Supplementary Material

The effect of mesenchymal stem cells on the host response in severe community-acquired pneumonia

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Supplementary Methods

Study design, patient population, and study treatment

SEPCELL (prospectively registered in the European Union Clinical Trials Register, EudraCT Number: 2015-002994-39; ClinicalTrials.gov Identifier: NCT03158727) was a randomized, multicentre, double-blind, placebo-controlled phase Ib/IIa trial primarily aimed at determining the safety profile of Cx611 (allogeneic adipose-derived cryopreserved mesenchymal stem cells) as an adjunctive therapy for patients with severe CABP [1,2]. The study was approved by independent ethics committees of the participating hospitals and written informed consent was obtained from all patients, their legal representative or next of kin. The current study was part of the pre-defined exploratory biological endpoints of the trial [1]. The complete trial protocol and the results of the primary safety and secondary clinical efficacy endpoints have been published previously [1,2]. In brief, adult patients (≥18 years and ≤80 years old) were eligible for the study if there was a clinical suspicion of severe CABP (two of the following symptoms: fever, tachypnoea, leucocytosis, or hypoxemia, plus radiographic evidence of a new infiltrate), and if patients needed mechanical ventilation (including high-flow oxygen) and/or vasopressor treatment. Exclusion criteria included CAP of solely viral or fungal origin, immune deficiency and several other comorbidities. In- and exclusion criteria can be found below. Patients were randomized (stratified on need for vasopressors, need for mechanical ventilation, or both) to receive either two intravenous administrations of Cx611 ($160x10^6$ cells) or placebo (Ringer's lactate) at day 1 and 3 of the study. Viability of Cx611 was tested in batches, the viability specification for drug product release was not lower than 90% per batch. Viability after thawing was consistently high (mean 95.2%). Individual vials of the study drug were not retested for viability prior to administration.

Inclusion Criteria

1. Adult patients of either sex (aged ≥ 18 and ≤ 80 years).
2. Body weight from 50 kg to 100 kg.
3. Clinical diagnosis of acute (developed within past ≤ 21 days) community-acquired bacterial pneumonia based on the presence of two relevant signs (fever, tachypnoea, leucocytosis, or hypoxemia) and radiographic findings of new pulmonary infiltrate(s).
4. Patients with pneumonia of sufficient severity requiring management in the intensive care unit and with at least one to the two following major criteria of severity present for less than 18 hours:

(a) Required invasive mechanical ventilation for respiratory failure due to pneumonia, or

(b) Required treatment with vasopressors (i.e. dopamine > 5 μg/kg/min or any dose of epinephrine, norepinephrine, phenylephrine, or vasopressin) for at least 2 hours to maintain or attempt to maintain systolic blood pressure > 90 mmHg (or mean arterial pressure > 70 mmHg) after adequate fluid resuscitation (i.e. for shock). Patients who had a high-flow nasal cannula at ≥ 50 liters per minute and fraction of inspired oxygen (FiO2) ≥ 0.6 or under nonmechanical ventilation for ≥ 18 hours were not eligible for the study.

5. Female patients of nonchildbearing potential (nonfertile, premenarche, permanently sterile [had undergone hysterectomy, bilateral salpingectomy, or bilateral ovariectomy]) or postmenopausal (history of no menses for at least 12 months without an alternative medical cause),

or

Women of childbearing potential with a negative serum or urine pregnancy test (sensitive to 25 IU human chorionic gonadotropin) and agreed to use an adequate method of contraception for 3 months after the last dose of the investigational medicinal product according to her preferred and usual lifestyle. Adequate methods of female contraception for this study were: sexual abstinence (refraining from heterosexual intercourse); hormonal contraception (both progesterone-only or combined estrogen and progesterone, both with inhibition of ovulation or where inhibition of ovulation was not the primary mechanism of action); intra-uterine device; bilateral tubal occlusion; condom use by male sexual partner(s); or medically-assessed successfully vasectomized male sexual partner(s).

Male patients agreed to use one of the following methods of birth control according to his preferred and usual lifestyle for 3 months after the last dose of investigational medicinal product sexual abstinence (refraining from heterosexual intercourse), use of condoms or medically-assessed successful vasectomy, or having a female sexual partner(s) using an adequate method of contraception as described above.

6. Signed informed consent provided by the patient, the relatives, or the designated legal representative according to local guidelines.

Exclusion Criteria

A patient was not included in the study if he/she met any of the following criteria:

1. Patients who had hospital-acquired, healthcare-associated, or ventilator-associated pneumonia.
2. Patients who had pneumonia exclusively of viral or fungal origin.* Patients with bacterial pneumonia co-infected with viruses and/or other microorganisms were entered into the study.

*Owing to the short time window (up to 18 hours) between fulfilment of severity criteria (i.e. initiation of invasive mechanical ventilation or vasopressors, whichever came first) and the start of the first dose of the investigational medicinal product, patients with a pneumonia of suspected bacterial origin by any established standard diagnostic method routinely applied at the study site (e.g. urinary antigen test, real-time polymerase chain reaction were entered into the study (confirmation of bacterial origin must have been obtained afterwards).

3. Patients who had known or suspected *Pneumocystis jirovecii* (formerly known as *Pneumocystis carinii*) pneumonia.
4. Patients who had aspiration pneumonia.
5. Patients who had known active tuberculosis.
6. Patients who had a history of post-obstructive pneumonia.
7. Patients who had cystic fibrosis.
8. Patients who had any chronic lung disease requiring oxygen therapy at home.
9. Presence of infection in another organ location caused by the same pathogen (e.g. pneumococcal meningitis in the context of pneumococcal pneumonia).
10. Patients who were expected to have rapidly fatal disease within 72 hours after randomization.
11. Inability to maintain a mean arterial pressure ≥ 50 mmHg before screening despite the presence of vasopressors and intravenous fluids.
12. Patients who were not expected to survive for 3 months owing to other pre-existing medical conditions such as end-stage dementia or other diseases.
13. Patients who had a history of malignancy in the 5 years before screening, except for successful surgically-treated nonmelanoma skin malignancies.
14. Patients who had known primary immunodeficiency disorder or had HIV infection and AIDS with CD4 count < 200 cells/mm^3 or were not receiving highly active antiretroviral therapy for HIV.
15. Patients who were receiving immunosuppressant therapy (including long-term treatment with any anti-tumour necrosis factor alpha) or were on long-term high doses of steroids

(single administration of ≥ 2 mg/kg body weight for ≥ 2 weeks or 20 mg/day of prednisone or equivalent for ≥ 2 weeks).
16. Chronic granulocytopenia, not thought to be due to sepsis, as evidenced by an absolute neutrophil count < 500 per μL for > 21 days before onset of pneumonia symptoms.
17. Patients who had received stem cell therapy or undergone allogenic transplantation (organ or bone marrow transplant) within the past 6 months.
18. Patients who were receiving treatment with a biological agent (e.g. antibodies, cells), immunotherapy, or plasma exchange treatment within the past 8 weeks.
19. Patients who were receiving or had received another investigational medication within 90 days before start of the study (or 5 half-lives of the investigational compound, whichever was longer).
20. Had known allergies or hypersensitivity to penicillin or streptomycin and/or any component of CryoStor CS10.
21. Patients who had a known liver function impairment, associated with liver cirrhosis (Child Pugh C) or known oesophageal varices.
22. Patients who were hospitalized within the previous 15 days.
23. Conditions resulting in a New York Heart Association or Canadian Cardiovascular Society Class IV functional status.
24. End-stage neuromuscular disorders (e.g. motor neuron diseases, myasthenia gravis) or cerebral disorders that impair weaning.
25. Patients who had quadriplegia (traumatic or otherwise).

Time points for biomarker studies and sample collection

Samples for biomarker analyses were collected at up to six time points per patient: within 18 hours after initiation of vasopressors and/or mechanical ventilation but prior to the initiation of treatment (screening/SCR), 8-12 hours following the initial infusion of Cx611 or placebo on day 1 (visit 1/V1), day 2 (V2), 8-12 hours following the second infusion of Cx611 or placebo on day 3 (V3), day 7 (V7), and day 14±2 (V9) (Figure 1A). Sample collection continued after ICU and hospital discharge. Supplementary Table 1 and Supplementary Figure 1 show the total number samples at each time point and for each patient, and the total number of patients in the study in this time frame. For plasma biomarkers, blood was collected in ethylenediaminetetraacetic acid-anticoagulated (EDTA-)tubes and centrifuged (1750g, 10 minutes) to obtain plasma. For whole blood RNA, blood was collected in PAXgene RNA tubes (Becton-Dickinson, Breda, The Netherlands).

Protein biomarker assays

To obtain insight into the effect of Cx611 on host response pathways implicated in sepsis pathogenesis we measured 29 protein biomarkers reflective of five pathophysiological domains (inflammation, inhibition of inflammation, apoptosis, endothelial cell responses, and coagulation) before and at five time points after treatment (Figure 1A). These biomarkers included several molecules directly secreted by MSCs (e.g. neutrophil gelatinase-associated lipocalin, hepatocyte growth factor, galectin-1, and angiopoietin-1) [3–5], although these molecules may also be secreted by a various cells endogenous to the patient. Prothrombin fragment F1+2 was measured by ELISA (Siemens, Munich, Germany) according to the manufacturer's instructions. All other plasma biomarkers were measured using Luminex (R&D Systems, Abingdon, United Kingdom) on a BioPlex 200 (Bio-Rad, Hercules, California).

For Luminex, individual analytes with fewer than 25 beads measured, or samples with fewer than 25 beads measured in >50% of analytes, were removed. For both Luminex and ELISA, analytes below the limit of quantification were imputed to half the lower limit of quantification. Imputing to half the lower limit of quantification was necessary for 67/456 (14.7%) of measurements of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), 88/456 (19.3%) of measurements of interleukin (IL-)10, and a negligible number of samples for the remaining biomarkers (nine total measurements). Analytes above the upper limit of quantification were either diluted further and remeasured, extrapolated above the standard curve, or imputed to the highest value measured that day. Imputing to the highest value measured that day was necessary for only 33/13239 (0.25%) of total measurements.

Batch effects between assay plates in the Luminex assays were corrected as follows. For each run, the background fluorescence level was computed by taking the mean fluorescence of the blanks included on each plate ($F_{BG} = \sum f_B \,/n$). The background fluorescence was subtracted from each cell's fluorescence, and any background-subtracted fluorescence values that were less than 1 were set to 1 ($f_i' = \max(f_i - F_{BG}, 1)$). Quality control samples which were present in each Luminex run were used as bridging samples. A simple linear regression was used to fit log-transformed background-subtracted fluorescence values as a function of plate effects and quality control sample index. The regression formula was

$$\log f_i' = \sum \alpha_p P_{ip} + \sum \beta_q Q_{iq} + e_i$$

where α_p is the plate effect coefficient for plate p, P_{ip} is an indicator if sample i was on plate p, β_q is the sample coefficient for sample q, Q_{iq} is an indicator if sample i was on plate q, and e_i is the error term. Fluorescence values were then rescaled to plate 1 levels by backing out the plate effect coefficients and background fluorescence transformations.

$$f_i'^{(1)} = f' \exp\left(\alpha_1 - \sum \alpha_p P_{ip}\right)$$

$$f_i^{(1)} = f_i'^{(1)} + F_{BG,p}$$

A standard curve was run on each day, and a 5-PL model was used to compute concentrations for each day's samples. If the coefficient of variation of standard replicates exceeded 25% or if the standard recover was outside the limit ±20%, the standard was called outside the limit of quantification (LOQ). The LOQ was the set by taking the largest range of standards which contained no standards outside of the LOQ. For samples that were run at two dilutions, the lower dilution sample was preferred, unless that sample's concentration was below that run's lower limit of quantification, in which case the higher dilution sample was used.

RNA isolation and sequencing

PAXgene tubes (Becton-Dickinson, Breda, The Netherlands) were processed for RNA isolation using the PAXgene Blood miRNA Kit (QIAGEN, Hilden, Germany). RNA quality was assessed by bioanalysis (Agilent, Santa Clara, California), using RNA integrity (RIN) scores. Total RNA concentrations were determined by Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, California). Sequencing libraries were prepared by means of the KAPA RNA HyperPrep with RiboErase (Roche, Basel, Switzerland) as per manufacturer's instructions. Libraries were sequenced using the Illumina NovaSeq 6000 (Illumina, San Diego, CA, USA) to generate 150bp paired-end reads. The sequence read quality was assessed using FastQC methods (version 0.11.5; Babraham Institute, Babraham, Cambridgeshire, UK). Trimmomatic version 0.39 was used to trim the Illumina adapters and filter low quality reads and ambiguous nucleotide-containing sequences. After pre-processing, the remaining high-quality reads were aligned against the human Genome Reference Consortium Build 38 (GRCh38, Ensembl 84) using Hisat2 (2.2.0) with default parameters. Count data were generated by means of the FeatureCount. A further QC step was performed by applying principal component analysis (PCA) to the variance stabilized transformed data to detect for outlying samples. Any outliers were assessed for their FastQC metrics to ascertain whether technical issues were responsible for these outliers. After all quality control

steps, 30/454 (6.6%) samples were removed. RNAseq data is available from the NCBI Sequence Read Archive (SRA) under the BioProject accession PRJNA1097551.

RNA analysis

To take advantage of the repeated measure design of this study we implemented a linear mixed model (LMM) to compare intervention versus placebo groups. This approach evaluates within-subject changes and therefore controls for subject differences in gene expression at baseline. Specifically, we used the lmmSeq function from the glmmSeq package [6], to fit a Gaussian LMM to the variance stabilizing transformed expression values of each gene. The random effect was the subject ID to account for the within-subject correlation. The fixed effect variables were intervention group and time point (modelled as a categorical variable), and the interaction term between them. This interaction design allowed us to investigate the effect of intervention on gene expression at each time point by inspecting the interaction coefficient corresponding to each time-point (V1, V2, V3, V7, V9). Note that the screening (SCR) time point was always the baseline to which gene expression values were compared against. Then for the pathway analysis we constructed a standard score per gene per time point by dividing the interaction coefficient by the standard error of that interaction coefficient (z = beta / beta standard error). The standard scores were then ordered for Gene Set Enrichment Analysis (GSEA); this was performed on Reactome pathways focusing on Immune System (R-HSA-5357801), Hemostasis (R-HSA-109582) and Programmed Cell Death (R-HSA-5357801) [7]. Note that although we focused on presenting the above pathways we corrected for multiple testing (using the Benjamini-Hochberg method) of all Reactome pathways that contain more than 20 genes and are in the first 4 levels of the database hierarchy (a total of 778 pathways).

Statistical analysis

Tests were two-tailed throughout and a *P*-value <0.05 was considered statistically significant, adjusted for multiple testing using the Benjamini-Hochberg method where mentioned. Categorical data are displayed as count (percentage), normally distributed continuous data are displayed as mean (standard deviation), non-normally distributed continuous data are displayed as median (interquartile range). We assessed the distribution of continuous data graphically. For the heatmap, we first calculated the fold change of each protein biomarker within each patient from the SCR value to each time point after the initiation of the study drug (V1 through V9) and subsequently calculated the difference in fold change at each time point

between the Cx611 and control groups using Hedges' *g*, a commonly used effect size measure [8].

Protein biomarker levels were log-transformed prior to analysis. The overall effect of Cx611 on plasma biomarker levels was assessed using a linear mixed model in which the dependent variable was the concentration of a biomarker from time points V1 through V9, and the independent variables the baseline biomarker concentration prior to the first study drug administration (SCR) – to account for chance variation in pre-treatment biomarker levels – the time point (discrete time), the treatment arm, and an interaction term between the time point and the treatment arm. These models included a random intercept per patient. We then calculated the overall *P*-value for the difference in biomarker levels attributable to Cx611 from time points V1 through V9 (adjusted for variations in SCR levels) from these models using a type II Wald test. We used the same models to test differences between groups at each post-treatment time point by changing the reference category for time and subsequently assessing the *P*-value for the treatment coefficient.

R packages

Statistical analyses were performed using R (version 4.1.2). R packages used for statistical and bioinformatic analyses: rstatix, nlme, lme4, BioMart, DESeq2, and glmmSeq.

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Supplementary Tables

Supplementary Table 1. Number of samples per time point* and survival table.

	Plasma biomarkers			Blood transcriptomics	
Time point†	**Cx611**	**Placebo**		**Cx611**	**Placebo**
SCR	41	41		37	39
V1	40	41		37	33
V2	39	40		39	36
V3	37	40		35	37
V7	36	39		34	34
V9	33	35		31	32
	Survival table‡				
Days†	**Cx611**	**Placebo**			
0	41	41			
1	41	41			
2	41	41			
3	39	40			
4	39	40			
5	37	40			
6	37	40			
7	37	40			
8	36	40			
9	36	40			
10	36	40			
11	36	39			
12	36	38			
13	35	38			
14	35	38			
15	34	37			
16	34	37			

*The table lists the number of samples that passed all quality control steps and were subsequently used in analyses, as described in the Supplementary Methods.
† Please note that the time points (in days) for the biomarker studies may not correspond exactly to the time (in days) at which participation in the study was registered, because the biomarker time points are defined relative to inclusion time and study treatment administration. Time points at which samples were collected for plasma protein and blood transcriptomic analyses: within 18 hours of initiation of vasopressors and/or mechanical ventilation, prior to the initiation of treatment (screening/SCR), 8-12 hours following the initial infusion of Cx611 or placebo on day 1 (visit 1/V1), day 2 (V2), 8-12 hours following the second infusion of Cx611 or placebo on day 3 (V3), day 7 (V7), and day 14±2 (V9).
‡Number of patients in the study at each day since intensive care unit admission in the window relevant for biomarker analyses, a decrease indicates that patients have died or have been censored (e.g. due to withdrawing consent to participate).

Supplementary Table 2. Key findings from the SEPCELL trial

Patients
• 42 Cx611, 41 placebo • Population balanced for demographics and disease severity scores
Safety outcomes
• No differences in frequency or severity of treatment-emergent adverse events • No differences in thromboembolic events • No differences in anti-HLA antibodies after treatment
Clinical outcomes
• No differences in 90-day mortality • No differences in days free from mechanical ventilation and/or vasopressors • No differences in other clinical outcomes
For more details see the primary clinical report [2]. HLA = human leukocyte antigen.

Supplementary Table 3. Overview of plasma biomarker concentrations per time point for the Cx611 and placebo groups

	SCR n = 41 | n = 41	V1 n = 40 | n = 41	V2 n = 39 | n = 40	V3 n = 37 | n = 40	V7 n = 36 | n = 39	V9 n = 33 | n = 35
TNF (pg/mL)	38.5 [28.5, 53.1]	40.7 [30.3, 54.7]	37.8 [30.2, 45.1]	35.4 [28.0, 44.6]	29.5 [22.6, 34.3]	25.1 [20.8, 30.1]
	35.1 [26.2, 48.3]	30.9 [25.5, 40.5]	29.9 [24.7, 39.6]	26.0 [22.7, 34.0]	26.5 [19.7, 30.3]	25.5 [21.5, 31.1]
IL-1β (pg/mL)	42.9 [35.8, 58.9]	45.8 [33.7, 61.8]	47.7 [36.2, 58.3]	45.5 [30.9, 52.4]	36.2 [29.8, 43.3]	31.0 [28.4, 38.7]
	43.2 [31.3, 63.1]	45.8 [27.0, 58.0]	39.8 [28.0, 56.9]	37.1 [26.1, 49.1]	36.1 [24.5, 46.7]	33.5 [28.0, 40.6]
IL-6 (pg/mL)	169.7 [52.9, 1287.8]	172.2 [42.7, 698.2]	90.7 [32.8, 340.5]	62.2 [38.7, 243.2]	27.7 [12.2, 66.5]	18.0 [9.7, 34.6]
	146.2 [48.4, 727.7]	107.0 [45.4, 230.3]	71.9 [44.1, 207.5]	40.4 [21.1, 78.3]	21.0 [13.6, 51.2]	17.0 [9.7, 65.2]
IL-8 (pg/mL)	49.6 [24.4, 170.6]	40.7 [28.1, 126.1]	34.8 [22.0, 72.9]	32.7 [24.0, 56.1]	26.0 [18.5, 49.2]	26.5 [15.0, 34.0]
	39.6 [23.7, 51.6]	30.1 [19.1, 61.7]	30.6 [19.9, 47.3]	25.1 [16.8, 34.6]	21.6 [16.1, 30.0]	21.7 [16.4, 35.4]
IL-18 (pg/mL)	624.7 [344.8, 1015.6]	619.0 [391.3, 1457.9]	703.6 [361.1, 1727.3]	672.7 [409.7, 1375.2]	437.1 [309.2, 950.5]	365.4 [297.2, 641.8]
	542.9 [328.6, 777.5]	539.1 [376.1, 786.9]	566.3 [390.7, 850.5]	543.1 [334.4, 807.6]	478.3 [297.3, 694.7]	398.8 [328.2, 565.2]
CCL18 (ng/mL)	99.2 [55.1, 154.7]	99.7 [60.9, 137.7]	94.6 [49.6, 125.9]	72.6 [38.1, 104.6]	96.8 [64.5, 129.1]	95.3 [76.1, 158.5]
	90.9 [67.4, 197.1]	106.5 [66.5, 155.5]	101.8 [57.0, 153.2]	67.1 [46.4, 94.6]	82.5 [59.9, 125.3]	124.6 [87.4, 152.8]
MMP-8 (ng/mL)	37.3 [14.0, 71.8]	34.8 [12.6, 84.0]	26.2 [11.5, 75.9]	15.4 [6.6, 39.8]	5.4 [3.3, 12.1]	2.7 [1.6, 7.0]
	49.9 [11.0, 91.3]	37.0 [9.4, 90.1]	31.2 [5.8, 60.3]	14.2 [4.5, 24.0]	4.7 [2.9, 9.5]	2.6 [1.7, 4.4]
NGAL (ng/mL)	253.8 [151.3, 491.1]	219.2 [131.8, 608.1]	224.9 [122.9, 516.6]	198.1 [113.4, 452.9]	172.2 [102.3, 314.8]	114.9 [65.4, 197.7]
	303.2 [123.5, 381.3]	230.7 [148.3, 412.6]	205.7 [118.4, 351.9]	127.2 [96.1, 287.4]	166.5 [96.7, 247.1]	99.3 [81.6, 213.2]
TREM-1 (pg/mL)	604.5 [438.7, 1009.8]	525.5 [407.9, 895.4]	547.6 [383.4, 795.4]	539.9 [342.9, 768.6]	486.8 [324.6, 750.5]	418.6 [311.2, 594.3]
	608.3 [346.6, 776.5]	508.5 [392.3, 702.7]	479.9 [356.1, 648.9]	356.5 [289.2, 539.1]	397.8 [251.8, 548.4]	383.8 [274.4, 610.6]
Procalcitonin (ng/mL)	10.0 [1.8, 22.2]	7.4 [1.4, 22.0]	4.1 [1.1, 17.6]	2.8 [0.4, 7.0]	0.5 [0.1, 1.4]	0.1 [0.1, 0.3]
	4.6 [1.6, 10.7]	3.6 [1.4, 14.3]	3.4 [1.5, 10.6]	1.2 [0.6, 3.2]	0.3 [0.1, 0.4]	0.1 [0.0, 0.4]
IL-1RA (ng/mL)	26.2 [3.4, 57.9]	8.9 [3.4, 39.9]	7.3 [2.3, 20.0]	3.2 [1.6, 9.4]	2.5 [1.1, 3.7]	1.5 [1.1, 2.9]
	8.9 [2.9, 46.3]	5.5 [2.7, 13.8]	3.5 [2.1, 7.0]	1.7 [1.1, 4.0]	1.6 [0.8, 3.3]	1.7 [0.8, 2.4]
IL-10 (pg/mL)	18.5 [8.6, 45.9]	13.8 [6.2, 44.7]	10.4 [4.7, 20.3]	9.0 [5.1, 12.7]	4.7 [2.9, 11.8]	3.6 [1.8, 7.1]
	13.1 [8.3, 31.7]	9.3 [6.5, 17.3]	8.2 [5.7, 12.7]	5.2 [3.7, 7.2]	6.8 [2.3, 8.2]	4.9 [4.0, 7.3]
IL-27 (pg/mL)	1509.0 [1166.1, 2072.4]	1694.8 [1160.6, 2287.2]	1513.3 [1108.5, 2081.8]	1407.0 [1183.0, 1785.0]	1247.7 [890.5, 1396.2]	1184.1 [868.4, 1466.9]
	1348.3 [1120.3, 1956.9]	1392.8 [1071.7, 1875.7]	1352.5 [1090.8, 1699.0]	1160.6 [884.2, 1522.0]	1075.2 [872.1, 1298.5]	1162.7 [1073.3, 1394.5]
HGF (pg/mL)	1418.8 [661.2, 3339.6]	1799.1 [717.7, 3522.0]	1749.7 [613.0, 2901.9]	1447.9 [595.1, 6145.3]	445.4 [240.3, 1956.8]	266.8 [186.6, 949.6]
	1240.2 [661.7, 2737.6]	1251.8 [816.2, 2968.5]	1000.9 [513.8, 2492.1]	918.1 [493.2, 2499.9]	640.4 [228.1, 1541.9]	325.9 [119.6, 1664.9]
Galectin-1 (ng/mL)	43.5 [36.3, 59.5]	42.6 [31.7, 58.1]	37.1 [29.7, 59.0]	35.8 [25.8, 46.5]	44.4 [34.1, 51.9]	39.7 [33.5, 49.9]
	43.6 [29.7, 52.1]	36.0 [28.6, 48.7]	37.5 [25.8, 46.8]	30.8 [23.6, 40.2]	39.0 [27.7, 51.4]	42.3 [35.6, 54.1]

VCAM-1 (ng/mL)	2732.8 [1938.0, 3992.1]	3057.8 [2074.7, 4605.0]	3676.6 [2330.6, 4273.0]	2845.6 [1973.5, 4337.3]	2596.7 [1752.2, 3375.1]	2140.2 [1322.3, 2497.5]
	2248.7 [1677.1, 3581.0]	2253.5 [1482.0, 2998.3]	2090.8 [1550.3, 2823.7]	2475.3 [1949.4, 2754.8]	2161.8 [1762.2, 2926.1]	1826.8 [1436.4, 2486.3]
E-Selectin (ng/mL)	80.2 [41.7, 169.0]	77.8 [42.7, 194.4]	73.9 [44.2, 116.9]	72.7 [39.2, 104.2]	55.1 [36.2, 70.4]	45.4 [33.2, 60.6]
	63.4 [38.9, 204.0]	64.6 [33.3, 172.2]	58.8 [34.1, 155.6]	47.9 [36.3, 106.4]	46.3 [28.7, 85.9]	41.3 [34.4, 66.8]
vWF (pg/mL)	6591.7 [4282.0, 8683.1]	5807.7 [4048.0, 9585.7]	5345.7 [3495.1, 8112.4]	4969.6 [3040.8, 6351.9]	3491.1 [2566.5, 5155.7]	3596.0 [2121.8, 5160.6]
	6133.5 [5132.4, 7435.2]	5224.7 [3611.3, 6244.5]	4676.5 [3089.3, 5366.8]	3549.7 [2749.9, 4809.4]	3880.4 [2718.3, 4571.1]	3512.5 [2633.6, 4467.7]
Thrombomodulin (ng/mL)	8.7 [6.7, 11.6]	9.5 [7.8, 13.9]	9.7 [6.9, 13.6]	10.6 [7.7, 14.9]	9.1 [6.0, 13.6]	8.1 [6.2, 11.0]
	7.5 [5.3, 10.2]	7.6 [5.7, 10.0]	7.5 [5.2, 11.2]	6.9 [5.6, 10.3]	7.3 [5.1, 11.3]	7.2 [5.2, 11.0]
Syndecan-1 (ng/mL)	8.5 [6.3, 17.8]	11.4 [6.4, 18.3]	11.6 [6.3, 18.9]	14.2 [8.3, 23.6]	16.7 [11.9, 24.4]	15.2 [10.5, 20.4]
	9.8 [5.5, 12.9]	10.1 [5.8, 12.4]	11.6 [5.7, 13.9]	14.3 [7.6, 20.2]	15.8 [11.7, 19.6]	13.1 [9.3, 19.5]
Angiopoietin-1 (ng/mL)	4.2 [2.1, 7.3]	3.5 [1.6, 6.0]	3.3 [1.7, 5.5]	2.9 [2.0, 6.3]	2.8 [1.6, 6.5]	7.6 [3.5, 12.8]
	5.0 [2.9, 8.7]	3.8 [1.8, 7.0]	2.9 [1.9, 10.0]	4.1 [1.6, 6.8]	4.4 [2.5, 8.7]	7.2 [3.2, 17.5]
Angiopoietin-2 (ng/mL)	8.7 [5.7, 13.7]	10.7 [5.8, 21.2]	11.5 [5.4, 24.5]	8.1 [5.7, 16.2]	4.3 [3.1, 7.8]	3.2 [2.5, 6.3]
	6.3 [4.6, 13.5]	8.1 [4.5, 13.5]	7.6 [4.2, 13.5]	5.6 [3.7, 11.4]	4.8 [2.7, 7.2]	3.7 [2.2, 6.6]
Tissue Factor (pg/mL)	63.7 [54.4, 86.4]	71.8 [59.5, 89.1]	70.6 [61.1, 89.4]	79.3 [62.9, 112.1]	73.7 [50.2, 99.1]	54.4 [48.1, 88.9]
	52.4 [36.7, 79.6]	54.3 [41.2, 68.5]	55.5 [39.9, 91.7]	60.4 [43.4, 80.4]	54.5 [36.4, 75.4]	47.4 [37.5, 67.6]
Prothrombin fragment 1+2 (pg/mL)	472.1 [276.0, 875.5]	661.8 [365.3, 1100.3]	377.3 [280.7, 719.9]	863.3 [496.3, 1207.3]	373.0 [245.4, 652.6]	277.4 [168.9, 565.1]
	442.2 [227.8, 655.6]	271.3 [157.6, 448.4]	274.3 [182.3, 546.2]	335.6 [247.0, 610.1]	319.3 [242.5, 481.4]	316.8 [246.5, 412.8]
D-dimer (ng/mL)	9192.1 [6341.9, 12037.5]	14294.1 [12109.7, 21760.6]	14398.5 [9811.7, 20243.4]	16581.9 [11233.4, 21425.0]	8389.8 [6227.2, 10922.2]	7137.8 [4936.6, 9924.7]
	6787.0 [5598.2, 11836.6]	7500.1 [6928.2, 11127.7]	7600.4 [5939.7, 11121.7]	7909.2 [5997.1, 10520.6]	7757.9 [6551.2, 9874.6]	7911.1 [5389.4, 9409.7]
PAI-1 (ng/mL)	49.6 [26.4, 79.0]	33.4 [20.8, 84.7]	32.2 [16.7, 60.7]	26.3 [14.5, 41.6]	18.9 [11.9, 34.6]	29.9 [17.4, 42.5]
	36.8 [21.4, 70.9]	36.8 [21.2, 62.3]	29.4 [15.6, 48.2]	22.3 [12.0, 37.8]	21.0 [16.4, 38.0]	33.7 [16.9, 63.4]
Fas (ng/mL)	8.8 [7.3, 10.9]	8.9 [7.6, 13.6]	10.3 [8.1, 14.7]	11.4 [8.9, 16.6]	10.6 [8.3, 16.1]	9.3 [7.9, 12.2]
	7.8 [6.1, 10.5]	8.4 [6.5, 13.1]	10.1 [7.0, 13.6]	9.8 [6.5, 14.7]	10.1 [7.4, 14.3]	9.8 [7.1, 13.3]
Fas ligand (pg/mL)	25.8 [18.5, 35.0]	25.5 [17.7, 33.3]	26.0 [17.9, 36.8]	24.4 [18.4, 33.3]	18.2 [13.9, 26.3]	22.2 [19.1, 30.2]
	26.8 [19.0, 37.5]	28.3 [18.6, 37.8]	23.5 [18.9, 37.4]	23.2 [18.4, 37.4]	20.5 [15.0, 30.0]	22.5 [16.3, 25.8]
TRAIL (pg/mL)	13.7 [9.4, 24.1]	15.9 [10.0, 25.9]	15.9 [7.6, 24.3]	15.9 [12.9, 29.4]	21.2 [11.5, 33.6]	30.4 [18.4, 51.6]
	14.8 [2.9, 21.9]	12.9 [7.1, 15.9]	13.4 [7.6, 24.5]	15.9 [7.1, 24.2]	13.7 [10.0, 33.6]	26.3 [10.9, 46.6]

Median [interquartile range] concentration values for each plasma biomarker at each time point for the **Cx611** (top row per biomarker, light orange background) and **placebo** (bottom row per biomarker, light blue/grey background) groups

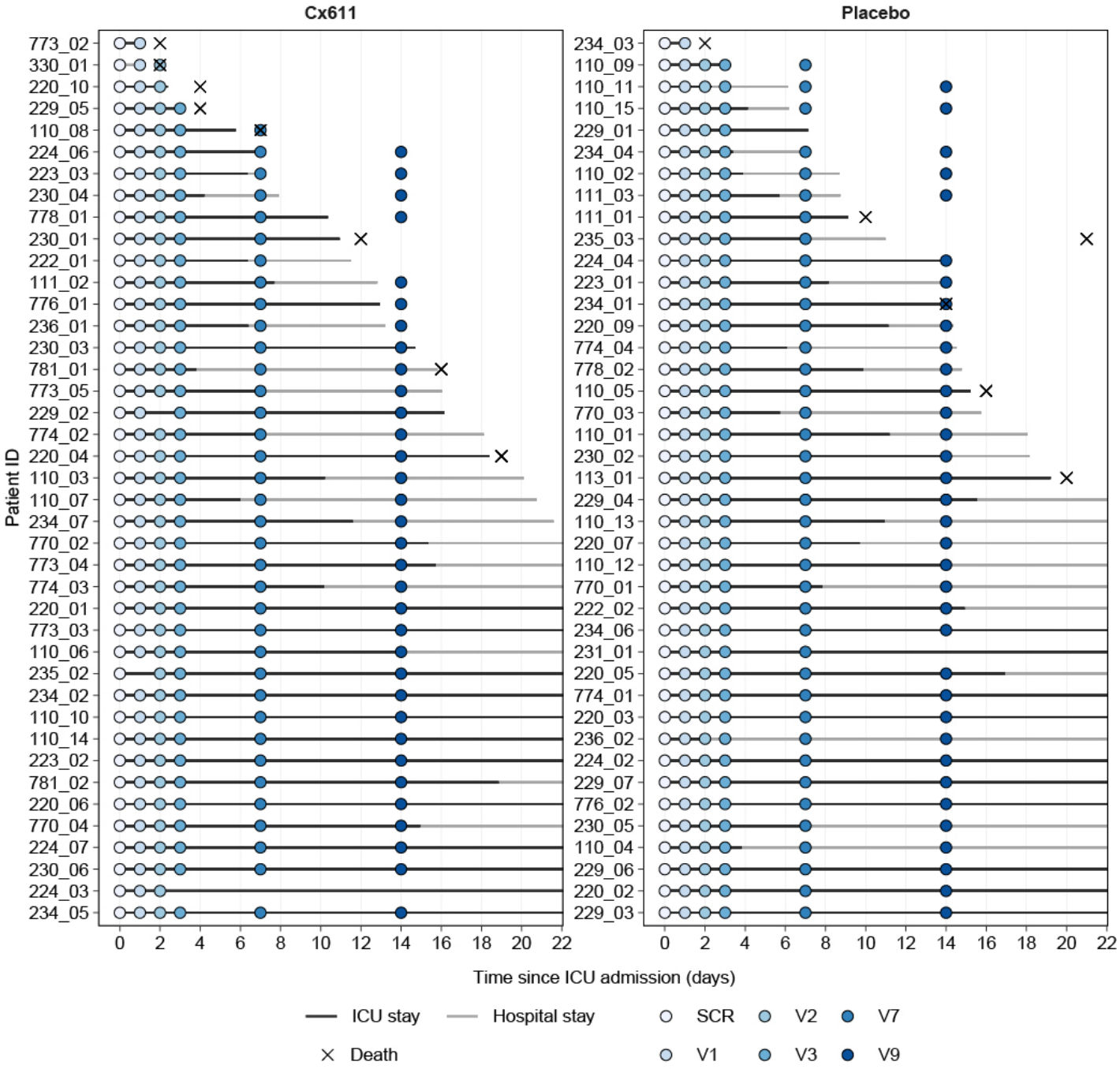
CL = CC chemokine ligand; HGF = hepatocyte growth factor; IL (RA) = interleukin (receptor antagonist); MMP-8 = matrix metalloproteinase 8; NGAL = neutrophil gelatinase-associated lipocalin; PAI-1 = plasminogen activator inhibitor 1; TNF = tumour necrosis factor; TRAIL = TNF-related apoptosis-inducing ligand; TREM-1 = triggering receptor expressed on myeloid cells 1; VCAM-1 = vascular cell adhesion molecule 1.

Supplementary Table 4. Overview of differentially expressed genes per timepoint derived from the interaction terms of the linear mixed models

	Upregulated	Downregulated	Total
V1	155	131	286
V2	165	148	313
V3	583	649	1232
V7	1596	794	2390
V9	474	238	712

Differentially expressed genes were defined as a Cohen's *d* effect size ≥0.5 for upregulated genes or ≤-0.5 for downregulated genes (generally considered a moderate effect).
The Cohen's *d* effect size for the interaction term was calculated by transforming the interaction term *t*-score using the following formula: *Cohen's d = 2t / sqrt(df)*.

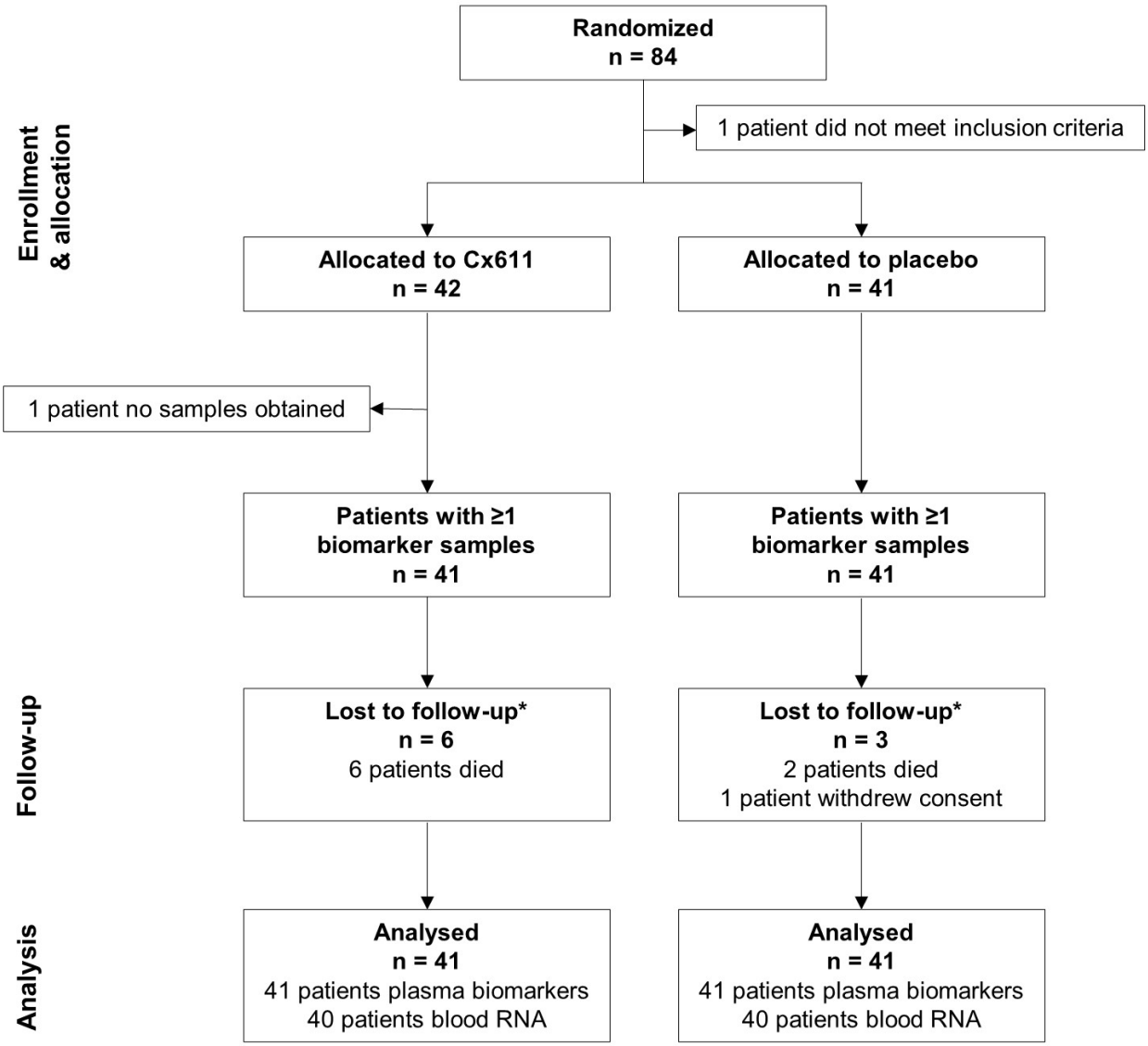
Supplementary Figures



Supplementary Figure 1. Swimmer plot of plasma biomarker time points.

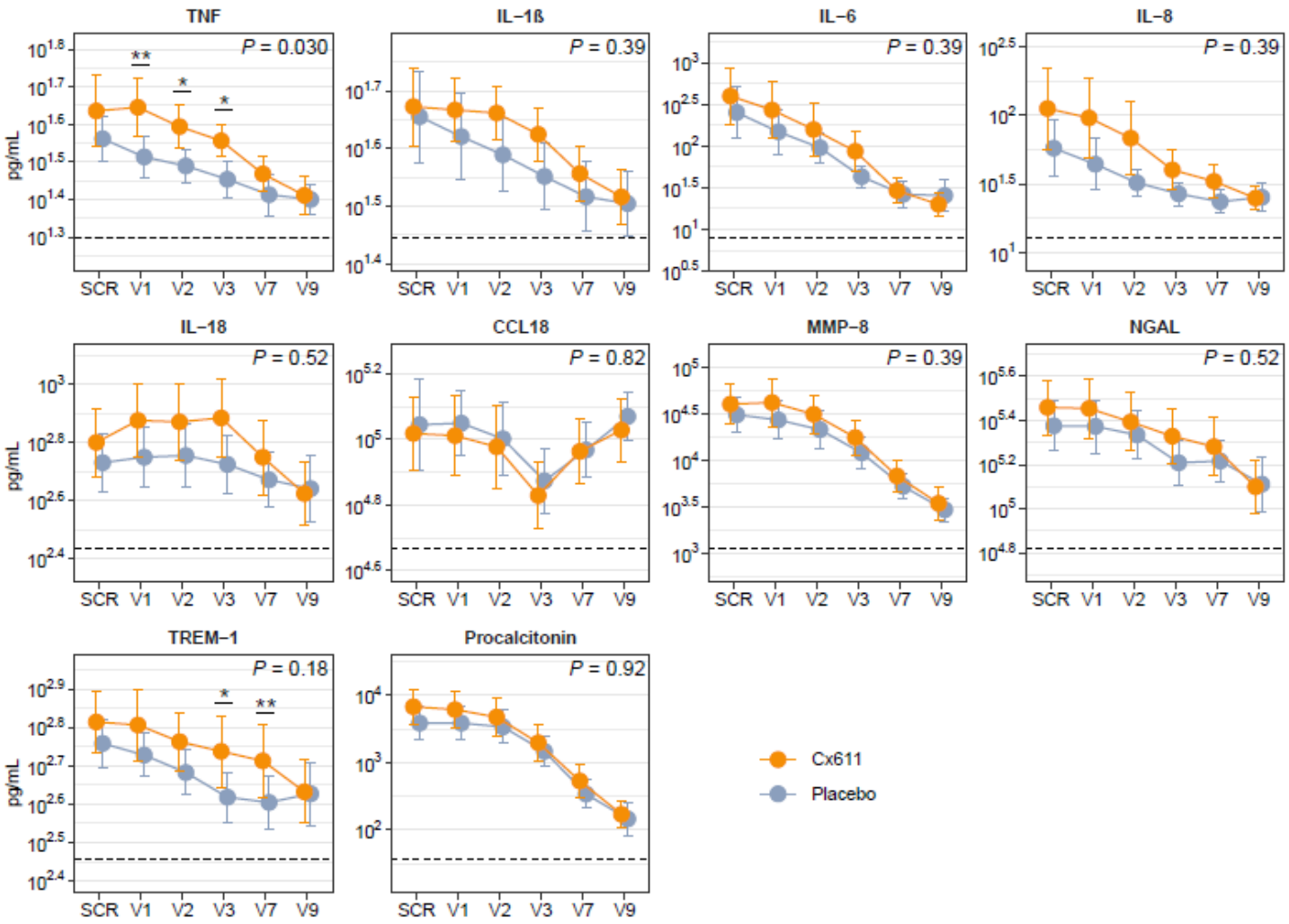
Each circle represents a plasma biomarker sample used in the analyses. Please note that the time points (in days) for the biomarker studies may not correspond exactly to the time (in days) at which participation in the study was registered, because the biomarker time points are defined relative to inclusion time and study treatment administration. For convenience, the time points are rounded down to whole days since intensive care unit (ICU) admission, or to day 14 in the case of V9 (which represents day 14±2). The timeline is cropped at day 22 to

facilitate interpretation. The horizontal dark grey lines depict the duration of ICU stay, the horizontal light grey line depicts the duration of hospital stay, lines that intersect the border at day 22 indicate an ICU or hospital stay ≥22 days. The "X" depicts the day on which a patient died, the graph does not depict deaths occurring >22 days after ICU admission.

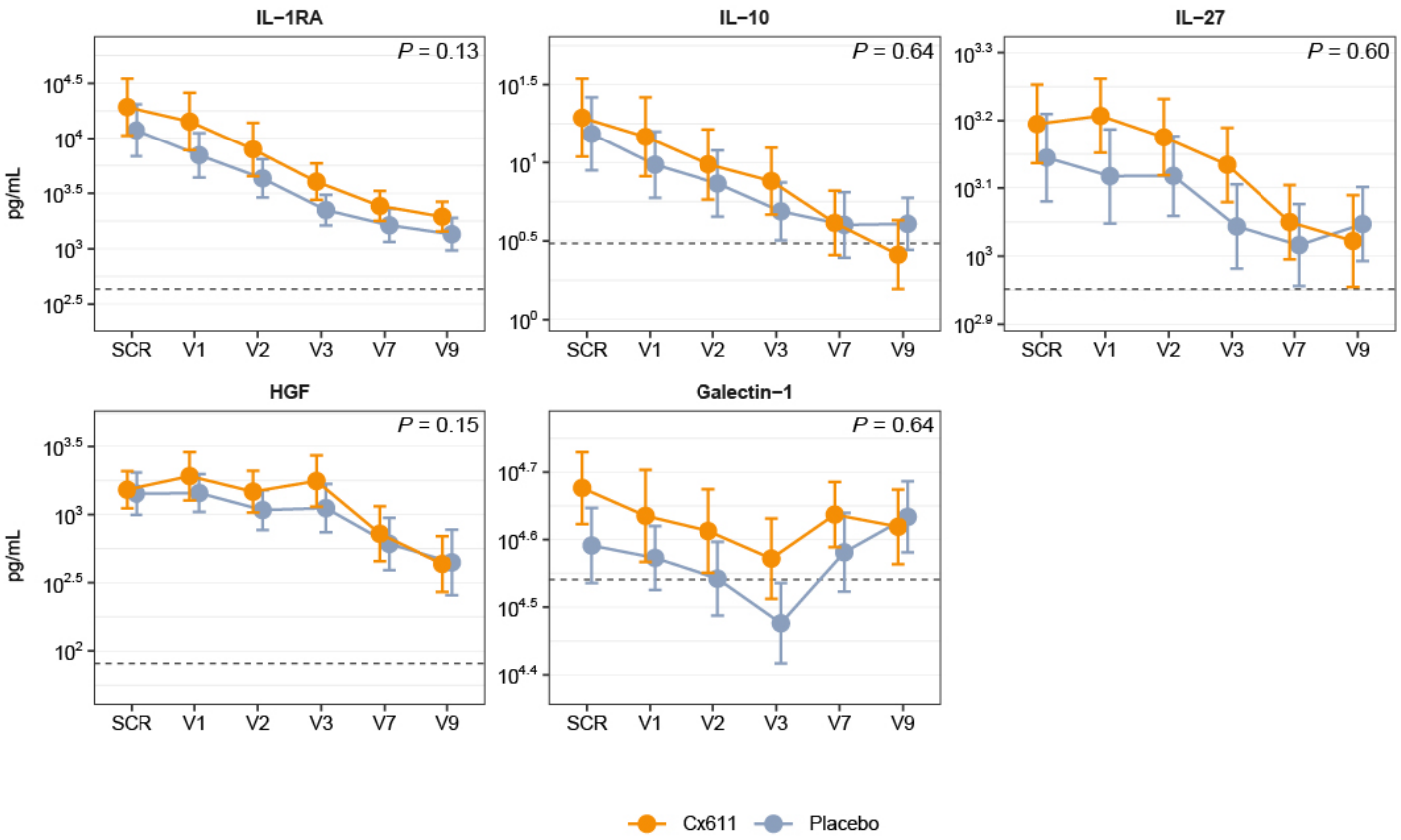


Supplementary Figure 2. Flow diagram of patient selection.

SEPCELL study flow diagram, adapted for the biomarker analyses presented here. *Lost to follow-up was defined here as any reason for discontinuation of study participation that prevented patients from completing the final biomarker timepoint (V9, day14±2). For more details, including how many patients were assessed for eligibility, please refer to the primary clinical report [2].

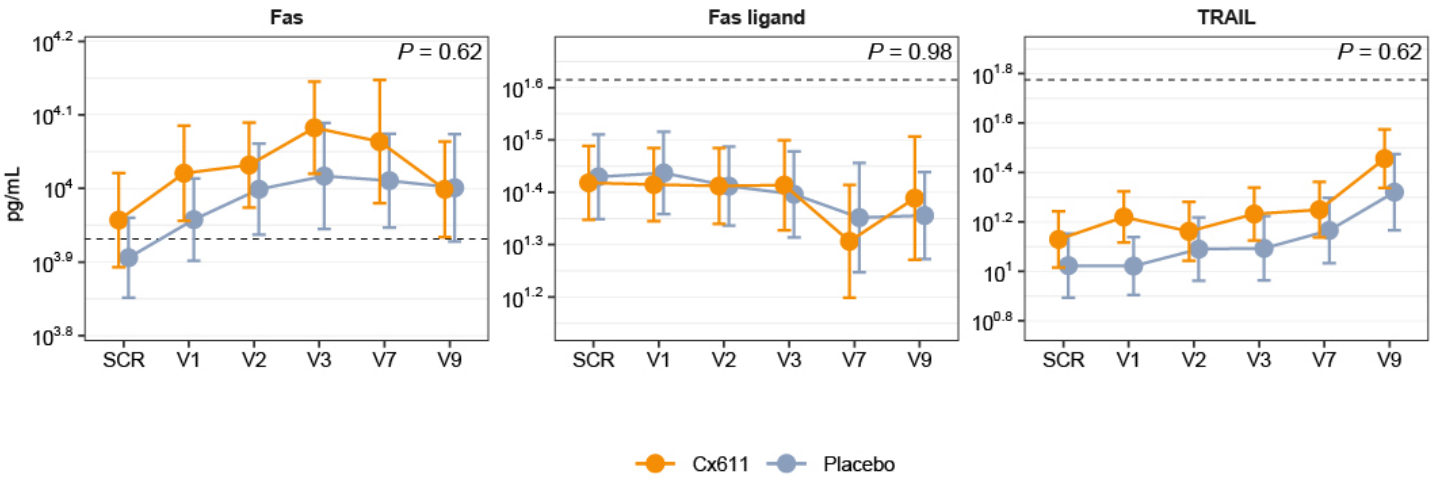


Supplementary Figure 3. Plasma host response biomarkers indicative of inflammation. Line charts depicting the levels of protein biomarkers related to inflammation over time for patients treated with Cx611 and placebo. Biomarker concentrations were log10-transformed and displayed as the mean with 95% confidence interval per group per time point. All biomarker concentrations are listed as picograms/millilitre. The Benjamini-Hochberg adjusted *P*-values listed in the upper right corner of each plot are derived from a type II Wald test on linear mixed models for each individual protein biomarker (as described in the statistical analysis paragraph), and correspond to the *P*-values displayed in Figure 1B. The *P*-values for the differences between groups at individual time points after initiation of treatment are derived from the same models and thus also adjusted for chance variation prior to treatment (i.e. the screening [SCR] time point). **P* <0.05, ***P* <0.01. CCL = CC chemokine ligand; MMP-8 = matrix metalloproteinase 8; NGAL = neutrophil gelatinase-associated lipocalin; TNF = tumour necrosis factor; TRAIL = TNF-related apoptosis-inducing ligand; TREM-1 = triggering receptor expressed on myeloid cells 1

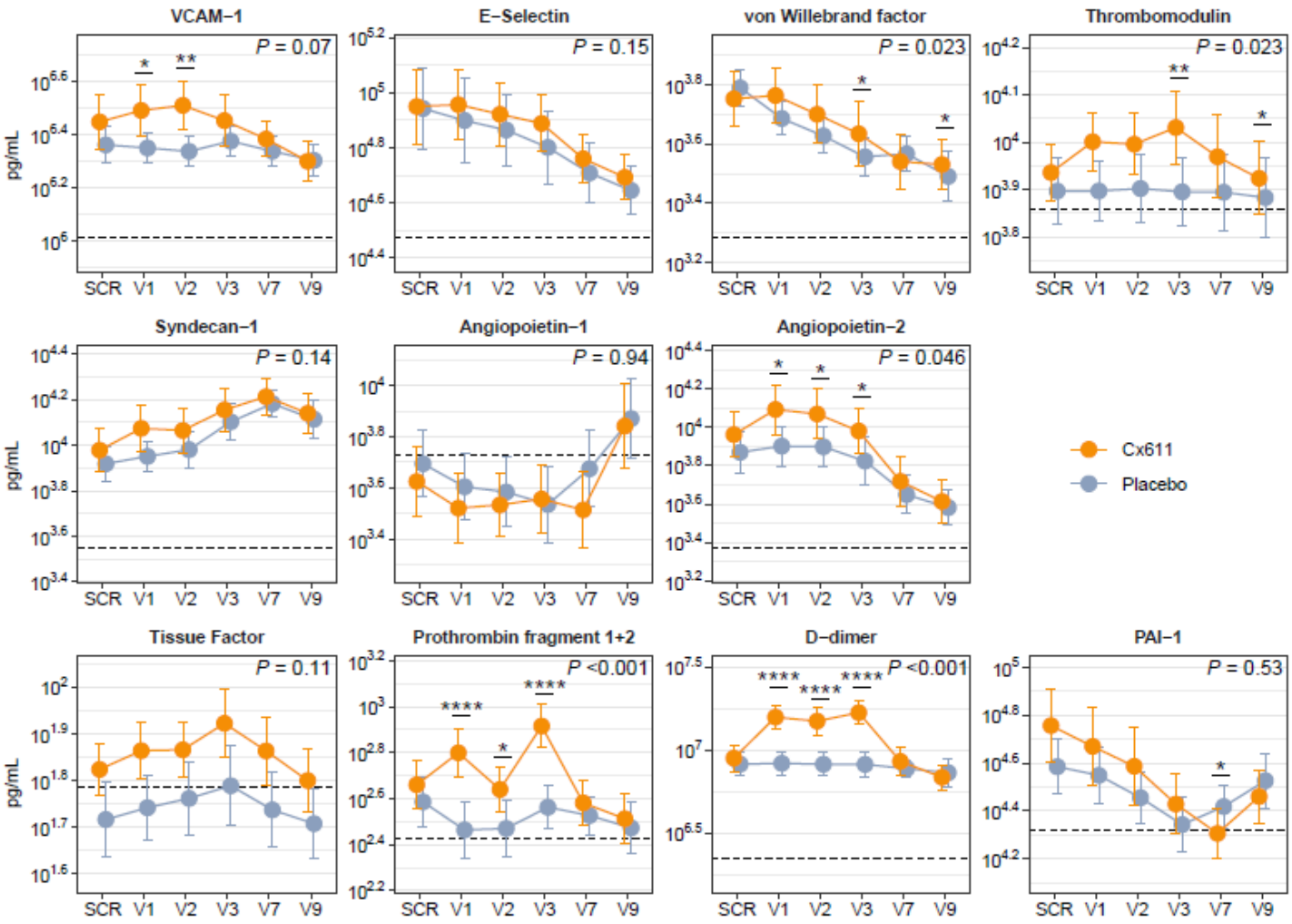


Supplementary Figure 4. Plasma host response biomarkers indicative of inhibition of inflammation.

Line charts depicting the levels of protein biomarkers related to inhibition of inflammation over time for patients treated with Cx611 and placebo. Biomarker concentrations were log10-transformed and displayed as the mean with 95% confidence interval per group per time point. All biomarker concentrations are listed as picograms/millilitre. The Benjamini-Hochberg adjusted *P*-values listed in the upper right corner of each plot are derived from a type II Wald test on linear mixed models for each individual protein biomarker (as described in the statistical analysis paragraph), and correspond to the *P*-values displayed in Figure 1B. The *P*-values for the differences between groups at individual time points after initiation of treatment are derived from the same models and thus also adjusted for chance variation prior to treatment (i.e. the screening [SCR] time point). HGF = hepatocyte growth factor; IL (RA) = interleukin (receptor antagonist);



Supplementary Figure 5. Plasma host response biomarkers indicative of apoptosis. Line charts depicting the levels of protein biomarkers related to apoptosis over time for patients treated with Cx611 and placebo. Biomarker concentrations were log10-transformed and displayed as the mean with 95% confidence interval per group per time point. All biomarker concentrations are listed as picograms/millilitre. The Benjamini-Hochberg adjusted *P*-values listed in the upper right corner of each plot are derived from a type II Wald test on linear mixed models for each individual protein biomarker (as described in the statistical analysis paragraph), and correspond to the *P*-values displayed in Figure 1B. The *P*-values for the differences between groups at individual time points after initiation of treatment are derived from the same models and thus also adjusted for chance variation prior to treatment (i.e. the screening [SCR] time point). TRAIL = tumour necrosis factor-related apoptosis-inducing ligand.



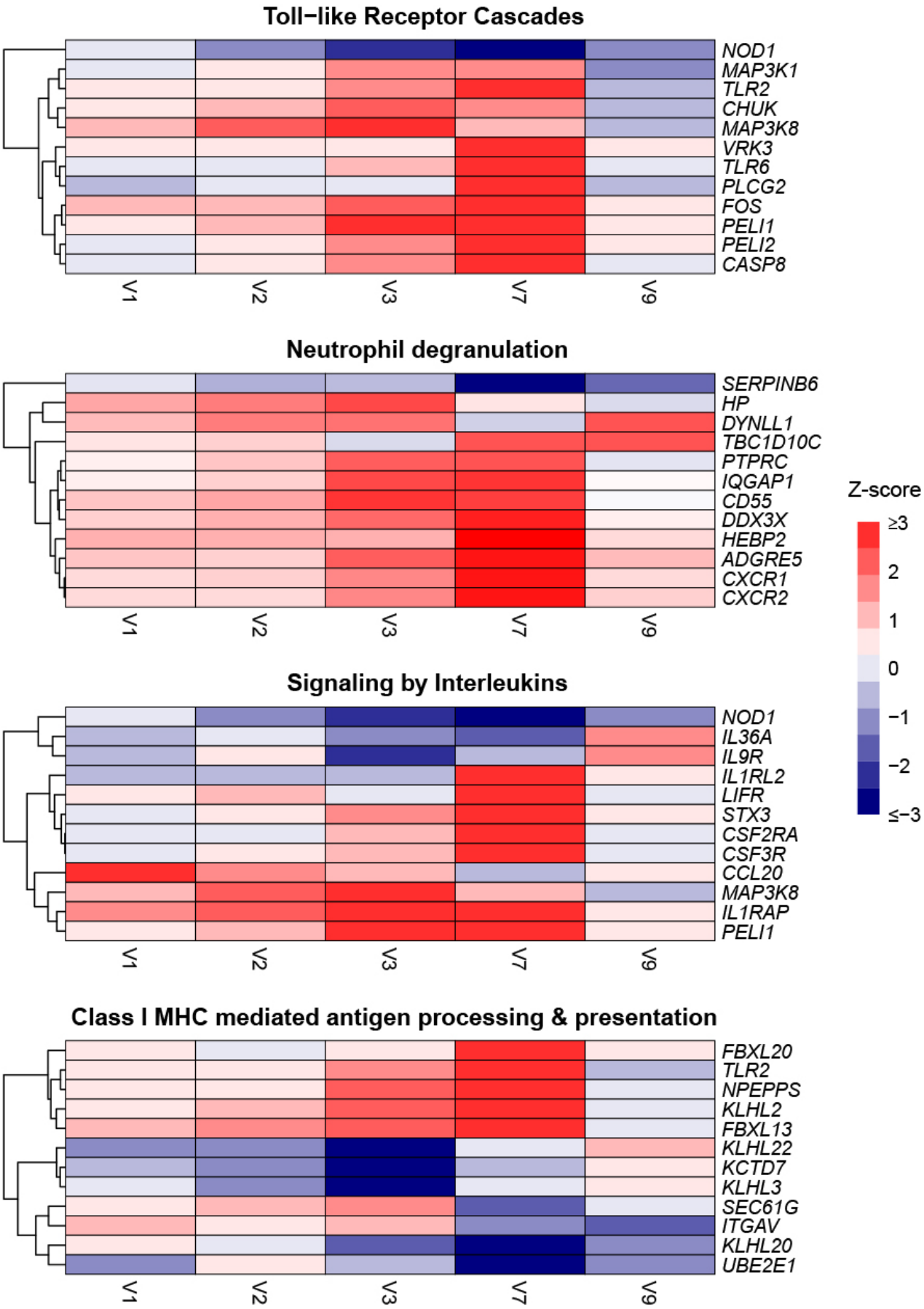
Supplementary Figure 6. Plasma host response biomarkers indicative of endothelial cell responses and coagulation activation.

Line charts depicting the levels of protein biomarkers related to endothelial cell responses and coagulation over time for patients treated with Cx611 and placebo. Biomarker concentrations were log10-transformed and displayed as the mean with 95% confidence interval per group per time point. All biomarker concentrations are listed as picograms/millilitre. The Benjamini-Hochberg adjusted *P*-values listed in the upper right corner of each plot are derived from a type II Wald test on linear mixed models for each individual protein biomarker (as described in the statistical analysis paragraph), and correspond to the *P*-values displayed in Figure 1B. The *P*-values for the differences between groups at individual time points after initiation of treatment are derived from the same models and thus also adjusted for chance variation prior to treatment (i.e. the screening [SCR] time point). **P* <0.05, ***P* <0.01, *****P* <0.0001. PAI-1 = plasminogen activator inhibitor 1; V1-V9 = visit 1 - visit 9; VCAM-1 = vascular cell adhesion molecule 1.


V1
V2
V3
V7
V9
Immune System
Innate Immune System
Antimicrobial peptides
C−type lectin receptors (CLRs)
CD209 (DC−SIGN) signaling
CLEC7A (Dectin−1) signaling
Dectin−2 family
Complement cascade
Initial triggering of complement
Regulation of Complement cascade
Cytosolic sensors of pathogen−associated DNA
ZBP1(DAI) mediated induction of type I IFNs
DAP12 interactions
DAP12 signaling
DDX58/IFIH1−mediated induction of interferon−alpha/beta
Negative regulators of DDX58/IFIH1 signaling
TRAF6 mediated NF−kB activation
Fc epsilon receptor (FCERI) signaling
FCERI mediated Ca+2 mobilization
FCERI mediated MAPK activation
FCERI mediated NF−kB activation
Fcgamma receptor (FCGR) dependent phagocytosis
Regulation of actin dynamics for phagocytic cup formation
Role of phospholipids in phagocytosis
Neutrophil degranulation
Nucleotide−binding domain, leucine rich repeat containing receptor (NLR) signaling pathways
Inflammasomes
NOD1/2 Signaling Pathway
ROS and RNS production in phagocytes
Toll−like Receptor Cascades
Regulation of TLR by endogenous ligand
Toll Like Receptor 10 (TLR10) Cascade
Toll Like Receptor 2 (TLR2) Cascade
Toll Like Receptor 3 (TLR3) Cascade
Toll Like Receptor 4 (TLR4) Cascade
Toll Like Receptor 5 (TLR5) Cascade
Toll Like Receptor 7/8 (TLR7/8) Cascade
Toll Like Receptor 9 (TLR9) Cascade
Cytokine Signaling in Immune system
FLT3 Signaling
Growth hormone receptor signaling
Interferon Signaling
Antiviral mechanism by IFN−stimulated genes
Interferon alpha/beta signaling
Interferon gamma signaling
Signaling by CSF3 (G−CSF)
Inactivation of CSF3 (G−CSF) signaling
Signaling by Interleukins
Interleukin−1 family signaling
Interleukin−10 signaling
Interleukin−12 family signaling
Interleukin−17 signaling
Interleukin−2 family signaling
Interleukin−20 family signaling
Interleukin−3, Interleukin−5 and GM−CSF signaling
Interleukin−4 and Interleukin−13 signaling
Interleukin−6 family signaling
Interleukin−7 signaling
Other interleukin signaling
TNFR2 non−canonical NF−kB pathway
NIK−−>noncanonical NF−kB signaling
TNFs bind their physiological receptors
Adaptive Immune System
Class I MHC mediated antigen processing & presentation
Antigen Presentation
Antigen processing
Antigen processing−Cross presentation
Costimulation by the CD28 family
CD28 co−stimulation
CTLA4 inhibitory signaling
PD−1 signaling
Immunoregulatory interactions between Lymphoid & non−Lymphoid cells
MHC class II antigen presentation
Signaling by the B Cell Receptor (BCR)
Antigen activates B Cell Receptor (BCR)
Downstream signaling events of B Cell Receptor (BCR)
TCR signaling
Downstream TCR signaling
Generation of second messenger molecules
Phosphorylation of CD3 and TCR zeta chains
−2 0 2
Normalized Enrichment Score
BH−adj P
NS
<0 05
<0 01
NES
−2
−1
1
2

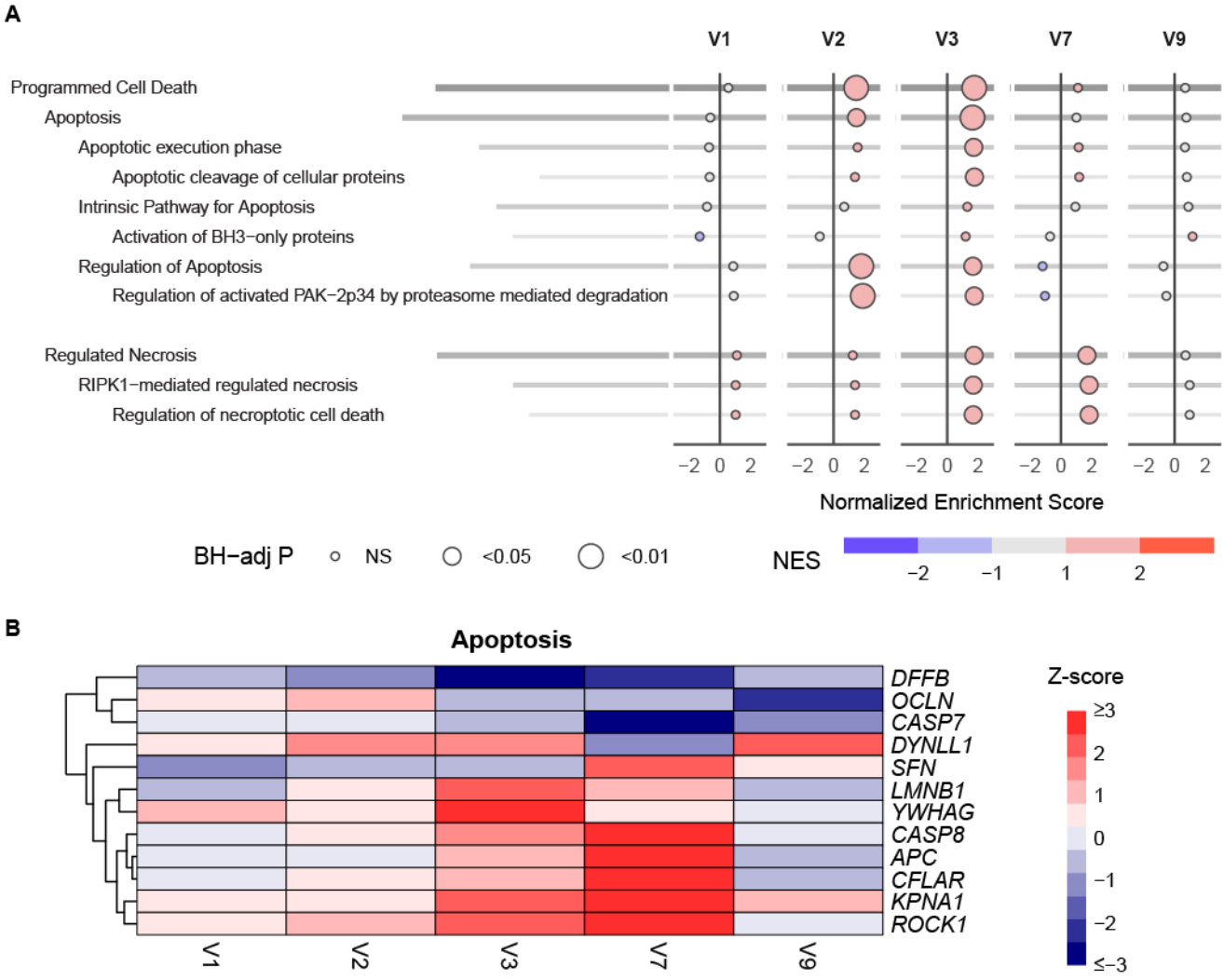
Supplementary Figure 7. All immune system pathways from gene set enrichment analysis of the blood transcriptome.

Bubble plot displaying the effect of Cx611 treatment on transcriptional pathways related to the immune system (as obtained from the Reactome knowledgebase [7]) for each time point after the initiation of treatment with Cx611 or placebo. To adjust for chance variation in baseline gene expression between groups, the differences in gene expression at each time point are derived from the interaction terms between Cx611 and time point in linear mixed models that included the SCR time point (prior to initiation as treatment) as the reference category, and can therefore be interpreted as the difference in gene expression levels between groups at each time point relative to the gene expression levels prior to initiation of treatment. The differences in expression of genes in the listed pathways are quantified as normalized enrichment scores (NES) which are reflected by the intensity of the colour: a red bubble means higher in the Cx611-treated group, a blue bubble means lower in the Cx611-treated group, and a grey bubble indicates a negligible difference. The size of the bubble is related to the BH-adjusted *P*-value for that pathway. This figure includes the same results as Figure 2, but also includes all pathways in which we found no significant differences between patients treated with Cx611 or placebo at all time points. CLEC7A = C-type lectin domain family 7 member A; (G-, or GM-)CSF = (granulocyte, or granulocyte macrophage) colony stimulating factor; CTLA-4 = Cytotoxic T-lymphocyte-associated protein 4; DAP12 = DNAX-activating protein of 12 kDa; DC-SIGN = Dendritic cell-specific intercellular adhesion molecule 3 grabbing non-integrin; DDX58/IFIH1 = DExD/H-box helicase 58/ interferon induced with helicase C domain 1; Fc = fragment crystallizable region (of an antibody); FLT3 = fms related receptor tyrosine kinase 3; IFN = interferon; MAPK = a mitogen-activated protein kinase; MHC = major histocompatibility complex; NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells; NIK = NF-κB inducing kinase; NOD = nucleotide oligomerization domain; PD-1 = programmed death 1; RNS = reactive nitrogen species; ROS = reactive oxygen species; TCR = T cell receptor; TNF(R) = tumour necrosis factor (receptor); TRAF6 = TNF receptor associated factor 6; ZBP1 (DAI) = Z-DNA-binding protein 1 (DNA-dependent activator of IFN-regulatory factors)


Toll−like Receptor Cascades
NOD1
MAP3K1
TLR2
CHUK
MAP3K8
VRK3
TLR6
PLCG2
FOS
PELI1
PELI2
CASP8
V1
V2
V3
V7
V9
Neutrophil degranulation
SERPINB6
HP
DYNLL1
TBC1D10C
PTPRC
IQGAP1
CD55
DDX3X
HEBP2
ADGRE5
CXCR1
CXCR2
V1
V2
V3
V7
V9
Signaling by Interleukins
NOD1
IL36A
IL9R
IL1RL2
LIFR
STX3
CSF2RA
CSF3R
CCL20
MAP3K8
IL1RAP
PELI1
V1
V2
V3
V7
V9
Class I MHC mediated antigen processing & presentation
FBXL20
TLR2
NPEPPS
KLHL2
FBXL13
KLHL22
KCTD7
KLHL3
SEC61G
ITGAV
KLHL20
UBE2E1
V1
V2
V3
V7
V9
Z-score
≥3
2
1
0
−1
−2
≤−3

Supplementary Figure 8. Top twelve differentially expressed genes in four immune system pathways

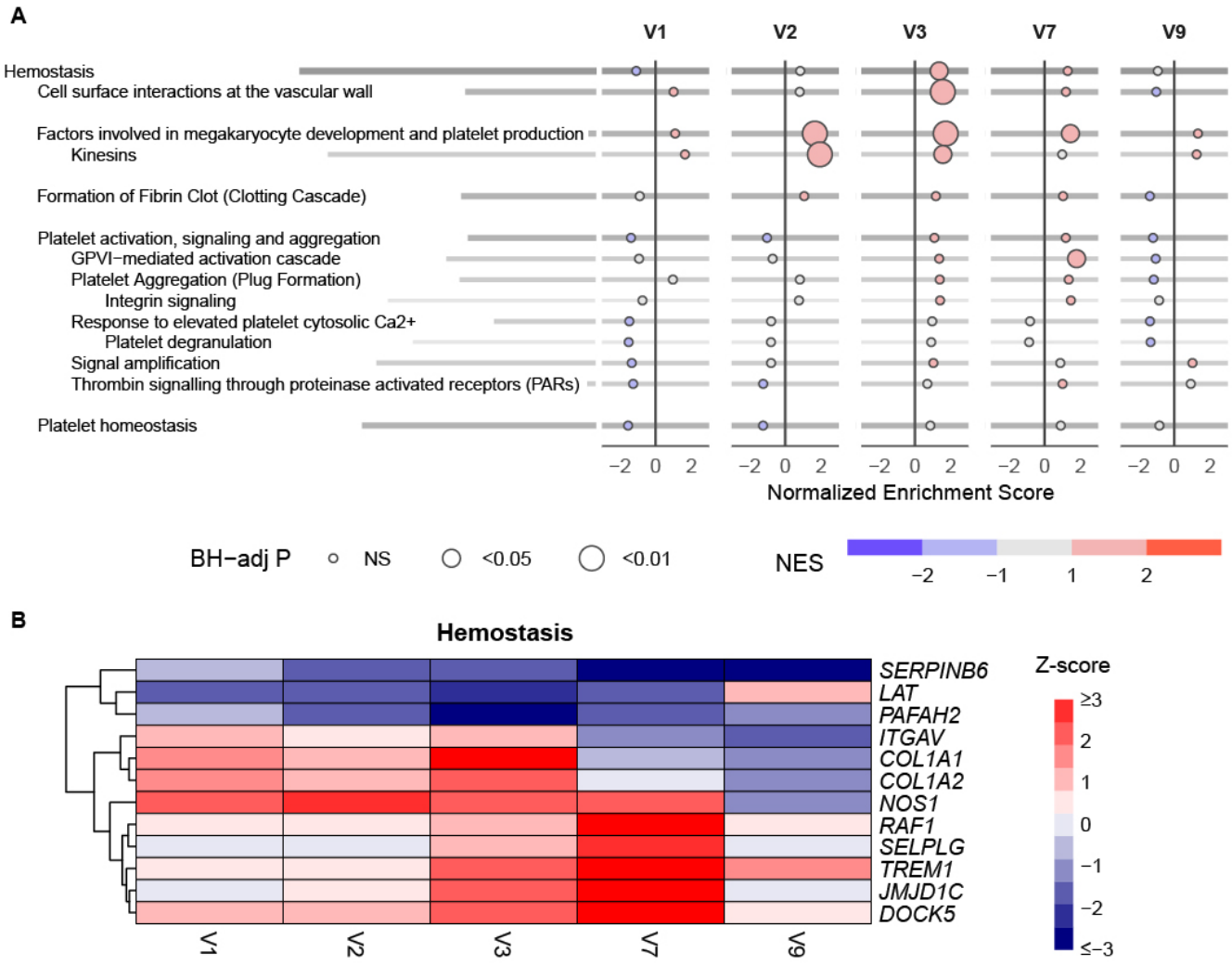
Hierarchically clustered heatmaps displaying the top twelve genes in four immune system pathways most differentially expressed between patients treated with Cx611 when compared with patients treated with placebo. The differences are expressed as the standard score per gene per time-point, calculated by dividing the interaction coefficient of the linear mixed models by the standard error of that interaction coefficient (z = beta / beta standard error) where the screening time point is taken as the reference category. Red indicates higher in patients treated with Cx611, blue indicates higher in patients treated with placebo. MHC = major histocompatibility complex.



Supplementary Figure 9. Apoptosis pathways from gene set enrichment analysis of the blood transcriptome and top differentially expressed genes

(*A*) Bubble plot displaying the effect of Cx611 treatment on transcriptional pathways related to apoptosis (as obtained from the Reactome knowledgebase [7]) for each time point after the initiation of treatment with Cx611 or placebo. To adjust for chance variation in baseline gene expression between groups, the differences in gene expression at each time point are derived from the interaction terms between Cx611 and time point in linear mixed models that included the SCR time point (prior to initiation as treatment) as the reference category, and can therefore be interpreted as the difference in gene expression levels between groups at each time point relative to the gene expression levels prior to initiation of treatment. The differences in expression of genes in the listed pathways are quantified as normalized expression scores (NES) which are reflected in the intensity of the colour: a red bubble means higher in the Cx611-treated group, a blue bubble means lower in the Cx611-treated group, and a gray bubble indicates a negligible difference. The size of the bubble is related to the

BH-adjusted *P*-value for that pathway. At approximately the same time points at which Cx611 induced a predominantly pro-inflammatory response (V2 through V7), and in spite of the reported enhancement of cell survival in preclinical studies [9], pathways related to both apoptosis and regulated necrosis, particularly necroptosis, displayed enhanced expression in Cx611-treated patients. (*B*) Hierarchically clustered heatmaps displaying the top twelve genes in the apoptosis pathway most differentially expressed between patients treated with Cx611 when compared with patients treated with placebo. The differences are expressed as the standard score per gene per time-point, calculated by dividing the interaction coefficient of the linear mixed models by the standard error of that interaction coefficient (z = beta / beta standard error). Red indicates higher in patients treated with Cx611, blue indicates higher in patients treated with placebo. PAK-2p34 = p21-activated kinase 2p34; RIPK1 = Receptor-interacting serine/threonine-protein kinase 1



Supplementary Figure 10. Haemostasis pathways from gene set enrichment analysis of the blood transcriptome and top differentially expressed genes.

(*A*) Bubble plot displaying the effect of Cx611 treatment on transcriptional pathways related to haemostasis (as obtained from the Reactome knowledgebase [7]) for each time point after the initiation of treatment with Cx611 or placebo. To adjust for chance variation in baseline gene expression between groups, the differences in gene expression at each time point are derived from the interaction terms between Cx611 and time point in linear mixed models that included the SCR time point (prior to initiation as treatment) as the reference category, and can therefore be interpreted as the difference in gene expression levels between groups at each time point relative to the gene expression levels prior to initiation of treatment. The differences in expression of genes in the listed pathways are quantified as normalized expression scores (NES) which are reflected in the intensity of the colour: a red bubble means higher in the Cx611-treated group, a blue bubble means lower in the Cx611-treated group, and a gray bubble indicates a negligible difference. The size of the bubble is related to the

BH-adjusted *P*-value for that pathway. The effect of Cx611 on transcriptomic pathways of haemostasis – likely largely related to platelet RNA captured in the PAXgene tubes – was limited, although some pathways, such as those pertaining to platelet production, were upregulated around time of treatment (Supplementary Figure 6). Although limited evidence suggests MSCs may inhibit platelet activation *in vitro* [10], we did not find evidence for this and even found upregulation of glycoprotein (GP) VI-mediated activation at V7. Upregulation of the pathway 'cell surface interactions at the vascular wall' is consistent with increase of plasma biomarkers indicative of endothelial cell activation. (*B*) Hierarchically clustered heatmaps displaying the top twelve genes in the haemostasis pathway most differentially expressed between patients treated with Cx611 when compared with patients treated with placebo. The differences are expressed as the standard score per gene per time-point, calculated by dividing the interaction coefficient of the linear mixed models by the standard error of that interaction coefficient (z = beta / beta standard error). Red indicates higher in patients treated with Cx611, blue indicates higher in patients treated with placebo. GPVI = glycoprotein VI.