Electronic Supplementary Material

Efficacy and safety of vilobelimab (IFX-1), a novel monoclonal anti-C5a antibody, in patients with early severe sepsis or septic shock - a randomized, placebo-controlled, double-blind, multicenter, phase IIa trial (SCIENS study)

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eTable 1: Eligibility

Inclusion criteria

1. Male or female patients ≥ 18 years old
2. Written informed consent from patient, legal or authorized representative or a confirmation of justification of trial participation by an independent medical consultant
3. Occurrence of at least two criteria of a systemic inflammatory response syndrome (SIRS) not explained by other reasons. These criteria should be present within 12 hours prior to screening:
    a. Fever (≥ 38 °C) or Hypothermia (≤ 36 °C)
    b. Tachypnea (≥ 20 bpm) or hyperventilation (PaCO2 ≤ 33 mmHg [≤ 4.3 kPa] or mechanical ventilation
    c. Leukocytosis (≥ 12.000 / µl) or leukopenia (≤ 4.000 / µl) or ≥ 10 % immature forms
4. Suspected or confirmed abdominal or pulmonary infection at screening.
    a. For abdominal infection one or more of the following conditions must be present at screening
        i. Clinically apparent acute abdomen in conjunction with SIRS criteria 3a or 3d (SIRS criteria should be present within 12 hours prior to screening) or with serum lactate ≥ 3.5 mmol/L
        ii. Evidence for microbiological findings within peritoneal fluid (also drainage fluid)
        iii. Evidence of cloudy / dirty abdominal drainage fluid after an abdominal operation
        iv. Evidence for an inflammation, abscess or perforation of an abdominal organ by x-ray, CT-scan, MRI or surgery
        v. Other evidence for a syndrome with a high likelihood of abdominal infection (e.g., ischemic gut within an endoscopy or CT-scan, ascending cholangitis etc.)
    b. For pulmonary infection one or more of the following conditions must be present at screening
        i. Pulmonary infiltrates consistent with pneumonia in a chest x-ray or CT- scan that has been taken within 12 hours prior to screening
        ii. Purulent BAL, tracheal or pleural drainage fluid, or bronchoscopic finding consistent with a significant infection of the tracheobronchial system
        iii. Abscess formation or other evidence for infectious focus within the tracheal-bronchial or pleural system
        iv. Evidence for significant microbial finding in BAL or tracheal swipe or in pleural drainage fluids
5. Broad spectrum i.v. antimicrobial therapy to treat abdominal or pulmonary infection which is also effective against N. meningitidis. One of the following conditions must be present at screening:
    a. Intention to start i.v. antimicrobial therapy until randomization or
    b. Change of therapy within last 24 hours or
    c. Start of therapy within the last 24 hours
6. At least one of the following acute organ dysfunctions due to sepsis. Each organ dysfunction must have occurred within 12 hours prior to screening, cannot mainly be explained by other disease processes than sepsis and is judged by the investigator as

being caused or directly related to an abdominal or pulmonary infectious focus

a. **Respiratory**: one or more of the following conditions must be present at screening
    i. New requirement of ≥5 L O2 flow over any mask or similar device in order to maintain an SpO2 ≥90 %
    ii. Any doubling of existing O2 flow via any mask or similar device with resulting O2 flow ≥ 5 L in order to maintain an SpO2 ≥ 90 %
    iii. Newly required invasive mechanical ventilation for reasons of a non-obstructive dysfunctional oxygenation
    iv. Newly developed oxygenation deficiency with PaO2/FiO2 ratio ≤ 200 mmHg in patients with existing invasive mechanical ventilation
b. **Renal**: one or more of the following
    i. Newly developed urine output ≤ 20ml/h for more than two hours in the absence of known or suspected urinary tract obstruction and despite ≥ 500 mL crystalloid or equivalent fluid challenge
    ii. Serum creatinine greater than two times the upper limit of normal (ULN) of the local laboratory
    iii. Newly developed relative increase in serum creatinine of ≥ 100% within the preceding 36 h

    In the presence of pre-existing chronic kidney dysfunction or failure, the patient must display another organ dysfunction for inclusion in the trial.

c. **Hematologic**: one or both of the following present at screening
    i. Newly measured platelet count < 80,000/mm3
    ii. Decrease in platelet count of more than 30 % within the preceding 36h

    In the presence of pre-existing immunological disorder or thrombocytopenia due to other reasons than sepsis, the patient must display another organ dysfunction for inclusion in the trial.

d. **Metabolic**: one or both of the following that is not otherwise explained by ventilation or other disease processes
    i. Newly measured serum lactate ≥ 3.5 mmol/L
    ii. Newly developed metabolic acidosis with pH < 7.30
e. **Cardiovascular**: one or both of the following has occurred within past three hours:
    i. New requirement for vasopressors (norepinephrine, epinephrine) to maintain a mean arterial blood pressure > 65 mmHg or a systolic pressure > 90 mmHg
    ii. Doubling of a preexisting vasopressor therapy over a time period of less than four hours

    New requirement is defined as a dose of ≥ 0.1 µg/kg/min norepinephrine or equivalent in sedated patients and ≥ 0.03 µg/kg/min norepinephrine or equivalent in non-sedated patients. New doubling is defined as doubling the dose compared to previous dose and total dose is ≥ 0.1 µg/kg/min norepinephrine or equivalent.

Exclusion criteria

1. Body weight of more than 130 kg
2. Infections necessitating an antimicrobial therapy for more than two weeks
3. Patients with meningitis

4. Life expectancy less than six months due to concomitant diseases
5. Significant hepatic impairment
6. Active hepatitis
7. Severe congestive heart failure
8. Severe neurological impairment such as persistent vegetative state
9. Cardiopulmonary resuscitation within the last 4 weeks,
10. Patients receiving within the last 14 days calcineurin inhibitors, proliferation inhibitors, anti-metabolites, or >50 mg prednisolone equivalent per day
11. Patients receiving high dose immunoglobulins within three months prior to screening
12. Patients with a neutrophil count of less than 1,000/mm^3 unlikely due to sepsis
13. Pregnant or breast-feeding women
14. Women with childbearing potential not willing to practice appropriate contraceptive measures during the study
15. Participation in another clinical study, prior participation in this study,
16. Chronically bed bound patients, patients with intravenous drug abuse
17. Patients without commitment for full life support

eTable 2: Sampling schedule

		Active treatment period *hours after 1st dosing*									Follow up *days after randomization*				
	Pre	2	6	12	14	24	26	48	72	74	5	8	13	HD	28
Citrat plasma															
Complement factors	x	x	x	x		x			x		x	x	x	x	x
Cytokines	x			x		x			x		x	x			
Vilobelimab	x	x	x	x	x[1]	x	x[2]	x	x	x[3]	x	x	x	x	x
Vilobelimab activity[4]	x		x			x			x		x	x	x	x	x
ADA	x											x		x	x
Serum															
CH-50	x	x	x	x		x			x		x	x	x	x	x
EDTA and heparin plasma															
Retention sample	x					x		x	x		x	x	x	x	x

[1]: cohort 1 only, [2]: cohort 2 and 3 only, [3]: cohort 3 only; [4]: Measurement performed only in samples with a vilobelimab concentration ≥ 7.3 µg/mL; ADA: anti-drug antibody, CH-50: total hemolytic complement activity, HD: hospital discharge, vilobelimab: PK samples for monoclonal anti-C5a antibody (study drug), Pre: pre-dosing (0 – 30 min before dosing)

eTable 3: Major protocol violations

	Placebo (N = 24) n (%)	**Cohort 1 (N = 16) n (%)**	**Cohort 2 (N = 16) n (%)**	**Cohort 3 (N = 16) n (%)**
Any major protocol violation	**6 (25%)**	**4 (25%)**	**3 (18.8%)**	**2 (12.5%)**
Violation of inclusion criteria	---	1 (6.3%)	---	---
Violation of exclusion criteria	2 (8.3%)	2 (12.5%)	2 (12.5%)	1 (6.3%)
Deviation from doses scheme	2 (8.3%)	1 (6.3%)	1 (6.3%)	---
Deviation from visit schedule	2 (8.3%)	2 (12.5%)	---	---
Prohibited medication	3 (12.5%)	1 (6.3%)	1 (6.3%)	1 (6.3%)

Number of patients with at least one violation in respective category

Detailed description of major protocol violations

- Violation of inclusion and exclusion criteria
  - High doses of corticoid therapy within 14 days prior to screening (4 subjects)
  - Broad spectrum antibiotics were not started or changed within last 24 hours (2 subjects)
  - Violation of time window between start of cardiovascular dysfunction and randomization (2 subjects)
- Deviation from dose scheme
  - Omitted dosing of study medication (2 subjects)
  - Interruption of 1st infusion of study medication (1 subject)
  - Delay of 4 hours in start of 2nd infusion of study drug (1 subject)
- Prohibited medication
  - High dose corticosteroid therapy during the study (6 subjects)
- Deviation from visit schedule
  - Start of study drug was delayed for more than 8 hours after onset of cardiovascular dysfunction (2 subjects)
  - Start of study drug was delayed for more than 20 hours after onset of respiratory organ dysfunction (2 subjects)

eTable 4: Statistical significance against baseline of C5a inhibition in three dose cohorts

	Cohort 1 (N = 16)	**Cohort 2 (N = 16)**	**Cohort 3 (N = 16)**
2 h	<0.001	<0.001	<0.001
6 h	<0.001	<0.001	<0.001
12 h	<0.001	<0.001	<0.001
24 h	<0.001	<0.001	<0.001
72 h	0.011	<0.001	<0.001
Day 5	0.326	<0.001	<0.001
Day 8	0.110	0.791	<0.001
Day 13	0.320	0.078	0.685
Day 28	0.625	0.125	0.109

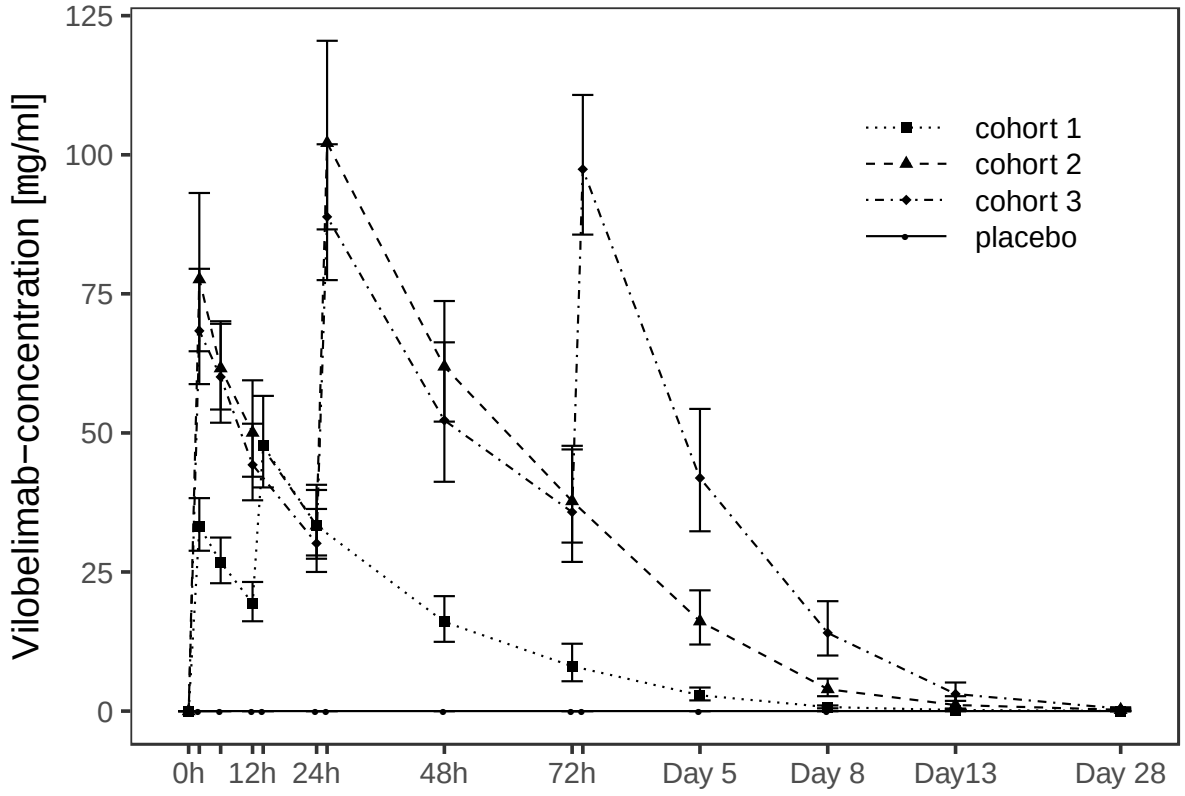
eTable 5: Overview of adverse events (AEs)

	Placebo (N = 24) n (%)	**Cohort 1 (N = 16) n (%)**	**Cohort 2 (N = 16) n (%)**	**Cohort 3 (N = 16) n (%)**
Overall Incidence	21 (87.5 %)	14 (87.5 %)	12 (75 %)	15 (93.8 %)
Related AEs[1]	2 (8.3 %)	3 (18.8 %)	1 (6.3 %)	1 (6.3 %)
Serious AEs	13 (54.2 %)	10 (62.5 %)	6 (37.5 %)	7 (43.8 %)
AEs with fatal outcome	4 (16.7 %)	6 (37.5 %)	3 (18.8 %)	2 (12.5 %)
Related AEs[1] with fatal outcome	-	1 (6.3 %)	-	-
AESI	0	0	0	0
AEs by System Organ Class and Preferred Term (≥ 10 % of patients in at least one cohort)				
System Organ Class Preferred Term				
Metabolism and nutrition disorders	9 (37.5 %)	9 (56.3 %)	5 (31.3 %)	6 (37.5 %)
Hyperkalaemia	2 (8.3 %)	3 (18.8 %)	1 (6.3 %)	1 (6.3%)
Hypokalemia	3 (12.5 %)	1 (6,3 %)	2 (12.5 %)	3 (18.8 %)
Hypernatraemia	4 (16.7 %)	2 (12.5 %)	1 (6.3 %)	1 (6.3 %)
Hyperglycaemia	3 (12.5 %)	1 (6.3 %)	-	2 (12.5 %)
Hypoalbuminaemia	2 (8.3 %)	-	1 (6.3 %)	2 (12.5 %)
Hypocalcaemis	-	1 (6.3 %)	2 (12.5 %)	-
Investigations	10 (41.7 %)	8 (50.0 %)	4 (25.0 %)	6 (37.5 %)
Haemoglobin decreased	6 (25.0 %)	2 (12.5 %)	1 (6.3 %)	1 (6.3 %)
Transaminases increased	-	2 (12.5 %)	2 (12.5 %)	1 (6.3 %)
Body temperature increased	1 (4.2 %)	2 (12.5 %)	-	-
Oxygen saturation decreased	-	1 (6.3%)	-	2 (12.5 %)
Platelet count decreased	3 (12.5 %)	-	-	-
Gastrointestinal disorders	9 (37.5 %)	7 (43.8 %)	5 (31.3 %)	6 (37.5 %)
Nausea	2 (8,3 %)	2 (12,5 %)	-	2 (12,5 %)
Small intestinal perforation	1 (4,2 %)	3 (12,5 %)	1 (6,3 %)	1 (6,3 %)
Constipation	1 (4,2 %)	-	-	2 (12,5 %)
Diarrhoea	1 (4,2 %)	-	2 (12,5 %)	-
Cardiac disorders	9 (37.5 %)	7 (43.8 %)	5 (31.3 %)	6 (37.5 %)
Atrial fibrillation	3 (12,5 %)	1 (6,3 %)	1 (6,3 %)	2 (12,5 %)
Tachyarrhythmia	5 (20,8 %)	3 (18,8 %)	-	-
Cardiac arrest	1 (4,2 %)	1 (6,3 %)	2 (12,5 %)	1 (6,3 %)
Bradycardia	1 (4,2 %)	-	2 (12,5 %)	1 (6,3 %)

Atrial flutter	-	-	-	2 (12,5 %)
Respiratory disorders	8 (33.3 %)	7 (43.8 %)	6 (37.5 %)	3 (18.8 %)
Respiratory failure	5 (20,8 %)	3 (18,8 %)	2 (12,5 %)	1 (6,3 %)
Pleural effusion	2 (8,3 %)	3 (18,8 %)	2 (12,5 %)	1 (6,3 %)
Blood and lymphatic system disorders	6 (25.0 %)	6 (37.5 %)	6 (37.5 %)	4 (25.0 %)
Anaemia	3 (12,5 %)	2 (12,5 %)	6 (37,5 %)	1 (6,3 %)
Leukocytosis	3 (12,5 %)	2 (12,5 %)	-	1 (6,3 %)
Thrombocytopenia	1 (4,2 %)	2 (12,5 %)	-	3 (18,8 %)
General disorders / administration site conditions	11 (45.8 %)	4 (25.0 %)	-	6 (37.5 %)
Multi-organ failure	2 (8,3 %)	2 (12,5 %)	-	1 (6,3 %)
Oedema peripheral	1 (4,2 %)	-	-	2 (12,5 %)
Pyrexia	3 (12,5 %)	-	-	-
Infections and infestations	8 (33.3 %)	4 (25.0 %)	2 (12.5 %)	3 (18.8 %)
Pneumonia	2 (8,3 %)	2 (12,5 %)	-	1 (6,3 %)
Septic shock	4 (16,7 %)	-	1 (6,3 %)	1 (6,3%)
Peritonitis	1 (4,2 %)	-	-	2 (12,5 %)
Psychiatric disorders	6 (25.0 %)	3 (18.8 %)	3 (18.8 %)	3 (18.8 %)
Delirium	4 (16,7 %)	2 (12,5 %)	2 (12,5 %)	1 (6,3 %)
Agitation	-	-	1 (6,3 %)	2 (12,5 %)
Injury, poisoning, procedural complications	5 (20.8 %)	4 (25.0 %)	2 (12.5 %)	3 (18.8 %)
Anastomotic leak	-	-	1 (6,3 %)	2 (12,5 %)
Hepatobiliary disorders	1 (4.2 %)	2 (12.5 %)	2 (12.5 %)	2 (12.5 %)
Vascular disorders	4 (16.7 %)	2 (12.5 %)	2 (12.5 %)	1 (6.3 %)
Nervous system disorders	5 (20.8 %)	2 (12.5 %)	1 (6.3 %)	1 (6.3%)
Renal and urinary disorders	-	3 (18.8 %)	-	-
Acute kidney injury	-	2 (12,5 %)	-	-
Skin disorders	2 (8.3 %)	2 (12.5 %)	-	1 (6.3 %)

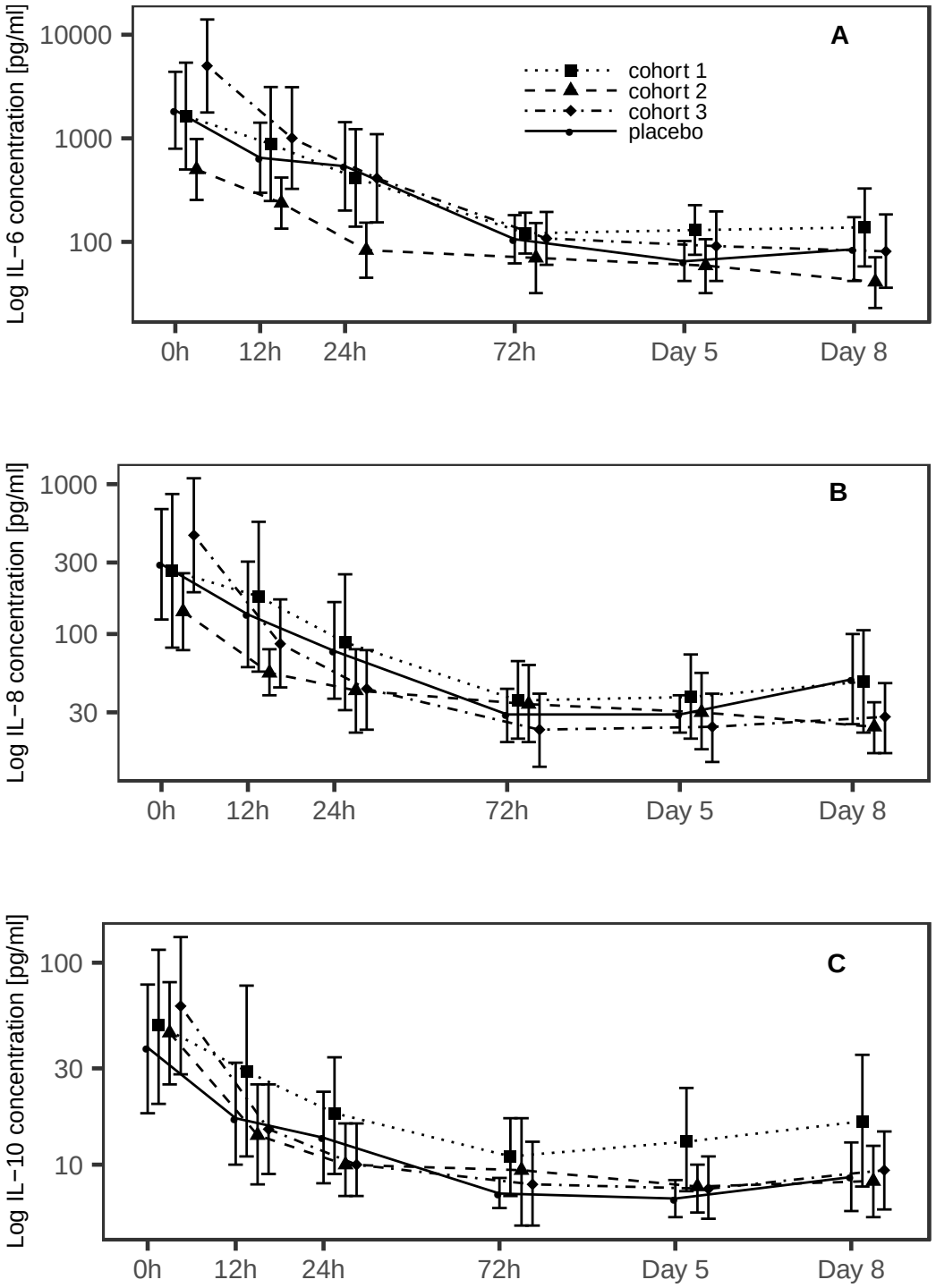
Number of patients with at least one event in respective category
Data are presented as absolute numbers and percentage. 1: Investigator assessed AE as at least possibly related

eFigure 1: Mean vilobelimab (IFX-1) concentrations over time



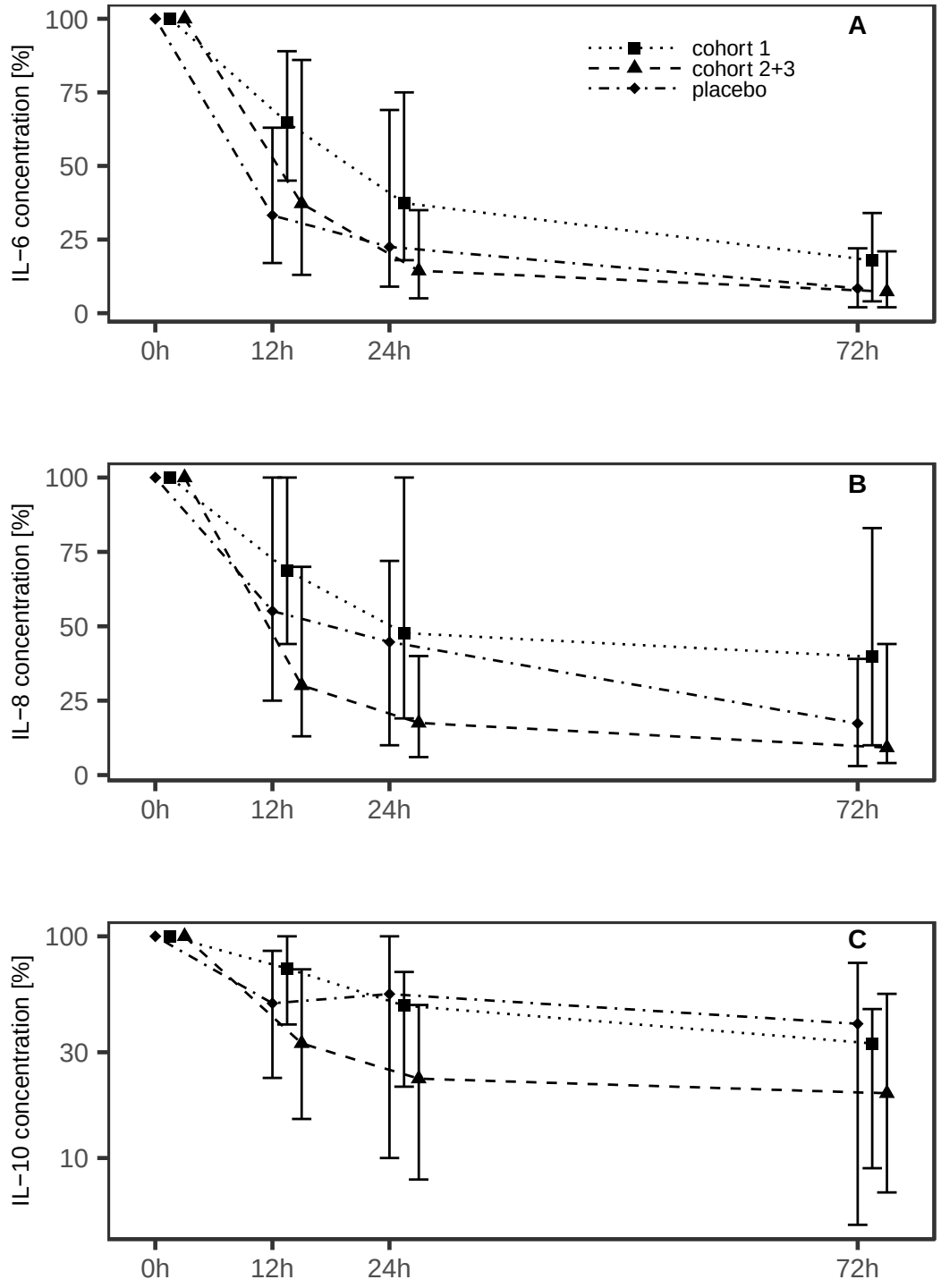
Geometric mean and 95% confidence intervals (statistical difference with $p<0.05$ is given when the confidence intervals do not overlap).

eFigure 2: Cytokine concentrations



Geometric mean and 95% confidence intervals (statistical difference with $p<0.05$ is given when the confidence intervals do not overlap).

eFigure 3: Cytokine concentrations (relative changes)



Relative change to baseline (%) with median and interquartile range. Dosing cohorts 2 and 3 were combined because they received the exact same dosing until 72h

eTable 6: Patient characteristics (placebo vs. cohort 2+3)

	Placebo (N = 24) n (%)	**Cohort 2+3 (N = 32) n (%)**
Male sex	16 (66.7 %)	20 (62.5 %)
Age [years]	65 (53, 75)	74 (59, 81)
BMI [kg/m^2]	25.3 (22, 27)	26.8 (24, 31)
APACHE II-score	22.5 (17, 27)	18.0 (16, 27)
SOFA-score	9.0 (8, 11)	9.0 (7, 11)
Modified SOFA-score	8.0 (7, 10)	7.0 (7, 8)
Acute renal insufficiency	6 (25.0 %)	12 (37.5 %)
Vasopressor use	22 (91.7 %)	27 (84.4 %)
Mechanical ventilation	20 (83.3 %)	22 (68.8 %)
Surgery	18 (75.0 %)	23 (71.9 %)
Primary focus Pulmonary Abdominal	 9 (37.5 %) 15 (62.5 %)	 13 (40.6 %) 19 (59.4 %)
Coexisting diseases Coronary artery disease Diabetes mellitus Arterial hypertension COPD	 5 (20.8 %) 2 (8.3 %) 10 (41.7 %) 3 (12.5 %)	 3 (9.4 %) 3 (9.4 %) 21 (65.6 %) 8 (25.0 %)
Lactate [mmol/l]	1.9 (1.6, 3.7)	3.5 (2.4, 4.4)
Time to IP (CD only)[h]	4.5 (4, 6)	3.6 (2, 6)
Time to IP (non-CD only)[h]	8.1 (5, 10)	6.8 (5, 10)

Data are presented as median and 25% and 75% percentiles or absolute numbers (percentage). APACHE: Acute Physiology And Chronic Health Evaluation; BMI: body mass index, CD: cardiovascular dysfunction, COPD: chronic obstructive pulmonary disease, IP: first infusion of investigational product (vilobelimab or placebo), SOFA: sequential organ failure assessment, modified SOFA: modified mean SOFA until day 10 with central nervous system subscore omitted and calculating renal subscore without considering urine output.

eTable 7: Secondary outcomes (placebo vs. cohort 2+3)

	Placebo (N = 24)	**Cohort 2+3 (N = 32)**
28-day mortality	3 (12.5 %)	5 (15.6 %)
ICU free days (until day 28)	2.5 (0, 20)	16.5 (0, 23)
Mean SOFA	5.7 (4.0, 8.5)	5.2 (4.0, 8.0)
Mean modified SOFA	5.1 (3.7, 7.9)	4.6 (3.3, 6.1)
Ventilator free days until day 14	6.0 (0.0,12.0)	10.0 (0.5,12.5)
Vasopressor free days (until day 14)	9.5 (4, 13)	11.5 (5, 13)
RRT free days (until day 14)	14.0 (14.0,14.0)	14.0 (13.5,14.0)
AT free days (until day 14)	0 (0, 4)	3 (0, 5)

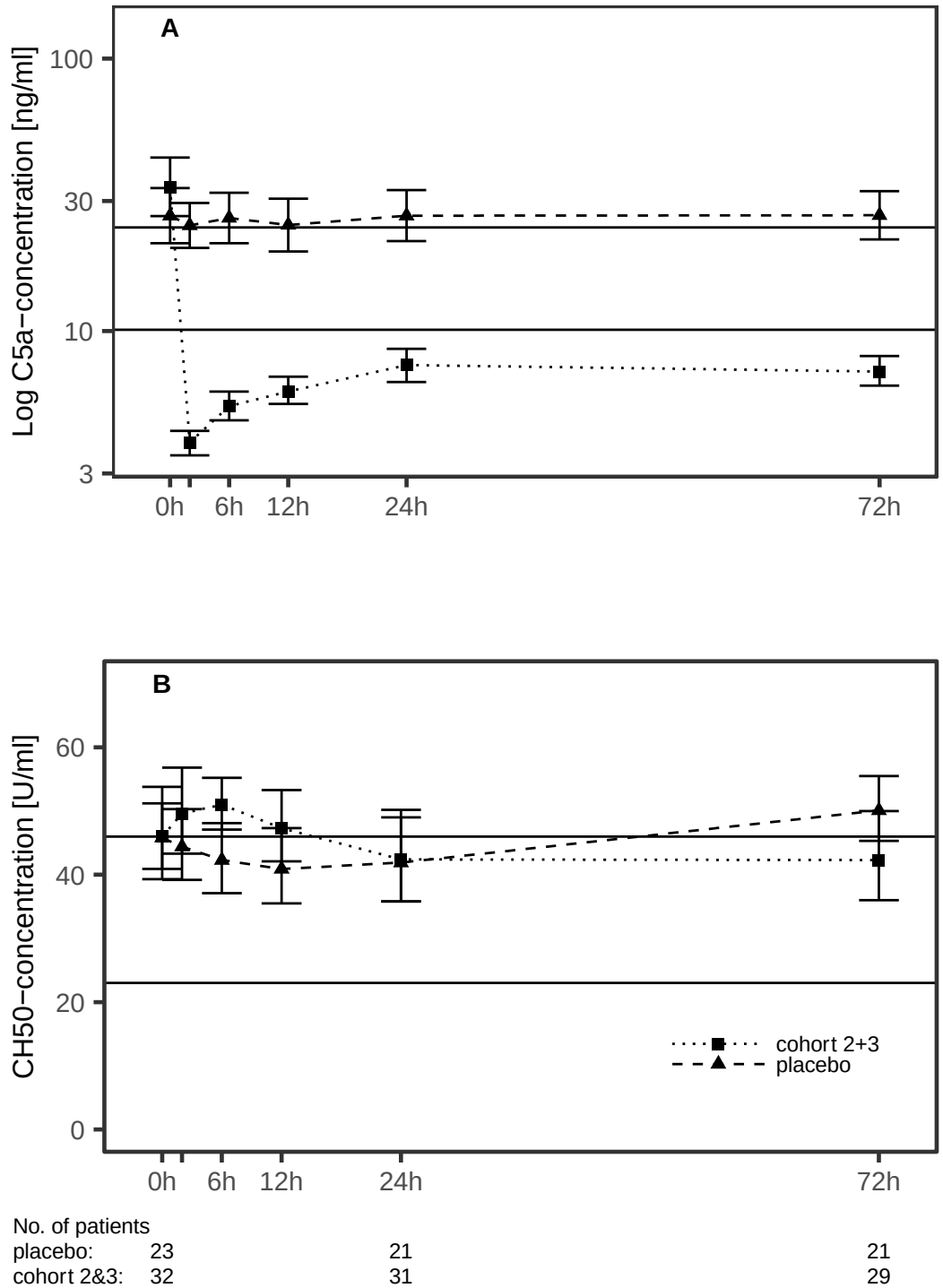
Data are presented as median and 25% and 75% percentiles or absolute numbers (percentage). AT: antimicrobial therapy, ICU: intensive care unit, RRT: renal replacement therapy, SOFA: sequential organ failure assessment. The mean SOFA-Score was calculated for each individual patient over 10 days based on the SOFA score for each study day.

eTable 8: Overview of adverse events (AEs; placebo vs. cohort 2+3)

	Placebo (N = 24) n (%)	**Cohort 2+3 (N = 32) n (%)**
Overall Incidence	21 (87.5 %)	27 (84.4 %)
Related AEs[1]	2 (8.3 %)	2 (6.3)
Serious AEs	13 (54.2 %)	13 (40.6 %)
AEs with fatal outcome	4 (16.7 %)	5 (15.6 %)
Related AEs[1] with fatal outcome	-	-
AEs by System Organ Class (≥ 10 % of patients)		
Metabolism and nutrition disorders	9 (37.5 %)	11 (34.4 %)
Investigations	10 (41.7 %)	10 (31.3 %)
Gastrointestinal disorders	9 (37.5 %)	11 (34.4 %)
Cardiac disorders	9 (37.5 %)	11 (34.4 %)
Respiratory disorders	8 (33.3 %)	9 (28.1 %)
Blood and lymphatic system disorders	6 (25.0 %)	10 (31.3 %)
General disorders / administration site conditions	11 (45.8 %)	6 (18.8 %)
Infections and infestations	8 (33.3 %)	5 (15.6 %)
Psychiatric disorders	6 (25.0 %)	6 (18.8 %)
Injury, poisoning, procedural complications	5 (20.8 %)	5 (15.6 %)
Hepatobiliary disorders	1 (4.2 %)	4 (12.5 %)
Vascular disorders	4 (16.7 %)	3 (9.4 %)
Nervous system disorders	5 (20.8 %)	2 (6.3 %)

Data are presented as absolute numbers and percentage. [1]: Investigator assessed AE as at least possibly related
Number of patients with at least one event in respective category

eFigure 4: Mean C5a concentrations and total hemolytic complement activity (placebo vs. cohort 2+3)



Mean C5a concentrations (panel A, black lines: normal range 10.1 – 24.0 ng/ml) and total complement activity (panel B, black lines: reference range of healthy subjects 23 – 46 U/ml) of patients alive. Data are shown as geometric mean ± 95% confidence intervals (statistical difference with $p<0.05$ is given when the confidence intervals do not overlap).

eTable 9: Type of Informed consent for eligible patients

Total (N)*	Medical consultant (n)	Authorized/legal representative (n)	Patient (n)
76	32	17	27

* Includes 4 patients who were registered, but not treated