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# Medical-Scientific Rationale for a Randomized, Placebo-Controlled, Phase 2 Study of Trabedersen/OT-101 in COVID-19 Patients with Hypoxemic Respiratory Failure

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## ABSTRACT

TGF-β was identified as the exclusive master regulator of the epithelial sodium channel (ENaC). ENAC internalization by alveolar epithelial cells and its upregulation in ARDS causes an ENAC trafficking defect with marked reduction in the cell-surface abundance of ENaC on lung epithelial cells thereby rapidly and substantially impairing alveolar fluid reabsorption in ARDS patients and contributing to the persistence of their pulmonary edema. A significant negative correlation existed between TGF-β levels in bronchoalveolar lavage fluid (BAL) samples from ARDS patients and ventilator-free days and ICU-free days. Furthermore, lower TGF-β levels correlated with better survival outcome indicating that that patients with higher TGF-β levels may have a higher and faster case mortality. The anti-TGF-β RNA therapeutic Trabedersen/OT-101 exhibited a favourable clinical safety profile in cancer patients and it also exhibited nanomolar in vitro potency against SARS-CoV-2. The correlation of lower BAL fluid TGF-β levels with improved survival of ARDS patients taken together with the potent anti-SARS-CoV-2 activity of Trabedersen/OT101 support the concept of reducing TGF-β levels with Trabedersen/OT-101 in COVID-19 patients with ARDS.

## Introduction

After infection with SARS-CoV-2, up to one third of COVID-19 patients develop an acute pulmonary inflammation with a fulminant progression to acute respiratory distress syndrome (ARDS) with a high fatality rate in high risk patient populations despite best available supportive care [1-3]. In these patients, the "burst release" of proinflammatory cytokines in massive amounts and in succession causes a severe form of systemic capillary leak syndrome with pulmonary edema, that can cause hypoxic injury and dysfunction of multiple organs, ultimately leading to an irreversible and fatal multi-organ failure [1-3]. This hyperacute inflammatory process is reminiscent of the cytokine release syndrome (CRS) observed in cancer patients treated with immunotherapeutic modalities such as CAR-T cells (e.g. tisagenlecleucel) or bispecific T-cell engagers (e.g. blinatumomab) that may hyperactivate elements of both the innate and acquired immune system [4-8]. Although an intensive research effort is underway globally to identify effective antiviral drugs or drug combinations against SARS-CoV-2, it is unlikely that such treatments, including treatments employing drugs such as Remdesivir (ClinicalTrials.gov Identifier: NCT04280705), Favipiravir (ClinicalTrials.gov Identifier: NCT04310228), Hydroxychloroquine (ClinicalTrials.gov Identifier: NCT04318444), albeit of very high clinical impact potential for post-exposure prophylaxis in early treatment settings, could effectively reverse or ameliorate the

pulmonary and systemic inflammation in COVID-19 patients with evolving or established ARDS. Therefore, there is an urgent and unmet medical need for treatments that can effectively reduce the risk of ARDS or its mortality rate in COVID-19 patients with pneumonia.

## Clinical Safety, Pharmacokinetics, and Activity of Trabedersen/OT-101

Antisense oligodeoxynucleotides are short strings of DNA that are designed to down-regulate gene expression by interfering with the translation of a specific encoded protein at the mRNA level. Trabedersen (OT-101; also known as OT101) is a synthetic TGFβ2-specific 18-mer phosphorothioate antisense oligodeoxynucleotide (S-ODN) that was originally designed to reduce the level of TGFβ2 protein within the tumor microenvironment (TME) of cancer patients in an effort to overcome the TGF-β-mediated immune-suppression, invasive tumor growth and generation of new blood vessels supplying tumor tissue (viz.: neovascularization) that contribute to progression of disease [9]. The safety and single agent anti-cancer activity of this RNA therapeutic was evaluated in Phase I and Phase 2 studies which demonstrated its high clinical impact potential for aggressive and difficult-to-treat advanced cancers, including high grade brain tumors, such as glioblastoma multiforme and Grade 3 anaplastic astrocytoma, and pancreas cancer [10-13]. Notably, in a randomized Phase IIb study (Clinicaltrial.gov identifier: NCT00431561), intratumoral delivery of OT-101 via extended convection-enhanced delivery (CED) in the absence of other therapeutic agents or radiation resulted in >3.5 year OS in more than half of a recurrent/refractory (R/R) high-grade glioma patient population [12, 13]. OT-101 showed remarkable single agent activity with more than a third of patients (26 of 77) receiving the intended 4-11 cycles of therapy achieving durable complete responses, partial responses, or prolonged stable disease and a median overall survival of >4.5 years.

Likewise, in a Phase I/II study (ClinicalTrials.gov identifier: NCT00844064) promising clinical activity and better than expected overall survival was observed in metastatic solid tumor patients, including a Stage IV pancreatic cancer patient who achieved a sustained complete response lasting >5 years [10, 11]. In the latter study, a total of 61 patients with Stage III/IV pancreatic cancer (n=37), malignant melanoma (n=19), or colorectal carcinoma (n=5) were treated with continuous intravenous (IV) infusion of OT-101 as 2nd to 4th-line therapy in escalating doses (40 mg/m$^2$/day up to 330 mg/m$^2$/day) of two treatment schedules (initial schedule: 7-days on, 7-days off (n = 17); modified schedule: 4-days on, 10-days off (n = 44); two cycles as core study and up to 10 cycles for expanded study). A summary of the number of patients per cohort and indication, together with the median number of cycles and daily trabedersen dose is provided in Table 1.

**Table 1: Number of Patients Participating in the Core Study, by Indication, Schedule, and Cohort (FAS)**

| | No. of Patients | | | |
|---|---|---|---|---|
| Treatment Schedule Cohort | Pancreatic cancer | Melanoma | Colorectal cancer | Total |
| Total | 37 | 19 | 5 | 61 |
| 7-days-on, 7-days-off | 11 | 2 | 4 | 17 |
| Cohort 1: 40 mg/m$^2$/day | 4 | 0 | 0 | 4 |
| Cohort 2: 80 mg/m$^2$/day | 2 | 1 | 0 | 3 |
| Cohort 3: 160 mg/m$^2$/day | 3 | 1 | 2 | 6 |
| Cohort 4: 240 mg/m$^2$/day | 2 | 0 | 2 | 4 |
| 4-days-on, 10-days-off | 26 | 17 | 1 | 44 |
| Cohort A1: 140 mg/m$^2$/day | 5 | 0 | 0 | 5 |
| Cohort A2: 190 mg/m$^2$/day | 2 | 1 | 0 | 3 |
| Cohort A3: 250 mg/m$^2$/day | 4 | 1 | 0 | 5 |
| Cohort A4: 330 mg/m$^2$/day | 1 | 1 | 1 | 3 |
| Last cohort: 140 mg/m$^2$/day | 14 | 14 | 0 | 28 |

The planned number of 3 patients per cohort of the dose-escalation period was reached for all cohorts: the number of patients in these cohorts varied between 3 to 6. Fourteen additional pancreatic cancer patients and 14 additional melanoma patients were enrolled in the last cohort. Consequently, the number of patients per cohort of the 7-days-on, 7-days-off and 4-days-on, 10-days-off dose escalation schedules varied between 3 and 6 patients. The last cohort treated with 140 mg/m$^2$/day with the 4 days on, 10-days-off schedule contained 28 patients (14 each with pancreatic cancer and melanoma). The median number of cycles per cohort varied between 1.5 and 5.0. The median trabedersen dose per day during the core study period reflected the respective dose cohort,

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with the highest median dose per day seen for cohort 4 of the 7-days-on, 7-days-off schedule (167.9 mg/m$^2$/day) (Table 2).

## Table 2: Extent of Exposure (FAS)

| Treatment Schedule Cohort | No. of patients Total (PanCa/MM/CRC) | No. of cycles Median (min; max) | Daily dose core study period (mg/m$^2$/day) Median (Q1, Q3) |
|---|---|---|---|
| **7-days-on, 7-days-off** | | | |
| Cohort 1: 40 mg/m$^2$/day | 4 (4/0/0) | 2.0 (1; 4) | 23.1 (20.2, 34.3) |
| Cohort 2: 80 mg/m$^2$/day | 3 (2/1/0) | 5.0 (4; 10) | 40.0 (39.9, 40.7) |
| Cohort 3: 160 mg/m$^2$/day | 6 (3/1/2) | 2.5 (1; 4) | 69.1 (50.4, 83.4) |
| Cohort 4: 240 mg/m$^2$/day | 4 (2/0/2) | 1.5 (1; 2) | 167.9 (133.6, 186.3) |
| **4-days-on, 10-days-off** | | | |
| Cohort A1: 140 mg/m$^2$/day | 5 (5/0/0) | 4.0 (1; 10) | 33.0 (21.5, 40.8) |
| Cohort A2: 190 mg/m$^2$/day | 3 (2/1/0) | 4.0 (2; 8) | 56.3 (56.1, 57.5) |
| Cohort A3: 250 mg/m$^2$/day | 5 (4/1/0) | 4.0 (1; 10) | 59.8 (58.2, 76.0) |
| Cohort A4: 330 mg/m$^2$/day | 3 (1/1/1) | 2.0 (2; 10) | 79.7 (75.3, 82.2) |
| **Last cohort (4-days-on, 10-days-off)** | | | |
| 140 mg/m$^2$/day | 28 (14/14/0) | 4.0 (1; 8) | 38.1 (31.8, 40.1) |

CRC = colorectal cancer; MM = malignant melanoma; No. = number; PanCa = pancreatic cancer; Q1 = first quartile; Q3 = third quartile

On average, patients in the first treatment schedule were exposed to study medication for 2.7 cycles, corresponding to a treatment period of 1.2 months. The total estimated average exposure was 2248 mg/m$^2$. In the second treatment schedule, patients on average were exposed for 4.4 cycles, corresponding to a treatment period of 2 months. The total estimated average exposure was 2734 mg/m$^2$.

Overall, Trabedersen was well-tolerated. The majority of the AEs occurring during treatment with OT-101 were most likely related to the underlying disease or associated symptoms rather than the study medication itself. A total of 30 patients (49.2%) reported 87 TEAEs considered possibly related to trabedersen by the Investigator, with slightly higher frequency in the 4-days-on, 10-days-off schedule group (24 patients [54.5%]) than in the 7-days-on, 7-days-off schedule group (6 [35.3%]) (Table 3).

Six patients (9.8%) overall reported 21 TEAEs considered related to trabedersen by the Investigator, 2 patients (11.8%) with the 7-days-on, 7-days-off schedule and 4 (9.1%) with the 4-days-on, 10-days-off schedule (Table 3, Table 4).

The only related TEAE reported by > 1 patient was thrombocytopenia that occurred in 2 patients of each schedule group. All cases of thrombocytopenia and decreased platelet count reported during the study were considered related or possibly related to trabedersen by the Investigator (Table 3, Table 4).

## Table 3: Possibly Related and Related Adverse Events, by Preferred Term and Schedule and Sorted by Frequency (FAS)

| | No. (%) of patients with TEAE / No. of TEAEs | | | | | |
|---|---|---|---|---|---|---|
| | Possibly related TEAEs | | | Related TEAEs | | |
| Preferred Term | 7-days-on, 7-days-off schedule (N=17) | 4-days-on, 10-days-off schedule (N=44) | Total (N=61) | 7-days-on, 7-days-off schedule (N=17) | 4-days-on, 10-days-off schedule (N=44) | Total (N=61) |
| Any TEAE | 6 (35.3)/12 | 24 (54.5)/75 | 30 (49.2)/87 | 2 (11.8)/2 | 4 (9.1)/19 | 6 (9.8)/21 |
| Fatigue | 1 (5.9)/1 | 5 (11.4)/6 | 6 (9.8)/7 | 0 | 1 (2.3)/1 | 1 (1.6)/1 |
| Nausea | 0 | 5 (11.4)/8 | 5 (8.2)/8 | 0 | 1 (2.3)/4 | 1 (1.6)/4 |
| Thrombocytpenia | 0 | 5 (11.4)/5 | 5 (8.2)/5 | 2 (11.8)/2 | 2 (4.5)/2 | 4 (6.6)/4 |
| Headache | 0 | 4 (9.1)/7 | 4 (6.6)/7 | 0 | 1 (2.3)/1 | 1 (1.6)/1 |
| Vomiting | 1 (5.9)/1 | 3 (6.8)/4 | 4 (6.6)/5 | 0 | 1 (2.3)/1 | 1 (1.6)/1 |
| Dyspnea | 0 | 3 (6.8)/4 | 3 (4.9)/4 | 0 | 0 | 0 |
| Pyrexia | 0 | 2 (4.5)/3 | 2 (3.3)/3 | 0 | 1 (2.3)/4 | 1 (1.6)/4 |
| Asthenia | 0 | 2 (4.5)/2 | 2 (3.3)/2 | 0 | 0 | 0 |
| Constipation | 0 | 2 (4.5)/2 | 2 (3.3)/2 | 0 | 0 | 0 |
| Decreased ppetite | 0 | 2 (4.5)/2 | 2 (3.3)/2 | 0 | 0 | 0 |
| Diarrhea | 0 | 2 (4.5)/2 | 2 (3.3)/2 | 0 | 0 | 0 |
| General physical health deterioration | 0 | 2 (4.5)/2 | 2 (3.3)/2 | 0 | 0 | 0 |
| Pain in extremity | 1 (5.9)/1 | 1 (2.3)/1 | 2 (3.3)/2 | 0 | 0 | 0 |
| Platelet count decreased | 1 (5.9)/1 | 1 (2.3)/1 | 2 (3.3)/2 | 0 | 1 (2.3)/3 | 1 (1.6)/3 |
| Pruritus | 0 | 2 (4.5)/2 | 2 (3.3)/2 | 0 | 0 | 0 |
| Chills | 0 | 1 (2.3)/1 | 1 (1.6)/1 | 0 | 1 (2.3)/1 | 1 (1.6)/1 |
| Metastasis | 0 | 0 | 0 | 0 | 1 (2.3)/1 | 1 (1.6)/1 |
| Skin Inflamation | 0 | 0 | 0 | 0 | 1 (2.3)/1 | 1 (1.6)/1 |

N = total number of patients; No. = number; TEAE = treatment-emergent adverse event; FAS = full analysis set

## Table 4.Grade ≥3 Adverse Events Assessed as Related or Possibly Related to Trabedersen (OT-101) by Investigator

| No. of AE | Preferred Term of AE | Grade | Seriousness | Outcome | Patient No. |
|---|---|---|---|---|---|
| 3 | Thrombocytopenia | 3 | Nonserious | Recovered | PC 1017/4 MM 1027/A3 MM 1055/LC |
| 1 | Thrombocytopenia | 3 | Nonserious | Recovered with sequelae | CC 1016/4 |
| 1 | ALAT increased | 3 | Nonserious | Recovered | PC 1022/A1 |
| 1 | GGT increased | 3 | Nonserious | AE still present | PC 1005/2 |
| 1 | Haemoglobin decreased | 3 | Nonserious | Recovered | MM 1055/LC |
| 1 | Rash maculo-papular | 3 | Nonserious | Recovered | CC 1014/4 |
| 1 | Gastrointestinal hemorrhage | 3 | Serious | Recovered | PC 1018/A1 |

Within Preferred Terms events are listed by seriousness, grade and outcome. Events are sorted by descending frequency.
AE = adverse event; CC = colorectal cancer; MM = malignant melanoma; Pat. No. = patient number; PC = pancreatic cancer

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A total of 4 patients experienced a DLT in the dose escalation part of the study (core study) as per regulations applied during the study conduct, with three of these patients treated at 240 mg/m$^2$/day (Cohort 3, Table 1) in the 7 days on, 7 days off schedule (2 Grade 3 thrombocytopenias, 1 Grade 3 maculo-papular rash). Therefore, a dose of 160 mg/m$^2$/day was declared to be the MTD for the 7-days-on, 7 days off schedule. The second schedule (4 days on, 10 days off) was completed without reaching the MTD. In the Phase II-part of the study additional pancreatic cancer and melanoma patients were treated with 140 mg/m$^2$/d according to the second schedule.

There were 2 SAEs which were considered as possibly related (1 SAE of gastrointestinal hemorrhage, 1 SAE of pyrexia, both with outcome recovered). The first related SAE was a case of gastrointestinal hemorrhage which occurred in a 65 year old male patient with pancreatic cancer. The event occurred 4 days after end of the first treatment cycle with 140 mg/m$^2$/day (4 days on, 10 days off). Gastroduodenoscopy showed an acute duodenal varix bleeding. The bleeding was stopped by sclerotherapy and the outcome was reported as recovered. The second related SAE was a case of pyrexia (verbatim term fever and chills) which occurred in a 66 year old male malignant melanoma patient. Pyrexia (NCI CTC grade 1) occurred about 12 hours after start of the second treatment cycle of OT-101 with 140 mg/m$^2$/day (4 days on, 10 days off) and got worse (NCI-CTC grade 2), requiring the patient's hospitalization on the third treatment day. The patient was treated with antibiotics (orally and intravenously) for suspected gastrointestinal infection and subsequently recovered from pyrexia. The patient had already suffered from gastrointestinal infection, from which he had recovered under antibiotic therapy prior to start of the second treatment cycle with OT-101. The investigator did not suspect the study drug to have caused pyrexia, but suspected that the treatment with OT-101 could have worsened these symptoms.

As thrombocytopenia has been identified as adverse drug reaction associated with trabedersen, the SOC Blood and lymphatic system disorders is looked at more closely. 12 of 61 patients (19.7%) suffered 14 AEs of either thrombocytopenia or platelet count decreased. All events were assessed as related/possibly related to trabedersen by the investigators and the vast majority (10 out of 14 events) was reported to have recovered without further treatment. None of these cases was assessed as serious. The intensity of 10 of the 14 occurrences was reported as mild or moderate whereas 4 AEs were reported to be of severe intensity. In 6 instances therapy with OT-101 was discontinued or temporarily interrupted. No bleedings were reported and no platelet transfusions became necessary. There were no signs of cumulative increases of thrombocytopenia in later treatment cycles.

Hence, thrombocytopenia is a known side effect of other phosphorothioate oligonucleotides and was identified as an expected adverse drug reaction during treatment with Trabedersen/OT-101. A close temporal relationship between the administration of Trabedersen/OT101 and the onset of the AE of thrombocytopenia and the fact that thrombocytopenia often occurs at the beginning of the treatment with trabedersen during cycle 1 or cycle 2 reinforce the existence of a causal relationship.

In PK analyses, the concentration time course of trabedersen/OT-101 was best described by a two-compartment model [14]. The distribution of trabedersen to tissues was not only rapid, but also extensive, with deep tissue penetration as demonstrated by the finding that the volume of the peripheral compartment far exceeded the total body water volume [14]. Plasma concentrations of trabedersen/OT-1011 began to be quantifiable up to 2 hours post start of infusion (SOI) for most subjects and generally fell below the lower limit of detection a few hours after the end of infusion, even for the high dose groups. PK profiles of trabedersen/OT-101 show sustained plasma concentrations throughout the dosing period (4 or 7 days) with similar PK exposure parameters (Cmax and Area under curve (AUC)) between Cycle 1 and Cycle 2. Exposure was dose proportional for trabedersen over the 7-day dose range (40, 80, 160 and 240 mg/mg$^2$/day) and the 4-day dose range (140, 190, 250, and 330 mg/m$^2$/day) (Table 5).

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**Table 5: Pharmacokinetic Parameters of Trabedersen after Intravenous Infusions of Trabedersen, by Cohort**

| | 7-days-on, 7-days-off schedule | | | | 4-days-on, 10-days-off schedule | | | |
|---|---|---|---|---|---|---|---|---|
| | 40 mg/m² | 80 mg/m² | 160 mg/m² | 240 mg/m² | 140 mg/m² | 190 mg/m² | 250 mg/m² | 330 mg/m² |
| **Cycle 1** | | | | | | | | |
| Tmax (h) | 168 | 114 | 139 | 119 | 58.0 | 70.2 | 63.5 | 62.3 |
| Cmax (µg/mL) | 0.961 | 1.46 | 2.86 | 4.45 | 2.17 | 3.24 | 2.95 | 3.90 |
| Tlast (h) | 170 | 171 | 173 | 173 | 134 | 181 | 101 | 102.0 |
| AUC (0-t) (µg*h/mL) | 93.0 | 141 | 383 | 559 | 173 | 278 | 254 | 324 |
| AUC (0-168) (µg*h/mL) | 92.4 | 140 | 379 | 593 | 160 | 240 | 249 | 316 |
| T1/2 (h) | 0.594 | - | 1.70 | 1.60 | 1.10 | 1.23 | 1.25 | 1.53 |
| Vd (mL/m²) | 1902 | - | 7855 | 6783 | 5644 | 5310 | 7682 | 9029 |
| Cl (mL/m²) | 2222 | - | 3309 | 2940 | 3728 | 2904 | 5126 | 4076 |
| **Cycle 2** | | | | | | | | |
| Tmax (h) | 169 | 114 | 119 | - | 66.3 | 47.9 | 95.0 | 59.8 |
| Cmax (µg/mL) | 0.482 | 1.36 | 2.38 | - | 2.43 | 3.31 | 3.68 | 3.50 |
| Tlast (h) | 170 | 171 | 179 | - | 138 | 124 | 236 | 102 |
| AUC (0-t) (µg*h/mL) | 53.1 | 127 | 356 | - | 193 | 271 | 523 | 305 |
| AUC (0-168) (µg*h/mL) | 52.4 | 125 | 354 | - | 185 | 253 | 298 | 298 |
| T1/2 (h) | - | - | 1.49 | - | 1.17 | 1.23 | 1.63 | 1.78 |
| Vd (mL/m²) | - | - | 6944 | - | 5097 | 4999 | 6327 | 11233 |
| Cl (mL/m²) | - | - | 3076 | - | 3118 | 2802 | 2801 | 4364 |

AUC = area under curve; CL = clearance; Cmax = maximum observed concentration; T1/2 = terminal elimination half-life; Tlast = time last quantifiable concentration observed; Tmax = time after dosing at which Cmax observed; Vd = volume of distribution

The mean Cmax values on Schedule 1, Cycle 1 were 0.96 µg/mL at 40 mg/m²/day, 1.46 µg/mL at 80 mg/m²/day, 2.86 µg/mL at 160 mg/m²/day, and 4.62 µg/mL at 240 mg/m2/day. The recovery phase at the end of the profiles declined in a linear manner for most subjects. The terminal elimination half-life (T1/2) was well determined from the recovery phase of most trabedersen profiles, and was approximately between 1 and 2 hours. The pharmacokinetic profile of trabedersen was in line with the short half-life expected for this class of agents. Observed plasma T1/2 was between 1 to 2 hours. This naturally requires a prolonged infusion as employed for this trial to ideally achieve extended target suppression, as for first-generation oligonucleotides a prolonged tissue half-life cannot be expected.

## Role of TGF-β in ARDS, SARS, COVID-19

ARDS is characterized by severe pulmonary edema caused by an endothelial cell damage with increased capillary permeability contributing to accumulation of a protein-rich viscous fluid in alveoli and interstitium combined with an impaired alveolar fluid clearance incapable of remove the edema from alveoli owing to dysregulated Na+ and fluid transport across the alveolo-capillary barrier caused by a functional defect of the epithelial sodium channel (ENaC). Alveolar barrier dysfunction in ARDS leads to more pulmonary edema and to the systemic release of biological mediators from the lung, contributing to the failure of other organs and potentially a multi-organ failure [15].

TGF-β has been implicated as an important pro-inflammatory cytokine in the pathophysiology of acute lung injury and ARDS that contributes to both increased permeability and failed fluid reabsorption in lungs leading to persistent and severe pulmonary edema [16-21]. Importantly, lavage fluid (BAL) samples from ARDS patients collected within 2 days after intubation showed higher TGF-β levels when compared to BAL samples from non-ARDS controls [22]. TGF-β can increase alveolar epithelial permeability [20] and pulmonary endothelial permeability by promoting adherens junction disassembly [23] as well as inhibiting pulmonary endothelial proliferation [24]. SARS-CoV has been shown to up-regulate pro-inflammatory cytokines, including TGF-β and TGF-β levels were markedly elevated in SARS patients with ARDS [25]. SARS-associated coronavirus (SARS-CoV) nucleocapsid (N) protein has

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been shown to potentiate TGF-β signalling via a Smad3-dependent induction of TGF-β1 expression [26]. 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 ubiquitin proteasome, p38 MAPK, and ERK1/2-mediated signaling [27, 28].

The contributing factors for the increased TGF-β levels in ARDS also include the activation of the complement signalling pathway with production of the complement cleavage product, C5a that triggers the formation of neutrophil extracellular traps (NETs) that are capable of activating platelets to release TGFβ. A recent study by Peters et al. demonstrated that TGF-β profoundly impacts alveolar ion and fluid transport by regulating the epithelial sodium channel (ENaC) activity and trafficking via a Tgfbr1-mediated unique signalling pathway [29]. TGF-β was identified as the exclusive master regulator of ENAC internalization by alveolar epithelial cells and its upregulation in ARDS causes an ENAC trafficking defect with marked reduction in the cell-surface abundance of ENaC on lung epithelial cells thereby rapidly and substantially impairing alveolar fluid reabsorption in ARDS patients and contributing to the persistence of their pulmonary edema [29]. A soluble recombinant TGF-β receptor protein capable of sequestering TGF-β has effectively attenuated the severity of pulmonary edema in experimental models of ARDS [20, 30]. Likewise, the anti-inflammatory isoflavone Puerarin has been shown to reduce the ARDS-associated inflammatory process in the lungs by inhibiting the expression of TGF-β [31]. Notably, a significant negative correlation existed between TGF-β levels in bronchoalveolar lavage fluid (BAL) samples from ARDS patients and ventilator-free days and ICU-free days. Furthermore, lower TGF-β levels correlated with better survival outcome indicating that that patients with higher TGF-β levels may have a higher and faster case mortality [22]. Notably, OT-101 exhibited nanomolar in vitro potency against both SARS-CoV and SARS-CoV-2 and it was at least 1 log superior to the antiviral agent Remdesivir (Table 6, Figure 1).

**Table 6. Antiviral activity of OT-101**

| Compound | Virus | $EC_{50}$ | $CC_{50}$ | SI |
|---|---|---|---|---|
| OT-101 | SARS-CoV-1 (Urbani strain)[1] | 7.6 (1.24 µM) | >1000 | >130 |
| OT-101 | SARS-CoV-1 (Urbani strain)[1] | 26 (4.23 µM) | >1000 | >38 |
| OT-101 | SARS-COV-2 USA_WA1/2020[2] | 2.0 (0.33 µM) | >1000 | >500 |
| RSV | SARS-COV-2 USA_WA1/2020[2] | 620.0 | >1000 | >1.6 |
| M128533 | SARS-COV-2 USA_WA1/2020[2] | 0.012 | >10 | >830 |
| Remdesivir | SARS-COV-2 (Wang M. et al., 2020, Cell Res. 30:269-271) | 1.06 (1.76 µM) | >10 | >830 |

RSV- Negative control antisense/M128533- positive control
EC50: 50% effective antiviral concentration (in µg/ml) / CC50: 50% cytotoxic concentration of compound without virus added (in µg/ml) / SI = CC50/EC50
[1]Source: Centers for Disease Control Stock 809940 (200300592)
[2]Source: The World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) at UTMB

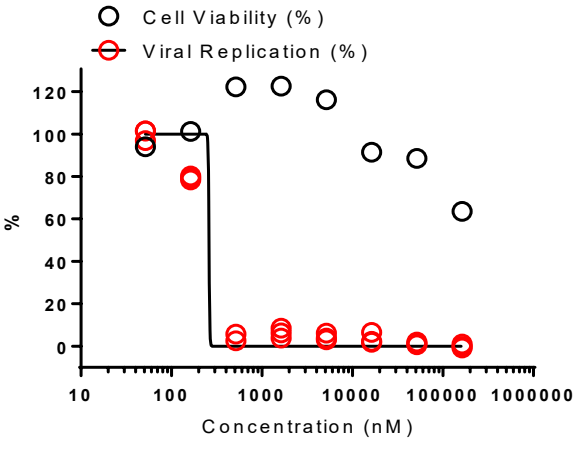


**Figure 1. Trabedersen is a Nanomolar Inhibitor of SARS-CoV-2 Replication.** The in vitro antiviral activity against SARS-CoV-2 (USA_WA1/2020 strain) was tested in Vero 76 cells in collaboration with Dr. Brett Hurst at Utah State University that is part of the NIAID Antiviral Testing Consortium. The cytotoxicity of OT101 against Vero 76 cells was also tested in the absence of SARS-CoV-2. See Table 6 for further details.

The correlation of lower BAL fluid TGF-β levels with improved survival of ARDS patients taken together with the potent antiviral activity of Trabedersen/OT101 against SARS-CoV-2 further support the concept of reducing TGF-β levels with Trabedersen/OT-101 in COVID-19 patients with ARDS [22].

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TGF-β is also involved in the pathogenesis of lung tissue remodelling and lung fibrosis that follows ARDS. Specifically, TGF-β contributes to the development of lung fibrosis by stimulating the pro¬liferation/differentiation of lung fibroblasts, accumulation of collagen and other extracellular matrix proteins in the pulmonary interstitial and alveolar space, leading to the occurrence and development of pulmo¬nary fibrosis [32, 33]. 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 signalling pathway [34]. Smad2, a key component of the canonical TGF-β signalling pathway was discovered to be regulated by miR-425 [34].Therefore, inhibition of the TGF-β signalling pathway also has the potential to prevent development of pulmonary fibrosis following ARDS and improve the pulmonary healing process [31].

## Clinical Development Strategy for COVID-19 Patients

Based on the clinical safety profile and PK of Trabedersen in cancer patients, we have designed our Phase I/II study in COVID-19 patients as a two-part study. Trabedersen will be used in combination with standard of care [35, 36] in COVID-19 patients with hypoxemic respiratory failure receiving either non-invasive positive pressure ventilation (NIPPV) or mechanical ventilation (MV). The eligibility criteria are listed in Table 7.

**Table 7. Main Inclusion and Exclusion Criteria**

**A. Inclusion Criteria**

1. Male or non-pregnant, non-lactating female subject with SARS-CoV-2 (previously known as 2019-nCoV) infection that is documented by an FDA-authorized diagnostic RT-PCR test (a) at or within 4 days of screening or (b) at baseline
2. ≥ 18 years AND ≤70 years of age
3. History of severe COVID-19 within the last 2 weeks prior to study enrollment as defined by: Respiratory distress ≥30 breaths/min, oxygen saturation ≤93% at rest in ambient air; supplemental oxygen requirement to keep oxygen saturation >90%
4. The patient or a legally authorized representative has provided written informed consent
5. The patient or the authorized representative is aware of the investigational nature of this study and willing to comply with protocol treatments, blood tests, and other evaluations listed in the informed consent form
6. Hospitalized with hypoxemic respiratory failure receiving either (a) non-invasive positive pressure ventilation (NIPPV) with Continuous Positive Airway Pressure (CPAP) or Bi-level Positive Airway Pressure (BiPAP) (clinical status: 3 on the 8-point ordinal scale) or (b) mechanical ventilation (clinical status: 2 on the 8-point ordinal scale)
7. History of worsening respiratory distress over the last 7 days
8. NIPPV or mechanical ventilation initiated ≤4 days prior to enrolment
9. Patients receiving mechanical ventilation must have acute respiratory distress syndrome (ARDS)
10. Patient's ARDS is MILD according to Berlin Definition with PaO2/FiO2 >200 AND ≤300 mmHg while on positive end-expiratory pressure (PEEP) or CPAP ≥5 cm H2O.
11. Bilateral opacities on a chest X-ray or Chest CT scan.
12. Ejection fraction on cardiac echo ≥40%
13. Grade 1 or less on CTCAE for red blood cells/haemoglobin, WBC and platelets.

**B. Exclusion Criteria**

1 Moderate or Severe ARDS by Berlin definition with PaO2/FiO2 ≤200 mmHg on a PEEP or CPAP ≥5 cm H2O; Extracorporeal membrane oxygenation (ECMO)

2 Uncontrolled hypertension (systolic blood pressure (BP) > 150 mmHg and/or diastolic BP > 100 mmHg), unstable angina, congestive heart failure (CHF) of any New York Heart Association (NYHA) classification, serious cardiac arrhythmia requiring treatment (exceptions: atrial fibrillation, paroxysmal supraventricular tachycardia), history of myocardial infarction within 12 months of enrollment;

3 Hypotension requiring vasoactive peptides, such as dopamine, norepinephrine, epinephrine, or dobutamine

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4 Renal function impairment with Creatinine >2 mg/dL; Liver function impairment with Bilirubin >2 mg/dL; Platelet count <50,000/µL; Multi-organ failure

5 Documented active infection with a bacterial pathogen requiring parenteral systemic antibiotics; Bacterial or fungal sepsis

6 History of live vaccination within the last 4 weeks prior to study enrollment; Patients must not receive live, attenuated influenza vaccine (e.g., FluMist) within 4 weeks before enrolment or at any time during the study

7 History of an allergic reaction or hypersensitivity to Trabedersen/OT101

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