CORONAVIRUS

Complement C3 inhibition in severe COVID-19 using compstatin AMY-101

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Complement C3 activation contributes to COVID-19 pathology, and C3 targeting has emerged as a promising therapeutic strategy. We provide interim data from ITHACA, the first randomized trial evaluating a C3 inhibitor, AMY-101, in severe COVID-19 (PaO2/FiO2 ≤ 300 mmHg). Patients received AMY-101 ($n = 16$) or placebo ($n = 15$) in addition to standard of care. AMY-101 was safe and well tolerated. Compared to placebo (8 of 15, 53.3%), a higher, albeit nonsignificant, proportion of AMY-101–treated patients (13 of 16, 81.3%) were free of supplemental oxygen at day 14. Three nonresponders and two placebo-treated patients succumbed to disease-related complications. AMY-101 significantly reduced CRP and ferritin and restrained thrombin and NET generation. Complete and sustained C3 inhibition was observed in all responders. Residual C3 activity in the three nonresponders suggested the presence of a convertase-independent C3 activation pathway overriding the drug's inhibitory activity. These findings support the design of larger trials exploring the potential of C3-based inhibition in COVID-19 or other complement-mediated diseases.

INTRODUCTION

Severe coronavirus disease 2019 (COVID-19) is a systemic, multiorgan, hyperinflammatory disorder that is characterized by high in-hospital mortality. Innate immune pathways are excessively activated during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, including neutrophil activation and the key defensive systems of complement and coagulation (*1*–*4*). SARS-CoV-2 can trigger all three pathways of complement activation, which converge at the proteolytic cleavage of the central complement protein C3 (*5*). C3-mediated pathways are broadly involved in a wide spectrum of pathogenic processes that exacerbate COVID-19 pathology (*1*, *6*, *7*). Moreover, systemic complement activation has been documented in hospitalized patients with COVID-19, and elevated C3 and C5 activation fragments have invariably been correlated with worse prognosis and disease severity (*5*). Accumulating evidence indicates that hyperactivation of the complement system in COVID-19 fuels the aberrant inflammatory response, leading to neutrophil-driven immunothrombosis, endothelial cell injury, blood-organ dysfunction, and acute respiratory distress syndrome (ARDS) (*3*, *7*–*9*). Therefore, targeting C3 may provide early control of the thromboinflammatory response that inflicts most of the tissue damage in patients with severe COVID-19.

AMY-101 is a clinically developed third-generation compstatin-based C3 therapeutic. Compstatins comprise a family of small peptidic compounds that block complement C3 activation exclusively in humans and nonhuman primates (*10*). Mechanistically, AMY-101 binds C3 and blocks its binding to and cleavage by the C3 convertases into its active fragments C3a and C3b. As a consequence, generation of the proinflammatory anaphylatoxins C3a and C5a and deposition of C3b, amplification via the alternative pathway, and all downstream complement-mediated actions are prevented regardless of the initiating trigger or pathway of complement activation (*11*).

Experimental studies indicated that there is no significant metabolism of the free drug or that any metabolites are very rapidly eliminated from circulation (*12*, *13*). In addition, biodistribution studies demonstrated a combination of hepatobiliary and renal clearance as routes of AMY-101 elimination (*14*).

The first evidence that C3 inhibition can effectively intercept COVID-19–associated thromboinflammation was provided in three previously described patients who received compassionate therapy with AMY-101 (*15*). We have recently shown that AMY-101 disrupts neutrophil-mediated tissue factor expression and attenuates

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the formation of thrombogenic neutrophil extracellular traps (NETs), supporting a key role for complement and NETosis in COVID-19 immunothrombosis (*3*).

Here, we describe key preliminary data from the ITHACA study, the first randomized, placebo-controlled, phase 2 trial targeting C3 in respiratory failure associated with severe COVID-19. We report clinical observations related to the therapeutic response to C3 inhibition and pharmacokinetic/pharmacodynamic profiles together with biological findings that may shed light on the global impact of C3 inhibition on complex pathogenic mechanisms underlying severe COVID-19.

RESULTS

Respiratory function and clinical outcomes

We analyzed patients affected by severe SARS-CoV-2 infection, as attested by a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) at ≤300 mmHg (*16*). The patients were randomized to receive either AMY-101 or placebo (1:1) in addition to standard-of-care (SOC) treatment. Detailed information for the design of the protocol can be found in Materials and Methods.

Administration of AMY-101 was safe and well tolerated, and no serious adverse events or adverse events related to drug administration were encountered (tables S1 and S2). On day 14 of the study, a higher proportion of AMY-101–treated patients (13 of 16, 81.3%) than placebo-treated patients (8 of 15, 53.3%) attained resolution of ARDS (PaO_2/FiO_2 > 300 mmHg) without oxygen requirement in room air; patients attaining resolution were characterized as "responders." However, the differential response rate between the two groups did not reach statistical significance (P = 0.097) (Fig. 1A). With regard to early changes in the severity of respiratory failure, as indicated by the PaO_2/FiO_2 ratio, the oxygenation status of a higher proportion (albeit nonsignificant, P = 0.103) of AMY-101–treated patients (12/16, 75%) than placebo-treated patients (5 of 13, 38.5%) improved from severe/moderate to mild (PaO_2/FiO_2 > 200) as early as day 7 (Fig. 1B). In line with this trend toward rapid improvement of respiratory function in the experimental group of the study, the mean time from treatment initiation (day 1) to discharge was almost 4 days shorter in the AMY-101–treated patients who survived than in the placebo-treated survivors (13.2 days versus 17.1 days, respectively, P = 0.089).

At baseline, none of the patients were in intensive care unit (ICU) or under mechanical ventilation. In total, six patients (three in the AMY-101 group) required ICU admission and mechanical ventilation during the study. Three patients from the AMY-101 (18.7%) group and two from the placebo group (13.3%) did not survive because of COVID-19–related complications such as refractory ARDS and/or pulmonary embolism (P = 1.000).

Systemic inflammation markers

Growing evidence indicates that severe COVID-19 is associated with marked neutrophilia and lymphopenia, as well as features of a pronounced systemic inflammatory response, including high serum C-reactive protein (CRP) levels and hyperferritinemia (*17*, *18*). To interpret the trend toward early improvement in respiratory function observed in the AMY-101–treated patients during the first 7 days of therapy, we sought to determine the impact of AMY-101 treatment on the blood counts and profile of key proinflammatory markers between days 1 and 7. There was no significant difference in peripheral blood counts, namely, absolute neutrophil count (ANC) or absolute lymphocyte count (ALC), between the two treatment groups (Fig. 2, A and B); however, administration of AMY-101 led to a significant reduction in CRP and ferritin levels on day 7 (P = 0.003 and 0.023, respectively), indicating an anti-inflammatory effect of C3 inhibition (Fig. 2, C and D).

C3 inhibition and thromboinflammatory response

Severe COVID-19 is characterized by a distinct thromboinflammatory response that affects the vasculature of vital organs, leading to micro- and/or macrothrombosis (*19*). In this context, increased plasma

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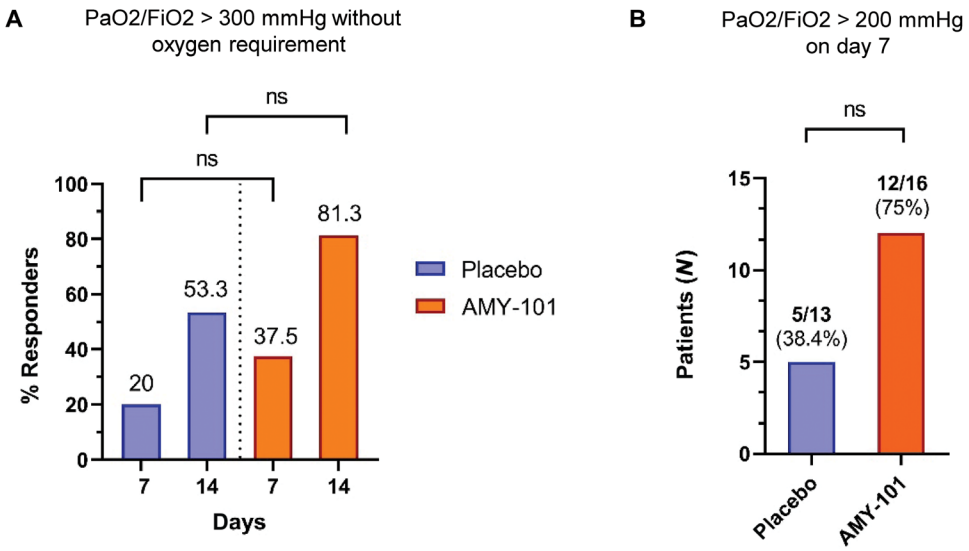

Fig. 1. Response rate and early changes in oxygenation need in study patients. (**A**) Proportion of patients who achieved resolution of respiratory failure (PaO_2/FiO_2 > 300 mmHg without oxygen requirement) on days 7 and 14 after treatment initiation and (**B**) number of patients whose oxygenation status had changed from severe/moderate to mild (PaO_2/FiO_2 > 200 mmHg) on day 7 after treatment initiation. For (A) and (B), chi-squared test. ns, not significant.

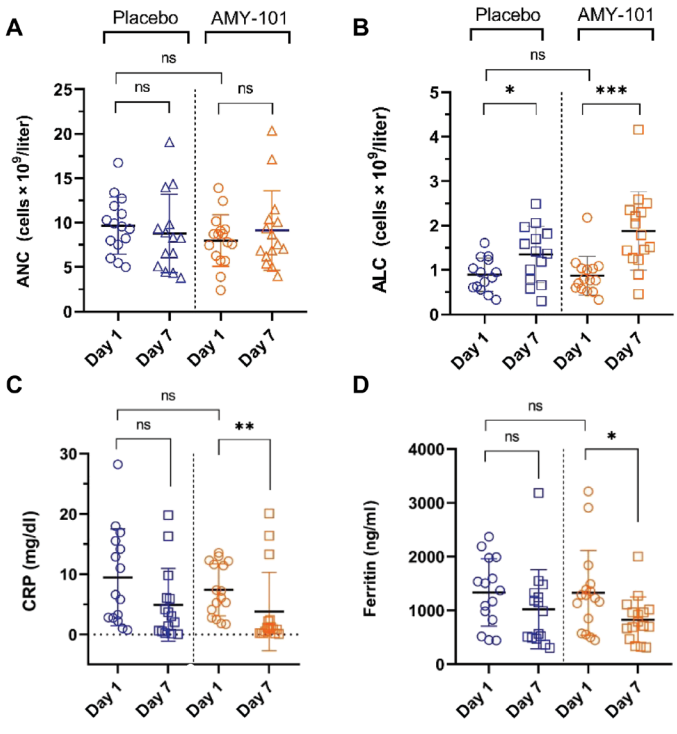

Fig. 2. Hematologic and inflammatory markers. (**A**) ANC, (**B**) ALC, (**C**) CRP, and (**D**) ferritin levels on day 1 (baseline) and day 7 after the initiation of treatment. For (A) to (D), a Mann-Whitney test was performed for unpaired analysis across groups, whereas paired analysis within a single group was performed using the Wilcoxon test; means ± SD. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

D-dimer concentration resulting from activated coagulation and consequent fibrinolysis has proven to be a clinically useful biomarker in patients with COVID-19, mainly in those who are severely ill. Moreover, several studies have indicated a key role for procoagulant NETs in the induction and propagation of thrombosis in COVID-19, and NET remnants such as circulating cell-free DNA, citrullinated histone H3 (CitH3), and myeloperoxidase-DNA complexes are known to be increased in the plasma and tissues of patients with COVID-19 (*20*). AMY-101 administration attenuated plasma D-dimer levels on day 7 and mean CitH3 levels in plasma through day 14 when compared to placebo ($P = 0.031$ and 0.024, respectively) (Fig. 3, A and B). In addition, the plasma of AMY-101–treated patients showed consistently lower thrombin generation, as indicated by thrombin–anti-thrombin (TAT) activity, than did that of the placebo-treated patients, although the difference was not significant (Fig. 3C).

Pharmacodynamic/pharmacokinetic analysis of AMY-101

To examine the pharmacodynamic effect of AMY-101, we measured the levels of C3a and sC5b-9 in plasma as markers of complement activation. C3a levels remained significantly lower in the AMY-101 group ($P = 0.002$) (Fig. 4A). In this group, a decrease in C3a was observed as early as day 2 ($P < 0.001$); C3a partly returned to pretreatment levels by day 14 ($P = 0.001$), reflecting the early discontinuation of AMY-101 administration because of the favorable response seen in most of the patients. In the AMY-101–treated patients, C3a reached baseline levels on day 21 ($P = 0.019$), remaining at these levels up to day 44 (last day of the study) (Fig. 4A). This measurement was coupled with pharmacokinetic analysis in plasma that demonstrated constant C3-saturating therapeutic levels of AMY-101, leading to complete and sustained inhibition of C3 activation in responders throughout the treatment window (Figs. 4, B and C, and 5, A and B). Moreover, sC5b-9 levels were significantly lower in the AMY-101 group ($P = 0.031$); no significant variability was observed between day 1 and day 44 time points ($P = 0.107$) (Fig. 4A). When markers of complement activation were compared in responders versus nonresponders to AMY-101, we found that C3a levels were significantly higher in nonresponders, as supported by analysis on day 2 ($P = 0.039$) (Fig. 6, A and B). In contrast, sC5b-9 levels remained higher than the upper normal values regardless of the response to treatment (Fig. 6C).

Together, these results suggest that C3a levels in plasma may be a more suitable and early (day 2) marker than sC5b-9 for monitoring disease activity and response to treatment during AMY-101 administration.

Residual C3 activity in AMY-101 nonresponders

Despite C3-saturating therapeutic levels of AMY-101 (Fig. 5, A and B), residual C3a generation was observed during AMY-101 treatment, mostly in nonresponders (Figs. 4C, 5B, and 6A). The difference between responders and nonresponders was observed as early as day 2 (Fig. 6B). Notably, in all three AMY-101 nonresponders, C3a generation seemed to exhibit a rebound effect after an initial inhibition (Fig. 5B).

To better understand the causes of residual C3 activity during AMY-101 treatment, we investigated potential AMY-101–induced immunogenicity related to the generation of antidrug antibodies (ADAs) that could alter pharmacokinetic and pharmacodynamic properties, reducing drug efficacy. However, even in severe cases, we did not detect any AMY-101–specific increase in ADAs that could neutralize the drug's therapeutic effects or cause severe adverse events in the patients.

Various proteases of the coagulation and fibribolytic pathways have previously been shown to directly activate C3 through proteolytic cleavage in a C3 convertase-independent manner (*21*). Recent studies confirm that the thrombin and kallikrein/kinin systems are strongly activated in severe COVID-19 (*22*). We found that a trend toward increased plasma thrombin and kallikrein activity, as assessed by TAT and prekallikrein-kallikrein conversion rate, respectively, was associated with the negative clinical outcome of the AMY-101 nonresponders (Fig. 7, A and B). While these results are only correlative and far from definitively demonstrating a direct impact of high thrombin/kallikrein activity on complement activation, they offer a plausible scenario supporting the bypass activation of C3 in the presence of a therapeutic C3 inhibitor. The aberrant expression of proteases of the coagulation system, such as thrombin and kallikrein, which has been documented during COVID-19 immunothrombosis, could likely contribute to the convertase-independent bypass activation of C3 in the small subset of patients who exhibit a more prominent hypercoagulable phenotype. Such a "bypass" pathway may override the inhibitory activity of AMY-101, even at target-saturating levels.

DISCUSSION

Several lines of evidence indicate that deregulated complement activation is a key driver of COVID-19–associated cytokine storm and

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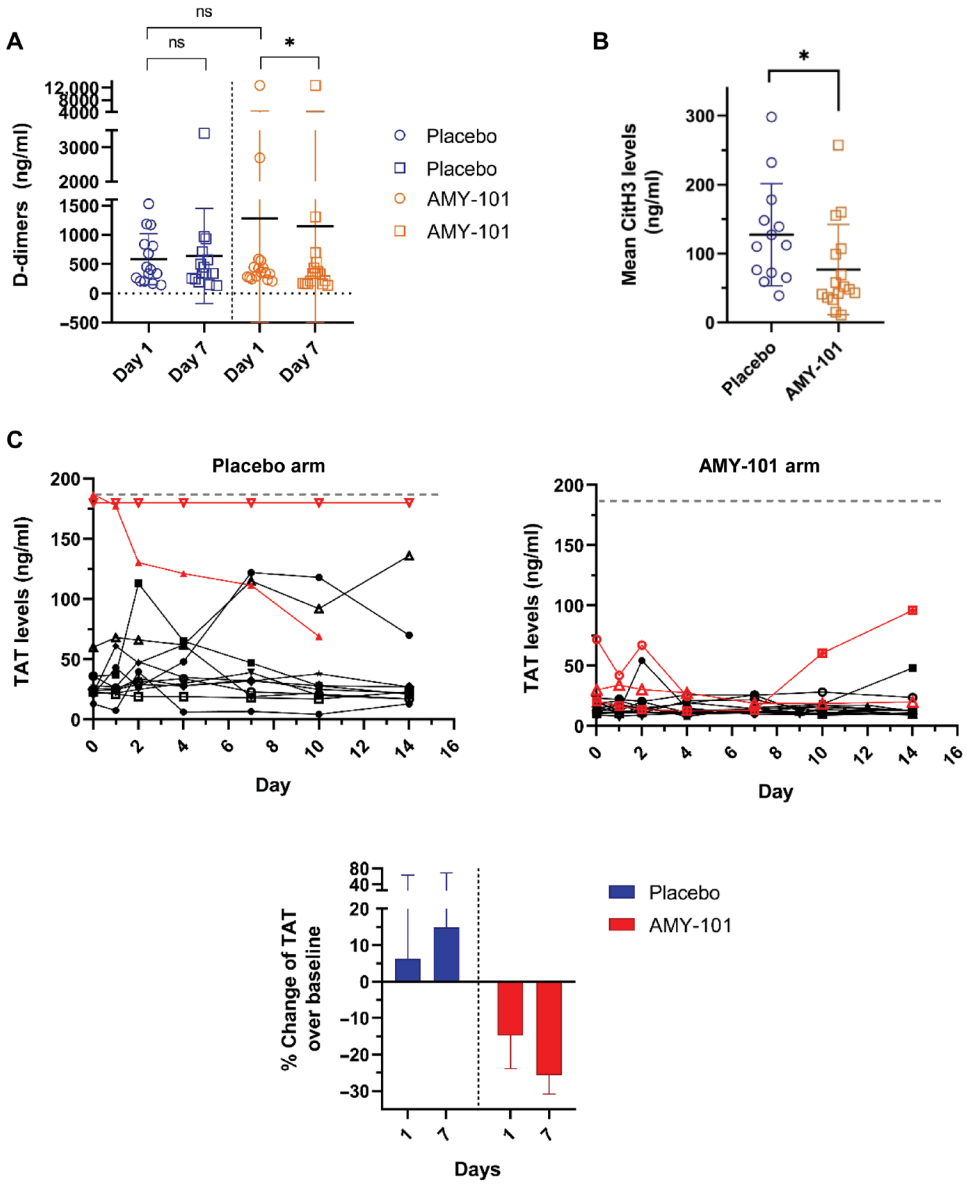

Fig. 3. Markers of thromboinflammation. (**A**) D-dimer levels in plasma on day 1 (baseline) and day 7 after treatment initiation, (**B**) Mean NETosis levels as denoted by the plasma concentrations of CitH3, and (**C**) TAT complexes in plasma after treatment initiation. For (A), paired analysis within the same group was performed using the Wilcoxon test. For (B), a Mann-Whitney test was performed for unpaired analysis across groups; means ± SD. *$P < 0.05$. For (C), upper graphs display the individual TAT profiles of each patient per group. The TAT profiles corresponding to nonsurvivors in both groups are shown in red. The gray dashed line denotes the upper cut-off value of the TAT assay. One placebo patient recorded plasma TAT levels, which were steadily above the upper cutoff value of the instrument, and therefore, all these values were arbitrarily set to the upper cutoff value. The lower graph indicates the percent change of mean TAT levels (±SD) over baseline for each group.

hyperinflammation, possibly leading to microvascular endothelial damage and immunothrombosis associated with progression to severe respiratory failure (*3*, *5*, *19*). Growing evidence supports the broader pathogenic involvement of C3 activation in COVID-19–related thromboinflammation, while C3 fragments have been implicated as prognostic factors for disease severity and adverse clinical outcomes (*15*, *23*). Accordingly, several case series using repurposed or experimental drugs targeting various complement components, including C3, have reported encouraging results (*24*). However, clinical experience with complement therapeutics in the context of randomized controlled trials (RCTs) remains limited, with only one phase 2 RCT, thus far having released the results of anti-C5a blockade in patients with severe COVID-19 (*25*).

The current study provides early data from the ITHACA phase 2 RCT regarding the efficacy and safety of a C3-targeted therapeutic, AMY-101, in patients with severe COVID-19. However, because of the low number of patients included, no definitive conclusion can be drawn with regard to clinical efficacy. Nevertheless, this study provides key biologic readouts about the impact of therapeutic C3 inhibition in severe COVID-19 that have been missing from the field and could better inform the design of future clinical studies using C3 inhibitors in similar clinical conditions.

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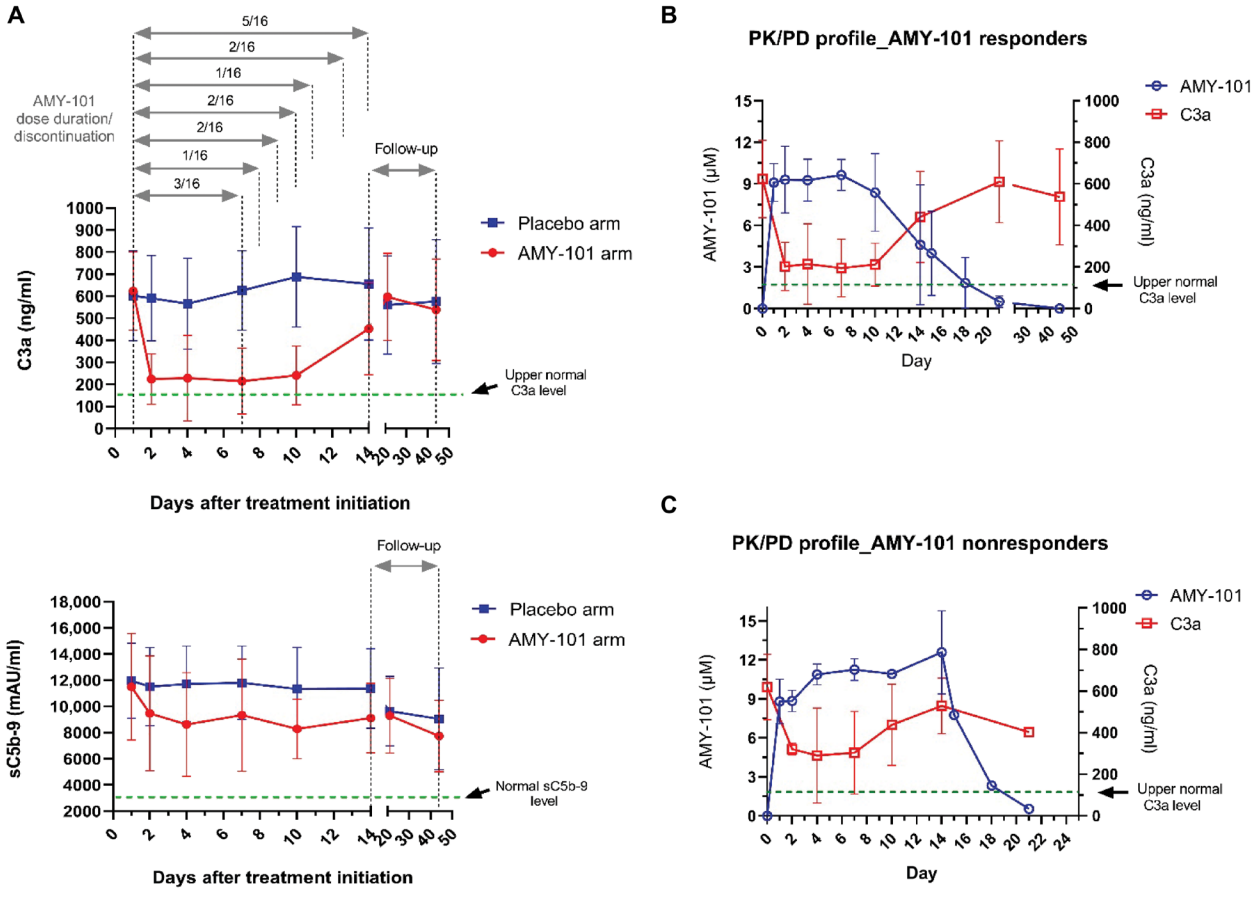

Fig. 4. Complete and sustained inhibition of C3 activation in AMY-101 responders throughout the treatment. (**A**) Plasma C3a and sC5b-9 levels during the treatment period and up to the last day of follow-up (day 44 of the study). (**B** and **C**) AMY-101 pharmacokinetic analysis and plasma C3a kinetics during the treatment and follow-up periods. Panel (B) shows the averaged pharmacokinetic/pharmacodynamic (PK/PD) profile of all AMY-101 responders. The duration of the AMY-101 infusion was 7 to 14 days, depending on the clinical outcome of the patient (i.e., time of resolution of COVID-19–associated ARDS or end of treatment, whichever came first). The proportion of patients receiving each AMY-101 dosing regimen and the various dosing regimens are indicated with gray-colored arrows over the graph in (A). (C) The averaged PK/PD profile of the three AMY-101 nonresponders. It should be noted that nonresponder #1 underwent treatment with AMY-101 for 7 days, while nonresponders #2 and #3 received AMY-101 infusions through day 14. For (A) to (C), means ± SD.

AMY-101 reached C3-saturating plasma levels in all patients who received the drug, resulting in complete and sustained inhibition of C3 in responders. Of note, most of the patients (randomized either to AMY-101 or placebo) displayed elevated plasma C3 concentrations at baseline (ranging from 7 to 9 μM), likely resulting from a pronounced acute phase response and immune activation after SARS-CoV-2 infection. Sustained C3 inhibition led to a favorable outcome in 81.3% of the AMY-101–treated patients, which was clinically observed in most cases by day 7 of the treatment. All patients in the experimental group exhibited rapid respiratory decompensation at the time of treatment initiation (mean $PaO_2/FiO_2 < 150$). Typically, these patients with COVID-19 are characterized by a severe disease course, lengthy hospitalization, and high mortality rate (*26*–*28*). In this "difficult-to-treat" group of patients with COVID-19, we observed a beneficial trend for AMY-101 therapy added to standard dexamethasone and low–molecular weight heparin (LMWH) treatment. Moreover, no serious adverse events related to drug use or drug-induced immunogenicity were observed throughout the 44-day follow-up period.

Results from two small clinical trials regarding C5 therapeutics in COVID-19 have recently been published. First, in a nonrandomized, single-institution trial, the anti-C5 monoclonal antibody eculizumab was administered as emergency treatment together with standard care in 35 ICU patients with severe COVID-19. The results were compared to 45 ICU control patients who were treated with SOC alone. Although eculizumab improved day 15 survival in comparison to SOC (82.9% versus 62.2%, log-rank test, $P = 0.04$) and reduced inflammatory markers, it was also associated with an increased risk of ventilator-associated pneumonia (51% versus 24% in SOC) and bacteremia (11% versus 2% in SOC). It was concluded that larger, RCTs are needed to corroborate the results of this study (*29*).

Second, in PANAMO, a RCT phase 2 study, the anti-human C5a monoclonal antibody IFX-1 was administered to patients with severe COVID-19. Patients received IFX-1 and best supportive care ($n = 15$) or best supportive care alone ($n = 15$). IFX-1 appeared to be safe in patients with severe COVID-19; however, the trial did not show statistically significant differences in clinical end points (*25*).

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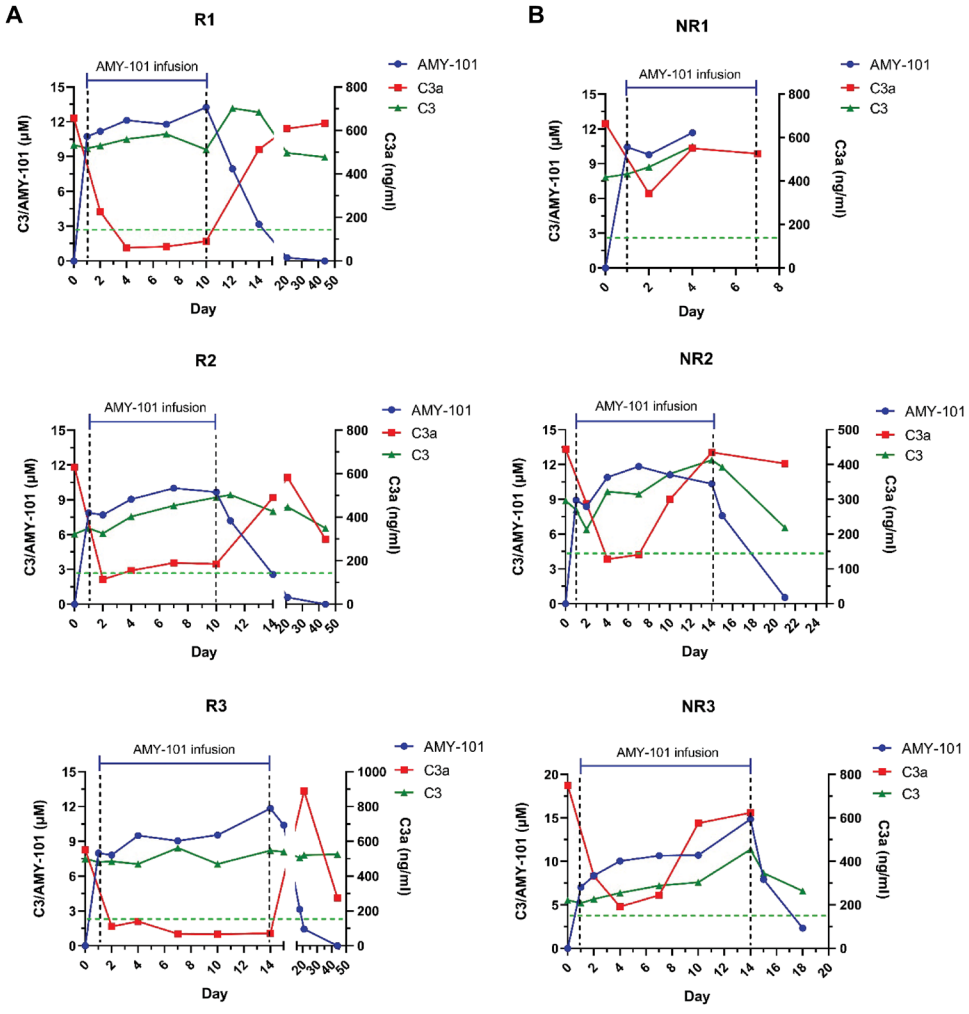

Fig. 5. C3-saturating therapeutic levels of AMY-101. AMY-101 pharmacokinetic analysis and plasma C3 and C3a kinetics during treatment and follow-up for (**A**) three representative responders (R) and (**B**) the three nonresponders (NR) to AMY-101. The green dashed line within the PK graphs denotes the upper normal C3a levels.

A comparative study involving small cohorts of patients with severe COVID-19 treated with the anti-C5 mAb eculizumab and the C3 inhibitor AMY-101 revealed distinct mechanistic features of complement C3 versus C5 inhibition and pointed to a broader therapeutic control of detrimental thromboinflammatory complications during C3-targeted intervention (*15*).

Severe COVID-19 is characterized by rapid progression of hypoxemic respiratory failure. Administration of AMY-101 led to a reduction in systemic biomarkers related to disease progression such as CRP, ferritin, D-dimer, and C3a, and it prevented excessive thromboinflammation by restraining thrombin generation and thrombogenic NET formation. This biological efficacy was reflected in the trend toward early improvement in the respiratory status on day 7 (PaO_2/FiO_2 > 200) that was observed in 75% of the AMY-101–treated patients. This improvement confirms previous findings in patients with COVID-19 who were treated with AMY-101 under a compassionate use program (*15*).

The absence of significant differences between the two cohorts in terms of peripheral blood counts, including neutrophils, may reflect the impact of corticosteroid treatment that was part of the SOC used in our patient cohort. Although previous results in a small series of patients had indicated an inhibitory effect of AMY-101 on neutrophil counts, these patients had not received dexamethasone or other corticosteroids (*15*).

Our analysis of complement activation markers indicated that plasma C3a levels can potentially be used as a candidate biomarker for treatment response in patients under AMY-101 therapy. The subgroup analysis of AMY-101–treated patients revealed that C3a reduction was more pronounced in responders, whereas residual C3a generation was detected in the three nonresponders despite sustained therapeutic levels of AMY-101 and the absence of ADAs. These findings suggest that in a subgroup of severely affected patients, an extrinsic pathway of C3a generation probably exists that leads to residual complement activity. Previous work has suggested that coagulation/fibrinolysis proteases such as thrombin, kallikrein, and plasmin may act as "noncanonical" C3 convertases, cleaving C3 directly to generate biologically active C3a and C3b (*21*, *30*). Accumulating evidence has demonstrated potent thrombin generation and strong activation of the kallikrein/kinin and fibrinolysis systems in patients with severe SARS-CoV-2 infection (*3*, *22*). This

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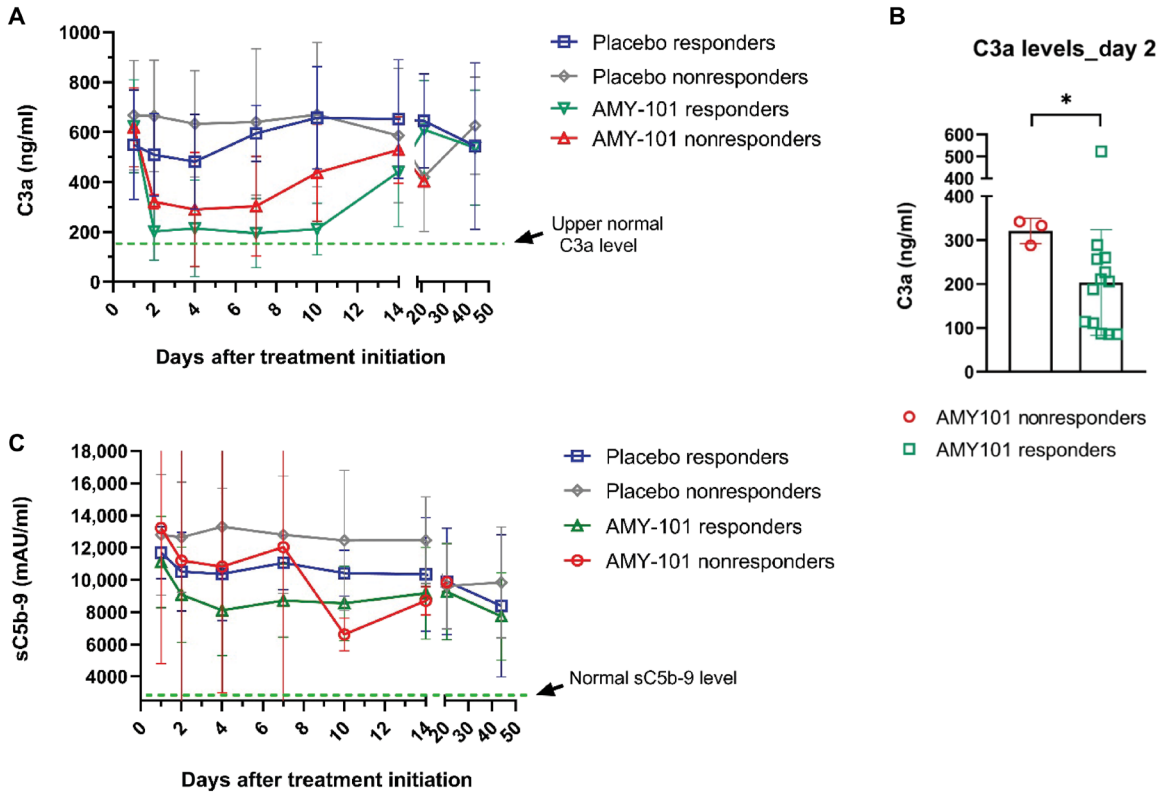

Fig. 6. Markers of complement activation and response to treatment. (**A**) C3a kinetics in plasma throughout the study and response to treatment. (**B**) Plasma C3a levels in AMY-101–treated patients on day 2 after treatment initiation. One of the responders showed high levels of C3a on day 2. This patient displayed a small decrease of C3a levels through day 4, fluctuating close to the baseline value through day 14. This PD pattern likely reflects the convertase-independent generation of C3a. The transient decrease of C3a in this patient through day 4, in a time frame associated with disease progression, likely represents the C3 convertase-targeted inhibitory effect of AMY-101. (**C**) sC5b-9 kinetics in plasma throughout the study and in relation to the response to treatment. For (A) to (C), means ± SD. For (B), statistical differences between the two groups were determined using a Mann-Whitney nonparametric test. *$P < 0.05$.

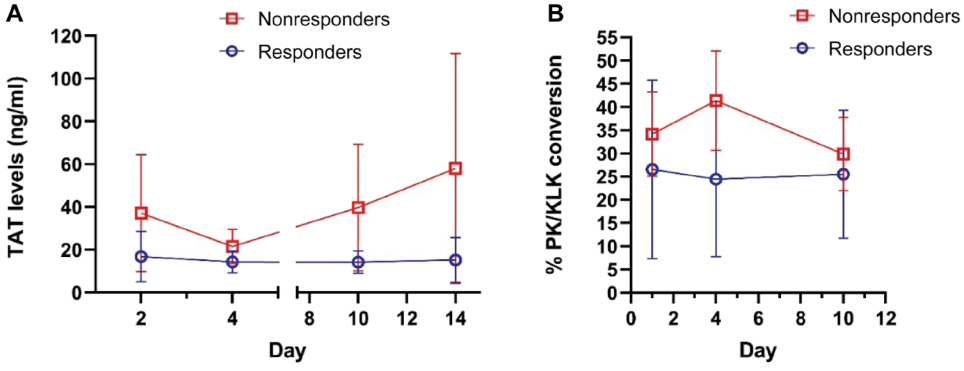

Fig. 7. TAT levels and kallikrein activity in the AMY-101–treated group. (**A**) TAT complexes and (**B**) prekallikrein (PK)–kallikrein (KLK) conversion rate in plasma after AMY-101 administration. Means ± SD.

complex network of proteases may operate in parallel with the canonical routes of complement activation, thereby serving as an extrinsic proteolytic pathway that links coagulation and complement, amplifying COVID-19–related thromboinflammation and tissue damage (*19*). In line with this concept, high plasma thrombin and kallikrein activity was observed in the three AMY-101–treated patients who showed partial C3 inhibition and a rebound of C3a levels in plasma during treatment. Consistent with their hypercoagulable state, two of the three AMY-101 nonresponders experienced fatal thromboembolic events (pulmonary embolism). The reasons for the occurrence of this hypercoagulable state remain elusive and may include both genetic and acquired factors driving an individual's

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thrombogenic propensity. Although several in vitro and preclinical studies have suggested a convertase-independent, "bypass" complement activation pathway (*21*, *30*), the results of the current study suggest a protease-mediated bypass of canonical C3 activation as a likely pharmacodynamic breakthrough mechanism in the clinical context of therapeutic C3 inhibition.

The return of plasma C3a to baseline levels after the discontinuation of therapy on day 14 and the prolonged detection of these levels up to day 44 indicate that probably a "second wave" of complement activation persists after the end of therapy. Whether this rebound of C3 activation contributes to long-COVID clinical sequelae (*31*) beyond the 44-day follow-up period of this trial remains to be determined in future studies. On the other hand, it can also be argued that a "second wave" of C3 activation could reflect the beneficial homeostatic actions of complement in facilitating the clearance of cell debris and/or the removal of immune complexes and other noxious by-products of severe COVID-19–related tissue damage (*32*).

Despite the attainment of C3-saturating therapeutic levels of AMY-101 and almost complete C3 inhibition in most patients, sC5b-9 generation was only partially inhibited in the presence of AMY-101. Thus, terminal pathway activation remained only partially suppressed, likely because of a "C3 bypass" mechanism that allowed C5 activation to proceed despite complete C3 inhibition. The most plausible explanation for this finding is that C5, similar to C3, can be directly activated by thrombin and perhaps other proteolytic enzymes of the coagulation/fibrolytic pathways that are highly expressed in severely infected COVID-19 patients (*33*, *34*). A C3 bypass activation of C5 can also be observed in cases of strong classical pathway (CP) activation, in which increased surface density of C4b may prime C5 for proteolytic activation, leading to C5b-9 formation (*35*). Consistent with this finding, more than 90% of critically ill patients with COVID-19 have complement-fixing autoreactive immunoglobulin M (IgM) and IgG with a promiscuous epitope recognition repertoire against a variety of tissue self-antigens (*36*). The presence of high levels of these autoantibodies could trigger the CP (*37*, *38*), thus allowing a continuous C3 bypass activation of C5, even in the presence of a C3 inhibitor.

Selecting the optimum timing of therapeutic intervention with complement blockers in patients with severe COVID-19, including C3 or C5 inhibitors, is a matter of paramount importance and should be integral to the design of relevant clinical trials. On the basis of the pathophysiology of the disease, complement inhibition is expected to exert its beneficial therapeutic effects during the host hyperinflammatory phase that follows the early virus replication phase and is responsible for the propagation of detrimental thromboinflammatory reactions to several vital organs besides the virus-infected lungs (*39*).

In conclusion, C3 inhibition with compstatin AMY-101 has potential as a safe therapeutic choice against severe COVID-19; AMY-101 elicited complete and sustained C3 inhibition throughout the treatment, leading to resolution of respiratory failure in more than 80% of patients. Nevertheless, residual C3 activity was found in a subgroup of AMY-101–treated patients, likely as the result of excessive thrombin/kallikrein activity that can overcome the C3 inhibitory effect of AMY-101, eventually compromising its efficacy. These observations suggest that combined therapeutic approaches targeting distinct components of the thromboinflammatory response may convey greater benefit in severe COVID-19 (*3*). Plasma C3a levels could serve as a reliable predictor of drug effectiveness and inform tailored therapeutic approaches. These clinical and mechanistic insights lay the groundwork for exploring the potential of C3-based inhibition in larger RCTs for COVID-19 and for other severe conditions characterized by complement-mediated hyper-(thrombo)inflammation.

MATERIALS AND METHODS

Study design

We analyzed initial data from the first 31 patients with COVID-19 enrolled up to the interim stage in ITHACA, a multicentered phase 2 randomized (1:1), placebo-controlled, clinical trial (EudraCT number: 2020-004408-32), from 19 November to 22 December 2020. The study was initially planned to enroll up to 62 patients in a period of 6 months. The original number of patients powered for superiority. The trial was discontinued prematurely by the sponsor at the interim stage of analysis because of funding constraints unrelated to futility or safety of the trial. The decision to terminate this trial was made by the sponsor before the disclosure and analysis of the interim results.

The primary end point of the study was the proportion of patients alive, without evidence of ARDS, as denoted by $PaO_2/FiO_2 > 300$ mmHg in room air, on day 14 from treatment initiation. We present early key data, together with biological observations included in the secondary end points of the study. Patients were eligible to participate in the trial provided that they fulfilled the following criteria: (i) age $\geq$ 18 years and (ii) diagnosed with severe SARS-CoV-2 infection, according to the following: (i) a positive reverse transcription polymerase chain reaction test for SARS-CoV-2 RNA in nasopharyngeal swab or bronchio-alveolar lavage (BAL), (ii) having a ratio of the partial pressure of oxygen (PaO_2) to the fraction of inspired oxygen (FiO_2) [PaO2/FiO2 (P/F ratio), $\leq$300 mmHg], and (iii) pulmonary infiltrates suggestive of SARS-CoV-2–related ARDS. Exclusion criteria were as follows: demonstrated or suspected uncontrolled systemic severe coinfection, local extrapulmonary abscess, ARDS due to cardiac failure or fluid overload, multiorgan failure, severe renal failure (glomerular filtration rate < 30 ml/min); concomitant treatment with immunomodulatory/immunosuppressive drugs, convalescent plasma, nonspecific intravenous immunoglobulins (IVIG), or SARS-CoV-2–specific monoclonal antibodies; administration of IVIG within 3 weeks before enrollment; chemotherapy within the last 3 months; and pregnancy.

Patients received AMY-101 (5 mg/kg; $n = 16$) or placebo ($n = 15$) for a maximum of 14 days (6-hour intravenous infusion loading dose, followed by a daily maintenance dose as a continuous 24-long iv infusion) in addition to the SOC, which included 6 mg of dexamethasone or equivalent doses of alternative glucocorticoids, and LMWH at therapeutic or prophylactic doses. AMY-101 treatment was discontinued before 14 days at the attending physician's discretion if the patient no longer required supplemental oxygen for at least 48 hours. Characteristics of the enrolled patients at baseline are presented in Table 1.

The study protocol design was approved by the National Ethics Committee of Greece (116/20/2020-10-22) and the National Organization for Medicines of Greece (EOF, IS107-20/2020-10-23). All subjects or legal representatives provided signed informed consent in accordance with the principles expressed in the Declaration of Helsinki.

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Table 1. Baseline characteristics of the patients studied ($n = 31$). BiPAP, bilevel positive airway pressure; COPD, chronic obstructive pulmonary disease; CPAP, continuous positive airway pressure; SOC, standard of care.

Parameter	AMY-101 ($n = 16$)	Placebo ($n = 15$)	P
Age (years)			
Means ± SD	52.4 ± 10.6	58.9 ± 11.0	0.105*
Sex, *n* (%)			
Male	14 (87.5)	13 (86.7)	1.000†
Female	2 (12.5)	2 (13.3)	
Body mass index			
Means ± SD	30.9 ± 5.3	29.8 ± 4.0	0.510*
Days from disease onset, *n* (%)			
≥8	12 (75.0)	14 (93.3)	0.333†
4 to 7	4 (25.0)	1 (6.7)	
Comorbidities, *n* (%)			
Obesity	9 (56.3)	7 (46.7)	0.594‡
Essential hypertension	6 (37.5)	8 (53.3)	0.376‡
Diabetes mellitus	5 (31.3)	4 (26.7)	0.779‡
Dyslipidemia	3 (18.8)	4 (26.7)	0.598‡
Coronary disease	0 (0)	2 (13.3)	0.226†
COPD/asthma	0 (0)	1 (6.7)	0.484†
Sleep apnea	1 (6.3)	1 (6.7)	1.000†
Heart failure	0 (0)	1 (6.7)	0.484†
Atrial fibrillation	2 (12.5)	0 (0)	0.484†
Hypothyroidism	1 (6.3)	1 (6.7)	1.000†
Epilepsy	1 (6.3)	0 (0)	1.000†
Stenting/aortic dissection	0 (0)	1 (6.7)	0.484†
Hyperuricemia	1 (6.3)	0 (0)	1.000†
Anxiety/depression	2 (12.5)	2 (13.3)	1.000†
Crohn's disease	0 (0)	1 (6.7)	0.484†
Thoracic aortic aneurysm	0 (0)	1 (6.7)	0.484†
Hepatic steatosis	0 (0)	1 (6.7)	0.484†
Mean number per patient ± SD	1.9 ± 1.6	2.3 ± 1.7	0.508*
PaO_2/FiO_2 (mmHg)			
Means ± SD	139.6 ± 38.6	124.7 ± 55.1	0.386*
$200 < PaO_2/FiO_2 \leq 300$, *n* (%)	0 (0.0)	2 (13.3)	
$100 < PaO_2/FiO_2 \leq 200$, *n* (%)	14 (87.5)	7 (46.7)	
$PaO_2/FiO_2 \leq 100$, *n* (%)	2 (12.5)	6 (40.0)	
High-concentration oxygen supply (≥15 liter/min), *n* (%)			
Nonrebreather facemask	8 (50.0)	9 (60.0)	0.576‡
High-flow nasal cannula	1 (6.3)	1 (6.7)	1.000†
CPAP/BiPAP	1 (6.3)	0 (0)	1.000†
SOC treatment, *n* (%)			
Dexamethasone	16 (100)	15 (100)	1.000‡
Low molecular weight heparin	16 (100)	15 (100)	1.000‡
Antibiotics	16 (100)	15 (100)	1.000‡
Fresh frozen plasma	2 (12.5)	0 (0)	0.484†

*Student's *t* test (variables considered as continuous). †Fisher's exact test. ‡Chi-square test.

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Plasma collection

To isolate plasma, venous blood was collected in BD Vacutainer EDTA tubes. Plasma was collected at day 1, before AMY-101 or placebo administration (baseline), and at days 2, 4, 7, 10, 14, 21, and 44. Blood was centrifuged at 500*g* for 15 min, and then plasma samples were stored at −80°C until analyzed.

SOC laboratory parameters

For all participants, ANC, ALC, CRP, ferritin, and D-dimer were analyzed in peripheral blood using routine laboratory techniques.

Complement proteins levels

The plasma concentration of C3 was quantified by nephelometry. C3a/C3a-desArg or sC5b-9 complexes were measured in EDTA plasma samples using commercial C3a (HK354, Hycult Biotech, Uden, Netherlands) or sC5b-9 enzyme-linked immunosorbent assay (ELISA) (HK328, Hycult Biotech) kits, respectively, according to the instructions of the manufacturer.

CitH3 ELISA

CitH3 levels in EDTA plasma were quantified with an H3Cit ELISA kit, according to the instructions of the manufacturer (501620, Cayman Chemical, Ann Arbor, MI, USA).

TAT assay

Thrombin–anti-thrombin complexes were analyzed in patients' sera by ELISA as previously described (*22*).

Kallikrein activity assay

Kallikrein activity was expressed as the percent ratio of prekallikrein to kallikrein. The relative protein abundance of prekallikrein and kallikrein in patients' plasma was determined by an automated Western blot–like assay (WES), as previously described (*22*).

AMY-101 levels in patients' plasma

Quantitation of AMY-101 was performed in patients' EDTA plasma samples collected at predetermined time points during treatment by using ultraperformance liquid chromatography–electrospray ionization mass spectrometry as described previously (*40*).

Antidrug antibodies

To detect ADAs in patients' plasma, an ADA ELISA was performed (*41*). In brief, 96-well ELISA plates were coated with the compstatin analog Cp40 H-[D]Tyr-Ile-[Cys-Val-Trp(Me)-Gln-Asp-Trp-Sar-Ala-His-Arg-Cys]-mIle-NH_2 (10 μg/ml). After a thorough wash with phosphate-buffered saline (PBS), blocking was performed using PBS/bovine serum albumin (BSA) for 1 hour. Next, serial dilutions of patients' plasma were added to the plate and incubated for 1 hour at room temperature. Wells incubated with PBS/BSA alone served as blanks. A diluted horseradish peroxidase–conjugated goat anti-human IgG (172-1050, Bio-Rad, Hercules, CA, USA) was added for 30 min. After washing with PBS, 100 μl of trimethylboron substrate was added. Color development was stopped with H_2SO_4, and the plate was read at optical density at 450 nm (Byonoy).

Statistical analysis

Chi-squared analysis was used to examine the differences between categorical variables; Fisher's exact test was alternatively used when >20% of cells have expected frequencies of <5. Student's *t* test was used to compare means between continuous variables; when the data deviated from a normal distribution, the nonparametric independent-sample Mann-Whitney *U* test was performed instead. Paired *t* tests were used to compare means of two measurements taken from same patient; when there was deviation from a normal distribution, the nonparametric-related sample Wilcoxon signed-rank test was used instead. The repeated-measure general linear model was used for analysis of within-subject and between-subject variance of the same variable measured several times in each patient. The level of statistical significance was set to $P = 0.05$. Statistical analysis was performed with SPSS 26.0.0.0 (IBM).

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at https://science.org/doi/10.1126/sciadv.abo2341

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