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BRIEF REPORT

The anti-C5a antibody vilobelimab efficiently inhibits C5a in patients with severe COVID-19

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Abstract

Recently, we reported the phase II portion of the adaptive phase II/III PANAMO trial exploring potential benefit and safety of selectively blocking C5a with the monoclonal antibody vilobelimab (IFX-1) in patients with severe coronavirus disease 2019 (COVID-19). The potent anaphylatoxin C5a attracts neutrophils and monocytes to the infection site, causes tissue damage by oxidative radical formation and enzyme releases, and leads to activation of the coagulation system. Results demonstrated that C5a inhibition with vilobelimab was safe and secondary outcomes appeared in favor of vilobelimab. We now report the pharmacokinetic/pharmacodynamic (PK/PD) analysis of the phase II study. Between March 31 and April 24, 2020, 30 patients with severe COVID-19 pneumonia confirmed by real-time polymerase chain reaction were randomly assigned 1:1 to receive vilobelimab plus best supportive care or best supportive care only. Samples for measurement of vilobelimab, C3a and C5a blood concentrations were taken. Vilobelimab predose (trough) drug concentrations in plasma ranged from 84,846 to 248,592 ng/ml (571 to 1674 nM) with a geometric mean of 151,702 ng/ml (1022 nM) on day 2 and from 80,060 to 200,746 ng/ml (539 to 1352 nM) with a geometric mean of 139,503 ng/ml (939 nM) on day 8. After the first vilobelimab infusion, C5a concentrations were suppressed in the vilobelimab group (median 39.70 ng/ml 4.8 nM, IQR 33.20–45.55) as compared to the control group (median 158.53 ng/ml 19.1 nM, IQR 60.03–200.89, $p = 0.0006$). The suppression was maintained on day 8 ($p = 0.001$). The current PK/PD analysis shows that vilobelimab efficiently inhibits C5a in patients with severe COVID-19.

Alexander P. J. Vlaar and Endry H.T. Lim contributed equally to this work.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

High concentrations of the potent anaphylatoxin C5a have been reported in patients with severe coronavirus disease 2019 (COVID-19), and the C5a–C5aR1 signaling axis has been suggested to be crucial in COVID-19 associated inflammation.

WHAT QUESTION DID THIS STUDY ADDRESS?

Does the anti-C5a antibody vilobelimab efficiently inhibit C5a in patients with severe COVID-19?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The current pharmacokinetic/pharmacodynamic analysis of the phase II part of the adaptive phase II/III PANAMO trial shows that vilobelimab efficiently inhibits C5a in patients with severe COVID-19. Our results confirm that C5a is strongly elevated in patients with severe COVID-19.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

These results suggest a potential important role of C5a-inhibition for patients with severe COVID-19. With our previous report on the clinical outcome, this underlines the need to investigate, within the currently enrolling phase III trial, whether C5a-inhibition may positively impact 28-day survival in critically ill patients with COVID-19.

INTRODUCTION

Vilobelimab is a chimeric monoclonal IgG4 antibody that specifically binds with high affinity to the soluble form of human complement split factor C5a. The potent anaphylatoxin C5a attracts neutrophils and monocytes to the infection site, and strongly activates these cells, causing tissue damage by oxidative radical formation and enzyme release but also inducing release of tissue factor from endothelial cells and neutrophils thereby activating the coagulation system. High concentrations of C5a have been reported in patients with severe coronavirus disease 2019 (COVID-19), and the C5a–C5aR1 signaling axis has been suggested to be crucial in COVID-19 associated inflammation.[1]

Recently, we reported the phase II portion of the adaptive phase II/III PANAMO trial exploring potential benefit and safety of selectively blocking C5a with the monoclonal antibody vilobelimab in patients with severe COVID-19.[2] Results demonstrated that C5a inhibition with vilobelimab was safe and other outcomes (kidney injury, pulmonary embolism, and survival) appeared in favor of vilobelimab. We now report the pharmacokinetic/pharmacodynamic (PK/PD) analysis of the phase II portion of the PANAMO trial.

METHODS

Between March 31 and April 24, 2020, 30 patients with severe COVID-19 pneumonia were randomized in three academic hospitals in the Netherlands: 15 to the vilobelimab plus best supportive care (BSC) group and 15 to the BSC control group. Severe COVID-19 was defined as severe pneumonia with pulmonary infiltrates consistent with pneumonia, a clinical history of severe shortness of breath within the past 14 days, or a need for noninvasive or invasive ventilation; severe disease defined as a ratio of partial pressure of arterial oxygen to fractional concentration of oxygen in inspired air (PaO2/FiO2) between 100 and 250 mm Hg in the supine position; and severe acute respiratory syndrome coronavirus 2 infection confirmed by real-time polymerase chain reaction. All patients in the vilobelimab group received at least one of the maximum seven scheduled (days 1, 2, 4, 8, 11–13, 15, and 22) doses of vilobelimab 800 mg i.v. treatment. The dose between days 11 and 13 was only given at the discretion of the investigator if signs of weakening of any clinical improvement were detected and the dose on day 22 was only administered to patients who were still intubated. Treatment with vilobelimab was discontinued if patients were discharged from the hospital. Three patients received seven infusions, three patients received six infusions, four patients received five infusions, and five patients received less than five infusions. Samples for measurement of vilobelimab, C3a, and C5a blood concentrations were to be taken up to seven times during treatment before vilobelimab administrations, including day 1, day 2, day 8, and day 29. Vilobelimab predose (trough) drug concentrations and C5a levels were measured by in-house developed and validated enzyme-linked

immunosorbent assays, and C3a concentrations in plasma was determined by commercialized MicroVue C3a Plus EIA (Quidel Corp.). All measurements were carried out by the laboratory menal GmbH.

Serial C5a levels in the vilobelimab plus BSC group were compared with baseline using the Wilcoxon test. All analyses were performed in SAS 9.4.

The study protocol of the phase II/III trial was approved by the institutional review board of the Academic Medical Center, part of Amsterdam UMC (Amsterdam, Netherlands; IRB 2020_067#B2020179).

RESULTS

Four of 15 (26.7%) patients died in the control group and two of 15 (13.3%) in the vilobelimab group. Baseline blood samples for vilobelimab measurement were available for 10 vilobelimab and 12 control patients, for C5a measurements for 10 and 12 patients, and for C3a measurement for eight and 13 patients, respectively. For seven patients, samples were available on days 1, 2, and 8 for vilobelimab and C5a measurements. Beyond day 8, sampling for PK and PD was too sparse to be representative. Vilobelimab predose (trough) drug concentrations in plasma ranged from 84,846 to 248,592 ng/ml (571 to 1674 nM) with a geometric mean of 151,702 ng/ml (1022 nM) on day 2 and from 80,060 to 200,746 ng/ml (539 to 1352 nM) with a geometric mean of 139,503 ng/ml (939 nM) on day 8 (Figure 1). The pharmacological activity of vilobelimab, the inhibition of C5a in patients with severe COVID-19, is confirmed by the suppression of C5a concentrations in the vilobelimab treatment group to the normal C5a blood level in healthy subjects (N = 40, median 41.07 ng/ml (4.9 nM, IQR 30.54–54.66, internal report).

Baseline concentration of C5a was elevated in all patients and were comparable between treatment groups: median 189.98 ng/ml (22.9 nM, IQR 109.81–272.62) for the vilobelimab group and 138.52 ng/ml (16.7 nM, IQR 70.81–210.84) in the control group (Figure 2). After the first vilobelimab infusion, C5a concentrations were suppressed in the vilobelimab group (median 39.70 ng/ml, 4.8 nM, IQR 33.20–45.55) as compared with the control group (median 158.53 ng/ml, 19.1 nM, IQR 60.03–200.89, $p = 0.0006$). In the vilobelimab group, the suppression was maintained on day 8 ($p = 0.001$). In the control group, no change of C5a concentrations were determined during the treatment period as compared to baseline. A summary of plasma concentrations of C5a by protocol-defined study day for both groups can be found in the Supplementary Information (Tables S1 and S2). PK/PD analysis based on arithmetic mean and SD showing the relationship of drug concentrations and C5a over time is plotted (Figure S1).

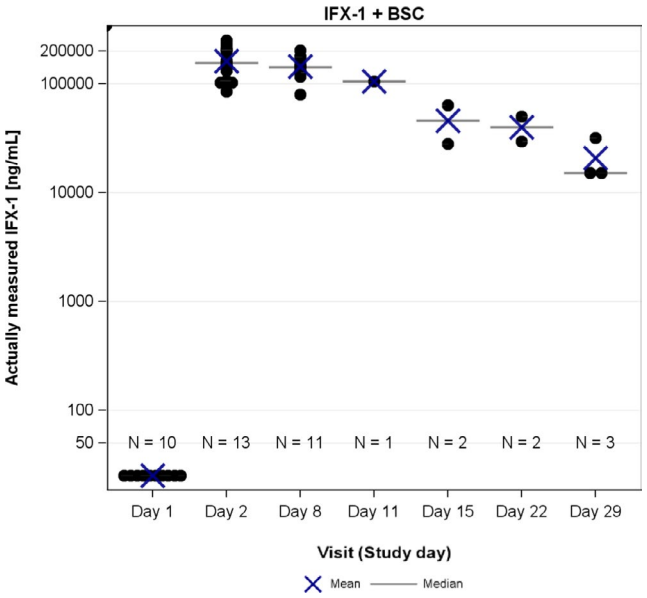

FIGURE 1 Vilobelimab drug concentrations in patients with severe COVID-19 treated with vilobelimab. BSC, best supportive care; COVID-19, coronavirus disease 2019; IFX-1, vilobelimab; *N*, number of patients with evaluations. Vilobelimab infusion scheme: Days 1, 2, 4, 8, and 11 (only in case of clinical worsening upon initial benefit at the investigator's discretion), days 15 and 22

As expected, vilobelimab did not have any effect on C3a concentrations (Figure S2). Although not significant, there was a trend of around 30% higher C5a levels at baseline in patients with any pulmonary embolism reported during the trial, compared with patients without pulmonary embolism ($n = 8$, median 180.08 ng/ml, 21.7 nM, IQR 126.41–288.81 vs. $n = 14$, median 139.88 ng/ml, 16.9 nM, IQR 98.60–231.68).

DISCUSSION

The current PK/PD analysis of the phase II part of the adaptive phase II/III PANAMO trial shows that vilobelimab efficiently inhibits C5a in patients with severe COVID-19. Our results confirm that C5a is strongly elevated in patients with severe COVID-19. The doses of 800 mg IFX-1 (vilobelimab) on days 1, 2, 4, and 8 immediately resulted in drug concentrations necessary for full pharmacological action of vilobelimab.

Several studies investigate complement inhibitors upstream of C5a in COVID-19. However, our recently published data from the phase II part of the PANAMO study suggest a potential important role of C5a-inhibition for this disease. It has been shown that upstream inhibition may not lead to complete blockade of C5a due to C5a generation through direct enzymatic cleavage (e.g., through thrombin and other enzymes), which cannot be inhibited

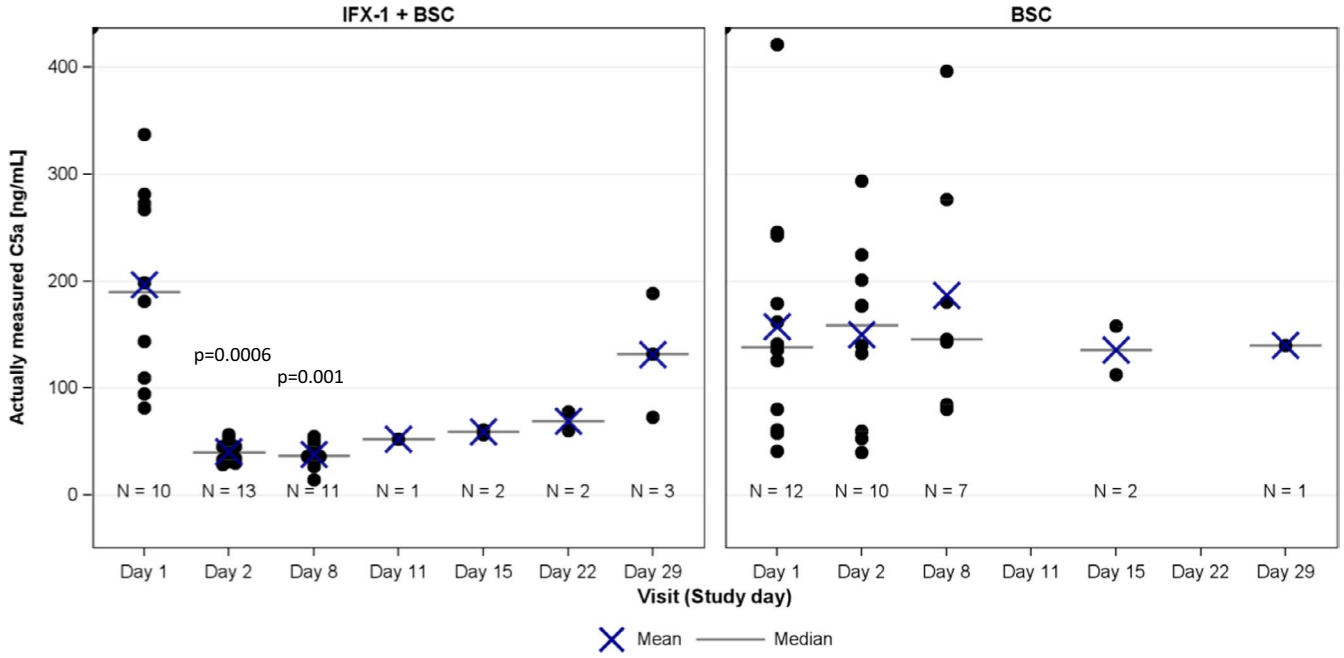

FIGURE 2 Serial C5a levels in vilobelimab-exposed patients (IFX-1 group, left) and control patients (right) with severe COVID-19 disease, *p* values are from Wilcoxon test comparing respective day with baseline (Day 1). BSC, best supportive care; C5a, complement factor 5a; COVID-19, coronavirus disease 2019; IFX-1, vilobelimab; *N*, number of patients with evaluations

by upstream complement inhibitors, such as C5 blocking antibodies.[3] This may be important in COVID-19 where hypercoagulation and subsequent thromboembolic events are particularly frequent. Especially thrombin, but also other enzymes will be abundant and highly active. A recently published case report demonstrated that C5a was not blocked in a patient who had been treated with the C5 inhibitor eculizumab.[4] Furthermore, a recently published study of eculizumab for treatment of patients with severe COVID-19[5] showed no difference in C5a concentrations between eculizumab-treated and eculizumab-free patients at day 1 and day 7.[6] Another potential advantage of direct inhibition of C5a compared with upstream inhibition, such as C5 or C3 inhibition, is that C5b and C5b-9 and the membrane attack complex (MAC) formation are left intact.[2,3] The MAC formation plays a crucial role in host defense through cell lysis. Indeed, the recently published results of upstream complement inhibition with the C5 inhibitor eculizumab in severely ill patients with COVID-19 demonstrated significantly increased secondary infection rates in the treatment arm compared with the control arm.[5] To the contrary, no increased secondary infection rates had been observed with direct C5a inhibitory antibody vilobelimab.[2,7] Given (a) the known link between complement and coagulation pathways, (b) the virus induced tissue damage and neutrophil activation, and (c) neutrophil induced organ damage by C5a activation, C5a inhibition could improve the microangiopathy and microthrombosis observed in patients with COVID-19. Along these lines, the observed relative increase of D-dimers from baseline after C5a inhibition by vilobelimab could be a sign of increased fibrinolysis.

Our previous report on the clinical outcome and the present report on PK/PD analysis underline the need to investigate, within the currently enrolling phase III trial, whether C5a-inhibition may positively impact 28-day survival in critically ill patients with COVID-19.

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The trial was designed by InflaRx representatives and academic advisors. Data were collected by investigators and associated site personnel, analyzed by statisticians employed by Metronomia, and interpreted by academic authors and InflaRx representatives. A.P.J.V., E.H.T.L., S.R., N.C.R., K.P., and D.B. take responsibility for the integrity of the data and the accuracy of the data analysis. All authors had full access to the data, and vouch for the completeness and accuracy of the data. The first draft of the manuscript was written by A.P.J.V., E.H.T.L., and D.B. with input from authors employed by InflaRx.

CONFLICTS OF INTEREST

A.P.J.V. reports personal fees from InflaRx, paid to Amsterdam UMC, during the conduct of the study. N.C.R. and R.F.G. are founders, active officers, and executive directors of InflaRx, and hold shares and stock options in InflaRx. K.P. is Global Head of Clinical Development of InflaRx and holds stock options in InflaRx. S.R. is an employee at Metronomia, a contracted statistical service

provider for InflaRx. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

A.P.J.V., E.H.T.L., S.B., K.P., N.C.R., M.C.B., R.F.G., and D.B wrote the manuscript. A.P.J.V., S.B., K.P., N.C.R., M.C.B., and D.B. designed the research. A.P.J.V., S.B., L.M.H., M.H.B., P.P., M.C.B., and D.B performed the research. A.P.J.V., E.H.T.L., S.B., S.R., K.P., N.C.R., M.C.B., and D.B analyzed the data.

Role of the funding source: The funder of the study had a role in study design of the trial, data collection, data analysis, data interpretation, and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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