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Early View

Original research article

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Immunomodulation and endothelial barrier protection mediate the association between oral imatinib and mortality in hospitalised COVID-19 patients

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'Take home' message

The effect of imatinib on mortality in hospitalised COVID-19 patients is mediated through modulation of innate immune responses and reversal of endothelial dysfunction, and possibly moderated by biological subphenotypes.

Abstract

Introduction: Imatinib reduced 90-day mortality in hospitalised COVID-19 patients in a recent clinical trial, but the biological effects that cause improved clinical outcomes are unknown. We aimed to determine the biological changes elicited by imatinib in patients with COVID-19, and what baseline biological profile moderates the effect of imatinib.

Methods: Secondary analysis of a randomised, double-blind, placebo-controlled trial of oral imatinib in hospitalised, hypoxemic COVID-19 patients. Mediating effects of changes in plasma concentration of 25 plasma host response biomarkers on the association between randomisation group and 90-day mortality were studied by combining linear mixed-effect modelling and joint modelling. Moderation of baseline biomarker concentrations was evaluated by Cox regression modelling. We identified subphenotypes using Ward's method clustering and evaluated moderation of these subphenotypes using the above-described method.

Results: 332 out of 385 participants had plasma samples available. Imatinib increased the concentration of surfactant protein D (SP-D), and decreased the concentration of interleukin-6, procalcitonin, angiopoietin 2 to 1 ratio, E-selectin, tumour necrosis factor (TNF)α, and TNF receptor I. The effect of imatinib on 90-day mortality was fully mediated by changes in these biomarkers.

Cluster analysis revealed three host response subphenotypes. Mortality benefit of imatinib was only present in the subphenotype characterised by alveolar epithelial injury indicated by

increased SP-D levels in the context of systemic inflammation and endothelial dysfunction (HR 0.29, 95%-CI: 0.10–0.92).

Conclusions: The effect of imatinib on mortality in hospitalised COVID-19 patients is mediated through modulation of innate immune responses and reversal of endothelial dysfunction, and possibly moderated by biological subphenotypes.

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Key words:

- Host immune response
- COVID-19
- Imatinib
- Cytokines
- Mediation analysis

Introduction

Acute hypoxemic respiratory failure is the most common reason for hospitalisation in patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The introduction of different treatment strategies, including steroids, interleukin (IL)-6 inhibitors and therapeutic anticoagulation [2-4], has resulted in improved clinical outcomes in hospitalised non-critically ill COVID-19 patients. However, with the best standard of care, mortality in hospitalised patients remains substantial with a rate of around 6.5% [5].

Observational studies have linked unfavourable outcomes in COVID-19 patients to dynamic changes in the plasma concentrations of biomarkers reflecting modulation of the innate immune response, endothelial barrier protection and epithelial injury [6, 7]. Imatinib, an ABL tyrosine kinase inhibitor, has been shown to improve the endothelial barrier by reversing the loss of cell-matrix adhesion and adherens junctions *in vitro* and in animals *in vivo* [8, 9], and in addition has immunomodulatory effects [10]. A randomised controlled trial of imatinib in hospitalised, hypoxemic patients with COVID-19 did not show statistical improvement in the primary endpoint (i.e. duration of oxygen therapy), but revealed a large decrease in 28-day mortality [11]. Imbalances in baseline characteristics between treatment arms were suggested to drive part of the protective effect, which could have led to a type I error. In an extended follow-up study, the survival benefit of imatinib at day 90 remained statistically significant in both the unadjusted and adjusted analysis, increasing the likelihood of a true protective effect [12].

Our current understanding of how pharmacological interventions improve outcomes in COVID-19 is limited. There is a general conception that immunomodulation of the innate immune

response and endothelial protection are important in the treatment of severe COVID-19 [13]. It is therefore assumed (1) that a change in these biological processes is required for the drug to work and (2) that patients with higher baseline activation of these pathways are more likely to respond [14-16]. However, this assumption has not been formally tested. Mediation analysis can be used to evaluate if a drug only results in improved outcomes when it elicits specific biological effects, thus testing if an intermediate response is required for the drug to work. Moderation analysis can be used to study whether the relationship between two variables is dependent on the value of a third variable, e.g. a baseline biomarker concentration.

In this study, we aimed to describe what biological changes are elicited by imatinib and how these changes relate to clinical outcomes. We hypothesized that the effect of imatinib on 90-day mortality was mediated by reversal of endothelial dysfunction and modulation of innate immune responses. We also postulated that the baseline biological profile of a patient moderated the effect of imatinib on 90-day mortality.

Methods

Study design and patient selection

This is a pre-specified secondary analysis of clinical data and biological material obtained from a randomised, double-blind, placebo-controlled, clinical trial that was done at 13 hospitals in the Netherlands. Details on study design and patient selection are described elsewhere [11]. In short, patients were eligible for inclusion if they were aged 18 years or older, had been admitted to the hospital with a SARS-CoV-2 infection (confirmed with a RT-PCR test), and required supplemental oxygen to maintain a peripheral oxygen saturation of greater than 94%.

The trial was approved by the Medical Ethics Committee of Amsterdam UMC (location VUmc, Amsterdam, Netherlands), and was done in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent before randomisation.

Study procedures

Patients were randomly assigned (1:1) to either placebo or oral imatinib treatment. After randomisation, patients in the imatinib group received a loading dose of 800 mg imatinib on day 0, followed by 400 mg once daily on days 1-9. Patients in the placebo group received placebo tablets in a similar dosing scheme. Heparin anticoagulated blood was collected right before first study drug administration (baseline), and at day 2, day 3 and day 5 thereafter. Plasma was harvested and stored at -80 °C within 4 hours after blood draw. Plasma was obtained from 20 healthy volunteers to obtain reference normal values.

Data collection

Measurements were done in heparin anticoagulated plasma. Twenty-five biomarkers were measured by Luminex multiplex assay (R&D Systems Inc., Minneapolis, United States), using the Bio-Plex 200 System (Bio-Rad Laboratories Inc., California, United States) in one batch at the end of the study (*table S1*). Ferritin and C-reactive protein (CRP) were not measured, due to differences in dilution. The data quality assessment is described in the supplementary methods.

Endpoints

For this secondary analysis focusing on the biological effects of imatinib, we used 90-day mortality as the primary endpoint and 28-day mortality as secondary endpoint.

Statistical analysis

All statistical analyses were performed in R (version 4.1.0) using RStudio (version 1.4.766).

Mediation analysis

All biomarkers were log10 transformed to better approach a normal distribution. The association between randomisation to imatinib and the longitudinal biomarker values were estimated using linear mixed-effects models (with the *lme4* package) [17]. The randomisation group, the measurement day and their interaction were included as fixed effects. Random intercepts were given to each subject. The effect of imatinib over time was determined by evaluating the interaction term and the 95% confidence interval (95% CI) of this term was calculated using bootstrapping. In a sensitivity analysis, baseline differences (i.e. age, body mass index (BMI), diabetes, and cardiovascular disease) were included as covariates.

Biomarkers that were statistically significant in the above described linear mixed-effects models were subsequently studied using Baron and Kenny's approach for mediation [18]. First, the abovementioned model was used to describe the effect of treatment on biomarker concentration (*nlme* package) [19]. Second, a joint model that combines a linear mixed-effects model and a Cox proportional hazards model was used to describe the effect of a change in biomarker concentration on mortality (using the *survival* and *JM* package) [20, 21]. Third, a Cox

proportional hazard model was used to describe the association between randomisation group and mortality. Next, the isolated effect of imatinib on mortality (i.e. the effect explained if no change in biomarker was observed) was calculated. As a sensitivity analysis, a mediation analysis using natural effects as described in the *medflex* package was performed [22]. All model assumptions are described in the supplementary methods.

Moderation analysis

To estimate the moderation of the baseline biological profile on the association between randomisation group and outcome, we performed Cox regression modelling (with the *survival* package) [20]. Randomisation group, baseline biomarker concentration and its interaction term were used as independent variables and 90-day mortality as time-to-event variable. Resulting p values were corrected for multiple testing using the Benjamini-Hochberg false discovery rate. A significant interaction term indicates that the effect between imatinib and mortality is influenced by the baseline biomarker concentration. In a secondary analysis, the baseline biomarker concentrations were dichotomised by maximally selected rank statistics (*survminer* package) [23]. This dichotomised variable was included in the above-described Cox models.

Lastly, we identified subphenotypes of patients with a similar baseline biological status using Ward's method clustering. For this, baseline (pre-treatment) host response biomarker concentrations were used. IL-10 and IL-17 were excluded from this analysis since these disproportionally affected the clustering due to a high proportion of values below the lower limit of quantification (*table S2*). The optimal number of clusters was determined using a majority ruling as described in the *NbClust* package [24]. This approach has been used previously to

identify and validate subphenotypes of acute respiratory distress syndrome (ARDS) [25]. To evaluate the effect of imatinib treatment on mortality within each cluster subgroup, a Cox regression model with randomisation group as only covariate was performed for patients within each cluster. A Cox regression model with randomisation group, age, BMI, diabetes and cardiovascular disease as covariates was performed as a sensitivity analysis.

Role of funding source

The funders of this study had no role in study design, data collection, data analysis, data interpretation or writing of the report.

Results

Patients

Between March 2020 and January 2021, 385 patients were included in the final analysis population, of which 197 patients were randomised to the imatinib group and 188 patients to the placebo group (*figure 1*). Baseline biomarker data were available for 154 imatinib patients (78%) and 142 placebo patients (76%). 169 imatinib patients (86%) and 163 placebo patients (87%) had at least one measurement during the study period (*table S3*). Patients included in the secondary analysis were comparable in terms of age and sex (*table 1*). Patients in the placebo group more often had obesity, diabetes mellitus and cardiovascular disease. Baseline routine laboratory values and medical treatments, both chronic medication use and medication initiated at hospital admission, in particular dexamethasone, were comparable between the groups. In line with the analysis of the total population [12], 90-day mortality was significantly lower in the imatinib group, with an unadjusted HR of 0.49 (0.26–0.92) and an adjusted HR of 0.47 (0.24–0.94). The

primary endpoint of the clinical trial (i.e. time to discontinuation of ventilation and supplemental oxygen for more than 48 consecutive hours, while being alive during a 28-day period after randomisation) was also comparable to the analysis of the total population, with an unadjusted HR of 0.99 (0.77–1.26). The comparison between patients included in the secondary analysis versus patients excluded in the secondary analysis did not demonstrate meaningful differences between the two groups, indicating that the patients in the secondary analysis are a representative reflection of the full study cohort (*table S4*).

Effect imatinib on biomarkers

In the linear mixed-effect models, imatinib was found to increase the log10 transformed concentration of the epithelial injury marker SP-D with 0.028 (95% CI: 0.003–0.054) per day (*figure 2B; figure S1*). Imatinib caused a decrease in the log10 transformed concentration of pro-inflammatory markers: IL-6 with 0.044 (0.015–0.073) per day, procalcitonin with 0.037 (0.016–0.058) per day, TNFα with 0.018 (0.006–0.030) per day, and TNFRI with 0.011 (0.001–0.021) per day. Imatinib also resulted in a decrease of endothelial markers: angiopoietin 2 to 1 ratio (Ang-2/Ang-1) with 0.025 (0.001–0.049) per day, and E-selectin with 0.018 (0.005–0.032) per day. The concentrations of other biomarkers were not affected by imatinib treatment (*figure S2A*). In a sensitivity analysis, corrected for age, BMI, cardiovascular disease and diabetes, the estimated effects remained the same (*figure S2B*), confirming that the results were not caused by baseline differences in these variables.

Mediation analysis

Mediation analysis was performed by estimating direct and indirect effects of imatinib on

mortality (*figure 2A*). For the above-described significant biomarkers, the association between the change in biomarker concentration and 90-day mortality was estimated. Higher concentrations of TNFRI, TNFα, E-selectin, Ang-2/Ang-1, procalcitonin and IL-6 and lower concentrations of SP-D were associated with a higher mortality (*figure 2C*). Incorporation of these models in mediation analysis showed that imatinib was not directly related to 90-day mortality when accounting for the indirect effect via IL-6, procalcitonin, Ang-2/Ang-1, E-selectin, TNFα, TNFRI and SP-D (*figure 2D*). In a sensitivity analysis using natural effects mediation, complete mediation via IL-6 and TNFRI was confirmed (*figure S3*). The above-described findings were replicated by a sensitivity analysis modelling 28-day mortality (*figure S4*).

Moderation analysis

Baseline characteristics of patients included in the moderation analysis (i.e. patients with baseline biomarker data) were comparable to those of patients in the mediation analysis (*table S5*). The baseline biomarker concentrations in this cohort were comparable between the randomisation groups (*table S6*). After correction for multiple testing, moderation analysis showed no significant interaction between baseline concentration of any single biomarker and treatment with imatinib on 90-day mortality. In a secondary analysis where patients were categorised by having a high or low baseline biomarker level, no moderation was found either (*figure S5*).

As none of the biomarkers could capture the biological complexity observed in the included patients, hierarchical clustering was used to group patients into biologically similar groups based

on baseline plasma biomarker levels. Majority rules showed that three clusters (10 out of 22 classifications) best explained the variation based on 22 plasma biomarkers (*figure 3*). 90-day mortality was highest in cluster 1 and 3 (17.6% and 13.9%, respectively), and lowest in cluster 2 (5.7%) (*table S7*). Plasma concentrations of all biomarkers were generally highest in cluster 1 and lowest in cluster 2, with plasma concentrations of patients in cluster 3 in between (*figure S6*). Patients in cluster 3 were distinct in their higher concentration of SP-D in plasma, indicative of alveolar epithelial injury. Longitudinal plasma concentrations of patients within each cluster are visualised in figures S7-9. Only in patients assigned to cluster 3, imatinib resulted in a 90-day mortality reduction (HR 0.30 (0.10–0.92)) (*figure 3*). There was no mortality difference in patients in cluster 1 or cluster 2. These results remained the same in a sensitivity analysis, correcting for baseline imbalances (*table S8*).

Discussion

In this study, we aimed to describe the biological changes elicited by imatinib and the relationship between these changes and clinical outcomes in hospitalised COVID-19 patients. Our results suggest that the benefit of imatinib is mediated through modulation of innate immune responses and reversal of endothelial dysfunction. None of the individual baseline biomarker concentrations showed evidence for predictive enrichment of patients benefitting from imatinib. Classification of patients into three subphenotypes suggested that a subgroup of patients with profound alveolar injury combined with systemic inflammation and endothelial dysfunction were selectively profiting from imatinib treatment. This information could aid in providing insight in the mechanism of action of COVID-19 related therapies, and in the relationship between biomarkers and a clinical intervention in general.

This is the first human study to assess the effect of imatinib on the host response, and – as far as we know – the first study that links data from a randomised controlled trial to detailed biological profiles. *In vitro* and *in vivo* studies showed that imatinib reinforces the endothelial barrier and mitigates alveolar inflammatory responses through nuclear factor kappa B mediated chemotaxis, resulting in lower IL-6 concentrations [10, 26, 27]. Anti-inflammatory effects and endothelial barrier protection were therefore a priori likely to mediate imatinib effectiveness in COVID-19. Although previous studies demonstrated that COVID-19 is not specifically associated with a strong cytokine release syndrome [28], therapy strategies targeting the release of cytokines (e.g. steroids [2], IL-6 inhibitors [3], IL-1 receptor antagonists [29], Janus kinase 1/2 inhibitors [30], and granulocyte macrophage-colony stimulating factor inhibitors [31]) have also shown to be effective in reducing COVID-19 related mortality. The mortality mediating mechanisms of these therapeutics remain uncertain, since only clinical outcomes and no biological data was collected in those studies.

The mediation analysis presented here strongly suggested that a reduction in IL-6 concentration completely mediated the mortality reduction of imatinib. In other words, mortality was only reduced when the plasma concentration of IL-6 decreased after imatinib treatment. Yet, baseline plasma IL-6 concentration did not moderate the effect of imatinib on outcomes, so we dispute the hypothesis that patients with a more pro-inflammatory starting position have more benefit. So why would patients with severe COVID-19 only benefit when systemic anti-inflammatory effects are seen, given that there is little evidence for innate immune responses compatible with cytokine release syndrome? A possible explanation is that imatinib primarily restores the

endothelial barrier function, compatible with the observed changes in Ang-2/Ang-1 seen in this study, which might have resulted in cytokine leakage from the alveolar compartment to the systemic compartment [32]. It has indeed been suggested that COVID-19 is characterised by an alveolar cytokine storm instead of a systemic cytokine storm [33, 34].

Given that biological complexity is insufficiently captured by single biomarkers, we used an established clustering method to identify three biological subphenotypes in hospitalised COVID-19 patients [25]. Separation into two biological subphenotypes has been described in ARDS [35] and in COVID-19 [36, 37]. Most of these studies relied on clinical data [38] or a combination of clinical data and biomarkers [35], while we used biological data alone to identify subphenotypes. Furthermore, we focused on patients admitted to the ward, while previous studies were restricted to a critically ill population admitted to the ICU. The inclusion of a comprehensive set of biomarkers provided separation by plasma concentration of SP-D within the subset of patients with an inflammatory profile and endothelial dysfunction (subphenotype 1 versus 3). SP-D is a biomarker of alveolar injury and is increased in patients developing ARDS [39], and an increase in plasma concentration is indicative of alveolar permeability [40]. Imatinib only decreased mortality in the subgroup with this biological profile, suggesting that a certain amount of alveolar permeability in the context of systemic inflammation and endothelial dysfunction needs to be present in order for imatinib to have a protective effect. When validated independently, patients with more alveolar injury in the setting of an inflammatory state could therefore preferentially be selected for imatinib treatment. It remains to be explained how a further increase in SP-D mediated the protective effect imatinib on mortality, as this is counter intuitive in light of the moderation analysis.

Our study has important strengths and some limitations. The use of randomised group allocation eliminates most forms of bias and therefore provides the best possible estimate of a causal treatment effect. Although mortality was a secondary endpoint and the protective effect of imatinib attenuated after correction for baseline differences, the long-term analysis at day 90 demonstrated a persistent survival benefit of imatinib, even after adjusting for baseline imbalances. Furthermore, in our study, the pre-treatment biomarker concentrations did not show any differences between the groups, confirming a comparable baseline biological profile and limiting the explanation that baseline differences were responsible for the observed mediating effects. Because the data was collected systematically with the performed analyses in mind, we obtained biomarker data of a large share of the study population. All patients in the biomarker cohort were alive at the time of the second measurement, excluding immortal time bias as explanation for our findings. Patients without biomarker data had similar baseline characteristics, but had fewer days of oxygen therapy. We assume that no bias occurred in the selection of patients for whom biomarker data was available. We selected 25 biomarkers representative of host response pathways implicated in COVID-19 and the mechanism of action of imatinib; nonetheless, we could have missed an important mediator. The absence of commonly measured biomarkers (e.g. ferritin or CRP) in our biomarker panel limits the ability to compare our dataset to other studies. Second, only the systemic host response was evaluated and the alveolar environment was not sampled nor studied because obtaining alveolar samples in non-intubated patients is infeasible. Last but not least, the study is likely underpowered to detect heterogeneity of treatment effects via moderation analysis and a larger sample might have yielded different results [41]. When examining the moderating effects of the three subphenotypes by an

interaction term instead of a stratified analysis, the HRs were comparable but confidence intervals were wide resulting in p values above 0.05, as expected. Therefore, future prospective testing is required to validate our results.

The findings of this study extend our biological understanding of how mortality can be reduced in patients with severe COVID-19. Changes in innate immune responses and endothelial barrier protection appear to mediate the reduction in mortality observed in the imatinib group. We speculate that this may translate to other immunomodulatory treatments as well. Furthermore, we illustrate that we should not assume that patients who have a high concentration of a single biomarker that is considered to be reflective of activation of the pathway that is targeted by the drug results in predictive enrichment within the context of severe COVID-19 pneumonia. Rather, identification of subphenotypes by comprehensive analysis of multiple pathways provided three clusters that responded differently to the tested intervention. This is in line with studies in ARDS [35, 42, 43], and shows that biological profiles should be used for predictive enrichment rather than single biomarker values. The subphenotype that responded favourably to imatinib treatment had a similar severity of illness compared to a subphenotype that did not have survival benefit from imatinib. This is in contrast to previous studies, and might suggest that this subphenotype could be used for predictive enrichment rather than prognostic enrichment.

To conclude, we here show that imatinib works as an effective therapy against severe COVID-19 only when circulating biomarkers confirm decreased systemic innate immune response and improved endothelial barrier function after treatment. Changes in these biomarker concentrations may be used as a surrogate endpoint when validated as mediators for therapy-related survival in

other randomised controlled trials. Three biological subphenotypes were identified and only patients classified as having alveolar injury by increased levels of SP-D in the context of systemic inflammatory response and endothelial dysfunction benefitted from imatinib treatment.

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Ethics approval

The ethical boards of the participating hospitals approved the collection of data for the study purposes.

Data sharing

De-identified participant data with data dictionary can be shared after approval of a proposal with a signed data access agreement and always in collaboration with the study group.

Author contributions

The authors designed the study together and were involved in collecting the data with the help of the study collaborators. JdB and LDJB had access to the raw data, did the analyses and drafted the manuscript. The other authors revised the initial draft. All authors approved the final version of the manuscript.

Declaration of interests

LDJB reports grants from the Dutch Lung Foundation, grants from the Dutch Lung Foundation and Health Holland (Public-Private Partnership grant), grants from the Dutch Lung Foundation (Dirkje Postma Award), grants from the IMI COVID19 initiative, and grants from Amsterdam

UMC fellowship, outside the submitted work. JA is inventor on a patent (WO2012150857A1; 2011) covering protection against endothelial barrier dysfunction through inhibition of the tyrosine kinase abl-related gene (arg). JA reports serving as a non-compensated scientific advisor for Exvastat. All other authors declare no competing interests.

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Tables

Table 1. Clinical characteristics of patients included in the cohort that was used for the presented secondary analyses.

	Imatinib group n = 169	**Placebo group** n = 163
Demographics		
Age, years, n (%)	65 [57-73]	64 [55-74]
Male gender, n (%)	127 (75.1)	107 (65.6)
BMI, kg/m^2, median [IQR]	27.3 [25.2-31.1]	29.8 [25.6-33.0]
Comorbidities, n (%) *		
Current or former smoker	63 (38.7)	67 (43.5)
BMI of > 30 kg/m^2	42 (28.2)	71 (49.0)
Diabetes	36 (21.3)	52 (31.9)
Cardiovascular disease †	32 (18.9)	45 (27.6)
Hypertension	56 (33.1)	66 (40.5)
COPD or asthma	30 (17.8)	32 (19.6)
Venous thromboembolism	3 (1.8)	2 (1.2)
Renal failure	5 (3.0)	7 (4.3)
Hepatic disease	1 (0.6)	1 (0.6)
Rheumatic disease	8 (4.7)	15 (9.2)
Heart failure	8 (4.7)	3 (1.8)
Medical treatments, n (%) ‡		
Glucose lowering drugs	35 (20.7)	48 (29.4)
Antihypertensive treatment	78 (46.2)	92 (56.4)
ACE or ARB	41 (24.3)	63 (38.7)
Statins	51 (30.2)	57 (35.0)
Platelet inhibitors	35 (20.7)	37 (22.7)
Oral anticoagulants	15 (8.9)	18 (11.0)
Laboratory values on admission, median [IQR]		
Hemoglobin, mmol/L	8.4 [7.8-9.1]	8.6 [7.9-9.1]
Leukocytes, x 10^9 cells/L	7.7 [5.6-10.5]	7.8 [5.9-10.0]
Neutrophils, x 10^9 cells/L	6.0 [4.2-8.6]	5.9 [4.4-8.3]
Lymphocytes, x 10^9 cells/L	0.86 [0.60-1.10]	0.91 [0.62-1.28]
Thrombocytes, x 10^9 cells/L	244 [185-321]	235 [190-311]
Urea, mmol/L	6.3 [4.5-8.5]	6.7 [5.0-8.9]
Creatinine, µmol/L	76 [65-88]	78 [66-94]
C-reactive protein, mg/L	104 [48-158]	92 [46-150]
Medication initiated on admission, n (%)		
Low-molecular-weight heparin	143 (84.6)	128 (78.5)
Oral anticoagulants	11 (6.5)	16 (9.8)
Antibiotics	68 (40.2)	63 (38.7)
Dexamethasone	125 (74.0)	117 (71.8)
Remdesivir	32 (18.9)	34 (20.9)
(Hydroxy)chloroquine	13 (7.7)	13 (8.0)
Disease severity on admission, median [IQR]		
qSOFA score	0 [0-1]	0 [0-1]

Data are median [interquartile range] or n (%). No p values are shown for baseline data, since data is obtained from a randomized controlled trial. ACE = angiotensin-converting enzyme, ARB = angiotensin receptor blocker, BMI = body mass index, COPD = chronic obstructive pulmonary disease, ICU = intensive care unit, IQR = interquartile range, qSOFA = quick sequential organ failure assessment. * Comorbidities as reported at admission or present in the patient's medical record. † Cardiovascular diseases included arrhythmias (predominantly atrial fibrillation), valvular disease, coronary artery disease and conduction disorders. ‡ Medical treatment (or home medication) as reported at admission or present in the patient's medical record.

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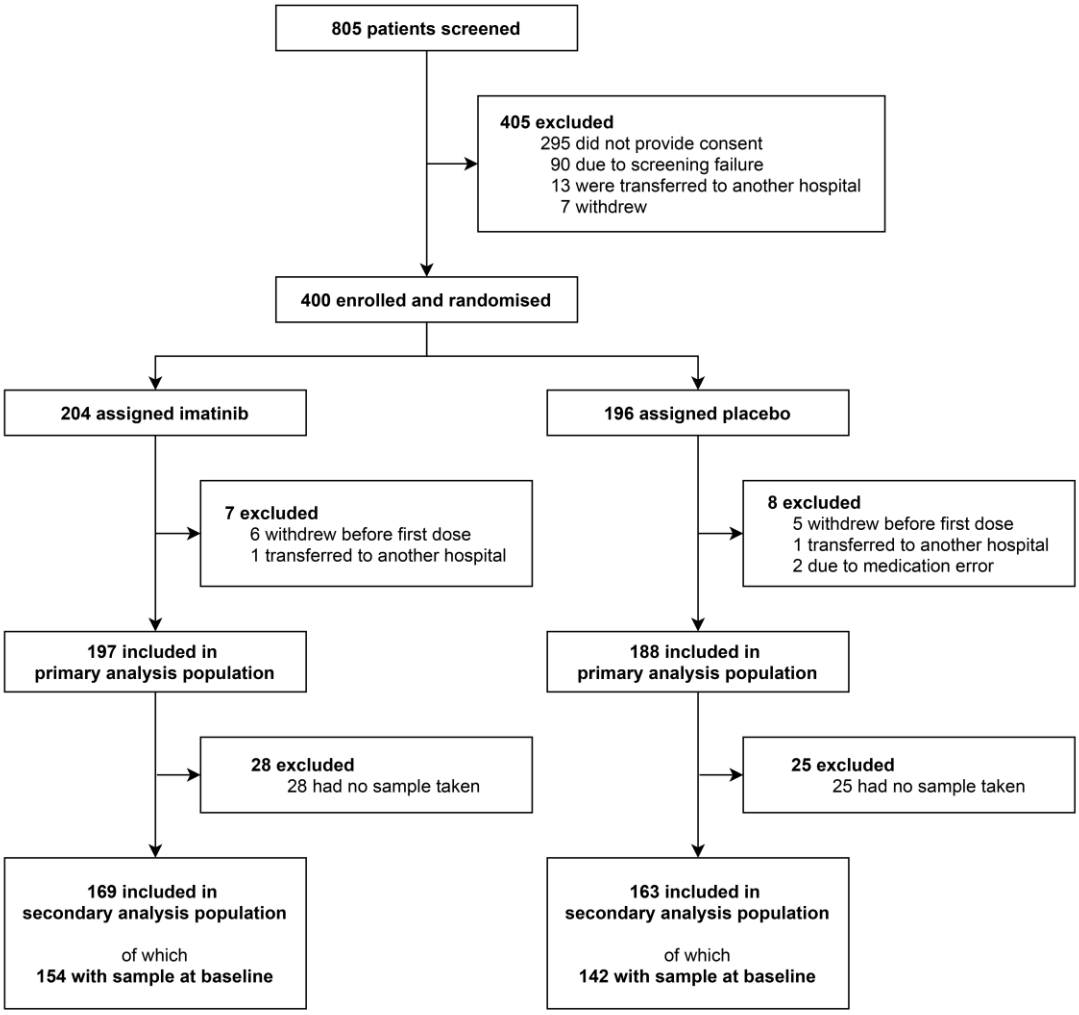

Figure 1: Flowchart of patient selection.

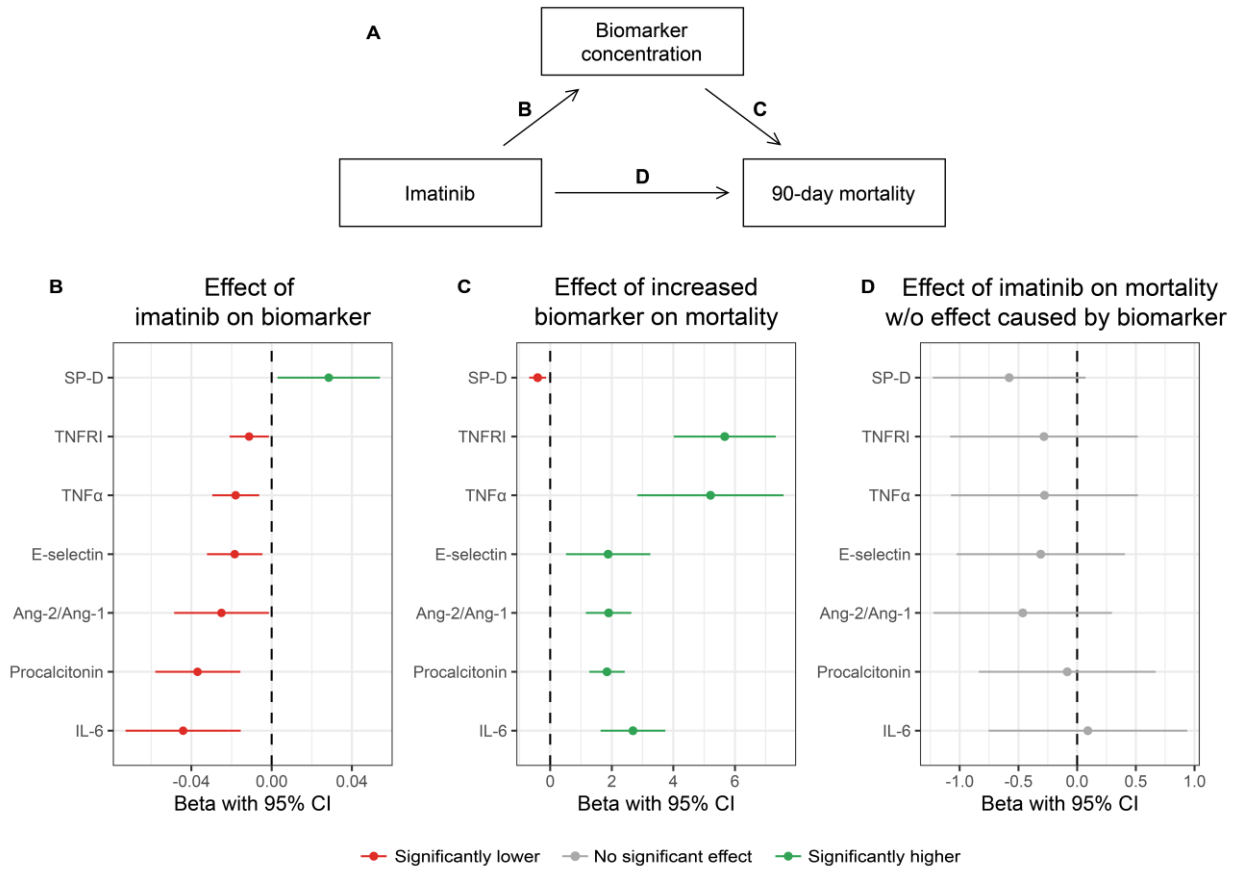

Figure 2A: Visualization of mediation analysis. 2B: The effect of imatinib on the biomarker concentration over time, when compared to placebo. 2C: The effect of an increased biomarker concentration over time on 90-day mortality. 2D: The effect of imatinib on 90-day mortality, when the effect of the biomarkers is left out. The effect of imatinib on 90-day mortality is completely mediated by changes in TNFRI, TNFα, E‑selectin, Ang-2/Ang-1, procalcitonin and IL-6. Abbreviations: SP-D = surfactant protein D, TNFRI = tumour necrosis factor receptor I, TNFα = tumour necrosis factor alpha, Ang-2/Ang-1 = angiopoietin 2 to 1 ratio, IL‑6 = interleukin-6.

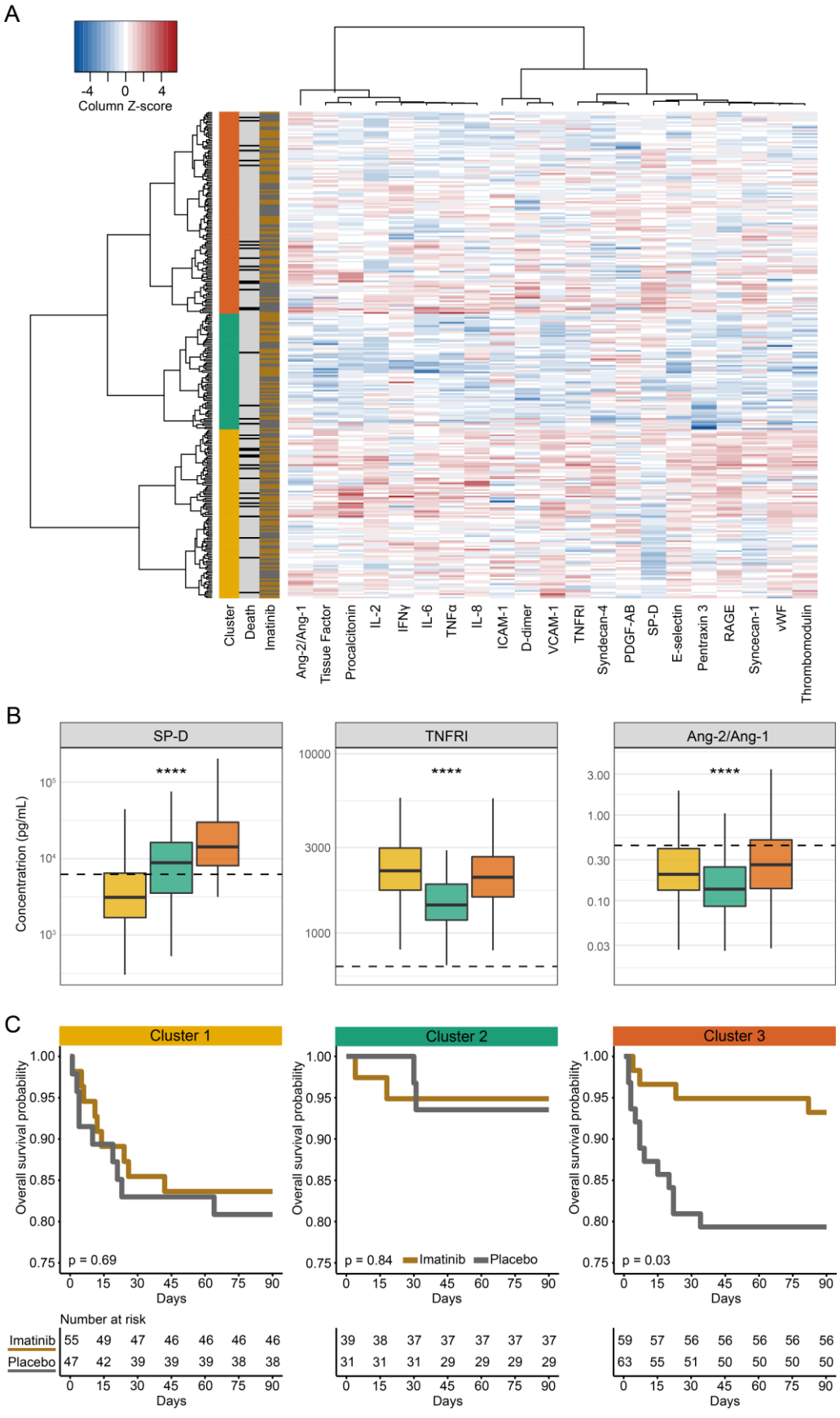
A
-4
0
4
Column Z-score
Cluster
Death
Imatinib
Ang-2/Ang-1
Tissue Factor
Procalcitonin
IL-2
IFNγ
IL-6
TNFα
IL-8
ICAM-1
D-dimer
VCAM-1
TNFRI
Syndecan-4
PDGF-AB
SP-D
E-selectin
Pentraxin 3
RAGE
Syncecan-1
vWF
Thrombomodulin
B
SP-D
TNFRI
Ang-2/Ang-1
****
Concentration (pg/mL)
C
Cluster 1
Cluster 2
Cluster 3
Overall survival probability
p = 0.69
p = 0.84
p = 0.03
Imatinib
Placebo
Days
Number at risk
Imatinib 55 49 47 46 46 46 46
Placebo 47 42 39 39 39 38 38
39 38 37 37 37 37 37
31 31 31 29 29 29 29
59 57 56 56 56 56 56
63 55 51 50 50 50 50

Figure 3A: Heatmap of subphenotypes, based on baseline biological profile. Rows represent patients, columns represent biomarkers. First column: three clusters; yellow is cluster 1, green is cluster 2, orange is cluster 3. Second column: patients that deceased within 90 days are indicated with black, surviving patients with grey. Third column: patients who received imatinib therapy are indicated with gold, placebo patients with grey. Heatmap: a higher concentration in comparison to the other included patients is indicated with red, while a lower concentration is indicated by blue. Ang-2/Ang-1 = angiopoietin 2 to 1 ratio, IL = interleukin, IFNγ = interferon gamma, TNFα = tumour necrosis factor alpha, ICAM-1 = intracellular adhesion molecule 1, VCAM-1 = vascular cell adhesion molecule 1, TNFRI = tumour necrosis factor receptor I, PDGF□AB = platelet-derived growth factor AB, SP-D = surfactant protein D, RAGE = receptor for advanced glycation end products, vWF = Von Willebrand factor. 3B: Baseline plasma concentrations of three biomarkers reflective of cluster analysis, stratified according to subphenotype. Data is depicted as box and whisker plots. Dotted lines indicate median values obtained in healthy controls. Asterisks indicates statistical significance by analysis of variance (ANOVA) tests. **** $p < 0.0001$. 3C: Kaplan Meier curves and risk tables for imatinib depicted in gold and placebo shown in grey stratified per biological subphenotype identified by cluster analysis shown in panel A. A Cox proportional hazards model was used to provide p values.

Online data supplement

Immunomodulation and endothelial barrier protection mediate the association between oral imatinib and mortality in hospitalised COVID-19 patients

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Table S1. Biomarkers measured.

	Abbreviation
Endothelial cell activation and function	
Angiopoietin-1	Ang-1
Angiopoietin-2	Ang-2
E-selectin	E-selectin
Fractalkine	Fractalkine
Intracellular adhesion molecule 1	ICAM-1
Syndecan-1	Syndecan-1
Syndecan-4	Syndecan-4
Thrombomodulin	Thrombomodulin
Vascular cell adhesion molecule 1	VCAM-1
Cytokine release	
Interferon gamma	IFNγ
Interleukin-2	IL-2
Interleukin-6	IL-6
Interleukin-8	IL-8
Interleukin-10	IL-10
Interleukin-17	IL-17
Tumour necrosis factor alpha	TNFα
Epithelial cell activation and function	
Receptor for advanced glycation end products	RAGE
Surfactant protein D	SP-D
Systemic inflammation	
Platelet-derived growth factor AB	PDGF-AB
Pentraxin-3	PTX-3
Procalcitonin	Procalcitonin
Tumour necrosis factor receptor I	TNFRI
Coagulation	
D-dimer	D-dimer
Tissue factor	TF
Von Willebrand factor	vWF

Table S2. Quality assessment of biomarker measurements.

Biomarker	Within all limits	> ULQ *	< LLQ †	< 25 beads measured ‡
Angiopoietin-1	695 (97.9)	8 (1.1)	..	7 (1.0)
Angiopoietin-2	701 (98.7)	..	..	9 (1.3)
D-dimer	614 (86.5)	61 (8.6)	..	35 (4.9)
E-selectin	703 (99.0)	..	..	7 (1.0)
Fractalkine	165 (23.2)	2 (0.3)	5 (0.7)	538 (75.8)
ICAM-1	702 (98.9)	..	1 (0.1)	7 (1.0)
IFNγ	692 (97.5)	..	11 (1.5)	7 (1.0)
IL-2	618 (87.0)	..	85 (12.0)	7 (1.0)
IL-6	696 (98.0)	..	7 (1.0)	7 (1.0)
IL-8	702 (98.9)	..	1 (0.1)	7 (1.0)
IL-10	519 (73.1)	..	184 (25.9)	7 (1.0)
IL-17	411 (57.9)	..	297 (41.8)	2 (0.3)
PDGF-AB	710 (100)	..	..	..
Pentraxin-3	693 (97.6)	..	11 (1.5)	6 (8.4)
Procalcitonin	690 (97.2)	5 (0.7)	..	15 (2.1)
RAGE	702 (98.9)	..	1 (0.1)	7 (1.0)
SP-D	706 (99.4)	2 (0.3)	..	2 (0.3)
Syndecan-1	675 (95.1)	..	1 (0.1)	34 (4.8)
Syndecan-4	703 (99.0)	..	..	7 (1.0)
Thrombomodulin	703 (99.0)	..	..	7 (1.0)
Tissue factor	703 (99.0)	..	..	7 (1.0)
TNFα	703 (99.0)	..	..	7 (1.0)
TNFRI	710 (100)	..	..	..
VCAM-1	524 (73.8)	183 (25.8)	..	3 (0.4)
Von Willebrand factor	701 (98.7)	..	..	9 (1.3)

Data are n (%). Total N = 710. Abbreviations: ULQ = upper limit of quantification, LLQ = lower limit of quantification, ICAM-1 = intracellular adhesion molecule 1, IFNγ = interferon gamma, IL = interleukin, PDGF-AB = platelet-derived growth factor AB, RAGE = receptor for advanced glycation end products, SP-D = surfactant protein D, TNFα = tumour necrosis factor alpha, TNFRI = tumour necrosis factor receptor I, VCAM-1 = vascular cell adhesion molecule 1. * Values above the upper limit of the calibration curve are extrapolated based on the standard curve. † Values below the lower limit of the calibration curve were set to the lower limit of quantification. ‡ Measurements with less than 25 beads measured were excluded from analysis.

Table S3. Number of samples measured per time point.

	Sample measured	Measurement missed *	Patient deceased	Patient discharged
Baseline	296 (76.9)	89 (23.1)	..	..
Day 2/3	256 (66.5)	100 (26.0)	2 (0.5)	27 (7.0)
Day 5	136 (35.3)	95 (24.7)	17 (4.4)	137 (35.6)

Data are n (%). Total N = 385. * Reasons for a missed measurement include: no blood withdrawal in participating center, failure of withdrawal, patient refusal, logistic difficulties.

Table S4. Comparison of clinical characteristics between patients included and excluded in the secondary analysis.

	Included in secondary analysis n = 332	**Excluded in secondary analysis** n = 53	**p value**
Randomisation, n (%)			
Imatinib group	169 (50.9)	28 (52.8)	0.91
Placebo group	163 (49.1)	25 (47.2)	0.91
Demographics			
Age, years, n (%)	64 [56-73]	62 [56-70]	0.65
Male gender, n (%)	234 (70.5)	30 (56.6)	0.06
BMI, kg/m^2, median [IQR]	28.4 [25.5-32.4]	28.7 [25.7-30.9]	1.00
Comorbidities, n (%) *			
Current or former smoker	130 (41.0)	23 (46.0)	0.61
BMI of > 30 kg/m^2	113 (38.4)	15 (30.6)	0.37
Diabetes	88 (26.5)	12 (22.6)	0.67
Cardiovascular disease †	77 (23.2)	6 (11.3)	0.08
Hypertension	122 (36.7)	23 (43.4)	0.44
COPD or asthma	62 (18.7)	9 (17.0)	0.92
Venous thromboembolism	5 (1.5)	5 (9.4)	**< 0.01**
Renal failure	12 (3.6)	2 (3.8)	1.00
Hepatic disease	2 (0.6)	0 (0.0)	1.00
Rheumatic disease	23 (6.9)	6 (11.3)	0.40
Heart failure	11 (3.3)	1 (1.9)	0.90
Medical treatments, n (%) ‡			
Glucose lowering drugs	83 (25.0)	11 (20.8)	0.62
Antihypertensive treatment	170 (51.2)	23 (43.4)	0.36
ACE or ARB	104 (31.3)	17 (32.1)	1.00
Statins	108 (32.5)	19 (35.8)	0.75
Platelet inhibitors	72 (21.7)	10 (18.9)	0.78
Oral anticoagulants	33 (9.9)	5 (9.4)	1.00
Laboratory values at admission, median [IQR]			
Hemoglobin, mmol/L	8.5 [7.9-9.1]	8.3 [7.5-9.0]	0.10
Leukocytes, x 10^9 cells/L	7.7 [5.7-10.3]	7.1 [5.7-9.3]	0.53
Neutrophils, x 10^9 cells/L	6.0 [4.3-8.5]	5.6 [3.9-8.1]	0.44
Lymphocytes, x 10^9 cells/L	0.90 [0.60-1.20]	1.10 [0.70-1.27]	0.11
Thrombocytes, x 10^9 cells/L	238 [189-315]	257 [190-322]	0.60
Urea, mmol/L	6.6 [4.8-8.8]	6.6 [4.9-8.1]	0.70
Creatinine, µmol/L	77 [65-91]	77 [65-90]	0.93
C-reactive protein, mg/L	99 [47-155]	95 [38-128]	0.41
Medication initiated at admission, n (%)			
Low-molecular-weight heparin	271 (81.6)	46 (86.8)	0.47
Oral anticoagulants	27 (8.1)	3 (5.7)	0.73
Antibiotics	132 (39.6)	29 (54.7)	0.05
Dexamethasone	242 (72.9)	34 (64.2)	0.25
Remdesivir	66 (19.9)	14 (26.4)	0.37
(Hydroxy)chloroquine	26 (7.8)	3 (5.7)	0.78
Clinical outcomes			
28-day mortality, n (%)	37 (11.1)	5 (9.4)	0.89

90-day mortality, n (%)	43 (13.0)	6 (11.3)	0.91
Time to discontinuation of oxygen support, days, median [IQR] §	7 [5-11]	5 [3-8]	< **0.01**
Duration of hospital admission, days, median [IQR]	7 [4-11]	5 [3-12]	0.19
Need for mechanical ventilation, n (%)	47 (14.2)	9 (17.0)	0.74
Duration of mechanical ventilation, days, median [IQR]	10 [5-18]	7 [4-10]	0.41
Duration of ICU admission, days, median [IQR]	9 [5-18]	9 [8-10]	0.92

Data are median [interquartile range] or n (%). ACE = angiotensin-converting enzyme, ARB = angiotensin receptor blocker, BMI = body mass index, COPD = chronic obstructive pulmonary disease, ICU = intensive care unit, IQR = interquartile range. * Comorbidities as reported at admission or present in the patient's medical record. † Cardiovascular diseases included arrhythmias (predominantly atrial fibrillation), valvular disease, coronary artery disease and conduction disorders. ‡ Medical treatment (or home medication) as reported at admission or present in the patient's medical record. § Time to discontinuation of ventilation and supplemental oxygen for more than 48 consecutive hours, while being alive during a 28-day period after randomisation.

Table S5. Clinical characteristics of patients with baseline biomarker data available (i.e. moderation analysis cohort).

	Imatinib group n = 154	**Placebo group** n = 142
Demographics		
Age, years, median [IQR]	65 [57-74]	64 [56-74]
Male gender, n (%)	116 (75.3)	93 (65.5)
BMI, kg/m^2, median [IQR]	27.4 [25.2-31.1]	29.7 [25.7-32.9]
Comorbidities, n (%) *		
Current or former smoker	59 (39.6)	58 (43.3)
BMI of > 30 kg/m^2	39 (28.5)	61 (48.4)
Diabetes	31 (20.1)	42 (29.6)
Cardiovascular disease †	31 (20.1)	38 (26.8)
Hypertension	52 (33.8)	54 (38.0)
COPD or asthma	28 (18.2)	30 (21.1)
Venous thromboembolism	2 (1.3)	2 (1.4)
Renal failure	5 (3.2)	5 (3.5)
Hepatic disease	1 (0.6)	1 (0.7)
Rheumatic disease	8 (5.2)	12 (8.5)
Heart failure	8 (5.2)	3 (2.1)
Medical treatments, n (%) ‡		
Glucose lowering drugs	30 (19.5)	38 (26.8)
Antihypertensive treatment	70 (45.5)	80 (56.3)
ACE or ARB	39 (25.3)	55 (38.7)
Statins	47 (30.5)	51 (35.9)
Platelet inhibitors	33 (21.4)	33 (23.2)
Oral anticoagulants	15 (9.7)	17 (12.0)
Laboratory values at admission, median [IQR]		
Hemoglobin, mmol/L	8.5 [7.8-9.1]	8.6 [7.9-9.1]
Leukocytes, x 10^9 cells/L	7.7 [5.6-10.4]	7.8 [6.0-10.1]
Neutrophils, x 10^9 cells/L	6.0 [4.2-8.6]	6.1 [4.4-8.2]
Lymphocytes, x 10^9 cells/L	0.90 [0.60-1.10]	0.90 [0.60-1.22]
Thrombocytes, x 10^9 cells/L	240 [184-322]	232 [189-311]
Urea, mmol/L	6.6 [4.7-8.8]	6.6 [5.0-8.7]
Creatinine, µmol/L	77 [64-89]	78 [66-92]
C-reactive protein, mg/L	104 [47-161]	91 [45-142]
Medication initiated at admission, n (%)		
Low-molecular-weight heparin	132 (85.7)	111 (78.2)
Oral anticoagulants	8 (5.2)	13 (9.2)
Antibiotics	61 (39.6)	51 (35.9)
Dexamethasone	115 (74.7)	103 (72.5)
Remdesivir	30 (19.5)	30 (21.1)
(Hydroxy)chloroquine	13 (8.4)	10 (7.0)

Data are median [interquartile range] or n (%). No p values are shown for baseline data, since data is obtained from a randomised controlled trial. ACE = angiotensin-converting enzyme, ARB = angiotensin receptor blocker, BMI = body mass index, COPD = chronic obstructive pulmonary disease, ICU = intensive care unit, IQR = interquartile range. * Comorbidities as reported at admission or present in the patient's medical record. † Cardiovascular diseases included arrhythmias (predominantly atrial fibrillation), valvular disease, coronary artery disease and conduction disorders. ‡ Medical treatment (or home medication) as reported at admission or present in the patient's medical record.

Table S6. Baseline (pre-treatment) plasma biomarker concentrations, stratified by treatment group.

	Imatinib group n = 154	**Placebo group** n = 142	**BH adjusted p value**
Endothelial cell activation and function			
Ang-2/Ang-1	0.20 [0.13 – 0.37]	0.20 [0.11 – 0.37]	0.78
E-selectin, ng/mL	23.32 [16.42 – 31.69]	26.11 [18.41 – 34.06]	0.30
ICAM-1, ng/mL	275.93 [213.70 – 388.37]	337.53 [243.18 – 496.03]	0.07
Syndecan-1, ng/mL	6.63 [4.65 – 9.35]	6.79 [4.60 – 9.65]	0.79
Syndecan-4, ng/mL	0.73 [0.49 – 1.11]	0.70 [0.49 – 1.03]	0.79
Thrombomodulin, ng/mL	5.11 [3.77 – 6.91]	5.50 [4.34 – 7.43]	0.29
VCAM-1, µg/mL	3.22 [2.08 – 6.53]	3.40 [2.15 – 5.96]	0.80
Cytokine release			
IFNγ, pg/mL	18.05 [10.79 – 24.83]	18.05 [11.20 – 30.27]	0.60
IL-2, pg/mL	6.64 [2.37 – 10.96]	7.68 [3.46 – 10.96]	0.48
IL-6, pg/mL	10.46 [6.03 – 17.00]	11.32 [7.47 – 24.31]	0.30
IL-8, pg/mL	12.99 [7.27 – 22.09]	14.47 [9.62 – 25.09]	0.29
IL-10, pg/mL	7.68 [1.09 – 13.24]	8.46 [2.68 – 13.24]	0.79
IL-17, pg/mL	2.49 [0.53 – 5.86]	2.49 [0.53 – 8.80]	0.59
TNFα, pg/mL	11.22 [8.58 – 13.84]	11.99 [9.64 – 15.41]	0.30
Epithelial cell activation and function			
RAGE, ng/mL	3.79 [2.00 – 7.28]	3.99 [2.50 – 7.85]	0.48
SP-D, ng/mL	8.15 [3.57 – 16.48]	9.31 [4.03 – 22.37]	0.48
Systemic inflammation			
PDGF-AB, ng/mL	0.91 [0.53 – 1.41]	0.92 [0.64 – 1.40]	0.80
Pentraxin-3, ng/mL	6.53 [3.39 – 1.11]	6.14 [3.25 – 10.83]	0.59
Procalcitonin, pg/mL	73.10 [44.95 – 121.03]	80.55 [49.21 – 138.50]	0.78
TNFRI, ng/mL	1.90 [1.37 – 2.36]	1.98 [1.50 – 2.68]	0.48
Coagulation			
D-dimer, µg/mL	3.17 [2.21 – 4.71]	3.59 [2.02 – 5.62]	0.48
Tissue factor, pg/mL	42.70 [30.05 – 61.79]	45.73 [34.19 – 68.27]	0.31
Von Willebrand factor, ng/mL	5.51 [3.68 – 7.88]	5.67 [3.94 – 7.80]	0.79

Results are presented as median [interquartile range]. Ang = angiopoietin, BH = Benjamini–Hochberg, ICAM-1 = intracellular adhesion molecule 1, IFNγ = interferon gamma, IL = interleukin, PDGF-AB = platelet-derived growth factor AB, RAGE = receptor for advanced glycation end products, SP-D = surfactant protein D, TNFα = tumour necrosis factor alpha, TNFRI = tumour necrosis factor receptor I, VCAM-1 = vascular cell adhesion molecule 1.

Table S7. Clinical characteristics of patients with baseline biomarker data available, stratified by subphenotype.

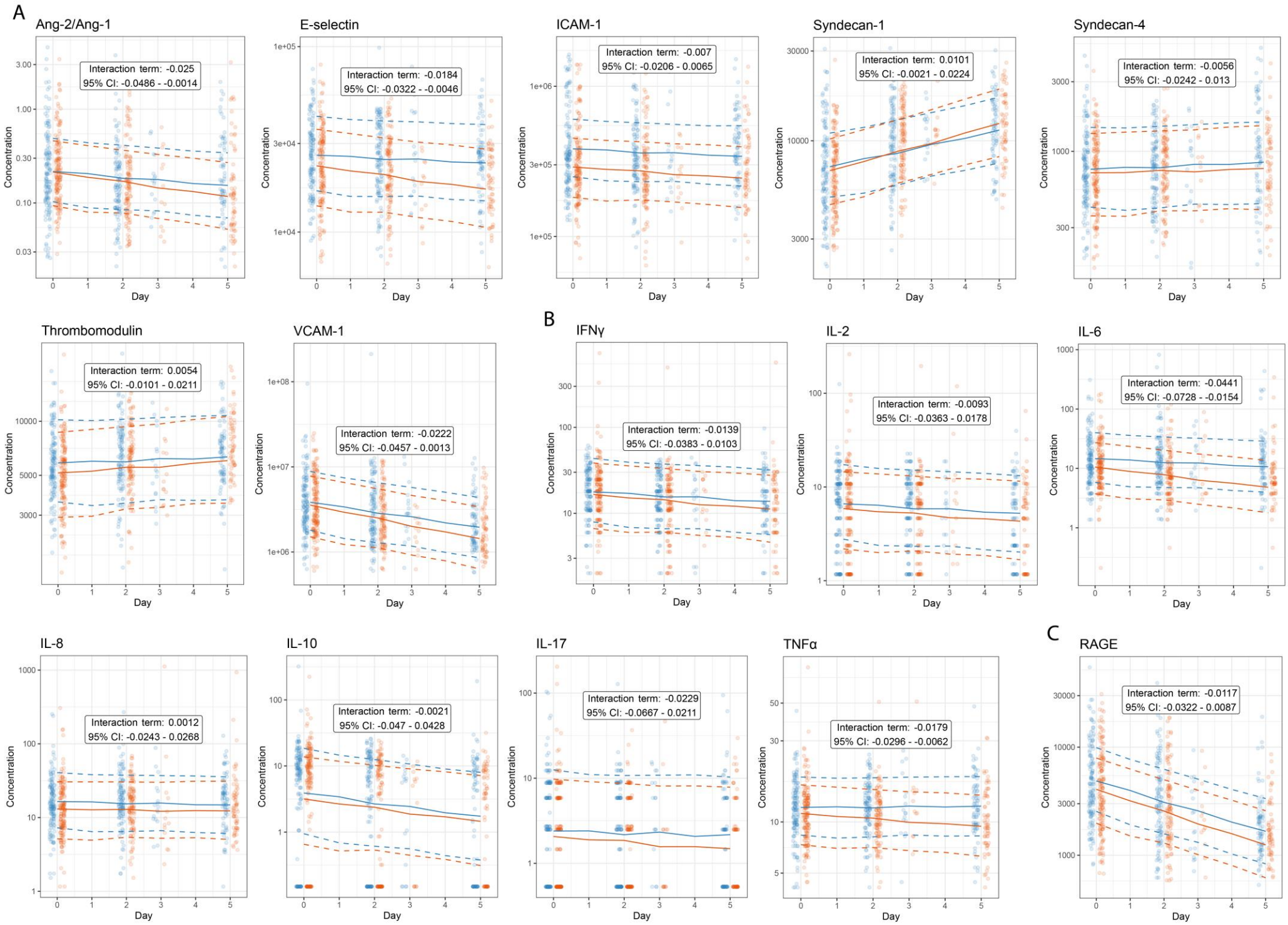
	Cluster 1 n = 102	**Cluster 2** n = 70	**Cluster 3** n = 122	**p value**
Randomisation, n (%)				
Imatinib group	55 (53.9)	39 (55.7)	59 (48.4)	0.55
Placebo group	47 (46.1)	31 (44.3)	63 (51.6)	0.55
Demographics				
Age, years, median [IQR]	65 [57-74]	61 [52-67]	68 [58-76]	**0.01**
Male gender, n (%)	78 (76.5)	37 (52.9)	93 (76.2)	< **0.01**
BMI, kg/m^2, median [IQR]	28.7 [25.6-31.9]	28.6 [25.6-33.1]	27.8 [25.2-31.6]	0.72
Medical treatments, n (%) *				
Glucose lowering drugs	30 (29.4)	13 (18.6)	25 (20.5)	0.17
Antihypertensive treatment	58 (56.9)	33 (47.1)	59 (48.4)	0.34
ACE or ARB	34 (33.3)	23 (32.9)	37 (30.3)	0.88
Statins	37 (36.3)	18 (25.7)	43 (35.2)	0.30
Platelet inhibitors	23 (22.5)	10 (14.3)	32 (26.2)	0.16
Oral anticoagulants	9 (8.8)	7 (10.0)	16 (13.1)	0.57
Vital signs at admission, median [IQR]				
Mean arterial pressure, mm Hg	90 [84-101]	90 [83-99]	90 [85-98]	0.80
Heart rate, beats per min	81 [70-91]	74 [66-83]	79 [69-87]	**0.01**
Peripheral oxygen saturation, %	94 [92-96]	94 [93-96]	94 [92-95]	0.23
Oxygen support, L/min	5 [3-10]	3 [2-5]	5 [3-10]	**0.01**
Respiratory rate, /min	22 [18-27]	18 [16-21]	20 [18-24]	< **0.01**
Temperature, °C	36.8 [36.3-37.5]	36.7 [36.4-37.1]	36.7 [36.3-37.2]	0.22
Laboratory values at admission, median [IQR]				
Hemoglobin, mmol/L	8.6 [7.9-9.2]	8.6 [8.0-9.1]	8.4 [7.8-9.1]	0.36
Leukocytes, x 10^9 cells/L	8.0 [5.6-10.8]	6.7 [5.2-9.0]	8.1 [6.3-10.7]	**0.01**
Neutrophils, x 10^9 cells/L	6.2 [4.0-9.0]	5.0 [3.7-6.5]	6.6 [4.6-9.3]	< **0.01**
Lymphocytes, x 10^9 cells/L	0.84 [0.60-1.03]	1.00 [0.83-1.37]	0.80 [0.60-1.10]	< **0.01**
Thrombocytes, x 10^9 cells/L	220 [178-270]	256 [199-331]	240 [192-330]	**0.01**
Urea, mmol/L	7.1 [5.5-10.4]	5.5 [4.2-7.0]	6.8 [5.0-8.6]	< **0.01**
Creatinine, µmol/L	81 [71-100]	69 [58-83]	76 [64-89]	< **0.01**
C-reactive protein, mg/L	116 [66-171]	63 [32-125]	104 [50-150]	< **0.01**
Medication initiated at admission, n (%)				
Low-molecular-weight heparin	85 (83.3)	58 (82.9)	98 (80.3)	0.82
Oral anticoagulants	5 (4.9)	3 (4.3)	13 (10.7)	0.14
Antibiotics	35 (34.3)	21 (30.0)	55 (45.1)	0.08
Dexamethasone	77 (75.5)	47 (67.1)	93 (76.2)	0.35
Remdesivir	25 (24.5)	13 (18.6)	21 (17.2)	0.37
(Hydroxy)chloroquine	6 (5.9)	7 (10.0)	10 (8.2)	0.60
Clinical outcomes				
28-day mortality, n (%)	16 (15.7)	2 (2.9)	15 (12.3)	**0.03**
90-day mortality, n (%)	18 (17.6)	4 (5.7)	17 (13.9)	0.07
Time to discontinuation of oxygen support, days, median [IQR] †	8 [5-13]	6 [4-9]	8 [5-11]	**0.01**
Duration of hospital admission, days, median [IQR]	7 [4-15]	4 [3-8]	7 [4-11]	< **0.01**
Need for mechanical ventilation, n (%)	21 (20.6)	2 (2.9)	14 (11.5)	< **0.01**
Duration of mechanical ventilation, days, median [IQR]	11 [6-19]	22 [21-24]	6 [3-11]	0.07
Duration of ICU admission, days, median [IQR]	12 [6-21]	23 [18-24]	7 [5-11]	0.07

Data are median [interquartile range] or n (%). ACE = angiotensin-converting enzyme, ARB = angiotensin receptor blocker, BMI = body mass index, COPD = chronic obstructive pulmonary disease, ICU = intensive care unit, IQR = interquartile range. * Medical treatment (or home medication) as reported at admission or present in the patient's medical record. † Time to discontinuation of ventilation and supplemental oxygen for more than 48 consecutive hours, while being alive during a 28-day period after randomisation.

Table S8. Clinical outcome of patients with baseline biomarker data available, stratified by subphenotype.

	Cluster 1 n = 102	**Cluster 2** n = 70	**Cluster 3** n = 122
90-day mortality			
Unadjusted	0.83 (0.33 – 2.09)	0.81 (0.11 – 5.78)	0.30 (0.10 – 0.92)
Adjusted for sex, BMI, diabetes and cardiovascular disease	0.61 (0.22 – 1.75)	1.06 (0.12 – 9.27)	0.20 (0.05 – 0.70)

Data are HR (95% CI). All HRs are for the imatinib group versus placebo group. HRs and 95% CIs were calculated by use of Cox regression analysis. HR = hazard ratio. CI = confidence interval. BMI = body mass index.


A
Ang-2/Ang-1
Interaction term: -0.025
95% CI: -0.0486 - -0.0014
E-selectin
Interaction term: -0.0184
95% CI: -0.0322 - -0.0046
ICAM-1
Interaction term: -0.007
95% CI: -0.0206 - 0.0065
Syndecan-1
Interaction term: 0.0101
95% CI: -0.0021 - 0.0224
Syndecan-4
Interaction term: -0.0056
95% CI: -0.0242 - 0.013
Thrombomodulin
Interaction term: 0.0054
95% CI: -0.0101 - 0.0211
VCAM-1
Interaction term: -0.0222
95% CI: -0.0457 - 0.0013
B
IFNγ
Interaction term: -0.0139
95% CI: -0.0383 - 0.0103
IL-2
Interaction term: -0.0093
95% CI: -0.0363 - 0.0178
IL-6
Interaction term: -0.0441
95% CI: -0.0728 - -0.0154
IL-8
Interaction term: 0.0012
95% CI: -0.0243 - 0.0268
IL-10
Interaction term: -0.0021
95% CI: -0.047 - 0.0428
IL-17
Interaction term: -0.0229
95% CI: -0.0667 - 0.0211
TNFα
Interaction term: -0.0179
95% CI: -0.0296 - -0.0062
C
RAGE
Interaction term: -0.0117
95% CI: -0.0322 - 0.0087
Concentration
Day

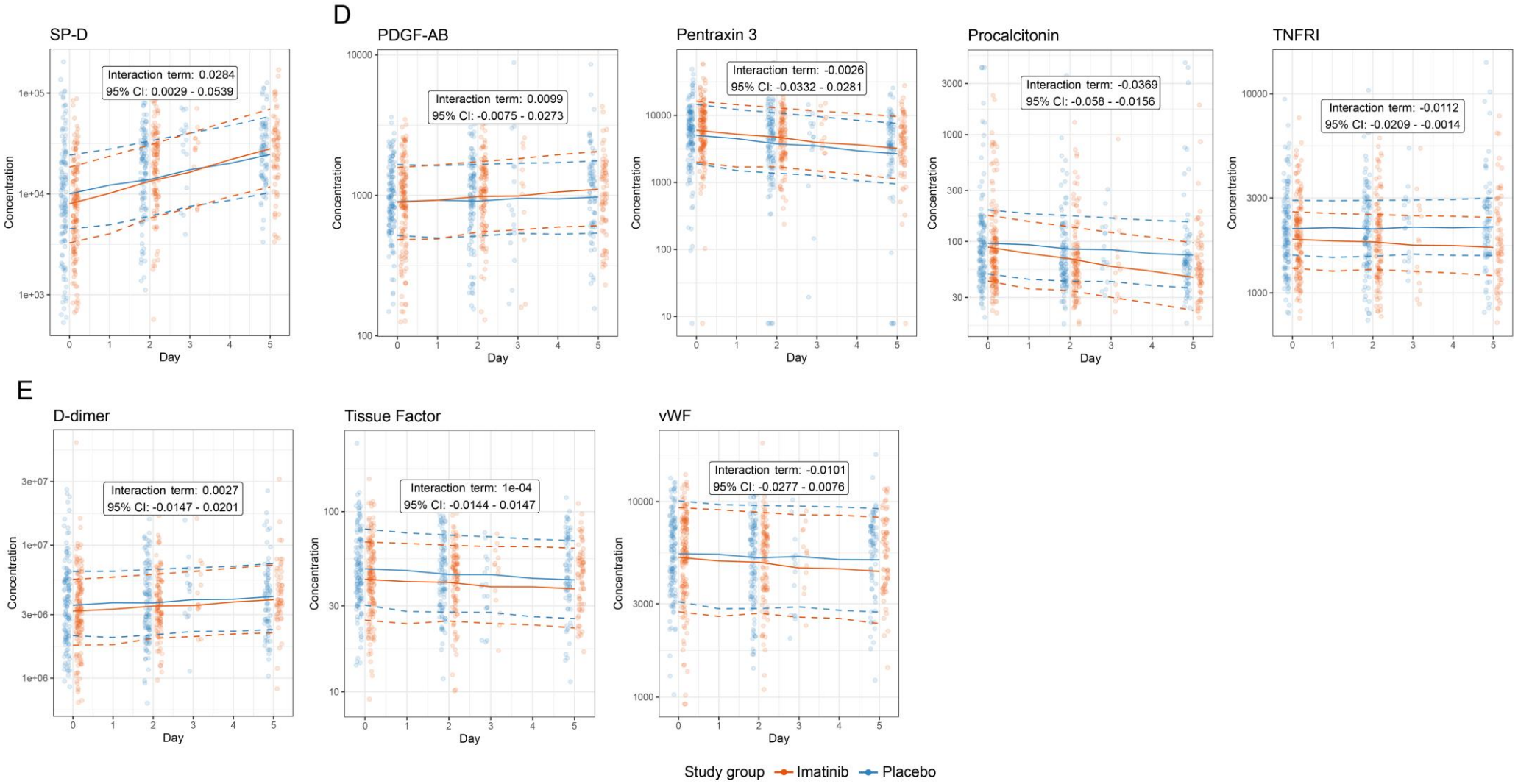


Figure S1. Longitudinal plasma concentrations of biomarkers reflective of host response pathways implicated in COVID-19 pathogenesis, stratified by treatment group. A = endothelial cell activation and function, B = cytokine release, C = epithelial cell activation and function, D = systemic inflammation, E = coagulation. Ang-2/Ang-1 = the ratio of angiopoietin 2 to 1, ICAM-1 = intracellular adhesion molecule 1, IFNγ = interferon gamma, IL = interleukin, PDGF-AB = platelet-derived growth factor AB, RAGE = receptor for advanced glycation end products, SP-D = surfactant protein D, TNFα = tumour necrosis factor alpha, TNFRI = tumour necrosis factor receptor I, VCAM-1 = vascular cell adhesion molecule 1, vWF = Von Willebrand factor.

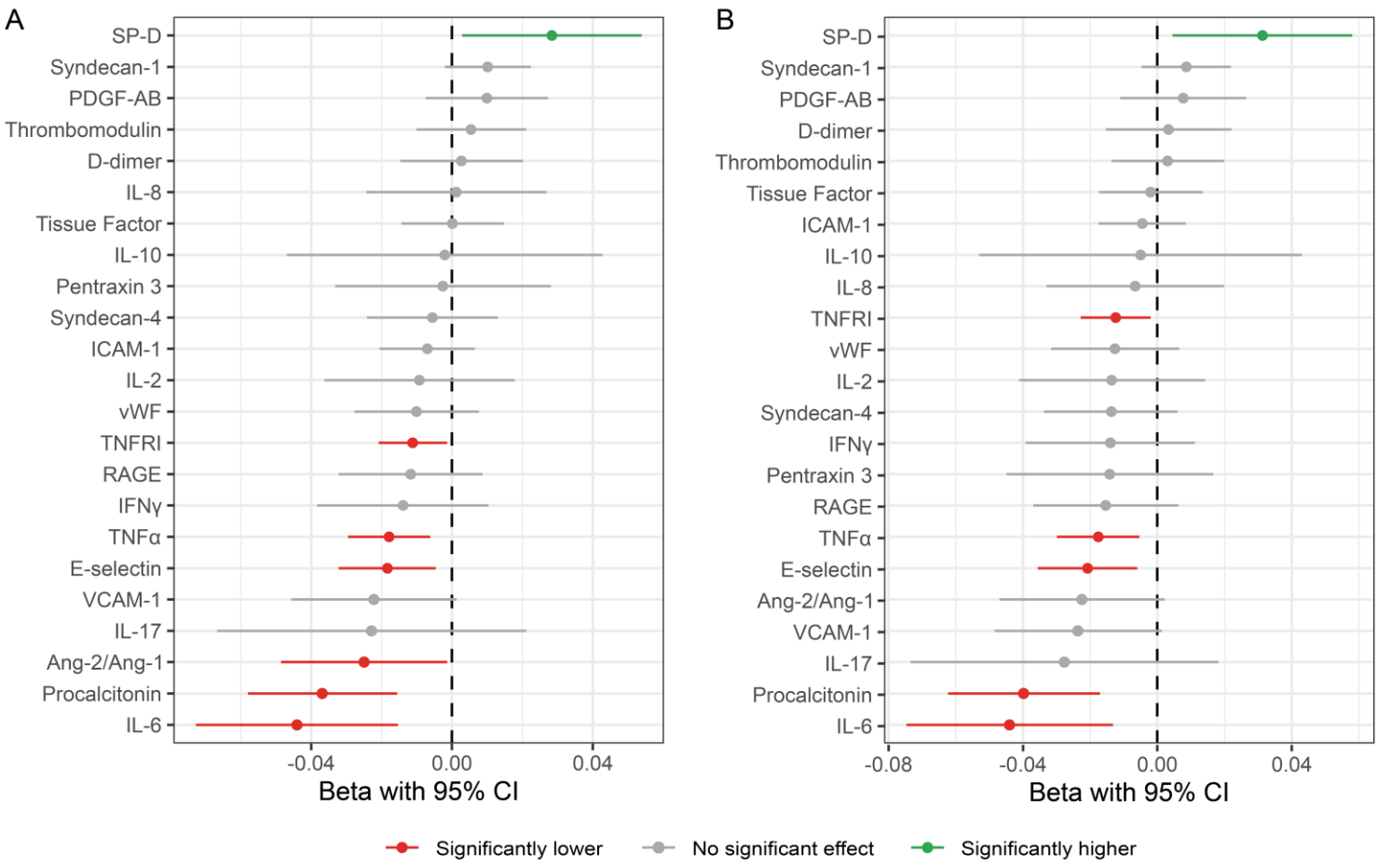


Figure S2A: The effect of imatinib on the biomarker concentration over time, when compared to placebo. **S2B**: The effect of imatinib on the biomarker concentration over time, corrected for body mass index, age, cardiovascular disease and diabetes. SP-D = surfactant protein D, PDGF-AB = platelet-derived growth factor AB, IL = interleukin, ICAM-1 = intracellular adhesion molecule 1, vWF = Von Willebrand factor, TNFRI = tumour necrosis factor receptor I, RAGE = receptor for advanced glycation end products, IFNγ = interferon gamma, TNFα = tumour necrosis factor alpha, VCAM-1 = vascular cell adhesion molecule 1, Ang-2/Ang-1 = the ratio of angiopoietin 2 to 1.

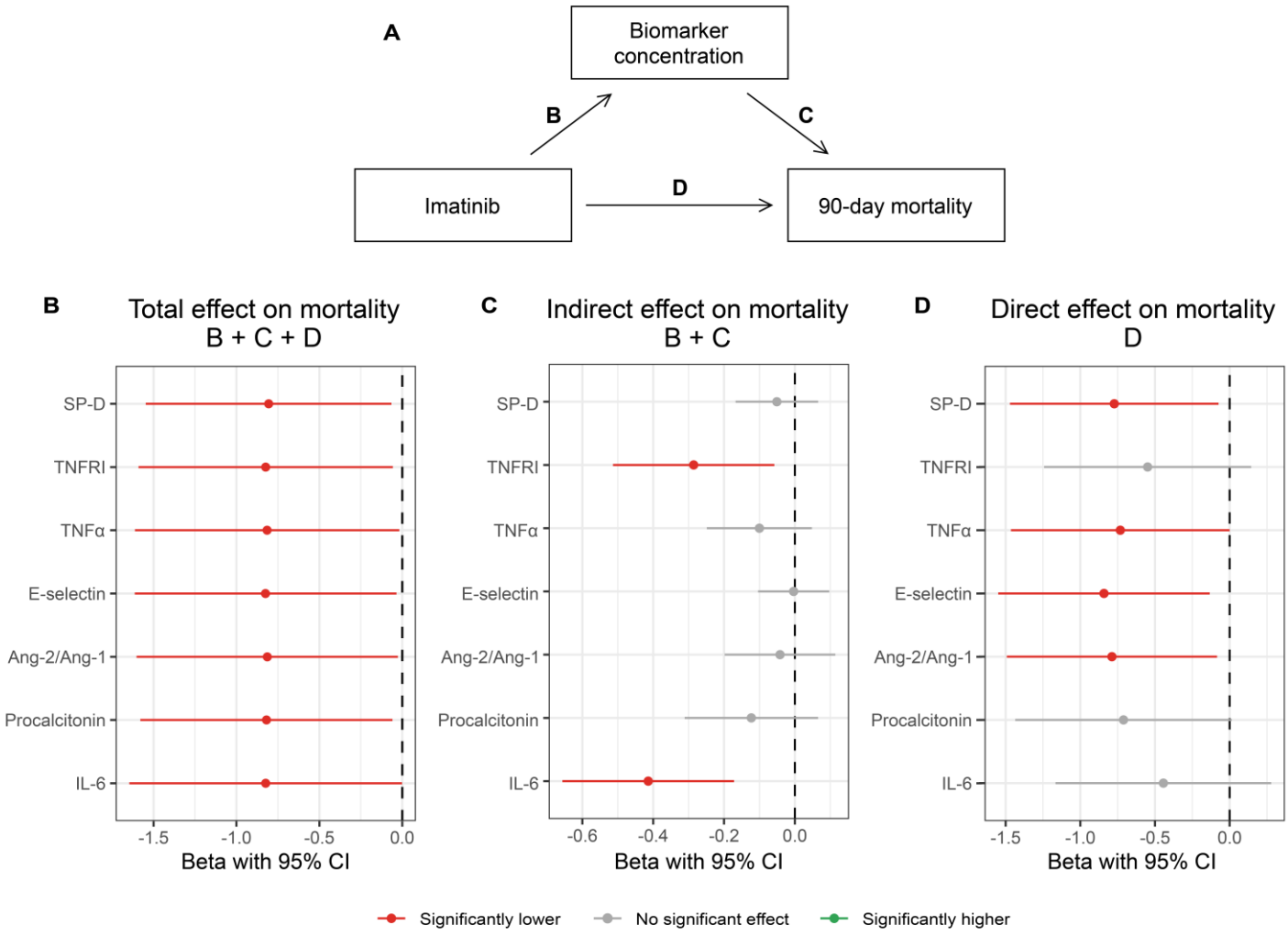


Figure S3A: Visualisation of mediation analysis with natural effects. **3B**: The total effect of imatinib on 90-day mortality, when compared to placebo, including its effects through the biomarker. **3C:** The indirect effect of imatinib on 90-day mortality, i.e. the effect of imatinib on 90-day mortality that is mediated by a change in biomarker concentration. **3D:** The effect of imatinib on 90-day mortality, when the effect of the biomarkers is left out. Abbreviations: SP-D = surfactant protein D, TNFRI = tumour necrosis factor receptor I, TNFα = tumour necrosis factor alpha, Ang-2/Ang-1 = the ratio of angiopoietin 2 to 1, IL-6 = interleukin-6.

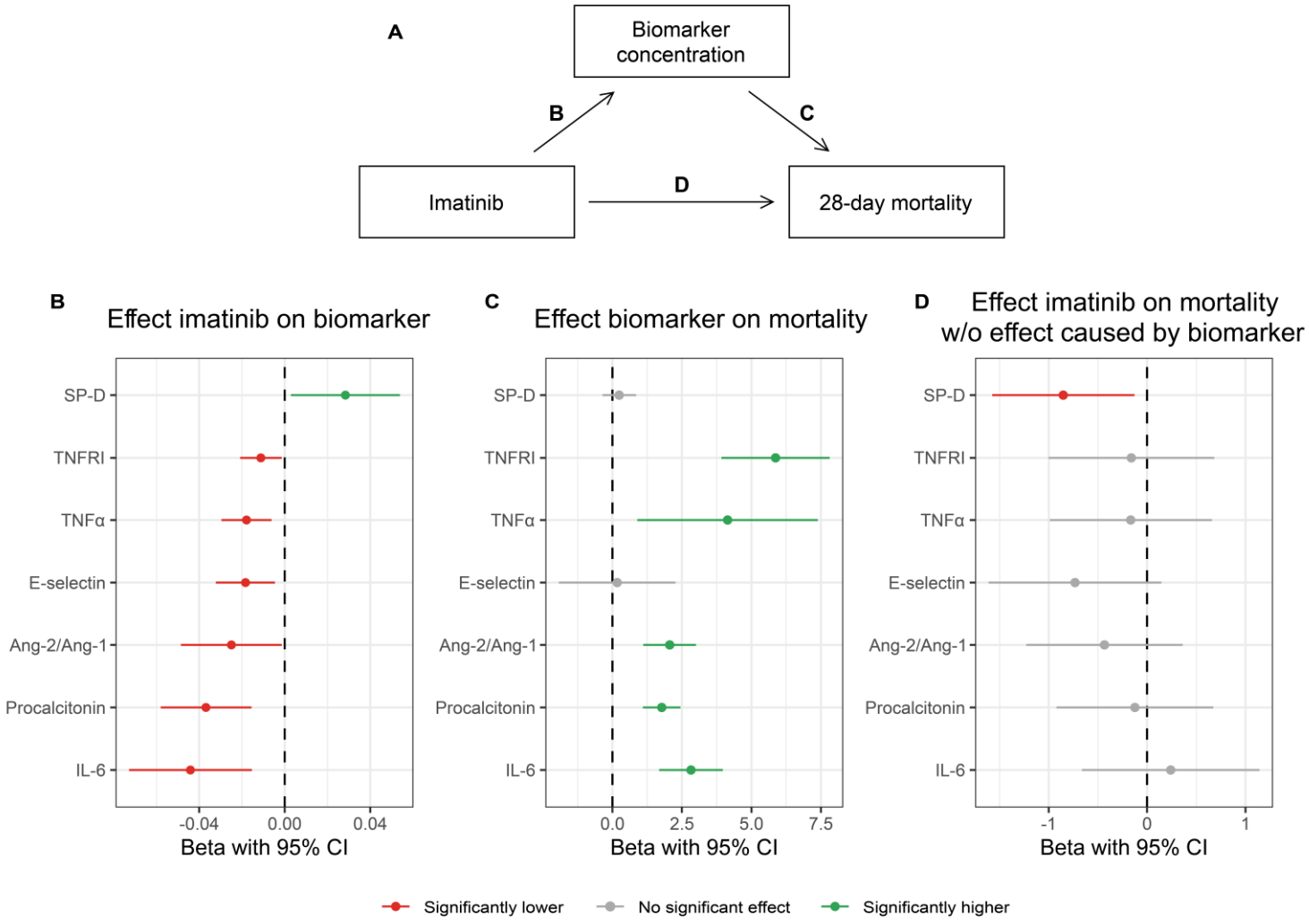


Figure S4A: Visualisation of mediation analysis. **4B:** The effect of imatinib on the biomarker concentration over time, when compared to placebo. **4C:** The effect of an increased biomarker concentration over time on 28-day mortality. **4D:** The effect of imatinib on 28-day mortality, when the effect of the biomarkers is left out. The effect of imatinib on 28-day mortality is completely mediated by changes in TNFRI, TNFα, E-selectin, Ang-2/Ang-1, procalcitonin and IL-6. Abbreviations: SP-D = surfactant protein D, TNFRI = tumour necrosis factor receptor I, TNFα = tumour necrosis factor alpha, Ang-2/Ang-1 = angiopoietin 2 to 1 ratio, IL-6 = interleukin-6.

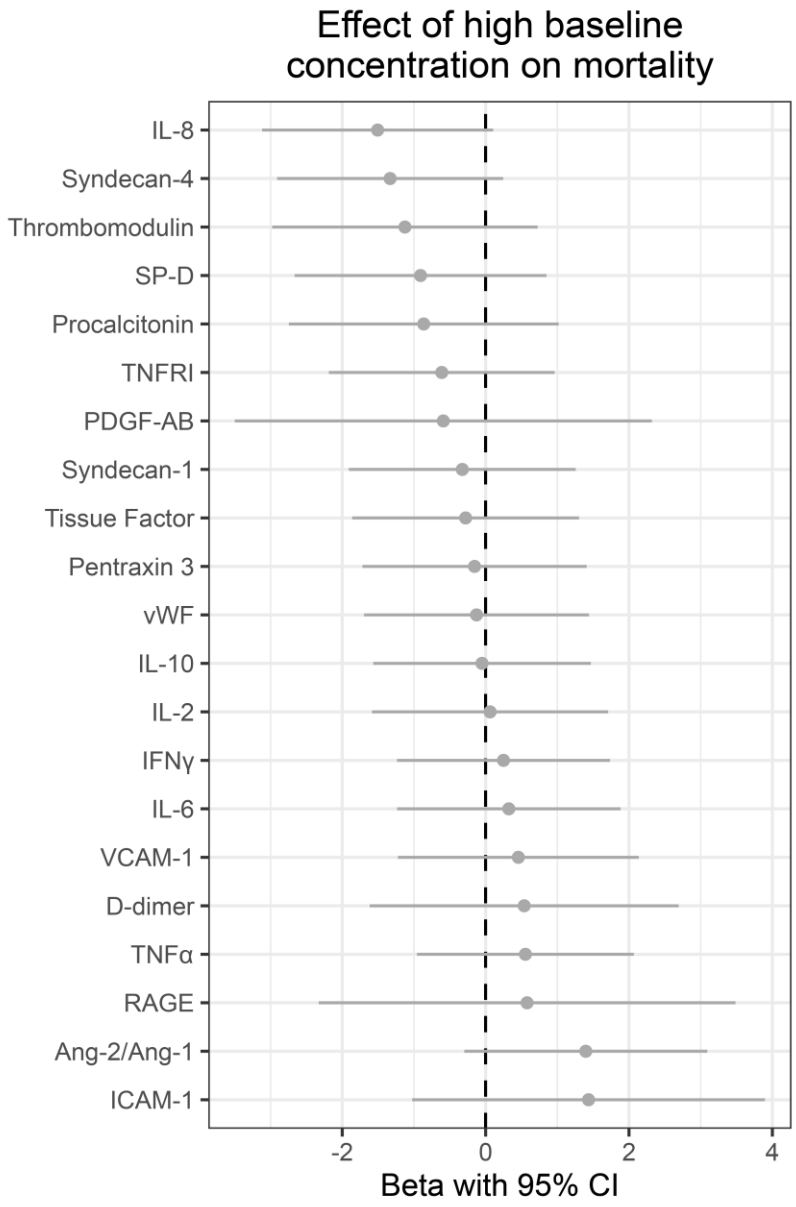


Figure S5. The effect of having a high baseline biomarker concentration on 90-day mortality in patients treated with imatinib, when compared to placebo. Dichotomisation between high and low biomarker concentrations was done by maximally selected rank statistics. Ang-2/Ang-1 = the ratio of angiopoietin 2 to 1, ICAM-1 = intracellular adhesion molecule 1, IFNγ = interferon gamma, IL = interleukin, PDGF-AB = platelet-derived growth factor AB, RAGE = receptor for advanced glycation end products, SP-D = surfactant protein D, TNFα = tumour necrosis factor alpha, TNFRI = tumour necrosis factor receptor I, VCAM-1 = vascular cell adhesion molecule 1, vWF = Von Willebrand factor.

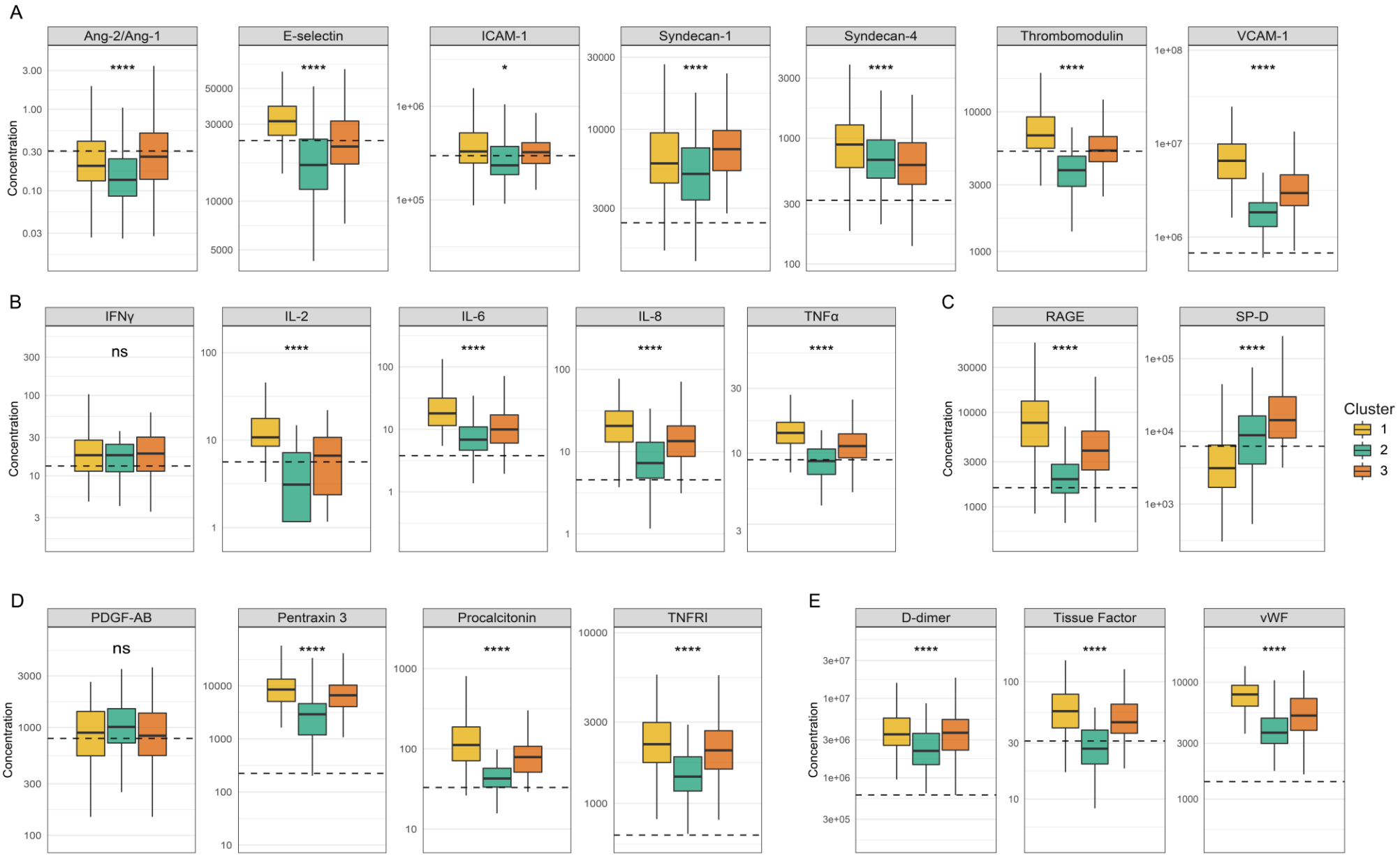
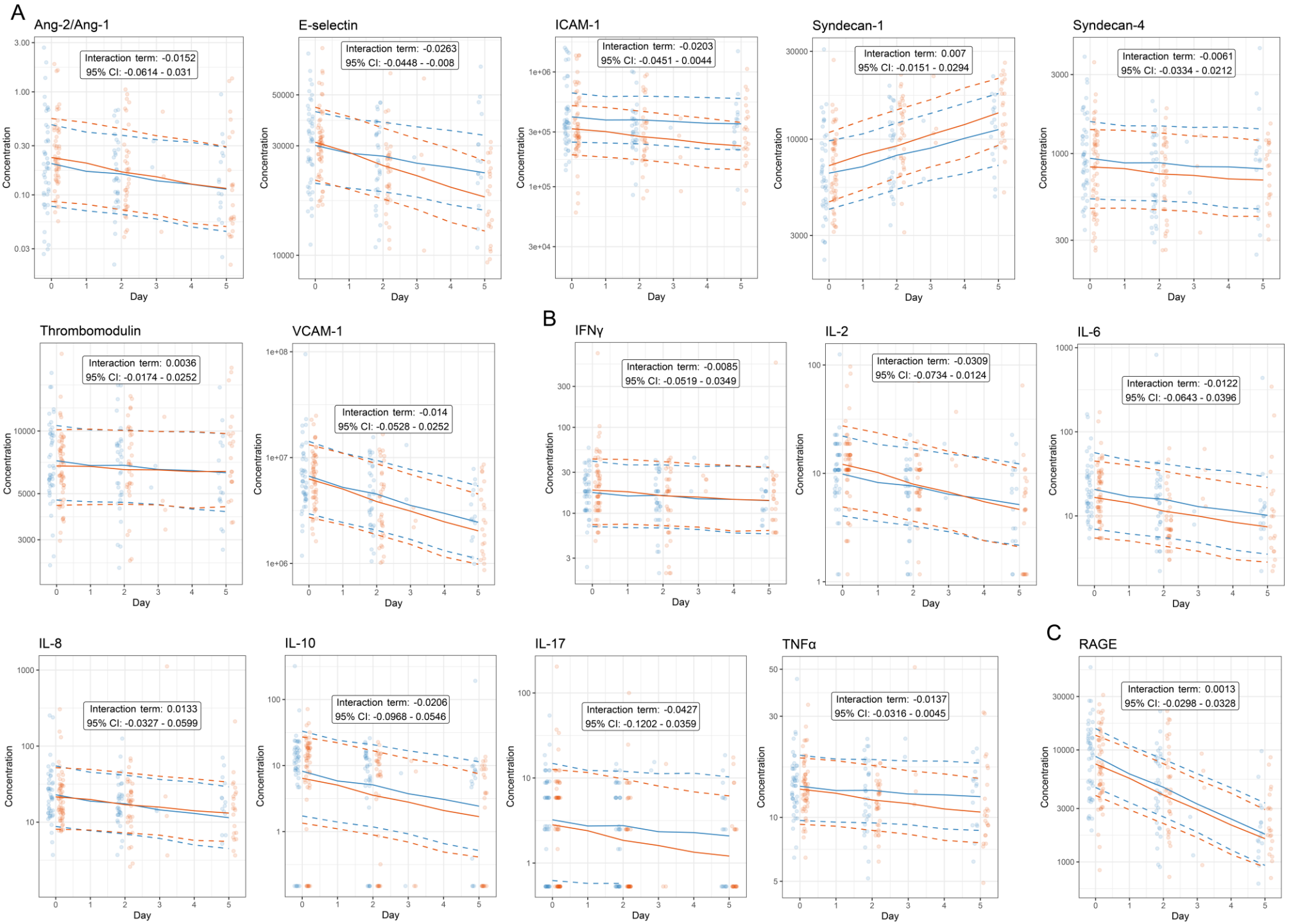


Figure S6. Baseline plasma biomarkers reflective of host response pathways implicated in COVID-19 pathogenesis, stratified according to subphenotype. Data is depicted as box and whisker plots. A = endothelial cell activation and function, B = cytokine release, C = epithelial cell activation and function, D = systemic inflammation, E = coagulation. Dotted lines indicate median values obtained in healthy controls. Asterisks indicates statistical significance by analysis of

variance (ANOVA) tests. **** $p < 0.0001$. *** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$. ns = not significant. Ang-2/Ang-1 = the ratio of angiopoietin 2 to 1, ICAM-1 = intracellular adhesion molecule 1, IFNγ = interferon gamma, IL = interleukin, PDGF-AB = platelet-derived growth factor AB, RAGE = receptor for advanced glycation end products, SP-D = surfactant protein D, TNFα = tumour necrosis factor alpha, TNFRI = tumour necrosis factor receptor I, VCAM-1 = vascular cell adhesion molecule 1, vWF = Von Willebrand factor.


A
Ang-2/Ang-1
Interaction term: -0.0152
95% CI: -0.0614 - 0.031
E-selectin
Interaction term: -0.0263
95% CI: -0.0448 - -0.008
ICAM-1
Interaction term: -0.0203
95% CI: -0.0451 - 0.0044
Syndecan-1
Interaction term: 0.007
95% CI: -0.0151 - 0.0294
Syndecan-4
Interaction term: -0.0061
95% CI: -0.0334 - 0.0212
Thrombomodulin
Interaction term: 0.0036
95% CI: -0.0174 - 0.0252
VCAM-1
Interaction term: -0.014
95% CI: -0.0528 - 0.0252
B
IFNγ
Interaction term: -0.0085
95% CI: -0.0519 - 0.0349
IL-2
Interaction term: -0.0309
95% CI: -0.0734 - 0.0124
IL-6
Interaction term: -0.0122
95% CI: -0.0643 - 0.0396
IL-8
Interaction term: 0.0133
95% CI: -0.0327 - 0.0599
IL-10
Interaction term: -0.0206
95% CI: -0.0968 - 0.0546
IL-17
Interaction term: -0.0427
95% CI: -0.1202 - 0.0359
TNFα
Interaction term: -0.0137
95% CI: -0.0316 - 0.0045
C
RAGE
Interaction term: 0.0013
95% CI: -0.0298 - 0.0328
Concentration
Day

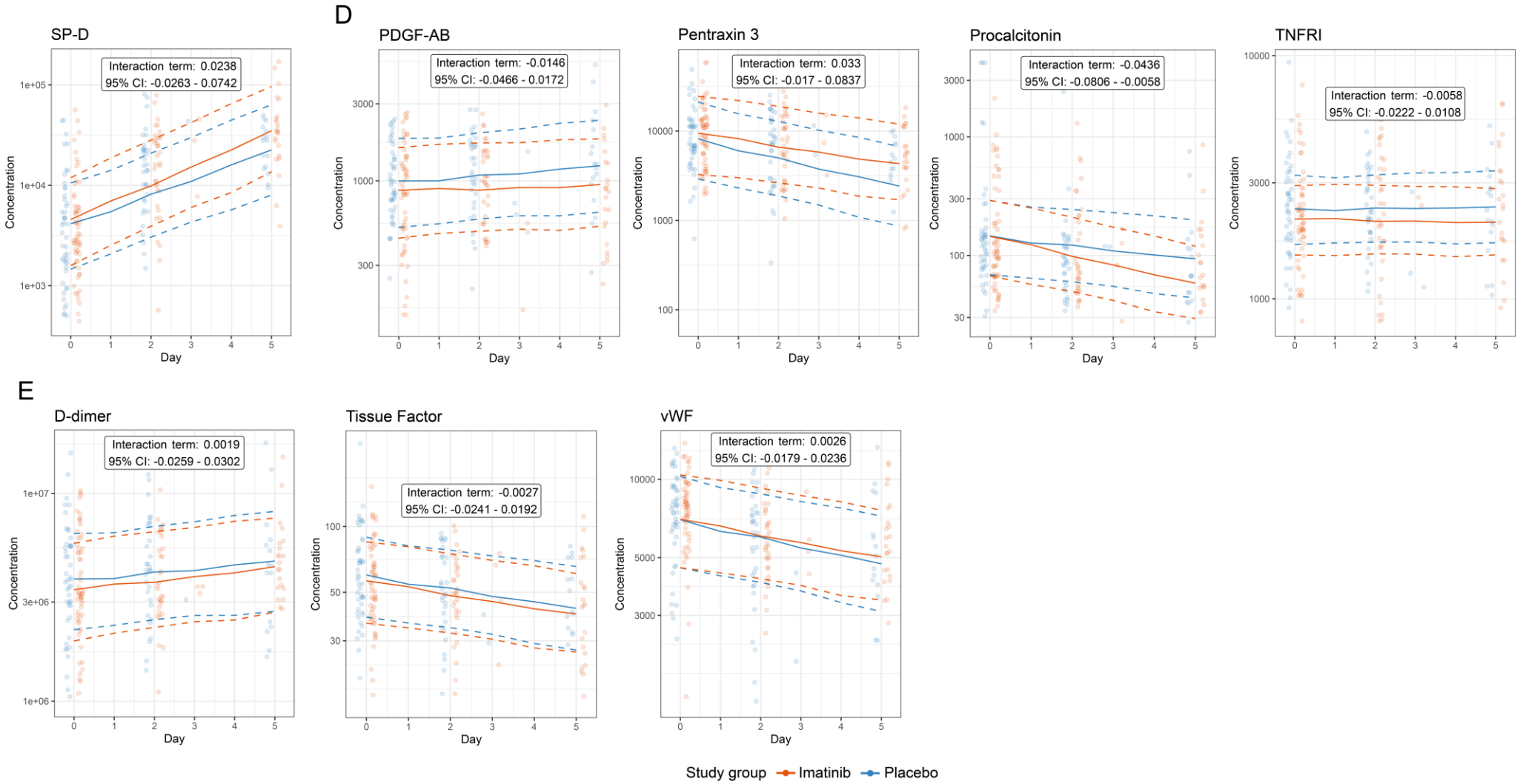
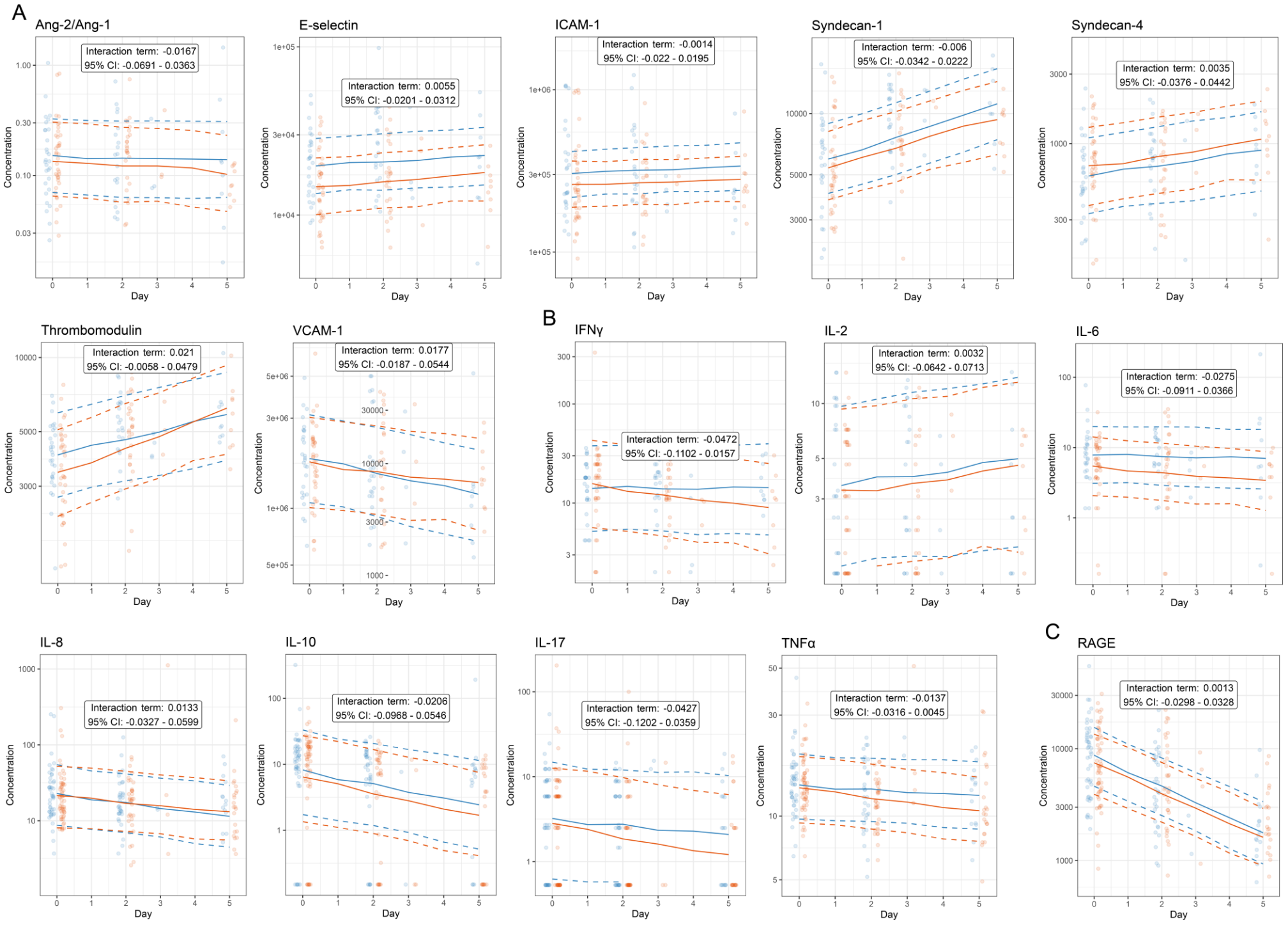


Figure S7. Longitudinal plasma biomarker concentrations in patients assigned to cluster 1, stratified by treatment group. A = endothelial cell activation and function, B = cytokine release, C = epithelial cell activation and function, D = systemic inflammation, E = coagulation. Ang-2/Ang-1 = the ratio of angiopoietin 2 to 1, ICAM-1 = intracellular adhesion molecule 1, IFNγ = interferon gamma, IL = interleukin, PDGF-AB = platelet-derived growth factor AB, RAGE = receptor for advanced glycation end products, SP-D = surfactant protein D, TNFα = tumour necrosis factor alpha, TNFRI = tumour necrosis factor receptor I, VCAM-1 = vascular cell adhesion molecule 1, vWF = Von Willebrand factor.


A
Ang-2/Ang-1
Interaction term: -0.0167
95% CI: -0.0691 - 0.0363
E-selectin
Interaction term: 0.0055
95% CI: -0.0201 - 0.0312
ICAM-1
Interaction term: -0.0014
95% CI: -0.022 - 0.0195
Syndecan-1
Interaction term: -0.006
95% CI: -0.0342 - 0.0222
Syndecan-4
Interaction term: 0.0035
95% CI: -0.0376 - 0.0442
Thrombomodulin
Interaction term: 0.021
95% CI: -0.0058 - 0.0479
VCAM-1
Interaction term: 0.0177
95% CI: -0.0187 - 0.0544
B
IFNγ
Interaction term: -0.0472
95% CI: -0.1102 - 0.0157
IL-2
Interaction term: 0.0032
95% CI: -0.0642 - 0.0713
IL-6
Interaction term: -0.0275
95% CI: -0.0911 - 0.0366
IL-8
Interaction term: 0.0133
95% CI: -0.0327 - 0.0599
IL-10
Interaction term: -0.0206
95% CI: -0.0968 - 0.0546
IL-17
Interaction term: -0.0427
95% CI: -0.1202 - 0.0359
TNFα
Interaction term: -0.0137
95% CI: -0.0316 - 0.0045
C
RAGE
Interaction term: 0.0013
95% CI: -0.0298 - 0.0328
Concentration
Day

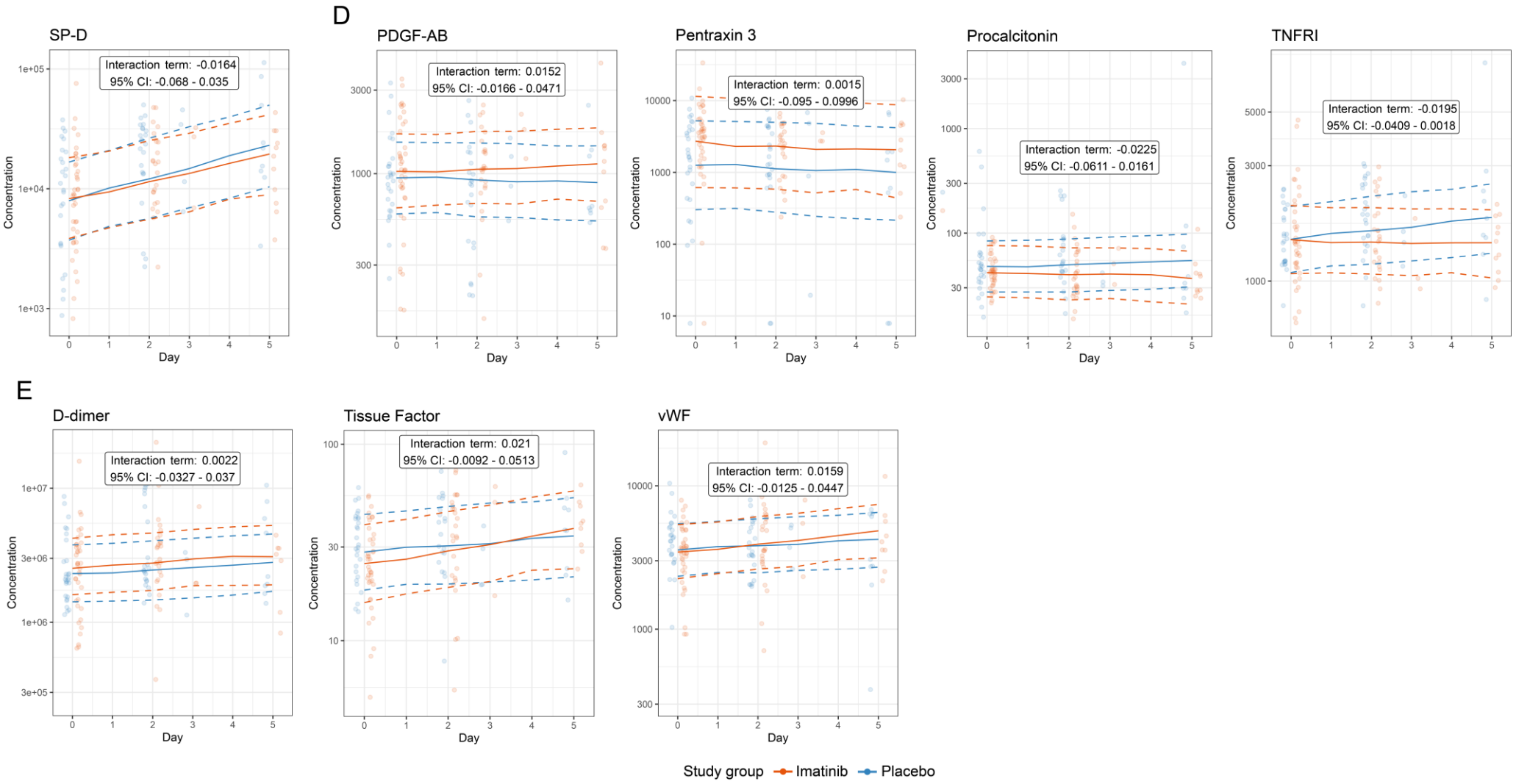
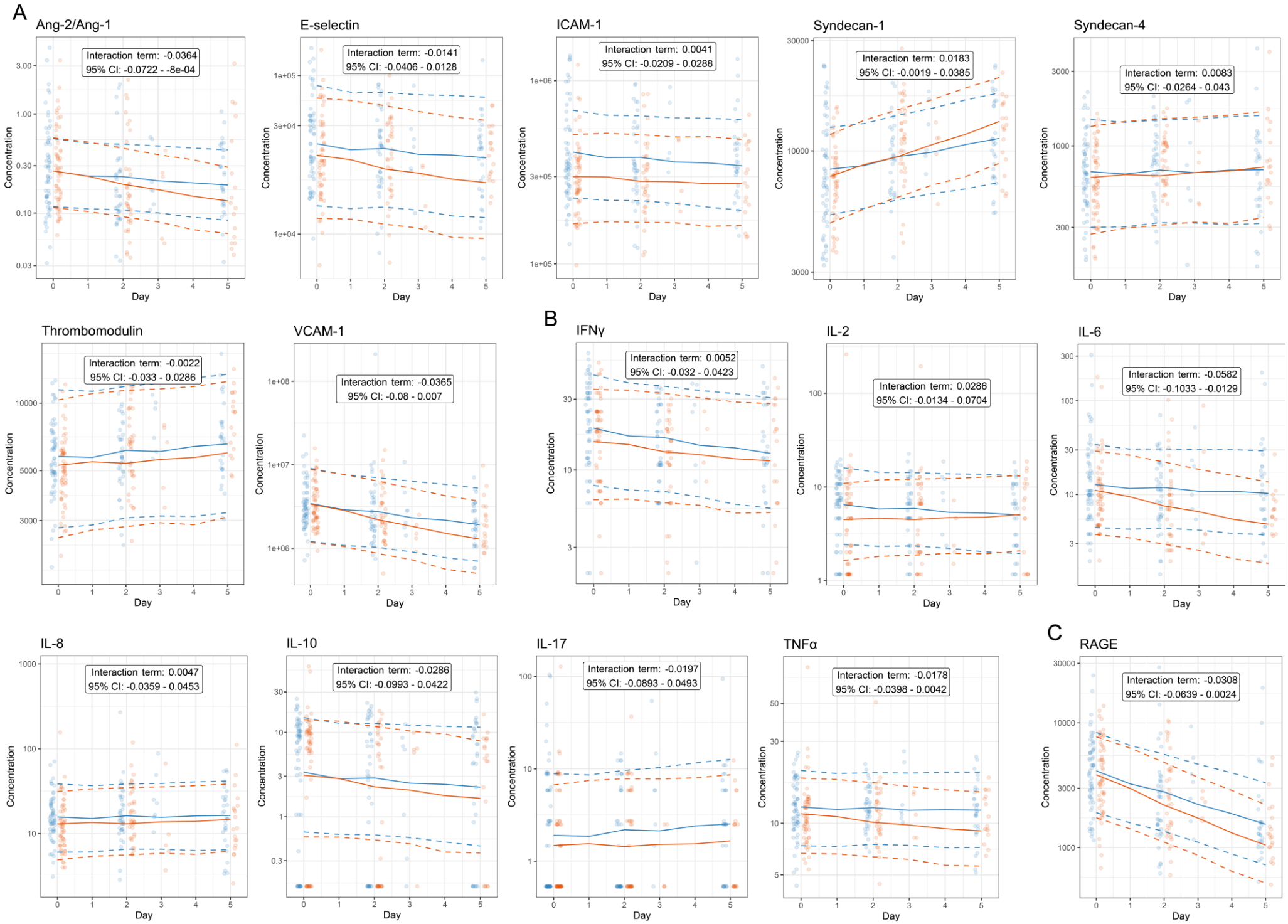


Figure S8. Longitudinal plasma biomarker concentrations in patients assigned to cluster 2, stratified by treatment group. A = endothelial cell activation and function, B = cytokine release, C = epithelial cell activation and function, D = systemic inflammation, E = coagulation. Ang-2/Ang-1 = the ratio of angiopoietin 2 to 1, ICAM-1 = intracellular adhesion molecule 1, IFNγ = interferon gamma, IL = interleukin, PDGF-AB = platelet-derived growth factor AB, RAGE = receptor for advanced glycation end products, SP-D = surfactant protein D, TNFα = tumour necrosis factor alpha, TNFRI = tumour necrosis factor receptor I, VCAM-1 = vascular cell adhesion molecule 1, vWF = Von Willebrand factor.


A
Ang-2/Ang-1
Interaction term: -0.0364
95% CI: -0.0722 - -8e-04
E-selectin
Interaction term: -0.0141
95% CI: -0.0406 - 0.0128
ICAM-1
Interaction term: 0.0041
95% CI: -0.0209 - 0.0288
Syndecan-1
Interaction term: 0.0183
95% CI: -0.0019 - 0.0385
Syndecan-4
Interaction term: 0.0083
95% CI: -0.0264 - 0.043
Thrombomodulin
Interaction term: -0.0022
95% CI: -0.033 - 0.0286
VCAM-1
Interaction term: -0.0365
95% CI: -0.08 - 0.007
B
IFNγ
Interaction term: 0.0052
95% CI: -0.032 - 0.0423
IL-2
Interaction term: 0.0286
95% CI: -0.0134 - 0.0704
IL-6
Interaction term: -0.0582
95% CI: -0.1033 - -0.0129
IL-8
Interaction term: 0.0047
95% CI: -0.0359 - 0.0453
IL-10
Interaction term: -0.0286
95% CI: -0.0993 - 0.0422
IL-17
Interaction term: -0.0197
95% CI: -0.0893 - 0.0493
TNFα
Interaction term: -0.0178
95% CI: -0.0398 - 0.0042
C
RAGE
Interaction term: -0.0308
95% CI: -0.0639 - 0.0024
Concentration
Day

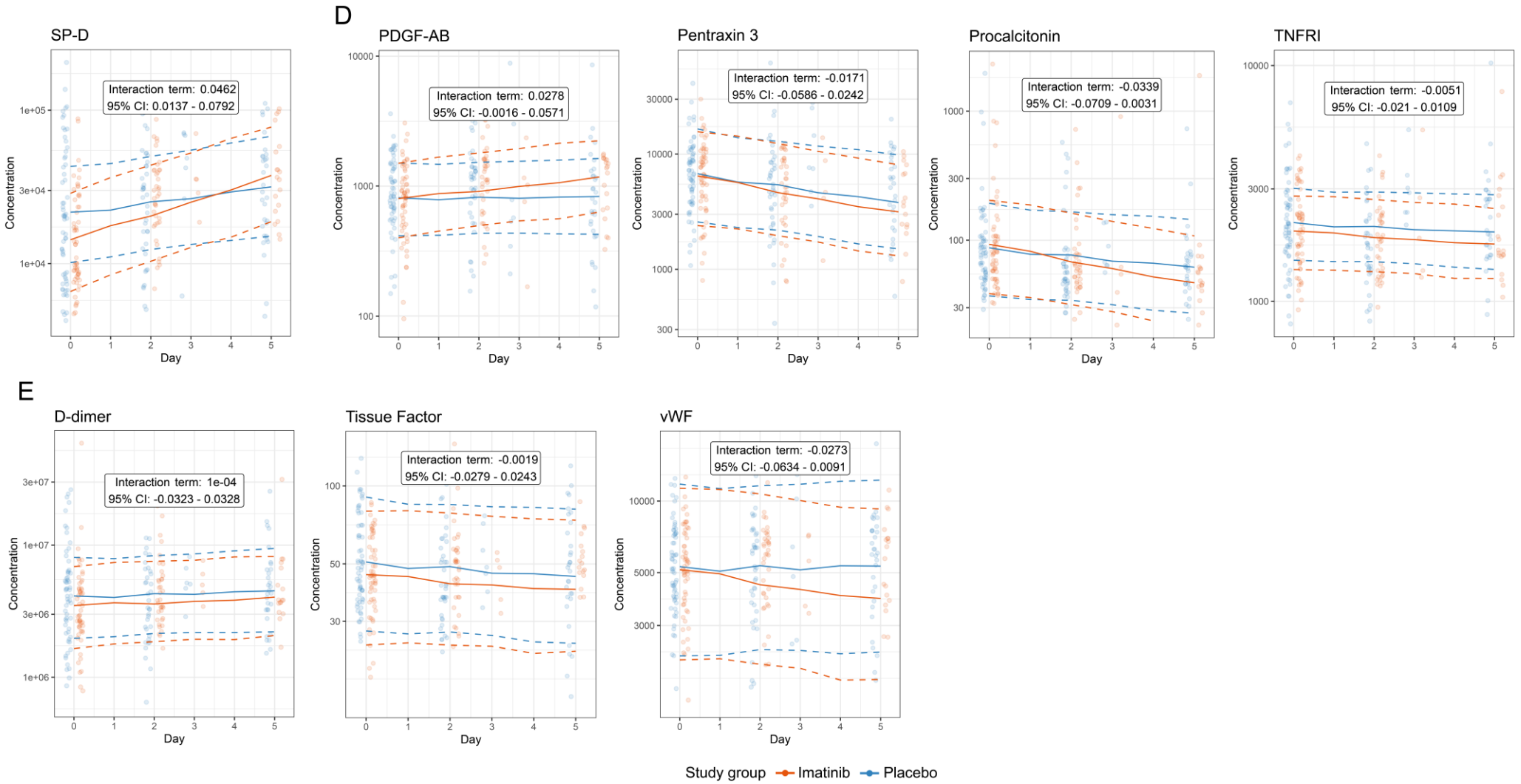


Figure S9. Longitudinal plasma biomarker concentrations in patients assigned to cluster 3, stratified by treatment group. A = endothelial cell activation and function, B = cytokine release, C = epithelial cell activation and function, D = systemic inflammation, E = coagulation. Ang-2/Ang-1 = the ratio of angiopoietin 2 to 1, ICAM-1 = intracellular adhesion molecule 1, IFNγ = interferon gamma, IL = interleukin, PDGF-AB = platelet-derived growth factor AB, RAGE = receptor for advanced glycation end products, SP-D = surfactant protein D, TNFα = tumour necrosis factor alpha, TNFRI = tumour necrosis factor receptor I, VCAM-1 = vascular cell adhesion molecule 1, vWF = Von Willebrand factor.

Supplementary methods

Luminex assay quality assessment

Data quality was assessed by evaluation of the beads count (the number of replicates counted per sample). A minimum bead count of 25 was considered to be acceptable. Values below the lowest point of the calibration point were set to the lower limit of quantification. Samples above the highest calibration point were extrapolated based on the algorithms available in the Luminex software. More than 50% of the fractalkine measurements were judged to be unreliable because of stringent quality criteria and were therefore excluded from analysis.

Model assumptions

- There is a linear change in biomarker concentration in the days after treatment with either placebo or imatinib.
- There is no unmeasured confounder between treatment allocation and mediator or outcome due to randomisation.
- There is no effect of the outcome or mediator on the treatment group due to randomisation.
- There is no measurement error in treatment allocation, the mediator and the outcome.
- There is no moderating effect of the mediators, which was tested.