Supplemental data

Supplemental methods

a) **Exclusion criteria** for the enrollment of COVID-19 patients in the Phase I/II trial of eculizumab were as follows:

Known hypersensitivity and / or previous eculizumab therapy; septic shock and / or multiple organ failure syndrome; history of infection by human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C (HCV) (with the exception of chronic infections treated or cured for HBV and HCV, which will be accepted); neoplasms in activity or under treatment, except for basal cell carcinomas; any major surgery, extensive radiation therapy, delayed toxicity chemotherapy, biological therapy, or immunotherapy within 6 weeks prior to clinical trial screening; any investigational medication other than the study drugs in the 6 weeks before the first dose of the study drug; history of chronic liver disease (Child-Pugh B or C); history of chronic kidney disease (glomerular filtration rate <30 mL / min / 1.73 m²); any contraindication to the use of penicillin for bacterial meningitis's prophylaxis during the use of eculizumab; any contraindications to the use of contraceptive methods in childbearing-age women.

b) **Exclusion criteria** for enrolling COVID-19 patients in the AMY-101 compassionate use program at San Raffaele Hospital (Milan, Italy) were as follows: evidence of bacterial infection; concomitant administration of other immunosuppressive biologic agents.

Supplemental Tables

Supplemental table S1. Patient demographics and clinical findings at baseline

a) AMY-101-treated COVID-19 patient group (N=3)

Demographics/clinical characteristics	**Patient #1**	**Patient#2**	**Patient #3**
Age range (decade)	70’s	60’s	60’s
Sex	Male	Male	Male
Ethnicity	Caucasian	Caucasian	Caucasian
Body Temperature (°C)	37.1	37.8	37.2
Baseline PaO_2/FiO_2 ratio (before start of therapy)	148 mmHg	142 mmHg.	PaO_2/FiO_2 =265 mmHg (at hospital admission)

Disease severity (NIH/ WHO guidelines)	Severe COVID-19	Severe COVID-19	Severe COVID-19
Body Mass Index (BMI)	32	25,26	29
Comorbidities	coronary artery disease/ atrial fibrillation	Hypertension, mild smoker	chronic hypertension
	hypercholesterolemia		spontaneous deep intraparenchymal hemorrhage/no neurological symptoms
	chronic hypertension		
	critical limb ischemia of the right leg requiring surgery.		
	mild renal impairment		
Other medications	N/A	chronic treatment with ACE inhibitor	chronic treatment with ACE inhibitor + amlodipine
Concomitant treatments during therapy with complement inhibitor (e.g. steroids, antivirals etc)	antibacterial prophylaxis: piperacilline/tazobactam	prophylaxis with LMWH Antibacterial prophylaxis with ceftriaxone	prophylaxis with LMWH Antibacterial prophylaxis with ceftriaxone
Drug dose, route of drug delivery, duration of therapy	5 mg/kg/daily, continuous IV infusion; **14 days**	5 mg/kg/daily, continuous IV infusion; **12 days**	5 mg/kg/daily, continuous IV infusion; **9 days**
Laboratory findings at baseline*			
White Blood cell, x109/L	11.3	11.7	8.2
Lymphocyte count, x109/ L	1.0	0.9	1.2
Neutrophil count, x109/L	9.2	10.4	6.2
Hemoglobin, g/dl	8.7	15.8	14.4
Platelet count, x109/L	203	372	354
Total bilirubin, mg/dl	0.72	1.06	0.53
Alanine Amino Transferase, U/L	78	50	53
Aspartate Transaminase, U/L	44	52	48
Creatinine, mg/dl	1.55	0.87	0.91
Glucose, mg/dl			

Lactate dehydrogenase, U/L	306	405	439
C-reactive protein, mg/L	94.5	61.2	35.3
Prothrombin time (sec)	14.3	13.8	14.2
Creatine kinase, U/L	97	236	106

b) Eculizumab-treated COVID-19 patient cohort (N=10)

Demographics/clinical characteristics	**Patient #1**	**Patient #2**	**Patient #3**	**Patient #4**	**Patient #5**	**Patient #6**	**Patient #7**	**Patient #8**	**Patient #9**	**Patient #10**
Age range (decade)	60's	30's	70's	30's	70's	70's	50's	50's	40's	30's
Sex	M	M	M	M	M	F	M	M	M	F
Ethnicity	Black	Caucasian	Caucasian	Pardo	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Body Temperature (°C)	35.4	38.2	35.6	38.2	35.0	37.5	36.2	37.6	36.3	37.5
Baseline PaO_2/FiO_2 ratio (before start of therapy)	128	73	73	380	54	122	126	140	104	81
Disease severity (NIH/ WHO guidelines)	Critical	Severe	Severe	Severe	Critical	Severe	Severe	Severe	Critical	Severe
Body Mass Index	23.8	36.9	19.82 (estimate)	39.18 (estimate)	26.51	23.51 (estimate)	31.21	45.2	33.8	41.9
Comorbidities	Epilepsy	Obesity	None	Obesity	benign prostatic hyperplasia	Asthma	Asthma	Obesity	Obesity	Obesity
	Schizophrenia	hypercholesterolemia		OSA			chronic hypertension	chronic hypertension		
Other medications	Carbamazepine, olanzapine, Clonazepam	None	None	None	Doxazosin	salbutamol	Enalapril, salbutamol	Losartan, Clonidine, aspirin	None	None
Concomitant treatments during therapy with complement inhibitor (e.g. steroids, antivirals etc)	Prophylaxis with LMWH Antibacterial drugs: Meropenem, vancomycin, ceftriaxone Steroids (metilprednisolone)	Prophylaxis with UFH Antibacterial drugs: Azithromycin ceftriaxone Steroids (metilprednisolone)	Prophylaxis with UFH Antibacterial drugs: Azithromycin ceftriaxone Steroids (metilprednisolone)	Prophylaxis with UFH Antibacterial drugs: Azithromycin ceftriaxone Steroids (metilprednisolone)	Prophylaxis with LMWH Antibacterial drugs: piperacillin/ tazobactam, Ceftriaxone Steroids (metilprednisolone)	Prophylaxis with UFH Antibacterial drugs: Clarithromycin, ceftriaxone Steroids (metilprednisolone)	Prophylaxis with UFH Antibacterial drugs: ceftriaxone Steroids (metilprednisolone)	Prophylaxis with LMWH Antibacterial drugs: Amoxicillin-clavulanate Steroids (metilprednisolone)	Prophylaxis with LMWH Antibacterial drugs: Meropenem, vancomycin, azithromycin, ceftriaxone Steroids (metilprednisolone, dexamethasone)	Prophylaxis with LMWH Antibacterial drugs: , azithromycin, ceftriaxone Steroids (metilprednisolone) Colchicine

Drug dose, route of drug delivery, duration of therapy	900mg, IV infusion, once a week (3 doses total).	900mg, IV infusion, once a week (2 doses total).	900mg, IV infusion, once a week (3 doses total).	900mg, IV infusion, once a week (1 dose total).	900mg, IV infusion, once a week (2 doses total).	900mg, IV infusion, once a week (3 doses total).	900mg, IV infusion, once a week (3 doses total).	900mg, IV infusion, once a week (2 doses total).	900mg, IV infusion, once a week (2 doses total).	900mg, IV infusion, once a week (1 dose total).
Laboratory findings at baseline*										
White Blood cell, x109/L	8.5	7.2	9.7	6.4	10.6	9.2	8.5	12.7	8.9	3.2
Lymphocyte count, x109/L	0.8	1.4	0.8	1.4	0.6	0.2	1	1.1	1.0	0.6
Neutrophil count, x109/L	7.4	5.2	9.7	4.1	9.3	7.9	7.1	10.7	7.1	2.4
Hemoglobin, g/dl	10.5	14.3	12.4	13.3	15.8	13.2	12.7	15.0	13.6	13.4
Platelet count, x109/L	168	231	536	202	267	348	279	329	387	159
Total bilirubin, mg/dl	0.73	0.5	0.3	1.14	0.44	0.5	0.31	0.4	0.8	0.29
Alanine Amino Transferase, U/L	110	141	28	99	53	25	187	43	38	41
Aspartate Transaminase, U/L	127	82	32	98	45	17	127	51	27	34
Creatinine, mg/dl	1.28	0.91	1	0.95	0.85	0.66	0.67	0.83	1.1	0.61
Glucose, mg/dl	93	128	155	94	177	169	168	130	117	160
Lactate dehydrogenase, U/L	568	390	356	675	373	235	321	808	353	477
C-reactive protein, mg/L	310	69	187.4	82	76	70	28	157.4	125	103
Lactate, mmol/L	2.6	1.8	2	2.3	3.3	2.3	2	2.1	1,5	1.4
Prothrombin time (sec)	14.7	12.5	15.5	13.6	14.4	15.3	17.3	16.2	1.14	1.1
Creatine kinase, U/L										

Supplemental Table S2. Severe adverse events in the cohort of patients receiving eculizumab.

Patient	Adverse Event	Grade (CTCAE)

UPN1	Disseminated intravascular coagulation	3
UPN1	Anemia	3
UPN5	Pulmonary thromboembolism	3
UPN6	Myocarditis	3

None of the severe adverse events were likely to be attributable to the study drug.

Supplemental Figures

Supplemental Figure 1: *Both C3 and C5 inhibition impact thrombogenic pathways that promote thrombin generation in COVID-19*. Complement inhibition resulted in reduction of thrombin-antithrombin complexes (TAT levels) in first 7 days of therapy. TAT complexes were measured in EDTA-plasma samples collected from COVID-19 patients dosed either with the C3 therapeutic AMY-101 (right panel) or with the C5-targeting mAb eculizumab (left panel). A similar reduction of TAT levels is observed in both patient groups from baseline through day 7, indicating a therapeutic effect of both inhibitors on COVID-19 associated coagulopathy. TAT complexes were quantified as described previously.

Supplemental Figure 2:

Supplemental Figure 2. The tetrahedral pathophysiology of COVID-19. Complement activation plays a key role in the pathophysiology of COVID-19, eventually leading to the cytokine storm (associated with lung disease) and thrombophilia (accounting for multi-organ thrombotic microangiopathies). Thrombo-inflammation has emerged as a hallmark of COVID-19 immunopathology and neutrophil-driven NETosis appears to be a key disease-exacerbating mechanism cross-linked with all other pathogenic events. Indeed, complement activation may trigger NETs generation, which in turn amplifies complement activation, thereby enhancing inflammation and thrombophilia. Complement activation, cytokine-driven inflammation, NETosis and thrombophilia are closely embedded in the pathophysiology of COVID-19, and therapeutic interventions aiming to 'defuse' this detrimental loop need to interfere with early pathogenic events, such as proximal complement activation, preceding the tissue damage associated with thrombo-inflammation.