SUPPLEMENTARY APPENDIX

Danicopan: an oral complement factor D inhibitor for paroxysmal nocturnal hemoglobinuria

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Presented as an oral presentation at the 24th European Hematology Association Congress, Amsterdam, NL, June 15, 2019. Presented as a poster presentation at the 61st annual meeting of the American Society of Hematology, Orlando, FL, USA December 8, 2019.

 doi:10.3324/haematol.2020.261826

Received: June 3, 2020.

Accepted: October 6, 2020.

Pre-published: October 29, 2020.

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Title: Danicopan: an oral complement factor D inhibitor for paroxysmal nocturnal hemoglobinuria

Short Title: Danicopan, oral factor D inhibitor for PNH

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Title character count: 88

Short title character count: 42

Abstract word count: 250

Text word count: 3861

Figures and tables: 2 tables and 4 figures

Supplementary figures and tables: 3 supplementary figures (separate file) and 6 supplementary tables (separate file)

References: 34

Presented as an oral presentation at the 24th European Hematology Association Congress, Amsterdam, NL, 15 June 2019. [31]

Presented as a poster presentation at the 61st annual meeting of the American Society of Hematology, Orlando, FL, 8 December 2019.[32]

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Risitano AM, et al. Danicopan: an oral complement factor D inhibitor for paroxysmal nocturnal hemoglobinuria.

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1. Assay Methods

The plasma concentrations of danicopan were determined by an LC/MS-MS method. The complement biomarkers were measured by the clinical laboratories. Serum complement C3 and C4 concentrations and serum classical pathway (CP) activity were measured with the pre-existing methods established in the clinical laboratories by using the Cobas (Roche) and Complement Activation EIA (Diasorin) kits. Serum alternative pathway (AP) activity was measured with AP Wieslab assay (Euro Diagnostica); Bb concentration was measured with MicroVue Complement Fragment Bb EIA (Quidel); serum FD concentration was measured with Quantikine® ELISA (R&D Systems, Inc.) by a single central laboratory after validating the assays according to the manufacturers' instructions. In normal individuals, the mean ± SD value of plasma Bb is 0.84±0.84 µg/mL, with a range of 0.553–1.357 µg/mL based on the data collected from three phase 1 studies in healthy volunteers (N=100; trial ID: ACTRN12617001521314, ACTRN12618001989235, ACTRN12618000896279) by the same single centralized lab with the same commercial kit.

C3 fragment deposition on erythrocytes was measured by a clinical laboratory with flow cytometry after validating the protocol described hereafter. After centrifugation of whole blood collected from patients, the pellet containing erythrocytes was transferred, washed (three times with phosphate buffer saline [PBS]), and resuspended in GVB^0 buffer (Complement Technology) at a density of erythrocytes about $1\text{-}2\times10^9$/mL. Prior to the test, an aliquot (5×10^6) of erythrocytes was transferred to a fresh tube, washed once with flow cytometry (FC) wash buffer (PBS + 15 mM EDA + 1% BSA), and incubated with 100 µl FC wash buffer containing FITC conjugated human C3d antibody (Assay Pro, Cat# 11294-05041), and PE conjugated human CD59 antibody (NOVUS, Cat # MEM-43) in the dark at room temperature for 1 hour. In the meantime, another aliquot of erythrocytes was processed in the same way but the antibodies in FC wash buffer were replaced with isotopic controls. At the end of incubation, erythrocytes were washed three times with FC wash buffer, resuspended in 200 µl FC buffer wash, and finally submitted to flow-cytometry analysis. Intact erythrocytes were gated based on physical parameters on forward and side scatter; gating for the fluorescence dye-conjugated antibodies was established with the isotypic controls. The following values were reported:

$$\frac{\text{erythrocytes stained negative for anti-CD59 antibody \& positive for anti-C3d antibody}}{\text{total erythrocytes}} \times 100\%$$

2. Dose Escalation Criteria

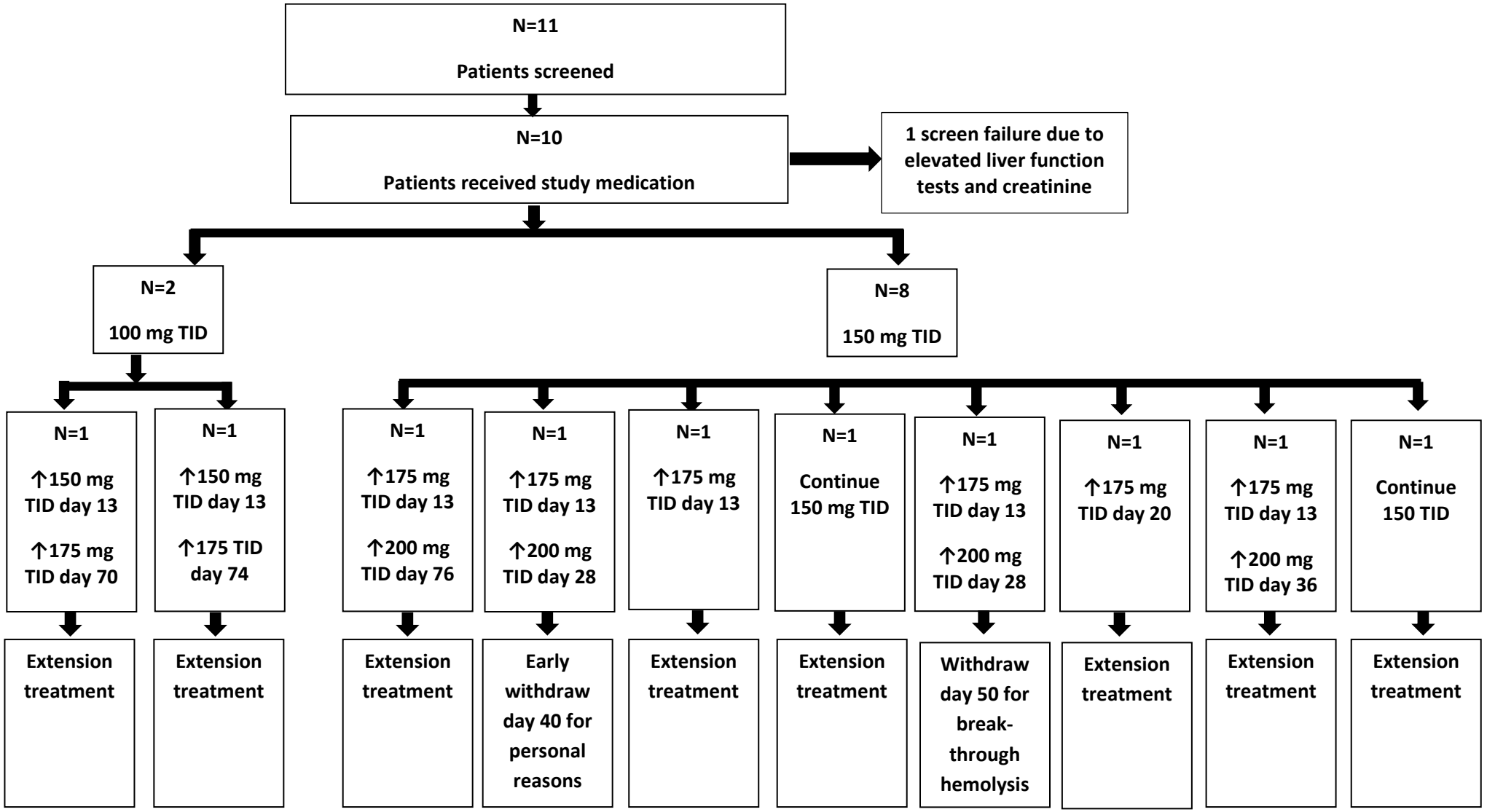
1st Dose Escalation Point (Day 7): On a patient-by-patient basis, if the starting dose of 150 mg TID is well tolerated and the available safety data are satisfactory, a patient may be escalated to 175 mg TID if his/her Day 6 LDH level, as measured locally, is still greater than 50% of his/her baseline value, unless the patient has achieved <1× upper limit of normal (ULN) for LDH. Depending on when these data are received and reviewed, if the patient is going to dose escalate, the site should contact the patient as soon as possible (Days 6-8) to provide new dosing instructions. If necessary, the patient may be asked to return to the clinic for new drug supplies.

2nd Dose Escalation Point (Day 14): On a patient-by-patient basis, if the patient was not dose escalated at Day 7, the 150 mg TID dose is well tolerated, and the available safety data are satisfactory, a patient may be escalated to 175 mg TID if his/her Day 13 LDH level, as measured locally, is still greater than 20% of his/her baseline value, unless the patient has achieved <1× ULN for LDH. Depending on when these data are received and reviewed, if the patient is going to dose escalate, the site should contact the patient as soon as possible (Days 13-15) to provide new dosing instructions. If necessary, the patient may be asked to return to the clinic for new drug supplies. In addition to the dose escalation evaluations defined above, the investigator, in consultation with the sponsor, may escalate dosing in increments of 25 mg to a maximum of 250 mg TID after evaluating the

clinical benefit and the available safety, PK, and PD data (including laboratory test results) in order to improve control of hemolysis.

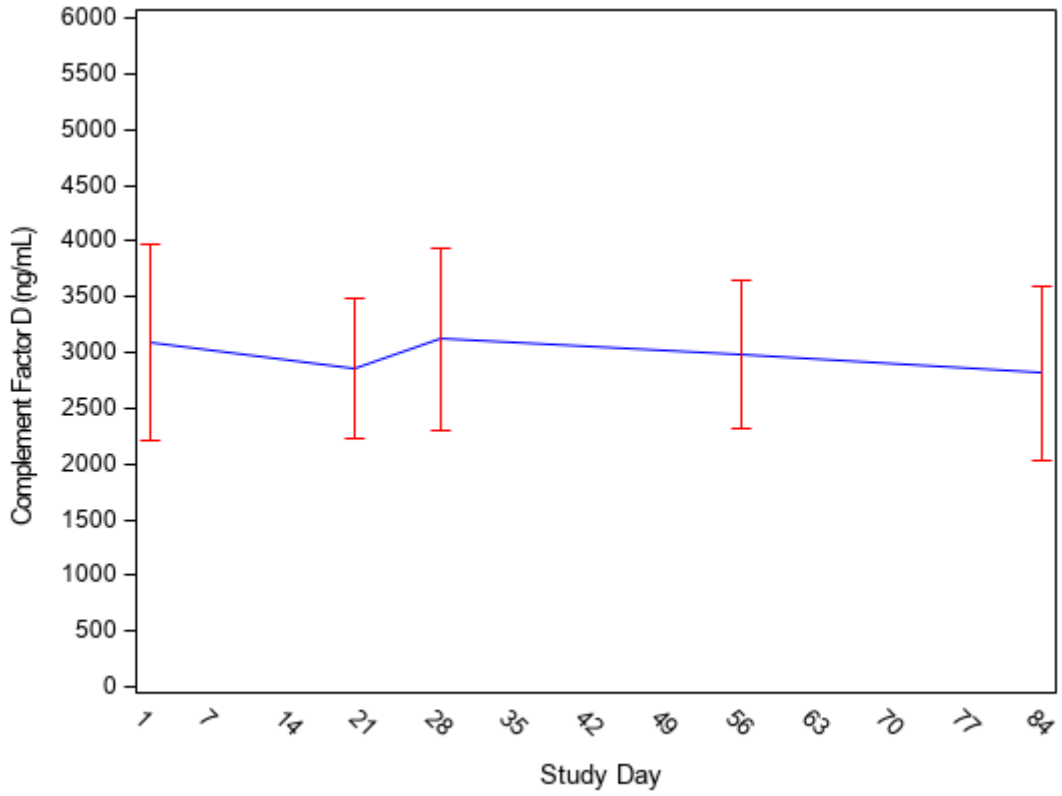
3. Supplementary Figures and Tables

Supplemental Figure 1: Patient disposition. TID=three times a day.



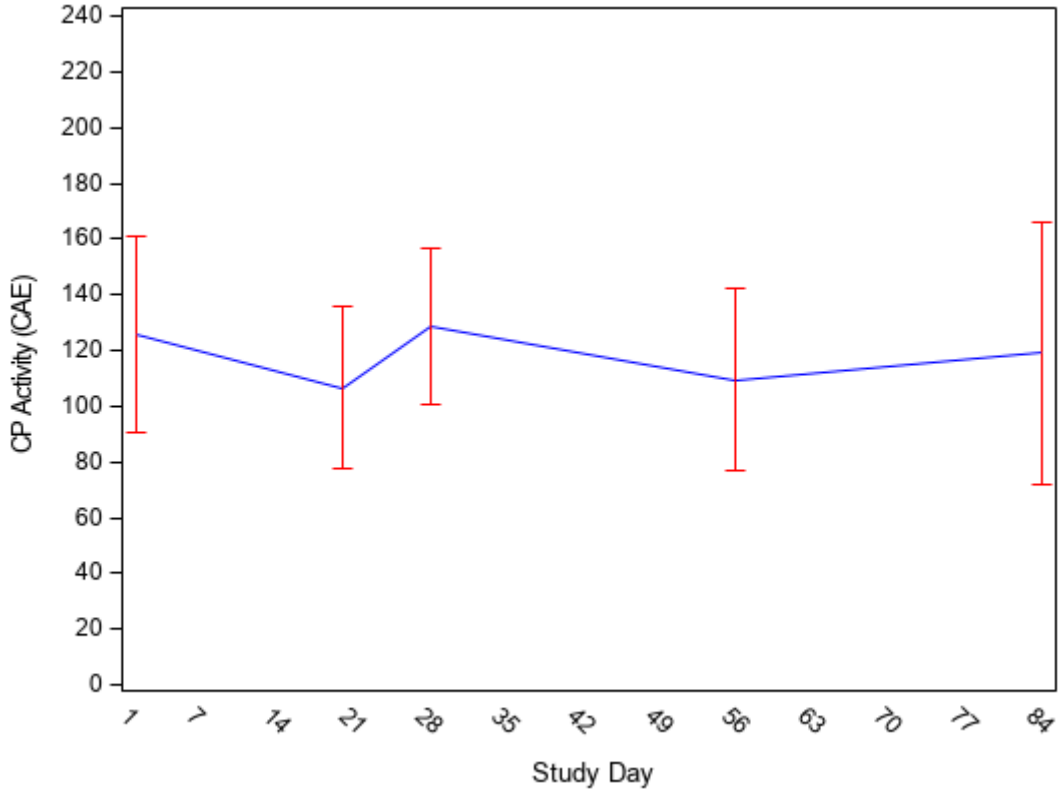
Supplemental Figure 2: Additional complement biomarker evaluation. The additional complement biomarkers as indicated in panels A, B, and C were monitored from baseline (day 1, predose) through the end of the study (day 84). The mean ± SD value at each time point is plotted over the study duration (baseline to day 84) along with more detailed statistical analyses at baseline and a few critical time points shown at the bottom of the chart. Panel D presents the results of quantitative relationship analysis between pharmacokinetic (PK; danicopan plasma concentration) and pharmacodynamic (PD; percent inhibition of AP activity relative to baseline in corresponding serum samples). In total, there are 211 paired data points with both the plasma danicopan concentration and the corresponding serum AP activity available from all patients enrolled in the study. Best fit values and 95% confidence intervals (CIs) are in ng/mL. Panel E displays the correlation between Bb concentration and LDH concentration. AP=alternative pathway. CAE=complement activity enzyme. CP=classical pathway. EC_{50}=half maximal effective concentration. LDH=lactate dehydrogenase. PD=pharmacodynamics. PK=pharmacokinetics. SD=standard deviation. *p<0.005.

A. Mean (± SD) of complement factor D



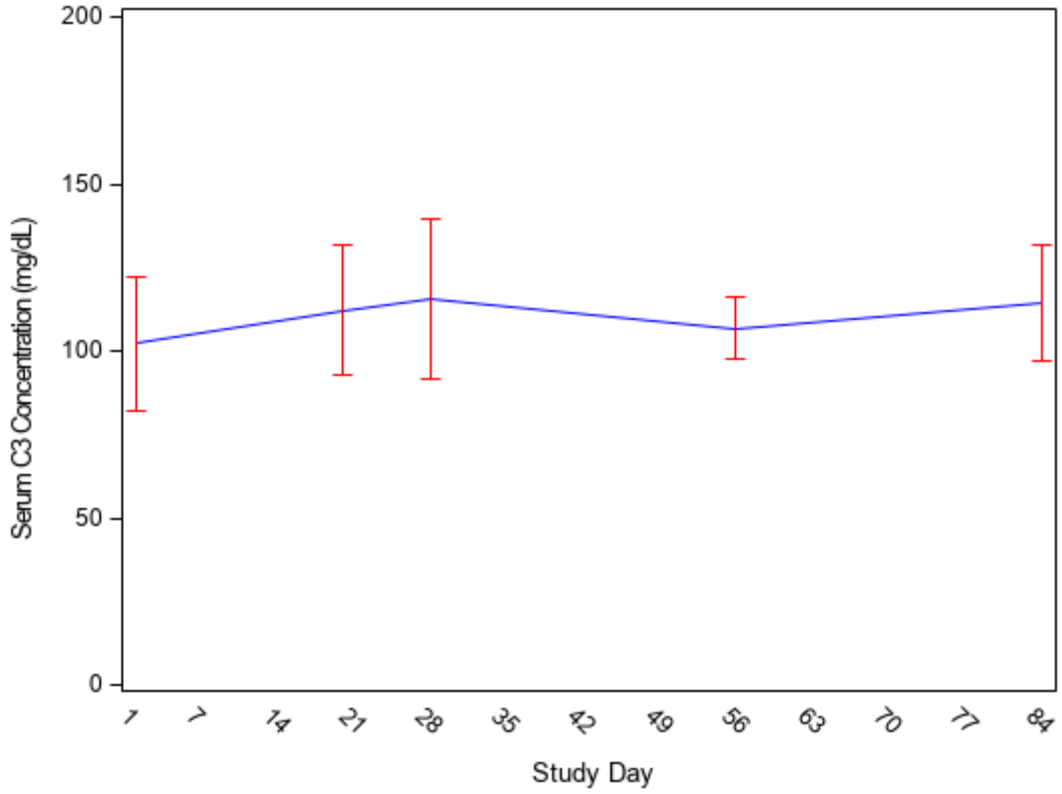
	Baseline (Day 1)	Day 28	Day 56	Day 84
N	10	10	5	8
Mean (SD)	3088 (883.5)	3122 (818.2)	2986 (668.3)	2816 (785.3)
Range	2019, 4976	1907, 4665	2125, 3620	1686, 3808

B. Mean (± SD) of CP activity



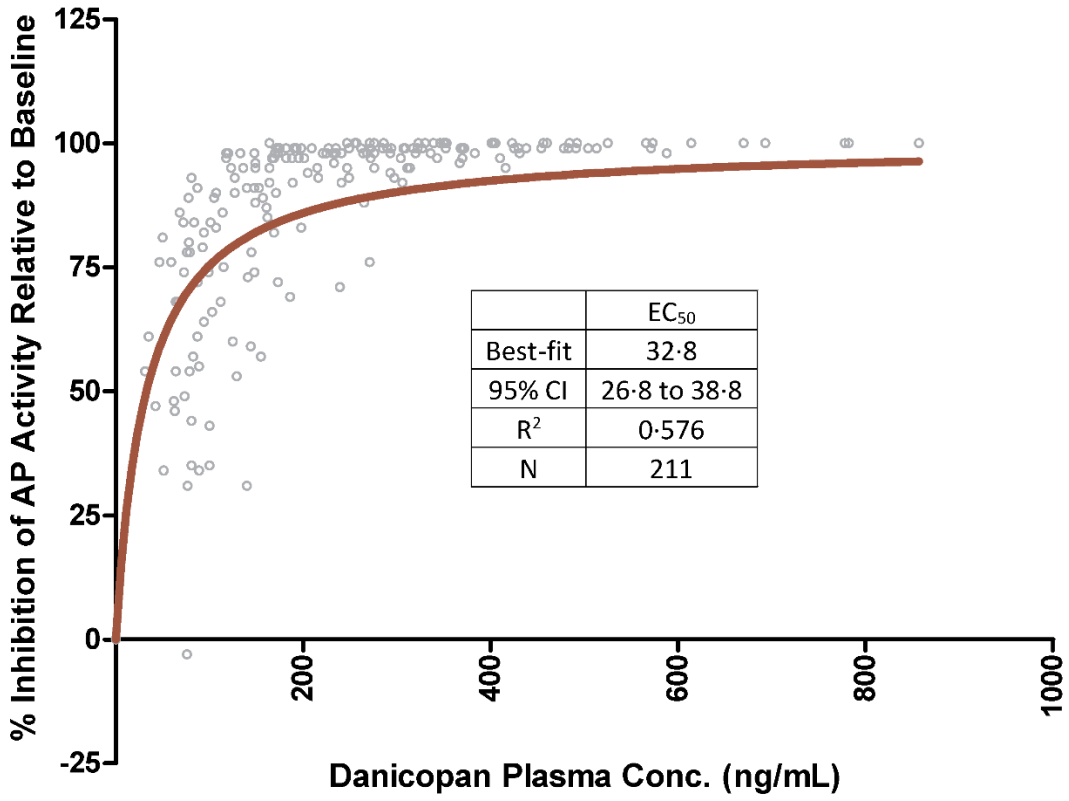
	Baseline (Day 1)	Day 28	Day 56	Day 84
N	10	8	5	6
Mean (SD)	126 (35.4)	129 (28.3)	110 (32.9)	119 (47.3)
Range	87, 195	89, 180	68, 155	66, 204

C. Mean ± SD of serum C3 concentration from days 1 to 84

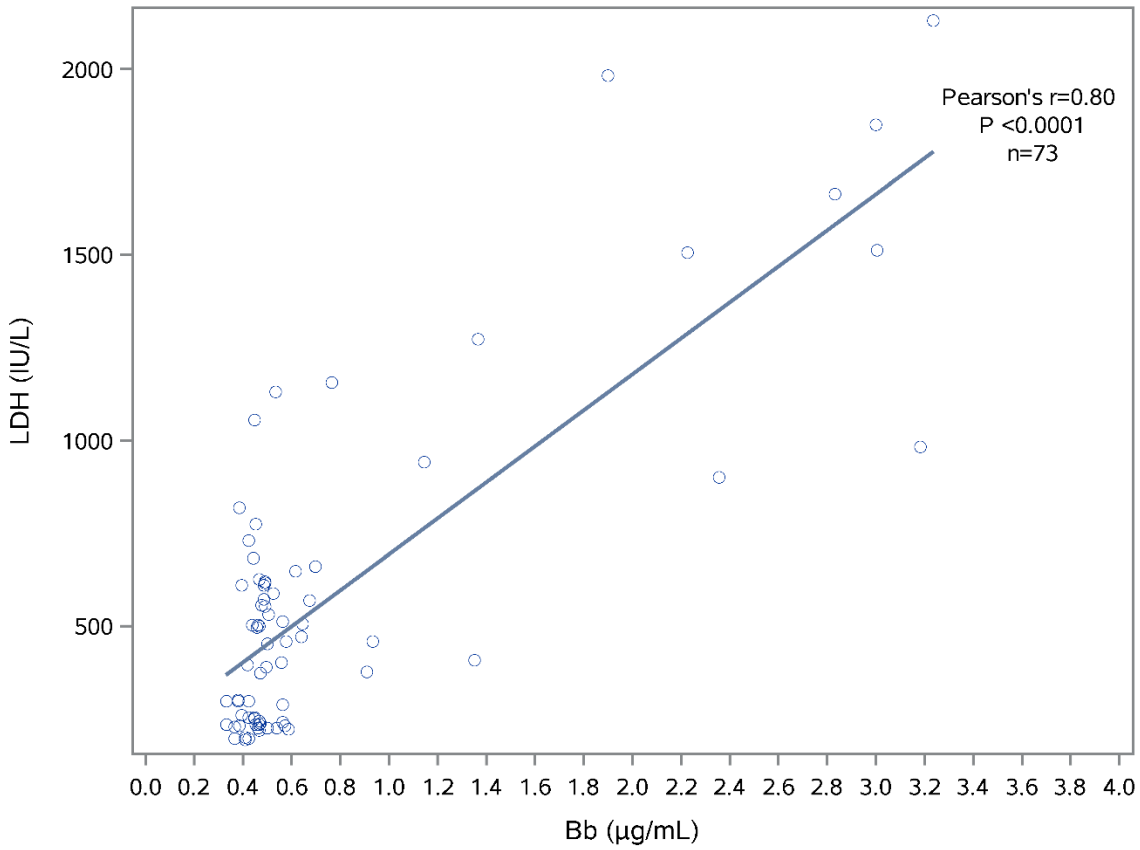


	Baseline (Day 1)	Day 28	Day 56	Day 84
N	10	10	5	6
Mean (SD)	102.2 (20.21)	115.5 (24.00) *	106.8 (9.15) *	114.2 (17.31)
Range	76.0, 144.0	83.0, 173.0	97.0, 120.0	99.0, 148.0

D. PK (danicopan plasma concentrations) and PD (AP activity inhibition) analysis

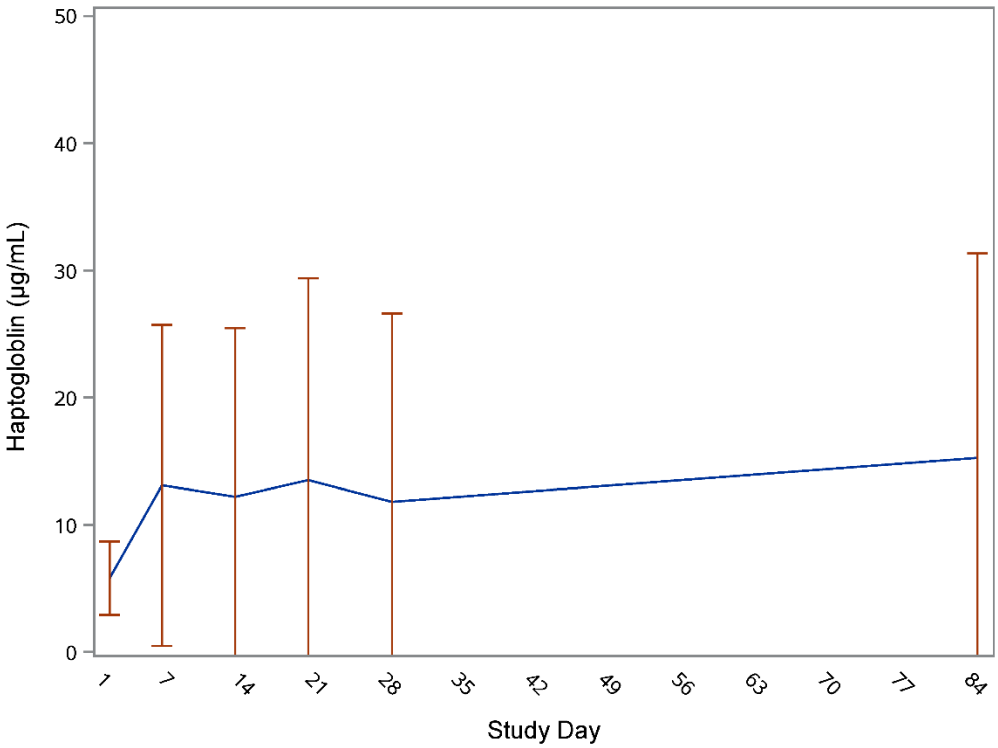


E. Correlation between plasma Bb concentration and LDH concentration



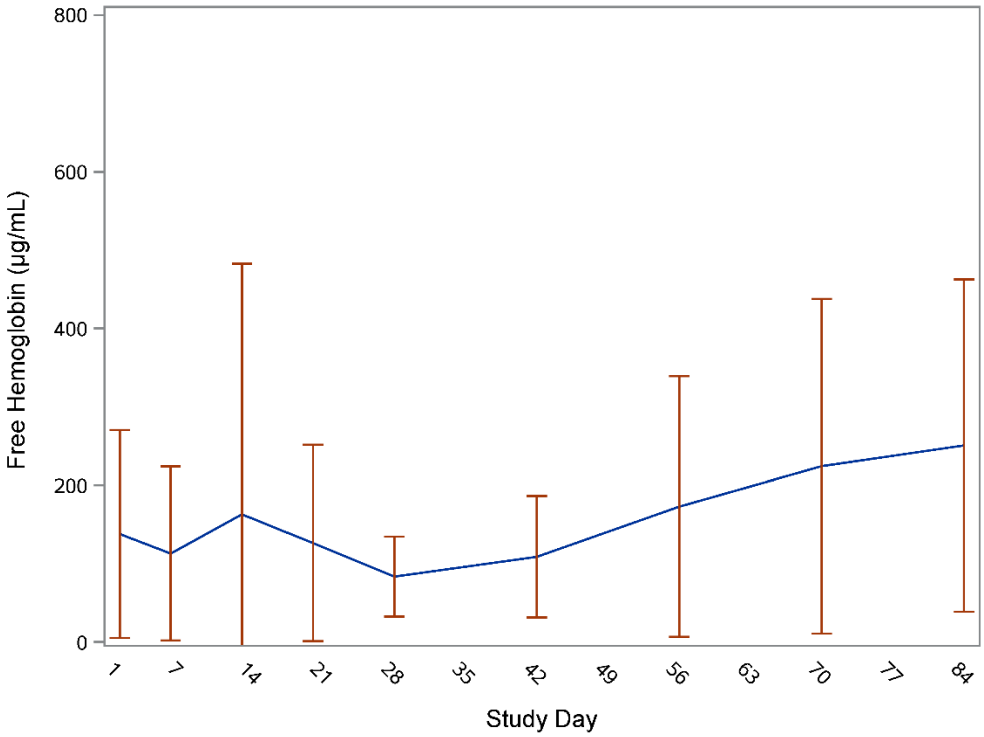
Supplemental Figure 3: Additional clinical endpoint evaluation. Additional clinical endpoints as indicated were monitored from baseline (day 1, predose) through the end of the study (day 84). The mean ± SD value at each time point is plotted over the study duration (baseline to day 84) along with more detailed statistical analyses at baseline and a few critical time points shown at the bottom of the chart. SD=standard deviation.

A. Mean (± SD) haptoglobin



	Baseline (Day 1)	Day 28	Day 84
N	10	10	8
Mean (SD)	5.8 (2.90)	11.8 (14.81)	15.3 (16.08)
Range	4.0, 10.0	4.0, 53.0	4.0, 42.0

B. Mean (± SD) free hemoglobin



	Baseline (Day 1)	Day 28	Day 42	Day 56	Day 84
N	10	10	9	7	8
Mean (SD)	138 (132.8)	83 (51.1)	109 (77.7)	173 (166.4)	250 (212.2)
Range	50, 500	40, 195	40, 270	30, 515	48, 670

Supplemental Table 1: Baseline Characteristics and Patient Disposition

Parameters (N=10)		Value
Sex, n (%)	Female	5 (50)
	Male	5 (50)
Ethnicity, n (%)	White	7 (70)
	Asian	1 (10)
	Indian	1 (10)
	Native Hawaiian or other	1 (10)
Age, years	Mean (SD)	35.9 (13.57)
	Median	32.5
	Range	16.9*–62.5
Body mass index (kg/m2)	Mean (SD)	25.1 (4.40)
	Median	25.3
	Range	18–34
Disease history	Aplastic anemia and PNH	2 (20)
	PNH	8 (80)
PNH duration, years	Mean (SD)	5.7 (4.41)
	Median	5.9
	Range	0–14
Transfusion history in the 3 years prior to the first dose	Number of patients	4
	Unit of RBC transfusions	..
	Mean (SD)	14.8 (13.65)
	Median	14.5
	Range	2–28
Subject disposition, n (%)	Completed 28-day treatment	10 (100)
	Completed 84-day treatment	8 (80)
	Reason for discontinuation	..
	Adverse event	1 (10)
	Withdrew consent	1 (10)

*Patient considered adult in country of enrollment.

Supplemental Table 2: Additional laboratory safety data

Test	Statistic	Baseline, Day 1	Day 28	Day 42	Day 56	Day 84
AST, U/L	**N**	10	10	9	7	8
	Mean (SD)	77 (31.1)	36 (24.6)	28 (13.3)	29 (13.0)	27 (11.5)
	Median	82	30	23	27	25
	Range	25–111	13–93	15–56	14–53	14–45
ALT, U/L	**N**	10	10	9	7	8
	Mean (SD)	27 (11.3)	26 (18.0)	27 (27.9)	20 (8.3)	15 (3.5)
	Median	27	20	19	21	14
	Range	12–43	10–58	9–98	9–32	11–22
Alk. phos., U/L	**N**	10	10	9	7	8
	Mean (SD)	68 (20.9)	72 (22.2)	76 (23.1)	74 (16.9)	84 (25.9)
	Median	64	70	66	74	82
	Range	42–113	42–119	45–125	53–102	45–112
GGT, U/L	**N**	10	10	9	7	8
	Mean (SD)	21 (22.8)	21 (22.4)	18 (14.1)	17 (10.4)	17 (10.2)
	Median	12	14	13	13	14
	Range	5–79	7–82	11–55	10–40	6–37
Creatinine, mg/dL	**N**	10	10	9	7	8
	Mean (SD)	0.76 (0.182)	0.77 (0.212)	0.75 (0.180)	0.84 (0.242)	0.78 (0.194)
	Median	0.69	0.71	0.73	0.79	0.68
	Range	0.50–1.02	0.47–1.07	0.49–0.99	0.50–1.18	0.61–1.14
eGFR, mL/min/1·73m^2	**N**	9	9	8	6	7
	Mean (SD)	102.0 (19.09)	100.2 (23.17)	100.8 (20.97)	89.0 (23.28)	100.2 (24.48)
	Median	101.0	97.0	94.9	80.6	97.0
	Range	76.0–131.9	72.0–135.0	78.0–129.9	69.0–130.0	67.0–146.0
INR, ratio	**N**	10	10	..	..	..
	Mean (SD)	1.34 (0.428)	1.45 (0.574)	..	..	..
	Median	1.14	1.12	..	..	..
	Range	1.00–2.19	1.00–2.40	..	..	..

Test	Statistic	Baseline, Day 1	Day 28	Day 42	Day 56	Day 84
APTT, seconds	**N**	10	10	··	··	··
	Mean (SD)	31.8 (6.71)	34.0 (8.68)	··	··	··
	Median	30.5	34.3	··	··	··
	Range	24.0–44.4	23.0–47.9	··	··	··
D-Dimer, ng/mL	**N**	10	10	··	··	6
	Mean (SD)	50.9 (41.05)	48.1 (47.68)	··	··	103.3 (194.49)
	Median	35.0	28.0	··	··	20.0
	Range	20.0–153.0	20.0–170.0	··	··	20.0–500.0
C-reactive protein, mg/dL	**N**	10	10	9	7	8
	Mean (SD)	2 (1.3)	2 (1.9)	1 (1.8)	1 (1.3)	3 (2.9)
	Median	1	1	1	1	1
	Range	0–4	0–6	0–6	0–4	0–9

Alk. phos. =alkaline phosphatase. ALT=alanine aminotransferase. APTT=activated partial thromboplastin time. AST=aspartate aminotransferase. eGFR=estimated glomerular filtration rate. GGT=gamma glutamyl transferase. INR=prothrombin international normalized ratio. SD=standard deviation.

Supplemental Table 3: Danicopan plasma pharmacokinetic parameters determined by intensive PK profiling

Dose*	Parameters	C_{max} (ng/mL)	T_{max} (hr)	AUC_{last} (hr•ng/mL)	C_8 (ng/mL)
100 mg TID	**Mean**	349	4.17	1476	47.7
(N=2, day 6)	**SD**	33.2	0.707	291	23.3
	CV%	9.5	17.0	19.7	48.9
	Median	349	4.17	1476	47.7
150 mg TID	**Mean**	465	3.30	2019	108.9
(N=8, day 6†;	**SD**	189	1.20	780	45.2
N=5, day 13;	**CV%**	40.6	36.7	38.6	41.6
N=4, day 20)	**Median**	438	3.67	1940	100.5
175 mg TID	**Mean**	543	3.60	2231	96.2
(N=5, day 13;	**SD**	200	1.30	861	31.3
N=6, day 20)	**CV%**	36.7	34.5	38.6	32.5
	Median	498	4.48	1894	86.9

*One of two patients who received 200 mg TID was not included on Day 56 because the sample was not available (missed study visit). PK sampling was performed as specified in the protocol on Day 6, 13, and 20; no patients were on 200 mg TID danicopan by day 20.

†One patient on day 7; N for C_8 is 7 as a patient left the site prior to hour 8.

AUC_{last}, area under the curve time 0 to last measurable time; C_8, trough concentration at hour 8; C_{max}, peak concentrations; CV%=percent coefficient of variation. PK=pharmacokinetics. SD=standard deviation. T_{max}=peak time. TID=three times a day.

Supplemental Table 4: Pre-dose AP activity and danicopan concentration from days 1 to 84

Time (Day)	PD: AP activity (%)												PK: danicopan concentration (ng/mL)											
	100 mg TID			150 mg TID			175 mg TID			200 mg TID			100 mg TID			150 mg TID			175 mg TID			200 mg TID		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Day 1	75.7	7.8	2	62.6	13.9	8	..	..	..	..	..	..	0.0	0.0	2	0.0	0.0	8	..	..	..	..	..	..
Day 6	14.3	18.2	2	11.6	9.3	8	..	..	..	..	..	..	91.6	79.8	2	169.3	92.0	8	..	..	..	..	..	..
Day 13	11.4	15.2	2	12.6	12.2	8	..	..	..	..	..	..	149.6	120.8	2	155.5	80.4	8	..	..	..	..	..	..
Day 20	..	..	..	16.9	18.1	5	9.4	10.6	5	..	..	..	..	..	..	159.7	79.6	5	193.8	71.9	5	..	..	..
Day 28	..	..	..	11.6	13.2	4	13.5	16.3	6	..	..	..	..	..	..	153.8	56.4	4	155.1	68.5	6	..	..	..
Day 42	..	..	..	27.7	32.1	2	4.8	1.50	2	59.0	30.6	2	..	..	..	194.5	77.1	2	279.7	157.2	3	82.9	8.3	2
Day 56	..	..	..	5.6	2.7	2	2.6	3.3	2	3.8	--	1	..	..	..	149.5	31.8	2	202.0	63.6	2	297.0	--	1
Day 70	..	..	..	11.6	9.50	3	8.3	11.3	3	..	..	..	..	..	..	203.3	65.2	3	299.0	223.1	3	..	..	..
Day 84	..	..	..	16.9	20.8	2	10.5	14.9	4	19.9	22.9	2	..	..	..	152.0	50.9	2	263.3	112.4	4	132.5	44.5	2

AP=alternative pathway. PD=pharmacodynamics. PK=pharmacokinetics. SD=standard deviation. TID=three times a day.

Supplemental Table 5: Correlation between PK, PD, and LDH

Pearson Correlation Coefficients, N=40 Prob > |r| under H0: Rho=0					
	Danicopan	**CP**	**AP**	**Bb**	**LDH**
Danicopan, ng/mL	1.00000	-0.11470 0.4810	-0.83666 <0.0001	-0.62271 <0.0001	-0.65284 <0.0001
CP activity, CAE	-0.11470 0.4810	1.00000	0.09553 0.5576	0.15734 0.3322	0.29287 0.0667
AP activity, %	-0.83666 <0.0001	0.09553 0.5576	1.00000	0.59026 <0.0001	0.67100 <0.0001
Bb, µg/mL	-0.62271 <0.0001	0.15734 0.3322	0.59026 <0.0001	1.00000	0.82860 <0.0001
LDH, IU/L	-0.65284 <0.0001	0.29287 0.0667	0.67100 <0.0001	0.82860 <0.0001	1.00000

AP=alternative pathway. CP=classical pathway. LDH=lactate dehydrogenase. PD=pharmacodynamics. PK=pharmacokinetics.

Supplemental Table 6: Summary of laboratory tests

Laboratory Tests	Statistics	Baseline (Day 1)	Day 28	Day 42	Day 56	Day 84
LDH, U/L	**N**	10	10	9	7	8
	Mean (SD)	1416 (540.3)	444 (255.8) **	435 (222.9) **	574 (351.9) **	537 (260.4) **
	Median	1509	417	389	610	607
	Range	407–2130	193–983	198–815	228–1131	226–818
LDH, x ULN	**N**	10	10	9	7	8
	Mean (SD)	5.7 (2.17)	1.8 (1.03) **	1.7 (0.89) **	2.3 (1.41) **	2.2 (1.04) **
	Median	6	1.7	1.6	2.4	2.4
	Range	1.6–8.6	0.8–3.9	0.8–3.3	0.9–4.5	0.9–3.3
AP functional activity, %	**N**	10	10	6	5	8
	Mean (SD)	65.2 (13.71)	12.7 (14.37) **	30.5 (31.39)	4.1 (2.59) **	14.4 (15.82) **
	Median	71.4	6.0	21.6	3.9	4.0
	Range	44.0–81.2	1.4–36.6	3.7–80.6	0.3–7.5	2.2–36.1
CP functional activity, CAE	**N**	10	8	··	5	6
	Mean (SD)	126 (35.4)	129 (28.3)	··	110 (32.9)	119 (47.3)
	Median	111	123	··	101	111
	Range	87–195	89–180	··	68–155	66–204
C3, mg/dL	**N**	10	10	··	5	6
	Mean (SD)	102.2 (20.21)	115.5 (24.00) **	··	106.8 (9.15) **	114.2 (17.31)
	Median	99.5	108.0	··	103.0	109.5
	Range	76.0–144.0	83.0–173.0	··	97.0–120.0	99.0–148.0
Bb, µg /mL	**N**	10	10	7	5	6
	Mean (SD)	2.24 (0.774)	0.84 (0.838) **	0.49 (0.205) **	0.47 (0.086) **	0.47 (0.059) **
	Median	2.29	0.58	0.45	0.49	0.47
	Range	1.15–3.24	0.41–3.18	0.33–0.94	0.37–0.56	0.38–0.54
Factor D, ng/mL	**N**	10	10	··	5	8
	Mean (SD)	3088 (883.5)	3122 (818.2)	··	2986 (668.3)	2816 (785.3)
	Median	2953	3144	··	3243	2887
	Range	2019–4976	1907–4665	··	2125–3620	1686–3808
Hemoglobin, g/dL	**N**	10	10	9	7	8
	Mean (SD)	9.8 (1.76)	10.9 (1.65) **	11.0 (1.72) **	10.9 (1.71) **	11.5 (1.41) **
	Median	9.8	10.7	11.0	11.1	11.5
	Range	6.9–12.0	8.4–14.1	8.2–13.3	8.5–13.1	8.7–13.7
Reticulocytes, 10^9/L	**N**	10	10	9	7	8
	Mean (SD)	154 (69.0)	70 (24.9) **	80 (32.5) *	101 (61.7)	81 (33.6) *
	Median	160	73	73	85	77
	Range	45–249	38–109	47–141	47–225	45–130
Haptoglobin, mg/dL	**N**	10	10	··	··	8
	Mean (SD)	5.8 (2.90)	11.8 (14.81)	··	··	15.3 (16.08)
	Median	4.0	8.5	··	··	8.5
	Range	4.0–10.0	4.0–53.0	··	··	4.0–42.0
Free hemoglobin, mg/dL	**N**	10	10	9	7	8
	Mean (SD)	138 (132.8)	83 (51.1)	109 (77.7)	173 (166.4)	250 (212.2)
	Median	105	67	90	160	180
	Range	50–500	40–195	40–270	30–515	48–670
Total bilirubin, mg/dL	**N**	10	10	9	7	8
	Mean (SD)	1.3 (0.74)	0.6 (0.29) *	0.6 (0.24) *	0.7 (0.32) *	0.6 (0.23) *
	Median	1.2	0.6	0.6	0.7	0.5
	Range	0.4–2.4	0.2–1.0	0.3–1.0	0.2–1.1	0.4–1.1

Laboratory Tests	Statistics	Baseline (Day 1)	Day 28	Day 42	Day 56	Day 84
Direct bilirubin, mg/dL	**N**	10	10	9	7	8
	Mean (SD)	0.3 (0.15)	0.2 (0.10)	0.2 (0.05)	0.2 (0.09)	0.2 (0.07) *
	Median	0.2	0.2	0.2	0.3	0.2
	Range	0.2–0.6	0.1–0.4	0.2–0.3	0.1–0.4	0.1–0.3
C3d$^+$ of total erythrocytes, %	**N**	10	3	9	··	6
	Mean (SD)	0.2 (0.15)	0.2 (0.12)	0.1 (0.03)	··	0.2 (0.12)
	Median	0.1	0.1	0.1	··	0.1
	Range	0.1–0.5	0.1 –0.3	0.1–0.2	··	0.1–0.4
ANC, 10^9/L	**N**	10	10	9	7	7
	Mean (SD)	2.1 (0.83)	2.0 (0.62)	1.9 (0.50)	1.9 (0.58)	2.4 (1.24)
	Median	2	2	1.8	2	2.2
	Range	0.8–3.8	1.1–3.0	1.3–2.7	1.1–2.5	1.2–4.4
Platelets, 10^9/L	**N**	10	10	9	7	8
	Mean (SD)	162 (61.7)	175 (70.9)	168 (78.6)	163 (78.4)	165 (83.8)
	Median	174	166	172	171	161
	Range	37–245	47–290	43–322	35–270	35–320
FACIT-FATIGUE score	**N**	10	10	··	7	8
	Mean (SD)	34 (11.5)	43 (9.6) *	··	39 (13.2) *	47 (7.5) **
	Median	36	47	··	46	50
	Range	20–49	23–52	··	18–51	31–52
GPI-deficient clone, erythrocytes %	**N**	10	9	7	5	8
	Mean (SD)	32 (24.6)	44 (15.6)	60 (20.1) **	59 (21.0)	56 (19.9) **
	Median	21	38	55	59	51
	Range	11–78	31–82	39–89	39–91	36–92
GPI-deficient clone, granulocytes %	**N**	10	9	7	5	8
	Mean (SD)	79 (16.9)	71 (23.5)	77 (21.9)	74 (20.3)	79 (13.3)
	Median	82	88	86	85	82
	Range	46–99	38–96	42–97	49–91	57–96

*p<0.05. **p<0.005.

ANC=absolute neutrophil count; AP=alternative pathway; CAE=complement activity enzyme; CP=classical pathway; FACIT=Functional Assessment of Chronic Illness Therapy; GPI- glycosylphosphatidylinositol; LDH=lactate dehydrogenase; ULN=upper limit of normal.