown that anti-viral immune reactions are inefficient and the contagion weakens after collapse in cytokine levels, specifically IL-6. Data from these studies reveal that IL-6 might be essential for virus removal through the modulation of lymphocyte responses, quenching inflammation, inducing the repair of lung tissue, restricting macrophage invasion and phagocytosis.

Lymphopenia and cytokine storms have been identified as two significant immunopathological consequences of coronavirus infection (i.e. SARS-CoV, MERS-CoV, and SARS-CoV-2). The amount of serum IL-6 was substantially elevated after the progression of the illness, which can be linked with a wide variety of pulmonary abrasions that have been reflected in the computed tomography (CT) scan findings.

Based on the study done, it is well known that IL-6 and its receptors are the major cytokines that are elevated in almost all the COVID-19 patients. So, it can aid as a budding therapeutic target for dealing with this viral infection.

### 1.7.2 Treatments involving blockage of IL-6 and IL-6 receptors

The therapeutic potential for COVID-19 signal inhibitors is unfolding rapidly because COVID-19 exhibits signs of both pro-inflammatory and autoimmune disorders. IL-6 has shown to be a key driver of cytokine storm in severely affected individuals of COVID-19 infection. To overcome this, it is critical that the selective blocking of cytokine is further explored with drugs such as the IL-6 inhibitors. This may provide therapeutic solutions to the cytokine storm and
lower the damages caused by inflammation\textsuperscript{44}. IL-6 receptor blockers are officially sanctioned for a variety of autoimmune disorders and in general are known to be well-tolerated and safe. Thus, they are also tested for the alleviating damages caused due to COVID-19 infection\textsuperscript{45}.

The brief mechanism of the action of these anti-IL-6 and anti-IL-6 receptor drugs is illustrated in Figure 4, which shows the blocking of IL-6 and its receptors leads to the inhibition of IL-6 signalling.

A handful of IL-6 and IL-6R blockers which are specifically targeted towards the remediation of COVID-19 are shown in Table 1.

1.7.2.1 Tocilizumab

A Japanese pharmaceutical company named, Chugai Pharmaceutical Ltd. developed this drug and named it Tocilizumab, an anti-IL-6R monoclonal antibody, freshly appeared as a substitute therapy for individuals suffering from COVID-19 exposed to cytokine floods\textsuperscript{15}. This is a humanized monoclonal antibody which works in contradiction to the human IL-6 receptors and restricts the biological purpose of IL-6R by constraining the attachment of IL-6 to its receptor.

Observations confirmed the efficacy of tocilizumab in stopping COVID-19 induced cytokine storms. Acute-phase reactant levels have declined in several patients and they have entered a steady state leading to decline in IL-6 levels. Several traditional agents such as corticosteroids are also used for the treatment of cytokine storm with limited success\textsuperscript{46}.

In a clinical trial (NCT04356937) the prescribed amount for tocilizumab is 4-8 mg/kg, delivered every 4 weeks as a single intravenous infusion which showed benefits to the patients. In several other trials, tocilizumab was shown to have a beneficial impact in combination with antiviral medications such as lopinavir/ritonavir (400 mg/100 mg twice daily) or remdesivir 100 mg/daily and corticoids that can boost patients’ clinical outcomes. The timing of the prescription of tocilizumab and the identification of eligible patients for treatment were the
important concerns. Patients in the serious disease process were involved in all of the trials analysed. Some researchers say that the best time for Tocilizumab to be administered is at the start of inflammation and the first stages of the decline in O$_2$ saturation. But the exact timing and phase of infection for initiating this prescription is still unknown$^{47}$.

1.7.2.2 Levilimab

Levilimab is an immunomodulatory drug with the name Ilsira®. Recently under investigation with the clinical trial number NCT04397562. The manufacturer i.e. Biocad, claims this drug to be helpful in the treatment of severe COVID-19 patients and some autoimmune disorders by blocking IL-6R$^{48}$. The drug was initially made for the cure of RA but latest reports have revealed that it is approved for the treatment of COVID-19 in Russia. It acts as an antagonist for IL-6R by neutralising the cytokine storm$^{49}$.

1.7.2.3 Olokizumab

It is an immunomodulatory drug which acts against the IL-6 in RA and recently in individuals with serious COVID-19 infection. The clinical testing of this drug is in phase 3 with the trial number NCT04380519. It was shown in the studies that this drug tends to nullify both cis and trans signalling of IL-6 in the cell. Nullification of cis signalling was done with CRPs and SAA established in primary human hepatocytes, where membrane gp80 was expressed. The neutralization of trans signalling was done in human umbilical vein endothelial cells where the soluble form of gp80 was obligatory for the phosphorylation of STAT$^{50}$.

1.7.2.4 Sarilumab

This is a fully human monoclonal antibody which deals in contrast to the IL-6R. This antibody was primarily developed for the treatment of RA and now it’s being tested for the remedy of COVID-19. The clinical testing number is NCT04327388. Sarilumab works by blocking the cis and trans signalling of IL-6 and hence, controlling the cytokine storm. It was developed by
Sanofi and Regeneron pharmaceuticals and US-FDA approved in the year 2017\textsuperscript{51}. It was launched in the market with the name Kevzara®. The drug can be used in the combination with methotrexate or as individually in case the patient is sensitive toward methotrexate\textsuperscript{52}.

1.7.2.5 Sirukumab

Sirukumab is a monoclonal antibody against IL-6, sponsored by Janssen Pharmaceutica N.V., Belgium. It binds to human IL-6 with high affinity, inhibiting IL-6-mediated signalling as well as IL-6’s biological effects. The phase II clinical trial (NCT04380961) of this mAb was completed on June 24, 2021, which included randomly selected 212 participants.

2 Conclusions and future directions

With the emergence of new mutations quite frequently in SARS-CoV2, it seems that the infection will continue for the upcoming years and there is a very paramount need for proper treatment for the disease. In this case, vaccines play a very cardinal role to fight against the virus. However, these new strains with novel mutations can escape and invade the immune system. As discussed in sections 1.2 and 1.7, IL-6 takes a foremost part in a cytokine storm, which is a major issue in COVID-19. Antibody therapies may also have potential applications not only against this virus but also with other deadly viruses inducing cytokine storm.

Recent studies have indicated that anti-IL-6R inhibitor Tocilizumab has been beneficial in preventing the progression of COVID-19\textsuperscript{53}. However, it did not improve the survival in a single treatment and so additional clinical interventions are required. The bulk of the studies have focused on using Tocilizumab. The effectiveness of the treatments could be evaluated with several other IL-6R or IL-6 inhibitors listed in Table 1.

Most of these inhibitors are full antibodies and there is a need for evaluating the safety of these antibodies in patients. Moreover, the cost of production of these antibodies are quite high. In addition, the demand for these inhibitors could be high given that the COVID-19 pandemic is
remaining and predicted to be affecting human societies for few more years. Since the costs of treatment are quite expensive, there is an urgent need for developing antibody fragments such as Fab or single-chain antibody fragments of anti-IL-6 or anti-IL-6R which can easily be produced in prokaryotic expression systems such as *E.coli*. In conclusion, the IL-6 and IL-6R inhibitors hold the promise in treating COVID-19 patients in combination with other clinical treatment options.

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**Conflict of interest-** Authors declares no conflict of interest.
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### List of Tables

**Table 1** Few IL-6 and IL-6R inhibitors targeting COVID-19

<table>
<thead>
<tr>
<th>Monoclonal antibody &amp; Brand name</th>
<th>Target</th>
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<td>(NCT04327388) Sanofi</td>
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<td>Human IgG1</td>
<td>COVID-19</td>
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</tr>
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</table>
**Figures**

**Figure 1** Effect of SARS-CoV2 on an individual leading to hypoxia. Here, the SARS-CoV2 directly targets the angiotensin converting enzyme (ACE2) receptors in the lungs. This leads to the excessive release of cytokines, majorly IL-6 which leads to cytokine release syndrome (CRS). Due to this, the oxygen saturation level in the blood drops drastically causing hypoxia and ultimately leading to organ failure.
**Figure 2** Mechanism of action of IL-6 when attached with mIL-6R and sIL-6R. The blue receptor is attached to the membrane and when IL-6 binds to this, it is called classic cis signalling. The yellow receptor is a soluble receptor and when IL-6 attaches to this receptor, it is called trans signalling. The signal transmission takes place via two pathways as shown.
IL-6 stimulation in liver

Induction
- Serum Amyloid A (SAA)
- C-reactive protein (CRP)
- Haptoglobin
- $\alpha_1$-antitrypsin
- Fibrinogen
- C3
- Hepcidin
- $\alpha_1$-acid glycoprotein

Inhibition
- Albumin
- Transferrin
- Fibronectin

Figure 3 Acute phase response of IL-6.
Figure 4 Mechanism showing the working of anti-IL-6 and anti-IL-6 receptor drugs. ‘A’ in the figure denotes anti-IL-6 drugs which go and bind to IL-6. Whereas, ‘B’ signifies anti-IL-6 receptor drugs, which binds to the IL-6 receptor and block the binding of IL-6 molecule to the receptor. Both kind of drugs ultimately stops the formation of the gp130 complex, resulting in the inhibition of IL-6 signalling.