Review Article

CLINICAL INVESTIGATION

Selectively targeting TGF-β with Trabedersen/OT-101 in treatment of evolving and mild ards in COVID-19

Abstract

Based on the role of TGF- β in the immunopathology of ARDS, we and others have proposed the use of TGF-β inhibitors for the treatment of COVID-19 pneumonia and ARDS. TGF-b targeting is employed as a strategy to stimulate the immune system of advanced-stage cancer patients in an attempt to overcome the immunosuppression and T-cell exhaustion within the tumor microenvironment. Nevertheless, we do not anticipate any worsening of existing ARDS or Cytokine Storm/Cytokine Release Syndrome (CRS) of COVID-19 patients as a treatment-emergent complication with our contemplated use of the anti-TGF-β RNA therapeutic OT-101. That is because (i) inhibitors of TGF-β signaling are not associated with ARDS, Cytokine Storm/CRS, or systemic capillary leak, (ii) OT-101 did not cause any pulmonary toxicity, non-infectious pneumonitis, CRS, systemic or pulmonary capillary leak or ARDS in any of the 61 patients with advanced solid tumors enrolled in Phase I/II study (ClinicalTrials.gov identifier: NCT00844064) who received much longer periods of OT-101 therapy, and (iii) OT-101 did not cause in human subjects an elevation of TNF-α, IL-6 or IL-10 levels associated with CRS and ARDS in COVID-19 patients-likewise, OT-101 did not induce production of these inflammatory cytokines in cultures of human white blood cells. We postulate that because of the significance of the TGF-β pathway on the development of ARDS and T cell exhaustion, treatment with OT-101 may prevent the progression of evolving or mild ARDS and help facilitate the recovery of lymphocytopenia and T-cell exhaustion in COVID-19 patients.

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Introduction

Clinical use of TGF-β pathway inhibitors are not associated with the systemic capillary leak, cytokine release syndrome/cytokine storm, or ARDS

The signaling of TGF-β can be modulated through three distinct strategies: using anti-sense nucleotides that block TGF-β an mRNA (Trabedersen/AP 12009-also known as OT-101), using monoclonal antibodies to block TGFβ isoforms (lerdelimumab, metelimumab) or using inhibitors of the TGF-β receptor. Several small molecules as well as large molecule inhibitors (e.g. neutralizing anti-TGF antibodies) of TGF-β signaling have been and are being evaluated in clinical trials. None of these drug candidates have caused CRS/Cytokine Storm, systemic capillary leak, or ARDS [1].

Fresolimumab is a pan-specific, recombinant, fully human anti- TGF-β monoclonal antibody. No patient experienced a systemic capillary leak, cytokine release syndrome/cytokine storm, or ARDS when treated in Phase 1 clinical trials for renal cell carcinoma, melanoma, or glioma [1]. Galunisertib is a small-molecule selective inhibitor of TGFβR1 (ALK5), the receptor that binds both TGF-β1 and TGF-β2. No patient enrolled in the open-label Phase II study in hepatocellular carcinoma (ClinicalTrials.gov: NCT01246986), experienced a systemic capillary leak, cytokine release syndrome/cytokine storm, or ARDS [2]. The observed treatment-emergent adverse events included fatigue, anemia, peripheral

edema, and abdominal pain and neutropenia, anemia, embolism, high bilirubin, and low albumin levels were encountered as Grade 3-Grade 4 treatment-related Adverse Events (AEs). In another Phase II clinical study of Galunisertib in patients with myelodysplasia (ClinicalTrials.gov, number NCT02008318), fatigue, diarrhea, fever, and emesis (5/41, 12%) were observed as treatment-emergent AEs. No patient experienced a systemic capillary leak, cytokine release syndrome/cytokine storm, or ARDS [3]. AVID200 is a rationally designed first-in-class receptor ectodomain trap that inhibits TGF-β1 and TGF-β3. Phase I studies of AVID200 were performed in patients with advanced cancers (ClinicalTrials.gov Identifiers: NCT03834662; NCT03094169). Diarrhea and elevation of the pancreas enzyme lipase were reported as drug-related Grade 3 AE but no patient experienced a systemic capillary leak, cytokine release syndrome/cytokine storm, or ARDS [4]. Pliant Therapeutics' proprietary small molecule PLN-74809 is an oral dual selective inhibitor of the tissue-specific αVβ6 and αVβ1 integrins blocking integrin-mediated stimulation of the TGF-β pathway that is currently in Phase 2 testing for lung fibrosis. The compound was well-tolerated in a randomized, double-blind, placebo-controlled study with no evidence of CRS/cytokine storm, systemic capillary leak, or ARDS [5].

The safety of the anti-TGF-β anti-sense Oligonucleotide Trabedersen (OT-101) was evaluated in Phase 1 and Phase 2 studies in cancer patients. In Phase I/II study (ClinicalTrials.gov identifier: NCT00844064), a total of 61 patients were treated with continuous intravenous (IV) infusion of OT-101 as 2nd to 4th-line therapy in escalating doses (40 mg/m2/day up to 330 mg/m2/day). Overall, OT-101 was well-tolerated and no patient experienced a systemic capillary leak, cytokine release syndrome/cytokine storm, non-infectious pneumonitis, or ARDS [6]. Thrombocytopenia, gastrointestinal hemorrhage, and fever observed as related/possibly related treatment-emergent AEs.

Interleukin 6 (IL-6) as the signature pro-inflammatory cytokine associated with cytokine storm/cytokine release syndrome (CRS) and ARDS in COVID-19

IL-6 is a pro-inflammatory cytokine that plays a pivotal role in the development, progression, and severity of CRS as well as its complications, including Disseminated Intravascular Coagulopathy (DIC) and multiorgan failure [7-10]. It is the main signature cytokine implicated in COVID-19 associated CRS and ARDS [7]. Detectable serum SARS-CoV-2 viral load (RNAaemia) is closely correlated with markedly elevated IL-6 levels in critically ill COVID-19 patients [11]. Diao et al. reported the serum cytokine concentration from inpatient data of patients with COVID-19 in Wuhan, admitted to hospital from December 2019 to January 2020, and healthy controls, who came to the hospitals for routine evaluation. IL-6 levels, along with IL-10 and TNF-α levels were markedly elevated [12]. Xu et al. also reported that IL-6 and TNF-α levels were increased in COVID-19 patients [13]. Subsequently, several additional groups likewise reported that IL-6 and TNF-α levels were increased in COVID-19 patients [14-19]. Early data from a single-arm, 21-patient Chinese trial indicated an anti-IL6 receptor monoclonal antibody may have significant clinical benefit in COVID-19 pneumonia patients. China has approved the use of that antibody in severe forms of COVID-19. In an open-label Phase 2 study (ClinicalTrials.gov Identifier: NCT04317092), Tocilizumab is being evaluated in patients with COVID-19 pneumonia and a related randomized study (ClinicalTrials.gov Identifier: NCT04306705), it is being evaluated for its efficacy in CRS associated with COVID-19. It is also being evaluated in combination with Favipiravir (ClinicalTrials.gov Identifier: NCT04310228). Likewise, Sarilumab (Kevzara), another monoclonal antibody that binds to the IL-6 receptor, is being evaluated in a Phase 2/3 study in hospitalized COVID-19 patients (ClinicalTrials.gov Identifier: NCT04315298). Based on the role of TGF-β in the immunopathology of ARDS, we and others have proposed the use of TGF-β inhibitors for the treatment of COVID-19 pneumonia and ARDS [20-22].

OT-101 does not increase IL-6 or TNF-α levels in human subjects when used at doses comparable to those proposed for the COVID-19 patient population

Cytokine levels of clinical plasma samples of 12 pancreatic cancer patients of the P001 study of OT-101 in advanced solid tumor patients were measured. These 12 pancreatic cancer patients were from the last cohort of the P001 study, with OT-101 treatment at 140 mg/m^2/day on 4-days-on, 10-days-off schedule. The daily dose level of OT-101 was comparable to the contemplated dose level of OT-101 for the proposed COVID-19 study (viz: 150 mg/m^2 on day 1, 100 mg/

m^2 on days 2-7; the selected dose level of 150 mg/m^2 on day 1 and 100 mg/m^2 on days 2-7 is lower than the MTD of 160 mg/m^2/day when OT-101 was administered via intravenous infusion according to a 7 day on, 7 day off schedule in the P001 patient population and caused no SAEs). The evaluated cytokine panel included IL-6, TNFα, IFNγ, MIP-1α, IL-10, IL-1β, IL-12p40, IL-17A, IL-2, and IL-8. Samples analyzed were acquired before the onset of OT-101 therapy and at selected 23-time points during the therapy. Most on-therapy samples were obtained at the following time points: Cycle 1 day 2 (time point 2), Cycle 1 day 5 (time point 3), Cycle 2 day 1 (time point 6), Cycle 2 day 2 (time point 7), Cycle 2 day 5 (time point 8), final visit (time point 9), exit period Cycle 3 day 5 (time point 11). As shown in Table 1, displaying the scatter plots for absolute IL-6 concentrations measured at different time points, OT-101 therapy was not associated with any significant treatment-emergent increase IL-6 levels. Examining the parameter estimates of mixed ANCOVA model across time points to identify individually affected cytokines showed that only Epidermal Growth Factor (EGF) increased significantly across time. In particular, neither IL-6 or TNF-α levels showed an increase in response to OT-101 therapy (Figure 1).

Table 1: Hematology laboratory values, by visit (FAS)

Laboratory value	Mean (STD)				
	Screening/Baseline	Cycle 1 Day 8	Cycle 2 Day 8	Cycle 3 Day 8	Cycle 4 Day 8
Hematocrit (L/L)	0.36	0.35	0.34	0.34	0.33
	0.04	0.04	0.04	0.04	0.04
Erythrocytes (10^12/L)	3.94	3.83	3.84	3.89	3.89
	0.53	0.5	0.44	0.49	0.57
Hemoglobin (g/L)	119.12	114.95	112.12	110.99	110.12
	15.31	14.18	13.15	13.36	13.5
Platelets (10^9/L)	268.18	192.18	188.81	201.86	212.24
	108.88	127.36	82.8	105.83	108.49
Leukocytes (10^9/L)	6.97	6.61	6.77	6.6	7.42
	2.04	2.73	3.44	2.75	4.32
Lymphocytes (%)	19.62	20.39	20.74	21.21	21.82
	8.84	8.47	9.85	7.5	10.26
Absolute neutrophils count	4.52	4.1	4.37	4.43	4.94
	1.6	2.1	2.18	2.5	3.47

STD=Standard Deviation

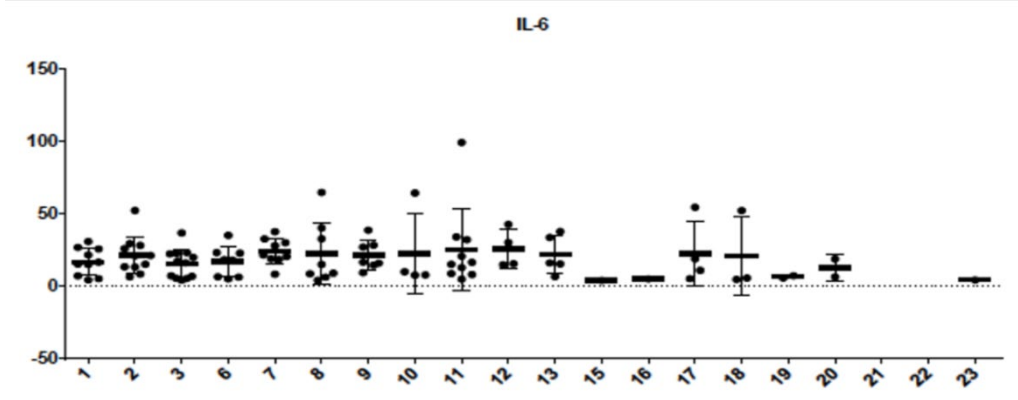

Figure 1: Scatter Plot for absolute IL-6 concentrations measured at different time points. Each dot represents a patient sample. The lines represent mean concentrations +/- standard deviation. Only concentrations that were in the quantifiable range are represented by a symbol. For time points where no quantifiable concentration was measured, no symbols are drawn. A timepoint was completely omitted from plotting if no measurable concentration for this and all the following time points were available.

OT-101 does not increase IL-6 levels in non-human primates (NHP) when used at doses comparable to as well as doses much higher than those proposed for the COVID-19 patient population

The objective of the NHP study was to determine the toxicity and toxicokinetic profile of the test item, OT-101 (Trabedersen), following a 13-cycle intravenous infusion (each 14-day cycle being 7-days of dosing followed by a 7-day washout period) to Cynomolgus monkeys and to allow assessment of reversibility of any changes following a 6-week recovery period. For each of the cycles, the test and control/vehicle items were administered as a continuous (i.e., 24 hours/day) intravenous infusion.

Notably, IL-6 or TNF-α levels showed no dose-response relationship suggestive of an OT-101 effect (Figure 2). There was no OT-101-related complement activation (i.e., C3a values), no changes in absolute counts of T cells, activated T helper and activated T cytotoxic T-cells, activated monocytes, B cells, or NK cells.

OT-101 does not cause IL-6 production at clinically relevant concentrations, and it does not amplify IL-6 production in stimulated whole blood cultures

At clinically meaningful concentrations of <5 μg/mL (C_{max} range in P001 study=1.46 μg/mL at 80 mg/m^2 OT-101-4.45 μg/mL at 240 mg/m^2 OT-101) [16], OT-101 did not induce above background IL-6 production. Even at clinically not applicable very high concentrations of 12 μg/mL or 48 μg/mL, the measured IL-6 concentrations in OT-101 treated cultures were ~0.1% of the IL-6 concentrations in positive control cultures stimulated with LPS/SE-B (Figure 3). Notably, the addition of OT-101 to cultures stimulated with LPS/SE-B did not result in amplified IL-6 production. These results demonstrate that OT-101 does not cause IL-6 production at clinically relevant concentrations, and it does not amplify IL-6 production in stimulated whole blood cultures [23].

The rationale for using OT-101 in COVID-19 patients with respiratory failure requiring non-invasive positive pressure ventilation (NIPPV) or mild ARDS requiring Mechanical ventilation (MV)

We postulate that the anti- TGF-β RNA therapeutic OT-101 will accelerate the resolution of respiratory failure of COVID-19 patients requiring NIPPV or Mild ARDS Requiring MV. Notably, TGF-β levels in Bronchoalveolar Lavage Fluid (BAL) samples from ARDS patients are inversely correlated with ventilator-free days and ICU-free days [22]. Furthermore, patients with higher TGF-β levels may have higher and faster case mortality [22]. Sequestering TGF-β has effectively attenuated the severity of pulmonary edema in experimental models of ARDS [22]. Furthermore, we do not anticipate any worsening of existing ARDS or Cytokine Storm/Cytokine Release Syndrome (CRS) of COVID-19 patients due to 7 days of OT-101 treatment. That is because no CRS or ARDS was observed as a treatment-emergent complication in a Phase I study of 61 patients with advanced solid tumors who were treated with more extended periods of OT-101 therapy. Additionally, OT-101 did not

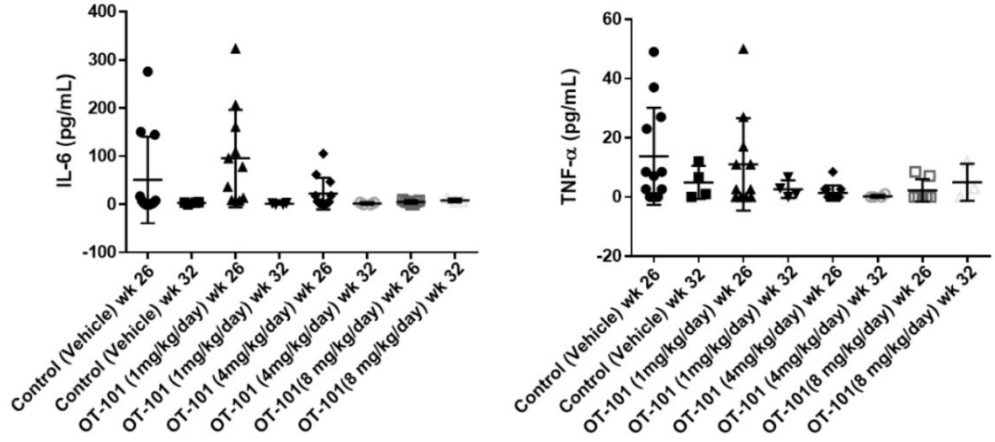

Figure 2: IL-6 and TNFα levels in cynomolgus monkeys treated with OT-101 (trabedersen), following a 13-cycle intravenous infusion (each 14-day cycle-7-days of dosing followed by a 7-day washout period. For each of the cycles, the test and control/vehicle items were administered as a continuous (i.e., 24 hours/day) intravenous infusion.

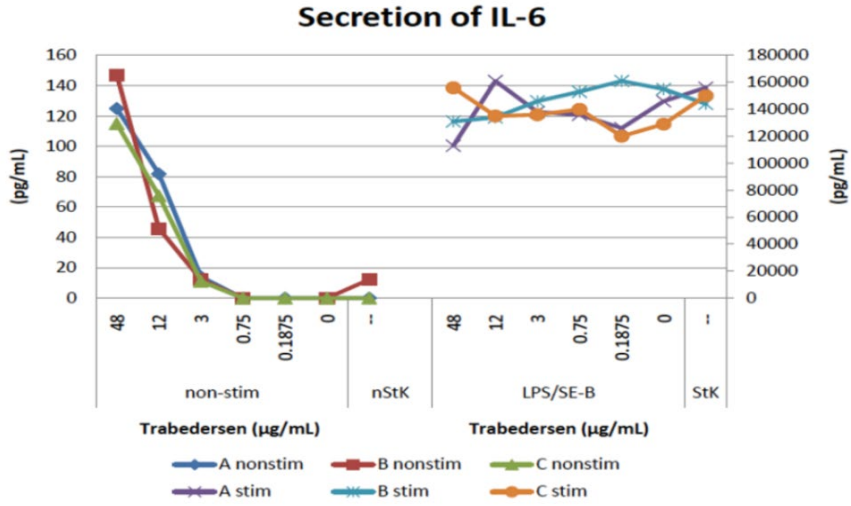

Figure 3: Effect of OT-101 on IL-6 production in human whole blood cultures. A whole-blood cell culture model was utilized to evaluate the cytokine response of blood cells to OT-101 treatment in vitro [23]. As a positive control, LPS [10 ng/mL] or SE-B [25 ng/mL]) were added to the blood cells in order to mimic inflammatory situations within the cell cultures. For each donor, one plate was prepared with all experiments being conducted in triplicate. Different dilutions of OT-101 (0.187 µg/mL, 0.75 µg/mL, 3 µg/mL, 12 µg/mL, and 48 µg/mL) were transferred into the plate and incubated with whole-blood cells of different donors for 1 hour. Thereafter, media for the unstimulated cultures or the different stimuli (LPS [10 ng/mL] or SE-B [25 ng/mL]) were added to the blood cells in order to mimic inflammatory situations within the cell cultures: the mixture of sample and capture microspheres were thoroughly mixed and incubated at room temperature (RT) for 1 hour. At the end of the 48-hour stimulation, the supernatants in triplicate were tested. Except for IFN-α, which was subsequently measured by Enzyme-Linked Immunoassay (ELISA), the concentration of each analyte to be tested in the supernatants from the blood culture was determined using a multiparametric bead-based readout system (Luminex™), called the Multi-Analyte Profile (MAP) test.

cause in human subjects an elevation of TNF-α, IL-6, or IL-10 levels associated with CRS and ARDS in COVID-19 patients. Likewise, OT-101 did not induce the production of these inflammatory cytokines in cultures of human white blood cells. Inhibition of the TGF-β signaling pathway also has the potential to prevent the development of pulmonary fibrosis following ARDS and improve the pulmonary healing process

TGF-β increases capillary permeability and impairs alveolar fluid reabsorption in ARDS patients, contributing to pulmonary edema's persistence

ARDS is characterized by severe pulmonary edema and an impaired alveolar fluid clearance incapable of removing the edema from alveoli caused by a functional defect of the Epithelial Sodium Channel (ENaC) [22]. Alveolar barrier dysfunction in ARDS leads to more pulmonary edema and the systemic release of biological mediators from the lung, contributing to the failure of other organs and potentially a multi-organ failure [24]. TGF-β is an important pro-inflammatory cytokine that affects the inflammatory process resulting from an acute lung injury, which contributes to the development of persistent and severe pulmonary edema in ARDS patients [25-30]. TGF-β can increase alveolar epithelial permeability [29] and pulmonary endothelial permeability by promoting adherens junction disassembly [31] as well as inhibiting pulmonary endothelial proliferation [32]. A recent study showed that TGF-β profoundly impacts alveolar ion and fluid transport by regulating the Epithelial Sodium Channel (ENaC) activity [33].

Elevated TGF-β levels in ARDS associated with SARS-CoV or SARS-CoV-2

Lavage fluid (BAL) samples from ARDS patients showed higher TGF–β levels when compared to BAL samples from non-ARDS controls [31]. The TGF-β levels in Bronchoalveolar Lavage Fluid (BAL) samples from ARDS patients were inversely correlated with the number of ventilator-free days or ICU-free days during hospitalization. Lower TGF-β levels were associated with better survival outcomes [34].

SARS-CoV up-regulates TGF-β expression, and TGF-β levels were markedly elevated in SARS patients with ARDS [35]. SARS-associated Coronavirus (SARS-CoV) nucleocapsid (N) protein

potentiates TGF-β signaling via a Smad3-dependent induction of TGF-β1 expression [36]. Further, the papain-like protease (PLpro) of SARS-CoV, a deubiquitinating enzyme and virulent factor in SARS pathogenesis has been reported to trigger TGF-β production via p38 MAPK, and ERK1/2-mediated signaling [37,38]. Xiong et al. showed that TGFβ genes are up-regulated in COVID-19 [39].

The contributing factors for the increased TGF-β levels in ARDS include the activation of the complement signaling pathway with the production of the complement cleavage product, C5a, that triggers the formation of Neutrophil Extracellular Traps (NETs). NETs are extracellular webs of chromatin, microbicidal proteins, and oxidant enzymes that are released by neutrophils to contain infections. However, when not appropriately regulated, NETs have the potential to propagate inflammation and microvascular thrombosis. That is because they are capable of activating platelets to release TGFβ. Recent studies have shown that sera from individuals with COVID-19 triggered NET release from control neutrophils in vitro, and high levels of NETs in many patients with COVID-19 may contribute to cytokine release and respiratory failure. Zuo et al. reported that sera from patients with COVID-19 have elevated levels of cell-free DNA, Myeloperoxidase (MPO)-DNA, and citrullinated histone H3 (Cit-H3); the latter two are highly specific markers of NETs [40]. Highlighting the potential clinical relevance of these findings, cell-free DNA strongly correlated with acute phase reactants including C-reactive protein, D-dimer, and lactate dehydrogenase, as well as absolute neutrophil count [40,41].

Sequestering TGF-β has effectively attenuated the severity of pulmonary edema in experimental models of ARDS [29,41]. Likewise, the anti-inflammatory isoflavone Puerarin ameliorates the ARDS-associated inflammatory process in the lungs by inhibiting the expression of TGF-β [42].

Contribution of TGF-β to coagulopathy in COVID-19 associated ARDS. As a platelet activator

TGF-β has been implicated in the pathophysiology of thrombosis and disseminated intravascular coagulopathy [43]. It is noteworthy that autopsies in COVID-19 cases have revealed micro-thrombi [44]. Stafford et al. postulated that TGFβ is a key molecule, along with TNFα, in the pathogenesis of severe COVID-19 [45].

Contribution of TGF-β to lung fibrosis post-ARDS

TGF-β is also involved in the pathogenesis of lung tissue remodeling and lung fibrosis that follows ARDS [22]. TGF-β stimulates lung fibroblasts and causes collagen production in the pulmonary interstitial and alveolar space, leading to the occurrence and development of pulmonary fibrosis [46,47].

Wang et al. reported that miR-425 reduction in lung fibroblasts contributes to the development of lung fibrosis post-ARDS through activation of the TGF-β signaling pathway [48]. Therefore, inhibition of the TGF-β signaling pathway could prevent the development of pulmonary fibrosis post-ARDS and improve the pulmonary healing process [42].

Contribution of TGF-β to lymphopenia and immune exhaustion in COVID-19

A consistent finding in COVID-19 is lymphopenia with low CD4 counts [49]. TGFβ has been shown to reduce T-cell numbers in disease states [50,51]. Xu et al. reported that a significant decrease of the T lymphocyte subset is positively correlated with in-hospital death and severity of illness based on the data from a total of 187 COVID-19 patients [52]. All patients exhibited a significant drop of T lymphocyte subsets counts with remarkably increasing concentrations of SAA, CRP, IL-6, and IL-10 compared to normal values. The median concentrations of SAA and CRP in critically-ill patients were nearly 4- and 10-fold than those of mild-ill patients, respectively. As the severity of COVID-19 getting worse, the counts of T lymphocyte dropped lower. 28 patients died in hospital, the median lymphocyte, CD3+ T-cell, CD4+ T-cell, CD8+ T-cell, and B-cell were significantly lower than other patients. Lower counts (/μL) of T lymphocyte subsets lymphocyte (<500), CD3+ T-cell (<200), CD4+ T-cell (<100), CD8+ T-cell (<100) and B-cell (<50) were associated with higher risks of in-hospital death of CIVID-19 [52]. TNF-α, IL-6, and IL-10 have been implicated in COVID-19 associated lymphocytopenia and T-cell reduction [52].

Similarly, Chen et al. [53] reported that compared with moderate cases, severe cases more frequently had lymphopenia with higher levels of alanine aminotransferase, lactate dehydrogenase, C-reactive protein, ferritin, and D-dimer as well as markedly higher levels of IL-2R, IL-6, IL-10, and TNF-α. Absolute numbers of T lymphocytes, CD4+ T cells, and CD8+ T cells were decreased in nearly all the

patients and were markedly lower in severe cases (294.0, 177.5, and 89.0 × 10^6/L, respectively) than moderate cases (640.5, 381.5, and 254.0 × 10^6/L, respectively). Diao et al. [54] reported that the T cell numbers in COVID-19 patients are inversely correlated with serum IL-6, IL-10, and TNF-α concentrations. T cells from COVID-19 patients also show increased expression of programmed cell Death Marker-1 (PD-1) and T cell Immunoglobulin and Mucin domain 3 (TIM-3) consistent with "exhaustion" [6]. Likewise, profound T cytopenia was reported by Chiappelli et al. [55]. The reduction of the T-cell numbers, as well as the expression of the T-cell exhaustion markers PD-1 and TIM-3, correlated with the progression of the disease from early stages to advanced ARDS requiring ICU management.

TGF-β has been implicated in regulating the size of the pathogen-specific T-cell responses and the propensity of these cells to undergo apoptosis [55-58]. Enhanced and sustained TGF-β/Smad signaling is a distinctive feature of virus-specific CD8 T cells during chronic *versus* acute viral infections *in vivo*. Selective attenuation of TGF-β signaling on T cells increases the function of CD8 T cells indirectly, rapidly eradicates the persistence-prone virus, and enables the generation of an effective memory response. Therefore, targeting TGF-β may favorably affect the immune function of host T-cells in COVID-19 patients.

OT-101 was evaluated in Phase I/II study (ClinicalTrials.gov identifier: NCT00844064), involving a total of 61 patients with advanced solid tumors [22]. OT-101 was well tolerated and had no significant effect on the lymphocyte counts and it did not cause lymphocytopenia. The results for 2 months of therapy, i.e., 14-day cycles × 4 cycles are shown in Table 1. Based on these findings, we postulate that because of the significance of the TGF-β pathway on the development of T cell exhaustion, treatment with OT-101 may help facilitate the recovery of lymphocytopenia and T-cell exhaustion in COVID-19 patients.

Conclusion

TGF-β inhibitors show potential for the treatment of COVID-19 pneumonia and ARDS. Inhibitors of TGF-β pathway may prevent or reduce the risk of development of ARDS and T cell exhaustion, halt or reverse the progression of evolving or mild ARDS and help facilitate the recovery of lymphocytopenia in COVID-19 patients.

Executive summary

Based on the role of TGF- β in the immunopathology of ARDS, we and others have proposed the use of TGF-β inhibitors for the treatment of COVID-19 pneumonia and ARDS. TGF-b targeting is employed as a strategy to stimulate the immune system of advanced-stage cancer patients in an attempt to overcome the immunosuppression and T-cell exhaustion within the tumor microenvironment. Nevertheless, we do not anticipate any worsening of existing ARDS or Cytokine Storm/Cytokine Release Syndrome (CRS) of COVID-19 patients as a treatment-emergent complication with our contemplated use of the anti-TGF-β RNA therapeutic OT-101. That is because (i) inhibitors of TGF-β signaling are not associated with ARDS, Cytokine Storm/CRS, or systemic capillary leak, (ii) OT-101 did not cause any pulmonary toxicity, non-infectious pneumonitis, CRS, systemic or pulmonary capillary leak or ARDS in any of the 61 patients with advanced solid tumors enrolled in Phase I/II study (ClinicalTrials.gov identifier: NCT00844064) who received much longer periods of OT-101 therapy, and (iii) OT-101 did not cause in human subjects an elevation of TNF-α, IL-6 or IL-10 levels associated with CRS and ARDS in COVID-19 patients-likewise, OT-101 did not induce production of these inflammatory cytokines in cultures of human white blood cells. We postulate that because of the significance of the TGF-β pathway on the development of ARDS and T cell exhaustion, treatment with OT-101 may prevent the progression of evolving or mild ARDS and help facilitate the recovery of lymphocytopenia and T-cell exhaustion in COVID-19 patients.

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