

Coronavirus Pandemic

The potential role of the combined PARP-1 and VEGF inhibition in severe SARS-CoV-2 (COVID-19) infection

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Abstract

Introduction: During the evolution of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, several drug candidates have been proposed for repositioning towards a quest for more effective treatments.

Methodology: We reviewed recent literature (Pubmed, Google, Clinicaltrials.gov), as of the middle of May 2021, for evidence regarding the potential benefit from poly(ADP-ribose)-polymerase inhibitors and vascular endothelial growth factor blockade in severe SARS-CoV-2 infection.

Results: poly(ADP-ribose)-polymerase inhibitors have been suggested as potential agents against coronavirus disease 2019 (COVID-19) by a variety of mechanisms. vascular endothelial growth factor-associated vascular permeability is implicated with increased vascular leakage and pulmonary oedema. Thus, anti-angiogenesis factors, such as bevacizumab are being investigated in critically ill COVID-19 patients.

Conclusions: The synergistic potential of these two classes of inhibitors in severe COVID-19 management could be beneficial. Further research should be carried out in order to support this hypothesis.

Key words: SARS-CoV-2; PARP-1 inhibitors; VEGF; bevacizumab; olaparib.

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Introduction

As per most recent World Health Organization (WHO) reports, the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected more than 160 million people and caused more than 3,000,000 deaths globally (as of middle of May 2021).

SARS-CoV-2 is a member of Coronaviridae family and its genome resembles with the severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA (ssRNA+) betacoronavirus [1]. The initial clinical manifestations of COVID-19 infection include cough, fever and fatigue, whereas gastrointestinal symptoms and headache are less common [2]. In the majority of the cases, the infection is self-limited and the symptomatology (if any) is mild. However, as the infection progresses, the virus moves and infects the lower respiratory tract and a considerable percentage of patients develop severe

symptoms that may require mechanical ventilation [3,4]. Several comorbidities such as cardiovascular diseases, cancer and diabetes mellitus, predispose for worst prognosis [5].

Although the exact pathogenetic mechanisms of SARS-COV-2 infection are not clear yet, a cytokine storm (also known as macrophage overactivation syndrome) is considered to be a detrimental event in COVID-19 progression. This overactivated immune response may lead to life threatening consequences, such as acute respiratory distress syndrome (ARDS) and multi-organ failure [6].

Since the initiation of the pandemic, several treatment approaches have been proposed for SARS-CoV-2 infection. Currently, the available therapeutic options are not highly effective and thus, as the pandemic keeps evolving, several drugs have been candidates for repurposing. Several studies support that vascular endothelial growth factor (VEGF) may be a therapeutic target in ARDS. On this basis, clinical trials

are conducted in order to investigate the role of bevacizumab, an anti-VEGF agent, in suppressing pulmonary oedema in severe or critically ill patients. Moreover, poly(ADP-ribose)-polymerase inhibitors (PARPi) have also been proposed as potential therapeutic agents, since they modulate the production of pro-inflammatory cytokines and immune response [7-9]. To date, four PARP inhibitors (olaparib, rucaparib, niraparib and talazoparib) have been granted FDA/EMA approval and are used in several cancer types that exhibit homologous recombination deficiency and replication stress. In May 2020, FDA approved of the combined use of bevacizumab and olaparib as first line maintenance treatment for epithelial ovarian, fallopian tube or primary peritoneal cancer, based on the results of a phase III clinical trial (PAOLA-1, ClinicalTrials.gov Identifier: NCT03737643). In terms of safety, the combination of the two agents was reported to be well tolerated.

Based on the above, the aim of this review is to investigate the repurposing of the combination of olaparib (PARP1/2 inhibitor) and bevacizumab as a potential treatment scenario for severe SARS-CoV-2 infection. In the present paper, we will focus on their potential synergistic effects and mechanisms of actions in suppressing the ARDS and cytokine storm in Covid-19.

Methodology

A literature search in PubMed database, clinicaltrials.gov and Google was conducted using the following keywords: “PARP inhibitors and SARS-CoV-2”, “VEGF and SARS-CoV-2”, “angiogenesis and SARS-CoV-2”, “pathophysiology and SARS-CoV-2 and ARDS”, “pathophysiology and SARS COV 2 and cytokine storm”. Original research articles, clinical trials and narrative reviews were collected and reviewed for their significant findings on the roles of PARP and VEGF inhibition in SARS-CoV-2 infection. Other related aspects that are implicated with acute lung injury and ARDS were also included and assessed until the middle of May 2021.

Brief overview of Immune response in SARS-CoV-2 infection

SARS-CoV-2 enters the host lung epithelial cells through the attachment of spike glycoprotein (S) to the angiotensin-converting enzyme 2 (ACE2) receptor. Type II transmembrane serine protease (TMPRSS2) co-assists the penetration, by activating the receptor-bound S protein [10]. Once the virus has entered the cells, it is free to replicate in the cytoplasm, triggering the host's

immune response. Antigen presenting cells (APCs) such as dendritic cells and macrophages have Pattern Recognition Receptors (PRRs) that recognize SARS-CoV-2 pathogen-associated molecular patterns (PAMPs) and stimulate signaling pathways to produce immune effector cells. NF- κ B activation induces the transcription of proinflammatory genes and thus regulates inflammation. The excessive production and release of proinflammatory cytokines (such as IL-6, TNF- α , TNF- β , IL-1 β , INF- α , INF- γ , IL-33) and chemokines (such as CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10) is directly associated with ARDS development [11]. Moreover, in SARS CoV-2 infection, antigen presentation is mostly mediated by MHC class 1 molecules. APCs presenting the CoV antigen, stimulate the activation of CD4+ T-helper cells and initiate an adaptive immune response. After Th-1 cell activation, cytotoxic CD8+ T-cells produce molecules with antiviral properties in order to promote virus clearance [12]. Meanwhile, CD4+ T-helper cells activate B-cells, which secrete IgM and IgG antibodies in response to the viral presence [13].

However, decreased levels of T- lymphocytes, CD4+ T cells, and CD8+ T cells were found in severe SARS-CoV-2 cases. This may be attributed to the increased levels of IL-10, IL-6 and TNF- α [3,14,15]. Interestingly, direct inverse associations have been reported between the absolute number of T-lymphocytes and IL-6 and TNF- α concentrations [15]. Another multifunctional cytokine, IL-17, has also been found to be increased in SARS-CoV2 infection [16]. The release of proinflammatory cytokines and chemokines promotes further the recruitment of neutrophils and monocytes to the site of inflammation, resulting in lung injury [12].

Brief overview of ARDS in SARS-CoV-2 infection

ARDS, the most severe form of acute lung injury (ALI), is characterized by the rapid onset of diffuse alveolar inflammation and impaired ability of gas exchange [17]. Despite that a variety of factors may trigger its development, ARDS follows a uniform pattern and the pathophysiological pathway includes 3 distinct phases. The initial stage, known as “exudative phase”, is attributed to an innate immune response, which results in a) the disruption of alveolar barrier functions and b) increased permeability. More specifically, the activation of alveolar macrophages leads to cytokine and chemokine production (*i.e.* TNF, IL-1 β , IL-6, IL-8), which induces an inflammatory reaction. Moreover, TNF- α triggers chemotaxis,

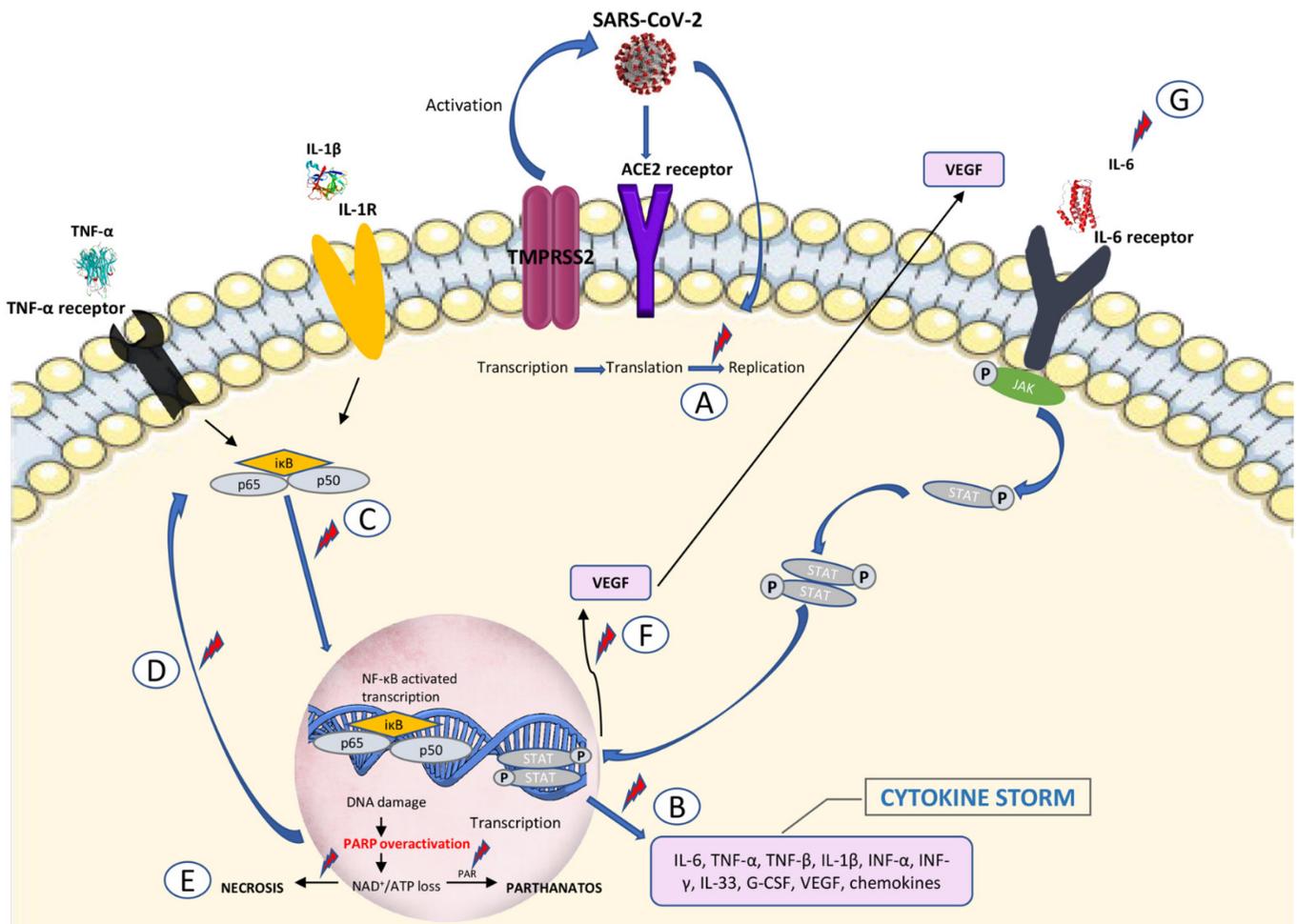
activates neutrophils and induces platelet aggravation and formation of microthrombosis. Another feature demonstrating the extended alveolar damage is the formation of hyaline-membrane in the alveoli. Subsequently, activated neutrophils further promote the inflammation process contributing to epithelial injury, interstitial and intraalveolar oedema. Furthermore, several endothelial injury-associated factors, such as VEGF, von Willebrand factor (vWF) and soluble intercellular adhesion molecule-1 (sICAM-1) are released, conferring further loss of barrier functions. In addition, type II alveolar epithelial cells are also damaged and the subsequent inactivation of surfactant amplifies the regional alveolar collapse. During the “proliferative phase” of ARDS several events take place in an attempt to reverse lung injury. This phase is generally characterized by a) the re-absorption of interstitial oedema fluid, b) type II alveolar epithelial

cell proliferation and c) the restoration of epithelial and endothelial barrier integrity, architecture and function. In many patients, ARDS resolves gradually, without evolving in the third, “fibrotic” phase. The latest includes a persistent damage in the basement membranes as well as extensive fibroblast proliferation. These events lead to increased extracellular matrix formation and intraalveolar and interstitial fibrosis [18].

PARP-1 and VEGF inhibition in severe SARS-CoV-2 infection

Poly(ADP-ribose) polymerases (PARPs) superfamily includes 18 different proteins and their corresponding genes [19]. PARPs play crucial roles in several cellular homeostasis mechanisms, such as in the regulation of chromosome structure and transcription, DNA damage repair, cell death signaling and inflammation [20]; PARP-1 and PARP-2 harbor the

Figure 1. Possible mechanisms of PARP-1 inhibition in SARS-COV-2 infection.



A) Inhibition of viral replication B) Modulation of the expression of pro-inflammatory cytokines and chemokines C-D) Regulation of NF-κB transcription factor. Inhibition of ROS/RNS-induced PARP overactivation further suppresses NF-κB E) Protection against cell necrosis associated with energy loss and pathanatos F-G) Regulation of VEGF expression via the inhibition of IL6/JAK/STAT3.

majority of PARP activity (85-90% and 10-15% respectively). Upon activation they act as mono-, oligo- or poly(ADP-ribose)-transferases, using NAD⁺ as an ADP-ribose unit provider. Poly(ADP-ribose)ylation or PARylation is a dynamic process that leads to the formation of long negatively charged, PAR-chained products that regulate the function of target proteins [19]. These polymer strands display short half-lives and the rapid PAR removal from its acceptor proteins is mediated by several PAR-degrading enzymes, including (poly ADP-ribose) glycohydrolase (PARG) and macrodomain-containing proteins [21]. Macrodomains are encoded by several virus families (*i.e.* Coronaviridae) and have been proposed to exert antiviral effects [22]. PARP overactivation is triggered in response to reactive oxygen species (ROS)- or reactive nitrogen species (RNS)-induced DNA damage [20].

Previous evidence shows that PARP-1 blockade may be implicated in SARS-CoV-2 infection by different mechanisms (Figure 1). A recently published comprehensive review summarizes the roles of PARP inhibition in SARS-CoV-2 infection [7]. In the following paragraphs we will epigrammatically refer to the role of PARP and VEGF inhibition in SARS-CoV-2 infection, focusing on the synergistic potential.

The interplay between PARPs and viruses is complex and several studies have demonstrated that specific PARPs can act either as promoters or as restrictors of viral replication [23]. PARP-1 has been reported to play important roles in viral propagation [24-26]. Recently, Ge *et al.* suggested that two PARP-1 inhibitors, olaparib and CVL218 can inhibit viral replication through targeting the nucleocapsid (N) protein of SARS-CoV-2 [8]. Another PARP 1/2 inhibitor, stenoparib, is also under investigation for the potential inhibition of SARS-CoV-2 *in vitro*. More specifically, the authors associated stenoparib with the depletion of SARS-CoV-2 replication and inhibition of the virus entry. Furthermore, the combination of stenoparib with remdesivir, may display higher synergistic effect compared to the efficacy of each compound alone [27]. PARP activation (mainly PARP-1, PARP-2 and PARP-3) in inflammation has been also reported in literature [28]. More importantly, a recent study suggested that SARS-CoV-2 infection induces PARP activity and downregulates its metabolism [29]. Several PARP-mediated mechanisms promote innate immune responses by modulating the expression of proinflammatory cytokines (*i.e.* IL-6, TNF- α , IL-1) and by regulating transcription factors such as NF- κ B [28]. Moreover, NAD⁺/ATP loss, secondary to PARP

activation, results in cell necrosis, which in turn triggers further inflammatory responses [30]. For example, nicotinamide (NAM) has been proposed as a promising PARP 1 inhibitor in several infections including COVID-19. NAM impedes NAD⁺ and adenosine triphosphate (ATP) depletion and thus SARS-CoV replication [29,31]. IL-6 inhibitors (*i.e.* tocilizumab) have also been proposed as therapeutic options for SARS-CoV-2 infection. COVID-19 severity and deterioration has been directly associated with the cytokine storm. Indeed, plasma levels of pro-inflammatory cytokines such as IL-6, IL-1B and TNF α were found elevated in severe stages of the infection [4,32,33]. Considering the above, PARP-1 inhibitors may restrain the inflammatory signal transduction and suppress the SARS-CoV-2-mediated cytokine storm by inhibiting pro-inflammatory cytokines and inflammatory responses.

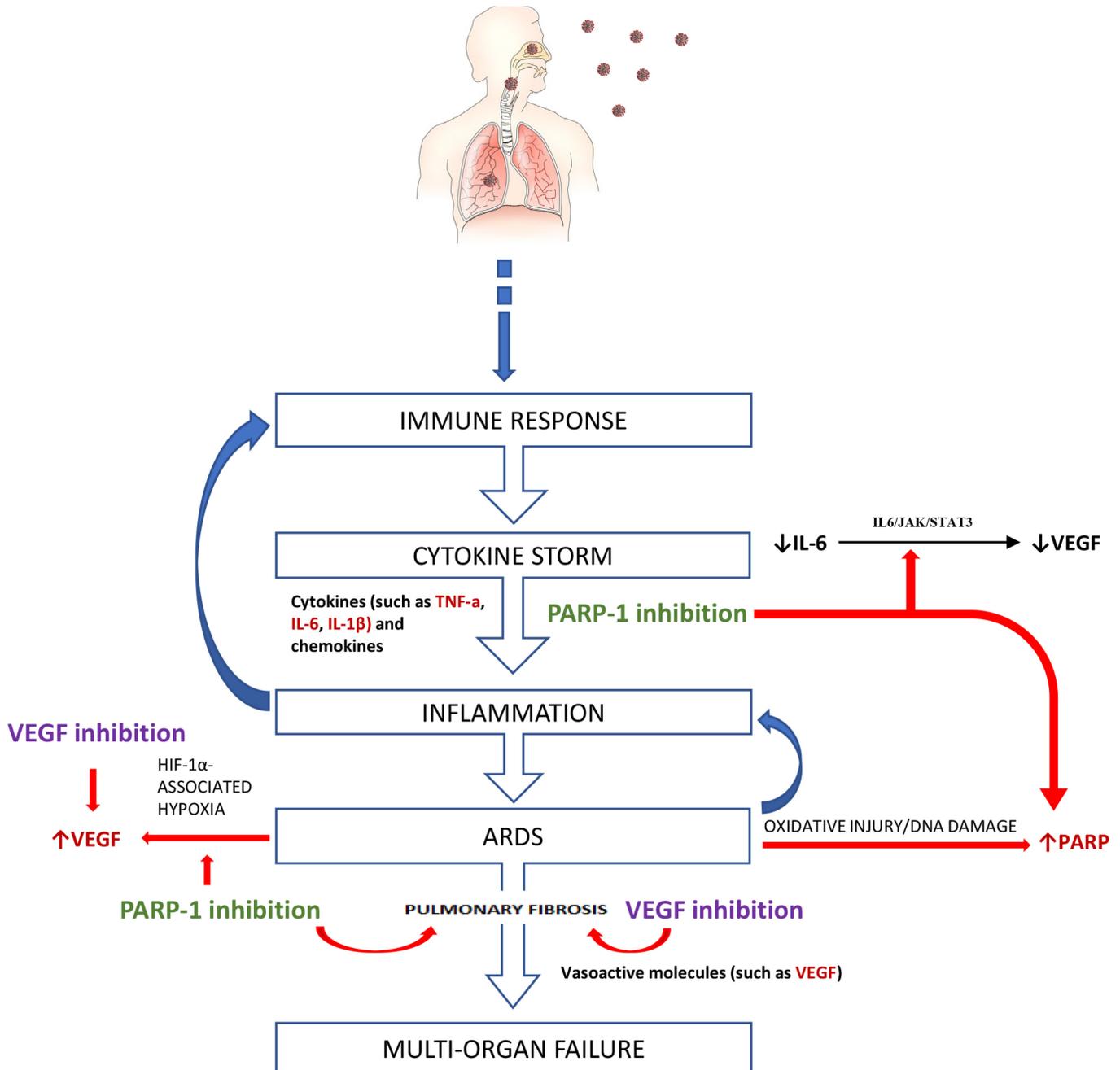
VEGF is a signaling protein produced by a variety of epithelial and non-epithelial cells in mammals, with a vital role in a variety of cellular processes. The family of VEGF consists of 5 proteins: VEGF-A (originally denoted as VPF, vascular permeability factor), VEGF-B, VEGF-C, VEGF-D and PGF (placenta growth factor). VEGF-A is located predominantly in alveolar type II cells, airway epithelial cells, mesenchymal cells and blood cells [34]. VEGFs bind to their receptors, namely VEGFR1 (flt-1), VEGFR2 (KDR in the human), and VEGFR3 (flt4). All three receptors activate intracellular downstream signaling pathways; the binding of VEGF to VEGFR-2 activates the PLC γ (phospholipase C γ) and the Raf/MEK/MAPK pathway (which initiates DNA synthesis and endothelial cell proliferation). PLC γ activation promotes vascular permeability and vasodilatation (via NO production) [35]. The activation of p38 MAPK further triggers the reorganization of actin and ultimately the migration of endothelial cells. Moreover, the activation of the phosphatidylinositol 3'-kinase (PI3K)-Akt pathway causes increased endothelial-cell survival and vascular permeability. This leads to the extravasation of enzymes and fibrin, which form the extracellular matrix that promotes the formation of new blood vessels [36].

VEGF-induced vascular permeability leads to vascular leakage and pulmonary oedema, by several mechanisms [37,38]. Furthermore, as mentioned above, ARDS, is characterized by impaired ability of gas exchange which leads to hypoxia both in lung tissues and other organs. Under hypoxic conditions, hypoxia inducible factor 1 alpha (Hif1 α) further induces angiogenesis, by upregulating the expression of VEGF

[39]. Moreover, severe inflammation promotes the apoptosis of endothelial and epithelial cells, triggering the expression of VEGF and deteriorating both pulmonary oedema and extravasation of immune cells [40]. Recent data report higher VEGF blood levels in

COVID-19 patients compared to healthy subjects [4]. Increased VEGF levels have also been correlated with COVID19 severity, supporting the role of VEGF in endothelial damage [41]. Ackermann *et al.* studied the morphogenetic features of intussusceptive angiogenesis

Figure 2. Schematic presentation of the potential synergistic scenario from the combined administration of bevacizumab and Olaparib in SARS-CoV-2 infection.



PARP-1 inhibitors modulate the expression of proinflammatory cytokines such as IL-6, TNF- α and IL-1 β . Reduced IL-6 levels suppress the IL6/JAK/STAT3 pathway and hence may result in VEGF downregulation in an indirect manner. The latest may enhance the anti-VEGF effect of concomitant bevacizumab administration. ROS/RNS production has been positively correlated with ARDS. PARP inhibitors protect the cells from PARP-induced oxidative/nitrosative stress, cell death and further inflammation. At the same point, bevacizumab decreases pulmonary permeability and oedema, which are key features of ARDS. In HIF-1-associated hypoxic conditions, VEGF is upregulated. PARP-1 is known to inhibit HIF-1 α accumulation. Thus, PARP-1 inhibition may suppress HIF-1-induced angiogenesis.

in the lungs of patients who died from Covid-19. Interestingly, the authors reported a unique expression pattern of angiogenesis-associated genes, such as VEGF [42]. Furthermore, miRNA computational analysis from SARS-CoV-2 genome, supported that mature miRNAs are targeting a large number of human target genes including VEGF-associated genes [43]. Based on the above, several studies have investigated the role of VEGF blockade in lung injury and ARDS [44-46].

Besides that the drugs under discussion act on different levels of SARS-CoV-2 infection, and thus can restrict severe infection through different mechanisms, we will try to support additional hypothetic scenarios regarding their synergistic potential (Figure 2).

PARP inhibition has been linked with the control of pulmonary fibrosis *via* several mechanisms. TGF β /SMAD pathway activation has been correlated with a) the proliferative and fibrotic phases of ARDS and b) accumulation of collagen 1 and other extracellular matrix molecules [such as alpha-smooth muscle actin (a-SMA) and fibronectin] in pulmonary fibrosis. Recently, PARP-1 inhibition was found to regulate TGF β expression and the TGF β /SMAD signaling pathway [47]. Moreover, PARP-1 inhibition has been reported to decrease the expression of matrix metalloproteinases, namely MMP-2 and MMP-9 [48]. In addition to the abovementioned, the interplay between VEGF and TGF β production has been also described in the pathogenesis of lung fibrosis [49]. Indeed, VEGF inhibition has been associated with TGF β regulation *via* the phosphoinositide 3-kinase/Akt pathway [50]. Therefore, we could assume that combined PARP and VEGF inhibition may be beneficial in terms of controlling interstitial and intra alveolar fibrosis during ARDS progression.

Recently, Afsar *et al.* reported that HIF-1 stabilization might be beneficial in SARS-CoV-2 infection by decreasing ferritin and ACE-2 expression and by halting the deterioration of hypoxia [51]. VEGF gene is a known target of HIF-1 α and it has been shown that, in hypoxic conditions, VEGF levels are increased *via* an HIF-1 α independent manner [39]. The role of VEGF in pulmonary oedema and lung injury has been mentioned in previous sections. Thus, HIF-1-induced angiogenesis may deteriorate pulmonary oedema. Moreover, generation of HIF-1 α -associated hypoxia has been correlated with resistance to angiogenesis inhibitors [52]. PARP-1 inhibition has been also previously reported to decrease HIF-1 α levels [53]. Considering the above, we could assume that in hypoxic conditions, PARP-inhibition may enhance the

anti-angiogenic effect of bevacizumab, by decreasing VEGF expression and by reversing the resistance to angiogenesis inhibitors. Moreover, another hypothesis could be that decreased HIF 1 α accumulation could also prevent hypoxia-induced cell apoptosis in pulmonary epithelia.

Another scenario implicates the suppression of the JAK/STAT signaling pathway. Mortality rates in SARS-COV-2 infection have been strongly connected with IL-6, a pleiotropic inflammatory cytokine that plays an important role in the associated cytokine storm. More importantly, increased IL-6 serum levels have been associated with poor prognosis [54]. Binding of IL-6 to its receptor leads to the activation of the IL6/JAK/STAT3 pathway that transduces downstream elements of critical inflammation-related targets. On the other hand, VEGF expression is regulated by several signaling pathways and transcriptional factors, including STAT3 [55]. VEGF has also been reported to increase IL-6 via a positive feedback loop [56]. As already mentioned, PARP inhibition can regulate IL-6 expression in the lung as well as in systematic circulation [57,58] Therefore, decreased levels of IL-6 may suppress the IL6/JAK/STAT3 axis and thus also modulate VEGF expression in an indirect manner. Consequently, coadministration of bevacizumab and olaparib could further control circulating VEGF levels.

The potential role of anti-VEGF treatment in ARDS/ALI has been described above. In addition, increased levels of neutrophil-derived reactive species (reactive oxygen species-ROS and reactive nitrogen species-RNS) were found in patients with ARDS and a positive correlation between those levels and disease severity has been also described [59]. Infection-induced ROS/RNS lead to extensive DNA damage, causing massive PARP-1 activation; subsequently, the latest causes NAD⁺ depletion and cell necrosis as well as PAR accumulation and parthanatos. Cell necrosis further triggers inflammation and macrophage activation, generating a vicious cycle of ROS/RNS production and additional PARP activation [7,8]. On this basis, combined VEGF and PARP inhibition could affect the progression of SARS-COV-2 infection in terms of suppressing ARDS.

Nevertheless, the abovementioned theoretical assumptions require more experimental and clinical evidence in order to be valid. For example, Ahmad *et al.* investigated the impact of olaparib treatment in a clinical model of sepsis in mice (cecal ligation and puncture, CLP model). The authors reported that despite that olaparib attenuated IL-6 levels, the concentration of VEGF in plasma remained unaffected

[60]. Moreover, there is also data that support the ambiguous role of VEGF in ARDS progression. Of note, Mura *et al.* concluded that increased levels of VEGF during the recovery phase of ARDS might be associated with lung recovery of the alveolar membrane [61]. Indeed, during recovery, VEGF levels increase, contributing to neoangiogenesis, membrane repair and clearance of the pulmonary oedema [62]. In addition, to date, literature data regarding the role of PARP-1 inhibitors in COVID-19 disease is scarce. For instance, as previously highlighted by Szabo *et al.*, some preclinical studies display various limitations, such as the use of first generation instead of third generation PARP inhibitors [63]. Thus, the use of PARPi in COVID-19 infection and the scenario of the proposed combination warrants further validation by properly designed randomized controlled trials.

The safety profile of Olaparib plus Bevacizumab combination

The approval timeline of Olaparib (Lynparza), a potent oral PARP1/2 inhibitor, started on December 2014 when FDA first approved of its use to treat patients with advanced ovarian cancer that were carriers of germline BRCA mutations (gBRCAm). Since then, the indications have expanded over the years to include several other types of cancers and/or settings such as a) maintenance treatment of germline and/or somatic BRCA1/2m advanced ovarian cancer, b) gBRCA1/2m, HER2 negative metastatic breast cancer following chemotherapy, c) gBRCA1/2m metastatic pancreatic adenocarcinoma, and d) homologous recombination repair (HRR)-mutated metastatic castration-resistant prostate cancer following prior treatment with enzalutamide or abiraterone. Olaparib is predominantly metabolized by CYP3A4/5 and thus concomitant therapy with potent CYP3A4/5 inhibitors or inducers should be avoided or used with caution after appropriate dose adjustments. Therefore, in case that a strong (such as itraconazole, aprepitant, boosted protease inhibitors, boceprevir, telaprevir, clarithromycin) or moderate CYP3A inhibitor (such as verapamil, diltiazem, erythromycin and fluconazole) must be co-administered, the olaparib dose should be reduced to 100 mg BID and 150 mg BID respectively. Similarly, co-administration of strong CYP3A inducers may have a substantial effect on olaparib efficacy. Thus, concomitant treatment with strong inducers (such as phenytoin, rifampicin, carbamazepine and phenobarbital) is not recommended. Moreover, olaparib has been reported to be a mild CYP3A (in vivo) and P-gp (in vitro) inhibitor itself, so appropriate

monitoring has been proposed in case of co-administration with CYP3A and P-gp substrates with a narrow therapeutic window [64]. Hence, the high drug-drug interaction (DDI) profile of olaparib should be taken under consideration, since critically ill COVID-19 patients may be also treated with several classes of medications. Further studies should investigate the possible interactions between olaparib and other drugs that are being currently used for SARS-CoV-2 infection. On the other hand, no dosing adjustments are required for patients with mild renal impairment. In cases of moderate renal impairment (CLcr = 31-50 mL/min) the dose should be modified to 200 mg BID whereas olaparib is not recommended in cases of severe renal impairment (CLcr \leq 30 mL/min), due to lack of safety and pharmacokinetic data in this group of patients. With regards to mild or moderate hepatic impairment (Child-Pugh classification A and B), no dosing modification is required. There are no data for severe (Child-Pugh classification C) hepatic impairment [64,65]. Furthermore, the most frequent adverse events observed with olaparib treatment are hematologic toxicities (mainly anemia), gastrointestinal toxicities (mainly nausea and vomiting) and fatigue.

Bevacizumab (Avastin) is a recombinant, humanized monoclonal antibody (IgG1) that inhibits vasculogenesis and angiogenesis by blocking the binding of VEGF-A to its receptors (VEGFR-1 and VEGFR-2). This agent first received FDA approval in 2004 for the treatment of metastatic colorectal cancer patients. Currently, bevacizumab is being used in combination with other anti-cancer treatments (or as maintenance therapy) in a variety of cancers such as advanced ovarian cancer, metastatic non-squamous non-small cell lung cancer, metastatic cervical cancer, metastatic renal cell carcinoma and recurrent glioblastoma. The clearance of bevacizumab does not rely on renal or liver elimination. More specifically, the metabolism of bevacizumab resembles endogenous IgG pathways (mainly through proteolytic catabolism). The long terminal half-life (approximately 20 days) of bevacizumab results from the binding of IgG to the neonatal Fc-receptor (FcRn). No special dosing adjustments are needed in renal or hepatic impairment since neither liver nor kidneys are implicated in bevacizumab clearance [66]. Therefore, pharmacokinetic DDIs are unlikely to occur with bevacizumab. The most common adverse events for bevacizumab are associated with VEGF inhibition and include hypertension, arterial and venous thromboembolism, proteinuria, increased risk of haemorrhage and gastrointestinal perforation.

Quite recently (May 2020), the combination of olaparib and bevacizumab was approved as first-line maintenance treatment for ovarian cancer patients who exhibit complete or partial response following platinum-based chemotherapy. This therapeutic option (olaparib 300 mg BID, per os plus bevacizumab 15 mg/kg, Q3W, intravenously) is currently limited for homologous recombination deficiency (HRD)-positive ovarian cancer and the approval was based on the results of PAOLA-1 clinical trial [67,68]. A total of 535 and 267 patients were randomized in the olaparib plus bevacizumab *versus* placebo plus bevacizumab arm, respectively (2:1 ratio). In terms of safety, the combination showed a generally consistent profile with olaparib monotherapy (assessed in SOLO-1 trial ClinicalTrials.gov number, NCT01844986). The three most common (all grades) adverse events that occurred in at least 10% of the patients that were allocated in olaparib plus bevacizumab arm were nausea (53%), fatigue (53%) and anemia (41%). Accordingly, more severe adverse events (\geq grade 3) were mainly anemia (17%), lymphopenia (7%) and fatigue. However, despite that the incidence of bevacizumab-related hypertension was more frequent in PAOLA-1 than in SOLO-1, we should reckon that critically ill Covid-19 patients can often develop ARDS in the context of sepsis, and thus hypotension may be present. More importantly, Ray-Coquard *et al.* suggested that the known toxicities related to bevacizumab treatment were not increased by adding olaparib [68].

We also conducted a research in clinicaltrials.gov and identified one randomized controlled clinical trial (currently recruiting) that investigates the potential role of bevacizumab (7,5 mg/kg in 100ml N/S 0.9%) in severe SARS-COV-2 pneumonia (BEST-RCT, ClinicalTrials.gov Identifier: NCT04305106). The pilot phase 2 study (BEST-CP) that recruited 27 patients, has now been completed. This single-arm, clinical trial revealed promising results in patients with severe SARS-COVID-19. According to the authors, adding bevacizumab to standard treatment significantly decreased the inflammatory response and improved the clinical condition of the patients. Compared to the control cohort, PaO₂/FiO₂ ratios, oxygen needs, chest X- ray imaging, CRP and lymphocyte levels were markedly ameliorated [69]. We also found another registered randomized controlled trial (CORIMMUNO-BEVA ClinicalTrials.gov Identifier: NCT04344782) that is not recruiting yet. Moreover, the results from a clinical trial conducted in Bangladesh exhibited a dramatic survival benefit (92%) in the subpopulation of critically ill patients that received a

single dose of bevacizumab ($p < 0.001$). However, it should be noted that only 25 patients were included and thus the results should be interpreted with caution [70]. To sum up and taking into consideration the existing scientific interest regarding the role of bevacizumab in severe COVID-19 infection, we could suggest the potential additional efficacy from the addition of olaparib in a selected patient population that suffers from severe SARS-COV-2 infection.

Conclusions

The interplay between SARS-COV-2-associated immune response and endothelial/epithelial injury has been a field of great investigation and offers several potential therapeutic targets. PARP-1 inhibitors exhibit pleiotropic functions and have emerged as promising agents for several non-oncological diseases, including COVID-19. Anti-angiogenesis factors, such as bevacizumab are also being currently investigated in critically ill COVID-19 patients. In this review, we tried to describe current evidence and discuss the synergistic potential of these two classes of inhibitors in severe COVID-19 management.

Authors' contributions

D.I.L. and G.A.: Conceptualization, D.I.L, V.M.B., E.Z. and E.P.: Methodology, data acquisition and writing, D.I.L.: Figures preparation, M.G., G.A., D.F.: review and editing, M.G., G.A., D.F.: supervision and approval of the submitted version. All authors have read and agreed to the published version of the manuscript.

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