Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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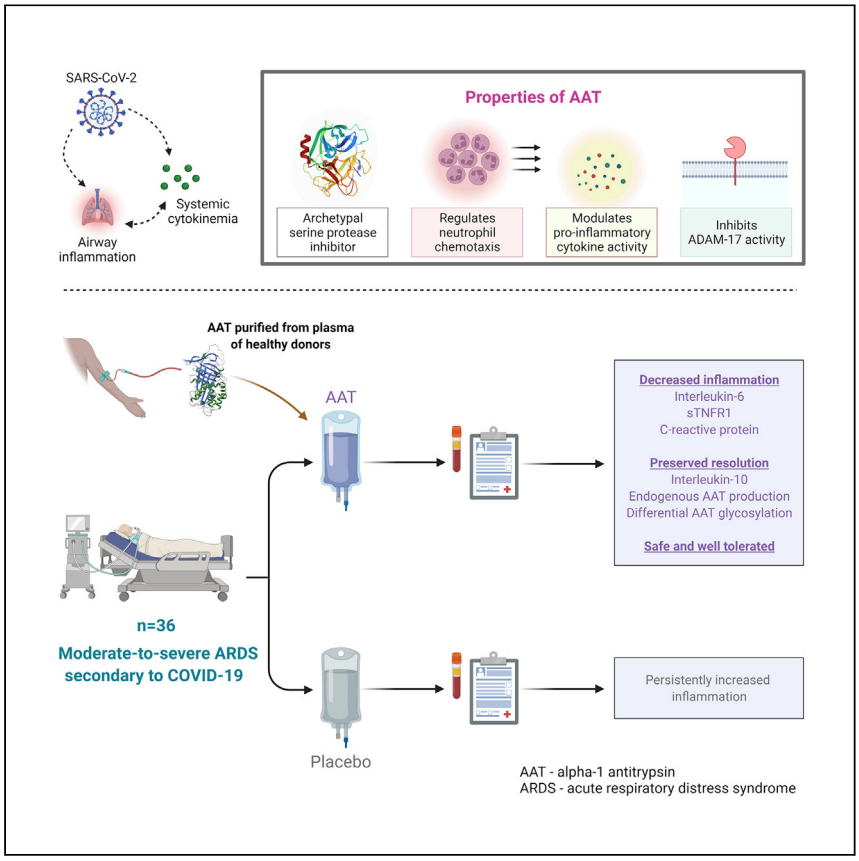
A randomized, double-blind, placebo-controlled trial of intravenous alpha-1 anti-trypsin for ARDS secondary to COVID-19



McElvaney and colleagues present the results of a randomized placebo-controlled trial of alpha-1 antitrypsin (AAT) for patients with acute respiratory distress syndrome secondary to COVID-19. Circulating levels of interleukin-6 and other pro-inflammatory mediators were decreased at 1 week in the treatment group, identifying a potential anti-inflammatory therapeutic for critical illness.

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Highlights

Patients with COVID-19-associated ARDS were randomized to receive IV AAT or placebo

Patients receiving IV AAT had decreased IL-6, sTNFR1, and CRP at 1 week

Differential glycosylation of endogenous AAT was preserved in the treatment group

Treatment with IV AAT was safe and well tolerated

Translation to Patients

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A randomized, double-blind, placebo-controlled trial of intravenous alpha-1 antitrypsin for ARDS secondary to COVID-19

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SUMMARY

Background: Patients with severe coronavirus disease 2019 (COVID-19) develop a febrile pro-inflammatory cytokinemia with accelerated progression to acute respiratory distress syndrome (ARDS). Here we report the results of a phase 2, multicenter, randomized, double-blind, placebo-controlled trial of intravenous (IV) plasma-purified alpha-1 antitrypsin (AAT) for moderate to severe ARDS secondary to COVID-19 (EudraCT 2020-001391-15).

Methods: Patients (n = 36) were randomized to receive weekly placebo, weekly AAT (Prolastin, Grifols, S.A.; 120 mg/kg), or AAT once followed by weekly placebo. The primary endpoint was the change in plasma interleukin (IL)-6 concentration at 1 week. In addition to assessing safety and tolerability, changes in plasma levels of IL-1β, IL-8, IL-10, and soluble tumor necrosis factor receptor 1 (sTNFR1) and clinical outcomes were assessed as secondary endpoints.

Findings: Treatment with IV AAT resulted in decreased inflammation and was safe and well tolerated. The study met its primary endpoint, with decreased circulating IL-6 concentrations at 1 week in the treatment group. This was in contrast to the placebo group, where IL-6 was increased. Similarly, plasma sTNFR1 was substantially decreased in the treatment group while remaining unchanged in patients receiving placebo. IV AAT did not definitively reduce levels of IL-1β, IL-8, and IL-10. No difference in mortality or ventilator-free days was observed between groups, although a trend toward decreased time on ventilator was observed in AAT-treated patients.

Conclusions: In patients with COVID-19 and moderate to severe ARDS, treatment with IV AAT was safe, feasible, and biochemically efficacious. The data support progression to a phase 3 trial and prompt further investigation of AAT as an anti-inflammatory therapeutic.

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Context and significance

Treatment options for patients with severe coronavirus disease 2019 (COVID-19), particularly those who progress to acute respiratory distress syndrome (ARDS), are limited. ARDS is a highly inflammatory state hallmarked by airway damage, respiratory failure, and increased mortality. Alpha-1 antitrypsin (AAT) is an anti-inflammatory protein produced by the liver and present the bloodstream. We investigated the use of AAT purified from the blood of healthy donors as a therapeutic option for patients with COVID-19-associated ARDS. Treatment with AAT resulted in decreased inflammation at 1 week, was safe and well tolerated, and did not interfere with patients' ability to generate their own protective response to COVID-19. The results suggest a potential role for AAT in treating COVID-19-associated ARDS and other inflammatory diseases.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a global threat to public health. As of October 2021, more than 250 million laboratory-confirmed cases have been documented, with over 5 million deaths.

Patients with severe COVID-19 typically develop a febrile pro-inflammatory cytokinemia with accelerated progression to acute respiratory distress syndrome (ARDS),[1–3] a pathological entity characterized by alveolar epithelial and lung endothelial injury, excessive protease activity, and dysregulated airway inflammation[4,5] that is associated with prolonged invasive mechanical ventilation, prolonged hospitalization, and long-term disability.[6]

Circulating concentrations of the master pro-inflammatory cytokine interleukin (IL)-6 have been shown to increase with disease severity and predict outcome in COVID-19, prompting consideration of therapies designed to counteract its well-described pathological effects. However, blanket inhibition of the cytokine in COVID-19 should be approached with caution, since IL-6 also regulates metabolism, is essential for innate and adaptive immunity, and facilitates pathogen clearance.[7–9] Critically, the physiological and pro-resolution properties of IL-6 are governed by classic signaling via the membrane-bound IL-6 receptor (IL-6R) on hepatocytes and select immune cells, while its pathological and pro-inflammatory effects are driven primarily via a process known as *trans* signaling.[7–9] In the latter, cleavage of IL-6R from the cell surface by the metalloprotease and disintegrin ADAM-17 generates a soluble receptor (sIL-6R) capable of binding circulating IL-6.[10] The resultant IL-6/sIL-6R complexes then interact with cell types that would otherwise be unresponsive to the cytokine.[7–9] In addition to orchestrating IL-6-mediated inflammatory damage, ADAM-17 drives autoimmunity by cleaving transmembrane tumor necrosis factor (TNF)-α to its active systemically available form,[11,12] and promotes neutrophil chemotaxis to IL-8.[13]

A number of nonspecific anti-inflammatories have been investigated in hospitalized patients with COVID-19.[14–21] To date, the number of prospective, double-blind trials of candidate therapeutics that have demonstrated benefit has been low, with the majority of positive results coming from open-label studies. Some drugs, such as dexamethasone, have been rapidly integrated into treatment algorithms based on data from large clinical trials.[14] Despite more mixed results,[16–21] specific anti-cytokine therapies, such as the anti-IL-6R monoclonal antibody tocilizumab, have also been implemented.

Alpha-1 antitrypsin (AAT) is a 52-kDa glycoprotein synthesized primarily in the liver, and is the archetypal serine protease inhibitor,[22–24] acting to protect the airway against damage by neutrophil elastase (NE), an omnivorous protease released by activated or disintegrating neutrophils that is increased in ARDS.[22,25–28] Moreover, AAT is a potent anti-inflammatory and immunomodulator, regulating the production and activity of several key pro-inflammatory cytokines, including IL-6, IL-1β, IL-8, and TNF-α,[29–34] while maintaining the anti-inflammatory cytokine IL-10.[35] In COVID-19, failure of the acute-phase AAT response to keep pace with increasing circulating IL-6 concentrations is associated with poor outcome in patients with severe disease requiring intensive care unit (ICU) admission.[3] Similarly, abrupt cessation of AAT augmentation therapy for patients with a hereditary deficiency of the protein results in increased systemic inflammation and subsequent progression to respiratory failure.[34] Further supporting its potential for use as a COVID-19 therapeutic, AAT also directly inhibits ADAM-17 cleavage activity[12,13] and TMPRSS2, the priming protease required for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection.[36,37] While recent open-label *in vivo* studies of AAT for hospitalized COVID-19 patients have shown evidence of an antiviral and anti-inflammatory effect,[38,39] randomized control trial data are required to support its use in clinical practice.

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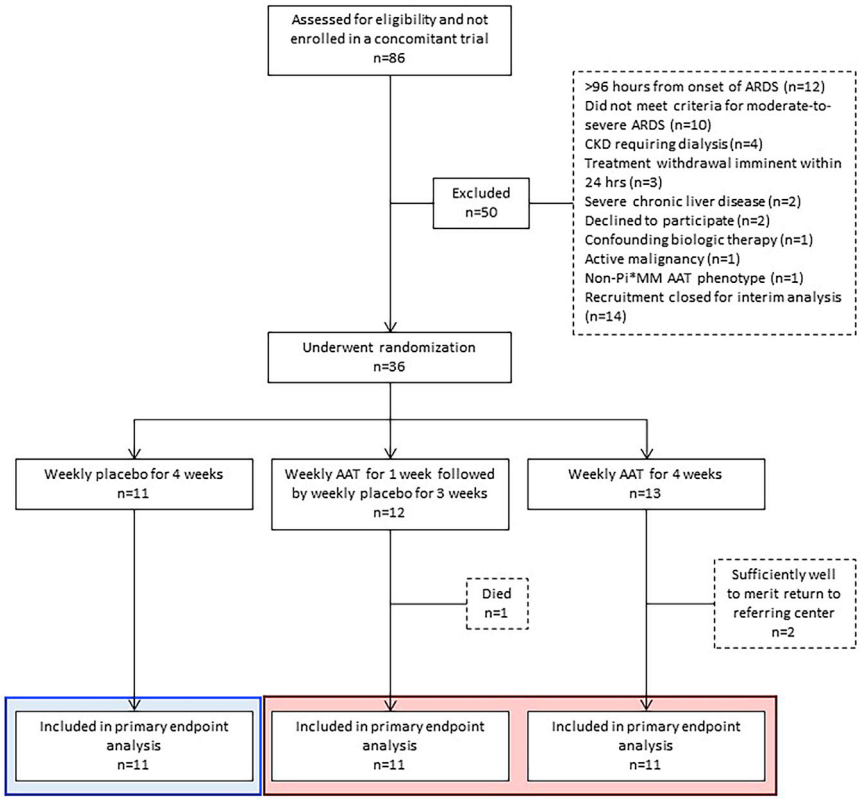

Figure 1. Consort diagram

Of 86 consecutive patients screened, 36 underwent randomization having satisfied the criteria for entry to the study. Of the 33 patients included in the primary endpoint analysis (change in circulating interleukin (IL)-6 concentration at 1 week), 22 had received a single infusion of alpha-1 antitrypsin (AAT) (highlighted in red) and 11 had received a single placebo infusion (highlighted in blue). Patients in the AAT treatment group subsequently received either weekly AAT or weekly placebo as part of a pilot safety and feasibility assessment.

In this study, based on biological plausibility, we conducted a phase 2, randomized, double-blind, placebo-controlled trial of intravenous (IV) plasma-purified AAT as an anti-inflammatory therapeutic for patients with ARDS secondary to COVID-19. The primary outcome was the change in plasma IL-6 concentration at 7 days after randomization. The secondary outcomes analyzed included the plasma concentrations of IL-1β, IL-6, IL-8, IL-10, and soluble TNF receptor 1 (sTNFR1).

RESULTS

Study participants

A total of 86 consecutive patients not enrolled in a concomitant clinical trial were screened, with 36 undergoing randomization having satisfied the criteria for entry to the study (Figure 1). Of these 36 patients, 25 were assigned to receive IV Prolastin and 11 were assigned to the placebo group. Of the 25 patients allocated to the treatment group, three discontinued the study prior to day 7. Of these three individuals, one died on day 6, and two had improved sufficiently for them to be returned to the center that referred to them. Although the latter two patients survived beyond 28 days, neither could be included in the final analysis, since the centers they were transferred to were outside the ethical jurisdiction of the trial.

Table 1. Characteristics of the cohort at randomization

	Placebo	AAT
Total number	11	25
Age (years)	57 ± 13	59 ± 11
Male/female	9 (82)/2 (18)	13 (52)/12(48)
BMI (kg/m^2)	33.4 ± 8.1	35.2 ± 11.0
SOFA score	7.8 ± 3.3	7.2 ± 3.4
Mean arterial pressure (mm Hg)	87.7 ± 12.1	87.6 ± 15.3
Tidal volume (mL)	411.4 ± 37.2	427.9 ± 64.6
Tidal volume (mL/kg IBW)	6.3 ± 0.6	6.5 ± 0.8
PEEP (cm H_2O)	13.8 ± 4.1	11.7 ± 2.9
Plateau pressure (cm H_2O)	27.7 ± 4.4	26.0 ± 5.7
PaO_2 (kPa)	10.9 ± 2.7	10.4 ± 1.8
FIO_2 (%)	70.9 ± 16.7	64.2 ± 16.8
PaO_2:FIO_2 (mm Hg)	122.5 ± 40.5	129.7 ± 38.2
Oxygenation index	12.3 ± 3.6	10.6 ± 4.4
Medications of interest		
Dexamethasone	8 (73)	18 (72)
Remdesivir	0 (0)	0 (0)
Tocilizumab	0 (0)	0 (0)
Hydroxycholoroquine	0 (0)	0 (0)
Receiving vasopressors at randomization	4 (36)	9 (36)
Circulating inflammatory markers		
Interleukin-6 (pg/mL)	259.9 ± 206.5	266.9 ± 220.9
Alpha-1 antitrypsin (g/L)	2.3 ± 0.6	2.3 ± 0.5
C-reactive protein (mg/L)	155.4 ± 118.4	154.4 ± 135.5
Known comorbidities		
Hypertension	4 (36)	12 (48)
Ischemic heart disease	2 (18)	4 (16)
Diabetes mellitus	4 (36)	6 (24)
Obesity	7 (64)	17 (68)
Chronic lung disease	3 (27)	8 (32)
Chronic kidney disease	3 (27)	7 (28)
Smoking history		
Current	0	1 (4)
Former	3 (27)	14 (56)
Never	8 (73)	10 (40)
Vaping history		
Current	0 (0)	2 (8)
Former	0 (0)	1 (4)
Never	11 (100)	22 (88)

Data presented as number (%) or mean ± SD.

BMI, body mass index; SOFA, sequential organ failure assessment; IBW, ideal body weight; PEEP, positive end expiratory pressure; PaO_2:FIO_2, ratio of partial pressure of oxygen in arterial blood to the fraction of inspired oxygen.

Patient characteristics

Baseline characteristics of the study groups at the time of randomization are available in Table 1. The study groups were adequately matched for age, body mass index (BMI), and clinical severity as assessed by the ratio of partial pressure of oxygen in arterial blood to the fraction of inspired oxygen (PaO_2:FIO_2) (122.5 ± 40.5 mm Hg in the placebo group versus 129.7 ± 38.2 mm Hg in the treatment group) and sequential organ failure assessment (SOFA) score (7.8 ± 3.3 versus 7.2 ± 3.4). Patients were receiving lung-protective ventilation with average tidal volumes of 6.3 ± 0.6 mL/kg/ideal body weight (IBW) in the placebo group and 6.5 ± 0.8 mL/kg/IBW in the treatment group. A large number of patients were either overweight

or obese, with an average BMI of 33.4 ± 8.1 kg/m^2 in the placebo group and 35.2 ± 11.0 kg/m^2 in the treatment group. Two-thirds of the total population studied had a BMI ≥30 kg/m^2. Just over one-third required vasopressors at the time of randomization. Almost three-quarters of patients were receiving dexamethasone, likely a consequence of the dissemination of preliminary results from the steroid arms of RECOVERY and REMAP-CAP on preprint servers while the present study was ongoing.

Patients in both groups had systemic inflammation, with comparable circulating levels of IL-6 (259.9 ± 206.5 pg/mL in the placebo group versus 266.9 ± 206.9 pg/mL in the treatment group), leukocyte count, C-reactive protein (CRP), lactate, D-dimer, and fibrinogen (Tables 1 and S3). AAT levels at randomization were identical between the groups (Table 1).

Plasma AAT concentrations in response to treatment with IV AAT

In patients receiving Prolastin, plasma AAT concentrations were significantly increased 2 days post infusion (Figure S1). Of note, AAT levels in the treatment group were still increased at day 7 compared with baseline, suggesting that weekly administration may result in a stacking effect. In contrast, no change in AAT levels was observed in those receiving placebo. At day 2 and day 7, plasma AAT concentrations were significantly higher in patients receiving Prolastin compared with those in the placebo group (both $p < 0.0001$).

Primary endpoint

Patients receiving Prolastin demonstrated a decrease in circulating IL-6 at day 7 compared with day 0 (day 0, 296.0 ± 219.7 pg/mL; day 7, 217.7 ± 168.7 pg/mL; Figure 2A; $p = 0.003$), whereas an increase in IL-6 levels was observed in the placebo group (day 0, 259.9 ± 206.5 pg/mL; day 7, 348.2 ± 264.0 pg/mL; Figure 2A; $p = 0.04$). Similarly, the change in plasma levels of IL-6 at 1 week compared with baseline was −78.3 ± 112.1 pg/mL in the treatment group compared with +88.3 ± 125.8 pg/mL in the placebo group (Figure 2B; $p = 0.002$), thereby satisfying the primary endpoint for the study. The percentage change in plasma IL-6 from baseline followed a similar pattern, with a mean reduction of 17.4% ± 42.3% in those receiving Prolastin versus a 37.8% ± 56.6% increase in patients assigned to placebo (Figure 2C; $p = 0.01$).

Secondary biochemical endpoints

We next investigated changes in other circulating cytokine concentrations in response to IV AAT. The measurement of TNF-α in blood is complicated by its short half-life and rapid turnover. In plasma, concentrations of sTNFR1 act as a reliable surrogate marker for TNF-α levels.[12,40] As for IL-6, a significant reduction in plasma sTNFR1 concentrations was observed in the treatment group at day 7 (day 0, 4,947 ± 2,605 pg/mL; day 7, 4,131 ± 2,207 pg/mL; Figure 3A; $p = 0.0009$). Circulating levels of sTNFR1 in the placebo group were unchanged. The absolute 7-day change in plasma sTNFR1 was +507.6 ± 1552 pg/mL (an increase) in the placebo group, compared with −815.8 ± 989.0 pg/mL (a decrease) in study participants receiving IV AAT (Figure 3B; $p = 0.02$). The percentage change in plasma sTNFR1 was also significantly different between the groups (placebo, +23.1% ± 37.7%; AAT, −15.3% ± 19.6%; Figure 3C; $p = 0.008$). These results are consisted with previous studies identifying AAT as a key regulator of TNF-α signaling and gene expression, and an inhibitor of TNF-α-induced nuclear factor κB (NF-κB) activation.[12]

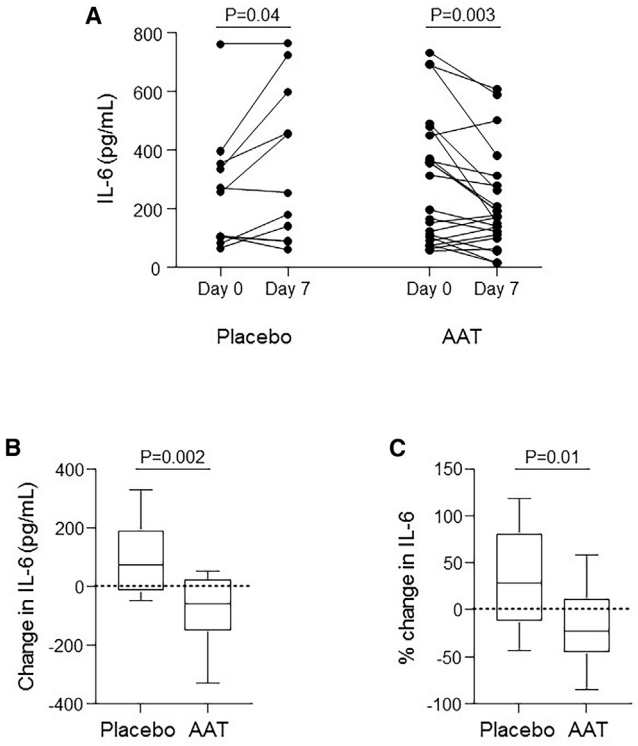

Figure 2. Decreased circulating IL-6 following treatment with IV AAT

(A) Plasma was obtained at day 0 and day 7 from patients receiving placebo (n = 11) and patients receiving AAT (n = 22). IL-6 levels were increased at day 7 compared with day 0 in the placebo group (day 0, 259.9 ± 206.5 pg/mL; day 7, 348.2 ± 264.0 pg/mL; p = 0.04) and decreased at day 7 in the AAT group (day 0, 296.0 ± 219.7 pg/mL; day 7, 217.7 ± 168.7 pg/mL; p = 0.003).

(B) The 7-day change in plasma levels of IL-6 from baseline was +88.3 ± 125.8 pg/mL in the placebo group compared with −78.3 ± 112.1 pg/mL in the treatment group (p = 0.002).

(C) Patients assigned to placebo demonstrated a 37.8% ± 56.6% increase in plasma IL-6 at day 7 compared with a mean reduction of 17.4% ± 42.3% in those receiving IV AAT (p = 0.01).

Definitive effects of AAT therapy on levels of IL-1β (Figures 3D–3F), IL-8 (Figures 3G–3I), and IL-10 (Figures 3J–3L) at 1 week were not observed. However, *post hoc* data analyses did demonstrate significant within-week differences in levels of IL-1β and IL-8 in response to IV AAT (Table S4). Within-week effects on IL-6 and sTNFR1 were also present, with the reduction in plasma levels of these cytokines greatest at day 2 post infusion, coinciding with peak circulating AAT levels.

Secondary clinical endpoints

The study was not powered to detect meaningful effects on clinical outcomes such as mortality, but data on these outcomes were collected as part of a safety and feasibility assessment. No difference in mortality was observed between patients who received placebo and those in the treatment group (Figure S3). IV AAT did not significantly reduce the time to extubation as assessed at the end of the 28-day study period compared with placebo (Figure S3), although the point estimates favored the treatment group, and this merits further investigation in a larger study. Similarly, the number of ventilator-free days, SOFA score, PaO_2:FIO_2, ICU length of stay, and hospital length of stay were numerically improved in patients receiving AAT, without reaching statistical significance (Tables 2 and S5). No increase in secondary bacterial infection was observed in the treatment group, and no rebound effect on safety or

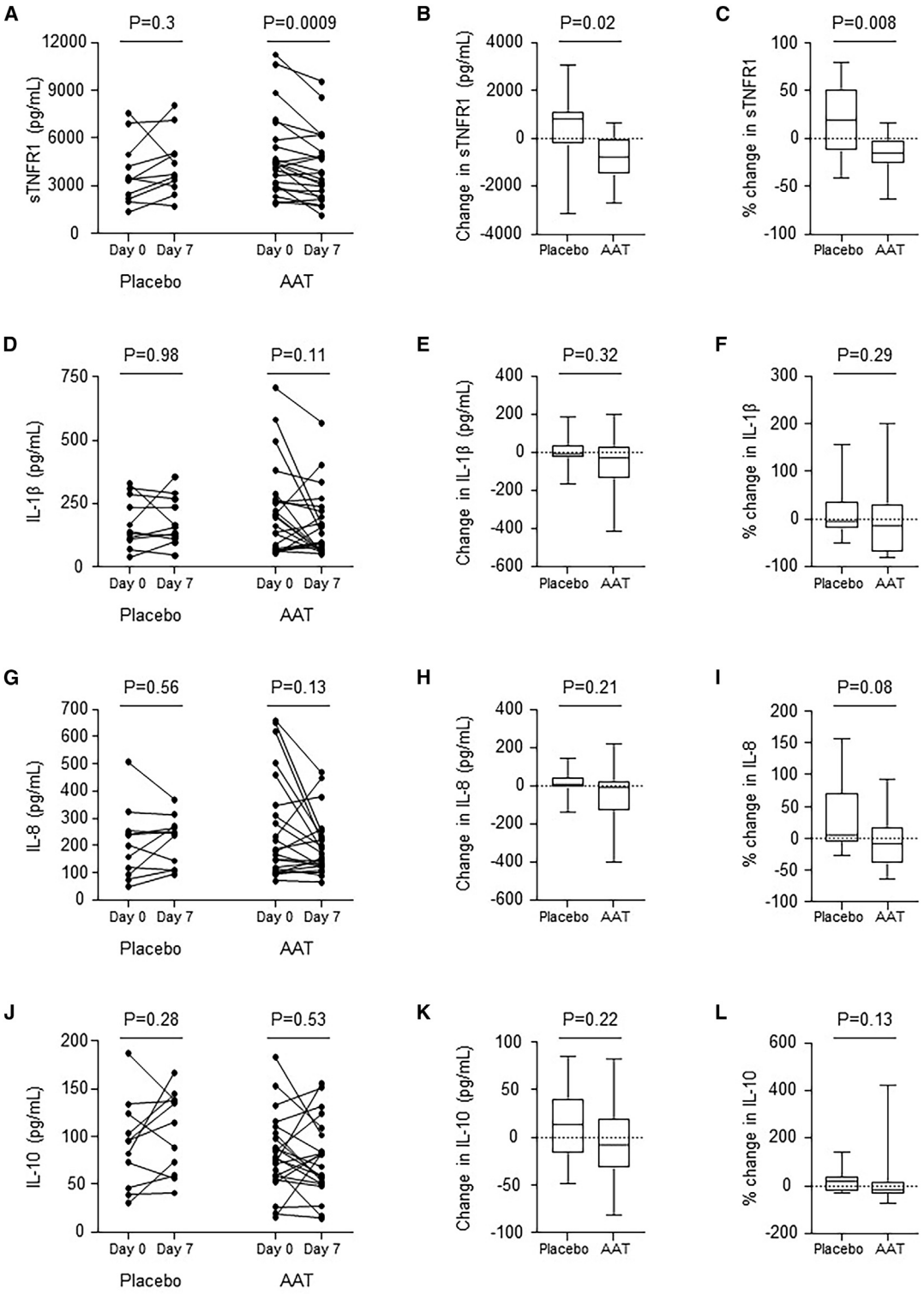

Figure 3. Secondary biochemical endpoints

(A) At day 7, levels of soluble tumor necrosis factor receptor 1 (sTNFR1) were not significantly different in the placebo group compared with day 0 (day 0, 3,808 ± 1,989 pg/mL; day 7, 4,315 ± 1,919 pg/mL; p = 0.3), in contrast to the AAT group, where sTNFR1 levels were decreased at day 7 (day 0, 4,947 ± 2,605 pg/mL; day 7, 4,131 ± 2,207 pg/mL; p = 0.0009).

Table 2. Clinical outcomes of interest

	Placebo	AAT once	AAT weekly	Any AAT
SOFA score at day 7	8.1 ± 3.5	7.3 ± 3.9	4.5 ± 4.0	5.8 ± 4.1
PaO_2:FIO_2 at day 7 (mm Hg)	127.7 ± 53.7	132.4 ± 69.1	221.4 ± 126.6	186.7 ± 135.1
Mortality	4 (36)	6 (50)	2 (15)	8 (32)
Ventilator-free days	3.6 ± 5.7	5.6 ± 10.1	10.7 ± 11.3	8.2 ± 11.3
Acute kidney injury[a]	8 (72)	9 (75)	8 (62)	17 (68)
Secondary bacterial pneumonia[a]	7 (63)	6 (50)	5 (38)	11 (44)
ICU length of stay (days)	21.4 ± 5.9	16.4 ± 9.3	15.6 ± 10.8	16.0 ± 9.9
Hospital length of stay (days)	24.6 ± 6.2	18.4 ± 8.2	20.1 ± 9.3	19.3 ± 8.7

Data presented as number (%) or mean ± SD. All variables assessed at day 28 unless stated otherwise.
ICU, intensive care unit.
[a]number of patients with an event over duration of study.

clinical outcomes was seen in patients transitioning from AAT to placebo at 1 week. A summary of clinical outcomes of interest is available in Table 2.

Safety and tolerability

No adverse events (AEs) or serious adverse events (SAEs) were considered to be related or probably related to the study drug. One AE (atrial fibrillation in a patient with known paroxysmal atrial fibrillation) was judged to be possibly related to IV Prolastin. One SAE was deemed to be possibly related to IV Prolastin (hypertension persistent for >30 min post infusion) and resolved without sequelae. No AE or SAE resulted in discontinuation of treatment.

Preservation of classic IL-6 signaling in COVID-19 ARDS patients treated with IV AAT

CRP is induced via physiologic classic IL-6 signaling—but not by pathologic *trans* signaling or *trans* presentation—as part of the acute-phase response,[7–9,41] and serves as an inflammatory biomarker in critical illness. At day 7 post infusion, patients treated with IV AAT displayed decreased levels of circulating CRP, proportional to the decreases observed for plasma IL-6 (Figure 4A). However, CRP levels post AAT were still elevated above the normal range (0–5 mg/L), indicating that the classic signaling pathway remained intact in patients receiving IV AAT at 120 mg/kg.

In addition to upregulating the production and release of endogenous AAT during the acute-phase response, IL-6 also induces a change in the glycosylation and sialylation of AAT.[30] This shift, which has previously been described in community-acquired pneumonia, results in the emergence of pro-resolution M0 and M1 AAT glycoforms on serum protein electrophoresis, a phenomenon that is specific for an IL-6-mediated acute-phase response via classic signaling.[30] Monoclonal antibodies against the IL-6 receptor such as tocilizumab do not discriminate between classic or *trans* signaling, and therefore abolish both the pathological and physiological effects of the cytokine. Immunofixation of plasma glycoforms from patients in the

(B and C) Compared with placebo, patients receiving AAT demonstrated a greater absolute change (placebo, +507.6 ± 1,552 pg/mL; AAT, −815.8 ± 989.0 pg/mL; p = 0.02) and the percentage change (placebo, +23.1% ± 37.7%; AAT, −15.3% ± 19.6%; p = 0.008) in sTNFR1 at day 7.
(D) No difference in IL-1β levels was detected at day 7 in either the placebo group (day 0, 174.7 ± 99.8 pg/mL; day 7, 179.9 ± 94.9 pg/mL; p = 0.98) or the AAT group (day 0, 224.7 ± 177.6 pg/mL; day 7; 164.9 ± 132.7 pg/mL; p = 0.11).
(E and F) Treatment with AAT did not result in a greater absolute change (p = 0.32) or percentage change (p = 0.29) at day 7.
(G–L) Similarly, no differences were observed for IL-8 on day 0 versus day 7 in either group (placebo, 205.2 ± 131.8 pg/mL versus 218.8 ± 91.3 pg/mL, p = 0.56; AAT, 264.3 ± 193.0 pg/mL versus 198.1 ± 110.1 pg/mL, p = 0.13) or for IL-10 (placebo, 92.1 ± 46.0 pg/mL versus 105.0 ± 42.9 pg/mL, p = 0.28; AAT, 82.3 ± 40.8 versus 76.9 ± 39.4 pg/mL, p = 0.53). For both IL-8 and IL-10, the 7-day change in concentration failed to reach statistical significance, either in absolute (IL-8, p = 0.21; IL-10, p = 0.22) or percentage terms (IL-8, p = 0.08; IL-10, p = 0.13).

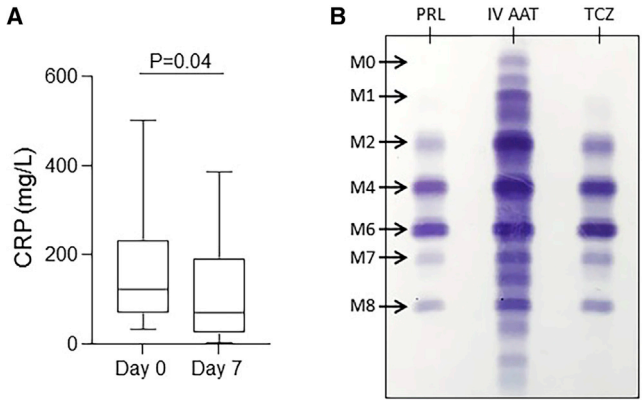

Figure 4. Physiological properties of IL-6 are preserved in patients treated with IV AAT

(A) Circulating CRP levels were measured in patients receiving IV AAT at study commencement (day 0) and again at 1 week post infusion (day 7, n = 22). CRP was decreased—but not abolished entirely—at day 7 compared with day 0 (day 0, 168.6 ± 138.0 mg/L; day 7, 110.4 ± 117.2 mg/L; p = 0.04).

(B) AAT protein phenotypes in plasma from patients in the treatment group (day 7 post infusion; IV AAT) and patients receiving tocilizumab for COVID-19 ARDS (TCZ) were determined by immunofixation of glycoforms via isoelectric focusing gel electrophoresis. Exogenous Prolastin (PRL) was also assayed, to demonstrate an absence of M0/M1 bands in the infused study drug. Endogenous M0/M1 AAT glycoforms were present in patients receiving exogenous IV AAT, but were absent in patients receiving tocilizumab (representative image).

treatment group by isoelectric focusing gel electrophoresis confirmed the presence of M0/M1 AAT glycoforms (Figure 4B), consistent with preservation of classic signaling. In contrast, when plasma from matched COVID-19 ARDS patients receiving tocilizumab was analyzed, M0/M1 glycoforms were absent, in keeping with inhibition of classic signaling.

DISCUSSION

Here we present results from a phase 2 randomized, double-blind, placebo-controlled trial of IV plasma-purified AAT for patients with moderate to severe ARDS secondary to COVID-19. Treatment with IV AAT was safe, feasible, and biochemically efficacious. Following administration of a single IV infusion of Prolastin, plasma concentrations of the pro-inflammatory cytokine IL-6 were significantly decreased, an effect mirrored by increases in circulating AAT levels. Levels of sTNFR1 were also substantially decreased at 1 week in patients receiving Prolastin. Levels of the anti-inflammatory cytokine IL-10 and differential M0/M1 glycosylation of endogenous AAT were preserved, indicating that the decrease in inflammation observed did not come at the cost of pro-resolution mediators.

While the data identify a potential role for AAT as an anti-inflammatory therapeutic in COVID-19 and ARDS, further studies are required to characterize the COVID-19 and ARDS subphenotypes[42] most likely to benefit from treatment, and the optimal method of administration. We opted for the IV route primarily because the study population had systemic inflammation with circulating cytokinemia. However, using aerosolized AAT may also prove effective. There are several potential advantages to aerosolized therapy: it limits the potential for volume overload, a relevant consideration in obese patients, and can achieve good deposition in the airways of spontaneously breathing patients (although this is less clear for patients who are intubated and mechanically ventilated).[43] Data

are also available regarding dose equivalency; in previous studies in cystic fibrosis (CF) for example, the AAT concentration in epithelial lining fluid following a dose of 3 mg/kg was roughly equivalent to that achieved by a 120 mg/kg IV dose.[43]

When designing future studies that incorporate aerosolized therapy, identifying the patient groups most likely to benefit from such an approach represents a key challenge. In COVID-19, aerosolized AAT might prove to be most effective in patients with inflammation mostly confined to the lungs. Although using an airway-directed therapy for a disease that initially takes hold in the respiratory tract seems intuitive, aerosolized AAT therapy may struggle to sufficiently regulate inflammation in patients who have progressed to severe cytokinemia. In these individuals, IV AAT or a combination strategy may be preferable.

To project the ability of IV AAT at 120 mg/kg to provide antiprotease protection in the airway, we can extrapolate from prior trials involving this therapy in other conditions,[22,28,38,43–49] and present-day investigations of protease activity in COVID-19 lungs.[50–53] In a previous study examining IV AAT in patients with CF, IV AAT at a dose of 120 mg/kg was capable of providing an antiprotease effect.[43] More recently, open-label use of IV AAT at the same dose for CF complicated by severe cytokinemic COVID-19 resulted in decreased NE activity in airway secretions, following a direct AAT/NE binding event.[38] The NE activity levels observed in these CF studies were higher than those reported in non-CF patients with COVID-19-associated ARDS, suggesting that the dose used here is likely to have provided an antiprotease effect not captured by the current study.

Our study is not without limitations. The population studied was small, and this prevents meaningful conclusions regarding clinical outcomes from being drawn. However, when it comes to studies with a biochemical endpoint, a more modest cohort size may in fact be desirable. Accurate measurement of IL-6 can be affected by a multitude of patient variables, including age, obesity, chronic disease, and medications such as IL-6 receptor antagonists.[9,54–56] Factors relating to processing and handling of samples also stand to pre-analytically influence assay measurements, in particular delays in processing and over-agitation during transit.[31,57–60] Furthermore, the cytokine displays significant diurnal variation, the most conspicuous effect being a trough in the morning,[61] making the timing of sampling especially important. In designing the present study, we were conscious of these potential confounders to our primary endpoint and decided to focus on a smaller number of well-matched patients with intensive follow-up and careful handling and processing of samples so as to maximize precision. Indeed, the concentrations of IL-6 reported here are in line with those described in prior studies of COVID-19-associated ARDS that used similar protocols.[3,62–67]

Although circulating IL-6 levels have been shown to correlate well with clinical outcomes, interpretation of an elevated IL-6 level in isolation when comparing disease severity, without consideration of the balance between physiological and pathological IL-6 signaling that determines its biological effects, may lead to conclusions that are somewhat misleading. In this study, this obstacle was mitigated by each patient serving as their own control. Moreover, in addition to baseline IL-6, CRP and AAT levels in each group were highly similar at randomization. Since both CRP and AAT are induced almost exclusively by classic IL-6 signaling via the liver, this indicates the balance of IL-6 signaling in patients receiving placebo was comparable with those receiving Prolastin. Furthermore, given that CRP was not abolished in

the treatment group, it can be concluded that classic signaling remained intact in these individuals. One potential explanation for this might be decreased cleavage of the IL-6R from the surface of hepatocytes by ADAM-17, the activity of which is directly inhibited by AAT. This represents a potential advantage over tocilizumab, which nonspecifically blocks all IL-6 signaling. Indeed, interventions that decrease IL-6-driven inflammation in a manner weighted toward blockade of ADAM-17-mediated *trans* signaling may represent a safer strategy, since they are more likely to preserve the physiological functions of IL-6, which include enhanced bacterial clearance.[68] As for most monoclonal antibody therapies, tocilizumab cannot be reversed once administered and, at high serum concentrations, has a terminal half-life of approximately 21 days. In contrast, the half-life of Prolastin is considerably shorter, at 4.6 days.

Over 70% of study participants were receiving dexamethasone, a glucocorticoid with anti-inflammatory effects that is now considered standard care for critically unwell patients with COVID-19. Although an anti-inflammatory effect can safely be attributed to AAT since an equal percentage of patients in each group were receiving dexamethasone therapy, further study is required to determine if the effect of AAT in steroid-treated patients is an additive or synergistic one. Similarly, it is also possible that steroid treatment masked modest anti-inflammatory effects of AAT on cytokines such as IL-1β and IL-8. The mid-study emergence of dexamethasone as a cornerstone of management in COVID-19-associated ARDS was an unavoidable consequence of an evolving situation and reflects the well-documented phenomenon of preprint data shaping policy during the current pandemic.[69] This underscores the difficulty of undertaking clinical trials in COVID-19, and highlights a need to account for the emergence of other novel therapeutics when projecting the sample size required for a phase 3 study.

Indeed, when applying findings such as these, the evolution of COVID-19 itself must be factored in. As new SARS-CoV-2 variants continue to surface, it is possible that the immune response to the subsequent respiratory syndrome will change. The impact of vaccination on the efficiency of immune responses to SARS-CoV-2 should also be considered: in this study, none of the patients studied were vaccinated against COVID-19, whereas, at the time of writing, Ireland has a national vaccine coverage of greater than 94%. However, the recent emergence of escape variants capable of infecting triple-vaccinated, healthy people highlights the enduring importance of identifying effective therapeutics for those who go on to develop severe disease despite taking the requisite precautions.

Moreover, the paradox of pandemic medicine is that, if a drug is actually found to be beneficial, its supply may be exhausted. The more options available to clinicians, therefore, the better. The data described here support progression to a larger phase 3 trial focusing on clinical endpoints, and prompt further investigation of AAT as an anti-inflammatory therapeutic in critical illness.

Limitations of the study

The present study demonstrates the anti-inflammatory effect of IV AAT in critically unwell patients with moderate to severe ARDS secondary to COVID-19. However, a larger trial is required to determine the effect of this therapy on clinical outcomes such as mortality or ventilator-free survival. Similarly, the data do not clarify the role of AAT in hospitalized patients outside the ICU. While the study groups were matched for age, healthcare system, baseline inflammation, and clinical severity as assessed by PaO_2:FIO_2 and SOFA score, they were not matched for sex. The

population had a variety of races and ethnicities, with an even distribution between groups, but the relatively small sample size meant that differences in outcomes or response to AAT could not be discerned.

At the time of study commencement, treatment with dexamethasone was not standard of care for severe COVID-19-associated ARDS. Although over 70% of patients in each group were receiving dexamethasone at randomization, further study is required to determine if the effect of AAT in steroid-treated patients is an additive or synergistic one. None of the patients studied were vaccinated against COVID-19. The impact of vaccination on immune responses to SARS-CoV-2 was therefore not captured.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.medj.2022.03.001.

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AUTHOR CONTRIBUTIONS

O.J.M., G.F.C., and N.G.M. conceptualized the study. O.J.M., N.L.M., F.B., G.F.C., and N.G.M. designed the study. O.J.M., J.G.L., I.M.-L., G.F.C., and N.G.M. contributed to design of the clinical trial protocol and study ethics submission. F.B. designed the randomization protocol and statistical analysis plan, randomized patients, and analyzed data. O.J.M., N.L.M., O.F.M., G.H., and T.P.C. performed the experiments. K.D., O.F., E.B., J.C., A.K., P.G., M.H., and I.M.-L. collected clinical data. J.C.H. was the study pharmacist. O.N.C., D.D.F., E.O., R.McG., A.M.C., A.C., and D.H. reconstituted and delivered blinded study drug and placebo. M.P.M., T.P.C., and D.D.F. carried out quality control. O.J.M., F.B., G.F.C., and N.G.M. co-wrote the manuscript. All authors read and approved the final article and take responsibility for its content.

DECLARATION OF INTERESTS

O.J.McE. has been an investigator in clinical trials for Vertex and Chiesi, reports speaking fees—all outside the present unfunded study—from AstraZeneca and Novartis, and reports current funding from the Elaine Galwey Memorial Research Bursary. N.G.McE. has been an investigator in clinical trials for CSL Behring, Galapagos, Chiesi, and Vertex, and reports personal fees—all outside the present unfunded work—from CSL Behring, Grifols, Chiesi, and Shire. G.F.C. currently receives funding from the Health Research Board via an Emerging Clinician Scientist Award (ECSA-2020-009). The remaining authors declare no competing interests.

INCLUSION AND DIVERSITY

One or more of the authors of this paper self-identifies as a member of the LGBTQ+ community. While citing references scientifically relevant for this work, we also actively worked to promote gender balance in our reference list. The author list of this paper includes contributors from the location where the research was conducted who participated in the data collection, design, analysis, and/or interpretation of the work.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human plasma	Study participants (Beaumont Hospital, St. James' Hospital)	N/A
Chemicals, peptides, and recombinant proteins		
Plasma-purified alpha-1 antitrypsin ("Prolastin")	Grifols S.A.	PA 1405/002/001
Critical commercial assays		
IL-1β ELISA	R&D	#DLB50
IL-6 ELISA	R&D	#D6050
IL-8 ELISA	R&D	#D8000C
IL-10 ELISA	R&D	#D1000B
sTNFR1 ELISA	R&D	#DRT100
Hydragel 18 A1AT Isofocusing kit	Sebia	#PN4357
Deposited data		
Deidentified raw and analyzed data file	This paper	Mendeley data: https://doi.org/10.17632/xrrkf8fr9y.1
Software and algorithms		
STATA 13.0	www.stata.com	N/A
Graphpad Prism 7.0	www.graphpad.com	sales@graphpad.com
SpectraMax M3 plate reader	Molecular Devices	N/A
Chemi Doc MP System	Bio-Rad	#17001402
Image Lab	Bio-Rad	https://www.selectscience.net/products/image-lab-software-(170-9690)/?prodID=115956

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Prof. Noel G. McElvaney (gmcelvaney@rcsi.ie).

Materials availability

This study did not generate new unique reagents.

Data and code availability

Individual participant data (including data dictionaries) will be made available in a manner compliant with European Union general data protection regulations (GDPR). Deidentified individual participant data and raw data derived from human samples that underlie the results reported in this article are deposited in Mendeley. The DOI is listed in the Key resources table. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Characteristics of the study groups at randomization, including total number, age, sex, body mass index (BMI), past medical history, clinical status and physiological parameters are reported in Table 1. Participant information on sex, age, ethnicity and race was self-reported. Information on gender and socioeconomic status was not collected. Biochemical characteristics at randomization are available in Tables 1 and S3.

The trial protocol was approved by the Health Products Regulatory Authority (HPRA) and the Health Research Consent Declaration Committee (HRCDC) of Ireland, Beaumont Hospital Ethics Committee (BHEC, REC #20/38), and institutionally at Beaumont Hospital and St. James' Hospital, both tertiary referral academic medical centers in Dublin.

Patients were recruited to the study using a next-of-kin assent/deferred consent framework developed in conjunction with and approved by the HRCDC and BHEC. In the event that a participant regained capacity, the investigators obtained written informed consent from each subject to continue in the study. This framework, which was designed specifically for the present study, also provided for the use of a remote virtual assent and consent process given the visitor restrictions and patient isolation protocols in place at the time of study commencement.

METHOD DETAILS

Trial design and oversight

The study was an investigator-initiated, randomized, double-blind, placebo-controlled, parallel-group clinical trial. The trial protocol was published prior to completion of enrolment.[70] A planned data and safety interim analysis was conducted after recruitment of the tenth study participant.

Patients (n = 36) were enrolled between April 20, 2020, and March 18, 2021. Hospitalized patients with a working diagnosis of ARDS secondary to COVID-19 were screened on arrival to the ICU. Eligible patients were aged $\geq$18 years, had a laboratory confirmed diagnosis of COVID-19, and were receiving ventilator support for moderate-to-severe ARDS with PaO_2:FIO_2 <200mmHg. In all patients diagnosed with COVID-19, SARS-CoV-2 infection was confirmed by RT-PCR of a nasopharyngeal swab specimen.

Patients were excluded if they were more than 96 hours from onset of ARDS, receiving extracorporeal life support, known to be pregnant or breastfeeding, or had a history of active malignancy requiring treatment within the last year, pulmonary embolus on a prior admission within the previous 3 months, chronic kidney disease requiring dialysis, severe chronic liver disease, WHO class III/IV pulmonary hypertension, or major trauma in the preceding 5 days. Patients were also excluded if they were receiving specific anti-cytokine therapies, had hereditary AAT deficiency (AATD), or had participated in a clinical trial of another immunomodulatory investigational medicinal product (IMP) within 30 days, given the potential confounding effects. A summary of inclusion and exclusion criteria for the study are available in Table S1.

Investigational medicinal product

Plasma-purified AAT (Prolastin) was provided free of charge by Grifols S.A., Spain, on compassionate grounds following a written request by the investigators. A weekly dose of 120 mg/kg was chosen given the half-life of IV AAT, and its successful inhibition of airway NE in AAT-deficient individuals.[44] IV administration was preferred to the aerosol route for two reasons. First, patients with severe COVID-19 frequently display significant systemic inflammation; second, the use of an aerosol-generating device may have introduced a safety hazard by increasing the risk of viral transmission. The placebo used in the study was 0.9% sodium chloride solution for infusion (normal saline).

Properties and administration of investigational medicinal product

Prolastin is a sterile, stable, lyophilized preparation of purified human AAT, prepared from pooled human plasma from healthy donors by modification and refinements of the Cohn cold ethanol plasma fractionation technique followed by a purification process. Prolastin contains small amounts of other plasma proteins, which may include IgA, haptoglobin, alpha1-acid glycoprotein, lipoprotein A-1, and albumin. Reconstituted Prolastin contains no preservatives and has a pH of 6.6 to 7.4. Unblinded members of the study team – who were not involved in the care of trial patients, the entry of outcome data or the statistical analysis – prepared the masked infusions at predesignated sites on each hospital campus prior to transporting them to the bedside. Unblinded team members were instructed not to reveal the treatment allocation for a study participant unless the participant was subject to emergency unblinding measures; no such instance occurred during the trial. Of note, IV AAT may exhibit a mild color tinge under certain lighting conditions. Furthermore, if unduly agitated, a minor – but visible – froth may develop on the liquid surface within an infusion bag. For these reasons, all study infusions (Prolastin and placebo) were covered using identical opaque infusion sets designed specifically for the study to ensure adequate blinding. A similar strategy was used in prior placebo-controlled double-blind RCTs of IV AAT for patients with hereditary AAT deficiency, including the RAPID study.

Randomization and patient groups

Randomization was performed using a centralized, computer-generated allocation sequence supervised by the trial statistician and stratified by trial site and age ($<$50 years and $\geq$50 years). Eligible patients were randomly allocated using blocks of size 3 to one of 3 groups: weekly AAT for 4 consecutive weeks, weekly AAT for one week followed by weekly placebo for 3 weeks, or weekly placebo for 4 weeks. This approach aimed to achieve a ratio of patients receiving a single AAT infusion to patients receiving a single placebo infusion of approximately 2:1 at one week. Similarly, at day 7 post-infusion, the subdivision of the AAT group (one half of the AAT group receiving weekly AAT for a further 3 weeks and the other half switching to placebo) was designed to investigate whether a safety signal would emerge with repeated dosing and to assess the feasibility of weekly infusions.

Treatment assignments were concealed from patients, clinicians involved in patient care, blinded investigators, and the committee performing the interim data and safety analysis.

Outcomes

The primary outcome was the change in plasma IL-6 concentration at 7 days after randomization.

The secondary outcomes analyzed included the plasma concentrations of IL-1β, IL-6, IL-8, IL-10 and sTNFR1, a plasma surrogate for circulating TNF-α. Safety and tolerability of Prolastin was defined by the number of adverse events (AEs) and serious adverse events (SAEs) related or possibly related to the active IMP. Changes in circulating AAT levels over the course of the study were also recorded.

Similarly, although the study was not powered to detect meaningful differences in clinical outcomes, data were collected on mortality, ventilator-free days (VFDs), time-to-extubation, sequential organ failure assessment (SOFA) score, pulmonary physiological parameters, development of shock, acute kidney injury and clinical relapse, since this information stands to inform the design of a larger study. Thus,

in addition to serving as a phase 2 trial with a biochemical primary endpoint, the present trial doubled as a pilot and feasibility study in advance of a phase 3 trial focusing on clinical endpoints. All outcome definitions appear in Table S2.

Plasma preparation and storage

Plasma was obtained by centrifugation of whole venous blood at 250 x *g* for 5 minutes at room temperature. Blood samples were obtained at the bedside under sterile procedures and transferred immediately to the laboratory for processing. The time from sample acquisition to completion of processing was capped at 30 minutes. Cytokine measurements were undertaken by a blinded investigator not involved in the clinical care of the patients. Similarly, results were not shared with the treating physicians so as not to bias or influence the clinical outcomes assessed. To minimize the potential for inter-assay variability, plasma supernatants were stored immediately at −80°C, with samples thawed and assayed on communal ELISA plates *en bloc*, with a selection of samples run on every plate as points of reference.

Measurement of AAT levels and circulating mediators of inflammation

AAT levels were measured centrally at Beaumont Hospital by immunoturbidimetric assay.[71] Regular blood sampling was undertaken immediately before the first dose (day 0), 2 days post-dose to coincide with peak circulating AAT levels (day 2),[28] and at 7 days post-dose (day 7), with further sampling at days 14, 21 and 28. AAT protein phenotypes in plasma were determined by immunofixation of glycoforms via isoelectric focusing gel electrophoresis prior to analysis.[72] Plasma levels of IL-1β, IL-6, IL-8, IL-10 and sTNFR1, were measured by ELISA (all R&D systems, Minneapolis MN, USA) in accordance with the manufacturer's instructions.

Reporting of adverse events and serious adverse events

Although plasma-purified AAT has an established safety profile in patients with hereditary AAT deficiency (AATD),[47,73–75] it has not been extensively studied in critically unwell populations. For patients recruited prior to the interim analysis, adverse event (AE) and serious adverse event (SAE) reporting was undertaken in line with guidelines specified by the HPRA for studies involving novel COVID-19 therapeutics. Continuation of the study beyond this probationary period was contingent on a favorable safety assessment as part of the interim analysis.

AEs and SAEs were reported to day 28. Events expected in this critically ill population were not reported as AEs unless considered to be related to the study drug, unexpectedly severe or frequent or atypical for a patient with ARDS. In addition, and in accordance with HPRA guidance, the following pre-specified adverse events occurring within 2 hours of the start of infusion were recorded:

1. New ventricular tachycardia (persistent tachycardia lasting >30 minutes)
2. Clinical scenario consistent with infusion reaction (e.g. urticaria, angioedema, new bronchospasm, anaphylaxis).
3. Persistent hypotension lasting >30 minutes and requiring intervention with vasopressors
4. Persistent hypertension lasting >30 minutes and requiring intervention with hypotensive agents
5. Any event occurring within this time period considered by the investigator to be atypical in a patient with ARDS.

Data entry

At each study visit, clinical characteristics, process variables and outcome data were inputted into electronic case report forms (eCRFs). AEs and SAEs were identified by treating clinicians and documented in real time and reported to the study sponsor. The relatedness of AEs or SAEs to the study drug was adjudicated by two blinded consultant physicians of requisite experience. Trial data were monitored at the sites (including consent and source data verification) by independent monitors according to a pre-specified monitoring plan and centrally by pre-assigned staff at each study center.

Testing availability and patient vaccination status at study commencement

During the study period, all individuals with a history of recent travel to a designated high-risk or endemic area, recent close contact with a confirmed case, or symptoms in the presence of clinical suspicion were eligible for COVID-19 testing. Testing via RT-PCR of nasopharyngeal swabs was made nationally available, and was free of charge. Similarly, medical treatment for patients with COVID-19 in Ireland, including treatment delivered in the intensive care unit, was provided free of charge by the Irish Health Services Executive. Although a national vaccination rollout has resulted in a population coverage of over 93% at the time of writing, this figure was lower at the time of trial commencement. Of the 36 patients recruited to the study, zero patients were vaccinated against SARS-CoV-2/COVID-19.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis

Results are reported as absolute numbers or means and standard deviations, as appropriate. Categorical variables are summarized as counts and percentages. No imputation was made for missing data. The primary efficacy analysis was on an intention to treat basis. Changes in the levels of inflammatory biomarkers within each patient group were analyzed using paired t-tests for normally distributed data and a nonparametric paired Wilcoxon signed-rank test in the event of data failing the test for normality. Changes between groups were analyzed using unpaired t-tests for normally distributed data and nonparametric Mann-Whitney testing for non-normally distributed data. Kaplein-Meier plots were also used to explore time-to-mortality and time-to-extubation over the 28 day follow-up period. The statistical analysis plan was approved prior to completion of the study. An independent data monitoring committee (DMEC) performed an interim data and safety analysis after recruitment of the 10th trial subject. Analyses and graphing of data were conducted using Stata 13.0 and GraphPad Prism 8.0 software for Windows. A value of $P < 0.05$ was considered statistically significant.

Sample size

The recruitment sample size was chosen based on prior studies evaluating the primary biochemical endpoint[22,28,76] and previous broad recommendations for similar studies pilot studies.[77–79] Since the present study served only as a pilot study with respect to clinical outcomes, a formal sample size calculation was not undertaken regarding these endpoints.

Study feasibility

With the exception of the initial AE/SAE reporting framework required, no substantial feasibility issues arose. No doses of study drug or placebo were delayed and none were missed. In 10 of the infusions administered over the course of the study, the infusion time came within 30 minutes of exceeding the allocated cut-off of 3 hours. In each case, the patient had a BMI ≥ 30 kg/m^2. In addition to a dysregulated

immune response, obese patients with COVID-19 are more likely to require ICU support, and are also more likely to have comorbidities when compared to patients of equivalent age with a normal BMI. A standard 1g vial of Prolastin is reconstituted in 40ml of sterile water before use – given the dosing regimen of 120 mg/kg/week, this equates to a total volume of 480ml per infusion for a 100kg person. Some of the patients on this study were as heavy as 200kg. Furthermore, given the set time allocated for infusion, larger volumes could not be spaced out under the study protocol. In clinical practice, this may risk volume overload in some patients and necessitate the use of diuretic therapy. At recruitment, venous access for each patient was established via a multi-lumen central venous catheter. However, following discharge of a patient to the ward, we relied on peripheral venous access for drug administration. Failure of peripheral venous access required temporary suspension of infusion on 3 occasions while access was established elsewhere.

Effects of age and sex on response to AAT therapy

Post-hoc analyses did not demonstrate a differential response to therapy in study participants <50 years compared to those ≥50 years (Table S7). Perhaps the most robust data available regarding the effect of sex on the response to AAT comes from the two largest clinical trials of IV AAT augmentation therapy (RAPID, its open-label extension study RAPID-OLE, and EXACTLE), and their respective subgroup analyses.[43,45,80] None of these studies demonstrated a differential response to AAT between sexes. Our trial was no different in this regard. In patients receiving IV AAT, no significant sex differences were observed regarding changes in cytokine levels following therapy (Table S7).

Levels of total circulating protein over time in patients receiving IV AAT

Given that AAT levels measured at one week post-infusion were elevated compared to pre-infusion levels, we examined whether an effect on total protein levels, and by extension a theoretical effect on oncotic pressure, would be observed over time in patients receiving weekly IV AAT compared to patients who transitioned from IV AAT to placebo at day 7 (Table S9). A statistically significant difference in total protein concentration did not emerge until day 28.

Although it would seem unlikely that weekly dosing would result in a clinically significant effect on oncotic pressure given the short half-life of IV AAT and the offsetting effect of exogenous AAT administration (and its anti-inflammatory effects) on endogenous AAT production and generation of other acute-phase plasma proteins, interpretation of the protein levels reported here is complicated by several factors. In patients with severe inflammation and ARDS due to COVID-19, hypoalbuminemia is common, not only because of hepatic injury and/or impaired hepatic perfusion, but also because of a downregulated production of albumin by the hepatocyte in favor of other acute phase proteins. Patient attrition in the cohorts also presents a challenge – some of these patients died, some recovered well enough to be transferred back to their referring center, and others improved sufficiently to be discharged home. Further study is therefore required to clarify.