Supplementary Table 1. Absolute and percent change from baseline for laboratory parameters at weeks 2, 4, 8, and 12 (pharmacodynamic analysis set)

		Iptacopan 25 mg/100 mg n=7			Iptacopan 50 mg/200 mg n=6		
		Raw	Absolute change	%Change	Raw	Absolute change	%Change
Lactate dehydrogenase (U/L)							
Baseline	n	7			6		
	Mean	2159.6			2172.6		
	90% CI	(1484.0, 2835.1)			(1561.9, 2783.2)		
Week 2	n	7	7	7	5	5	5
	Mean	531.9	-1627.7	-76.5	286.2	-1929.0	-85.0
	90% CI	(119.6, 944.1)	(-2240.0, -1015.5)	(-90.1, -63.0)	(227.2, 345.2)	(-2725.4, -1132.7)	(-92.8, -77.2)
	P-value	0.0461	0.0021	<.0001	0.0005	0.0067	<.0001
Week 4	n	7	7	7	5	5	5
	Mean	395.7	-1763.9	-79.7	198.8	-2016.4	-89.7
	90% CI	(170.6, 620.8)	(-2428.5, -1099.2)	(-91.5, -68.0)	(153.5, 244.1)	(-2799.9, -1232.9)	(-94.9, -84.5)
	P-value	0.0142	0.0021	<.0001	0.0007	0.0054	<.0001
Week 8	n	7	7	7	5	5	5
	Mean	300.3	-1859.3	-81.7	216.6	-1998.6	-88.3
	90% CI	(108.4, 492.1)	(-2617.8, -1100.7)	(-98.5, -65.0)	(138.7, 294.5)	(-2810.7, -1186.6)	(-96.6, -80.0)
	P-value	0.0228	0.0031	<.0001	0.0041	0.0063	<.0001
Week 12	n	6	6	6	4	4	4
	Mean	238.8	-1821.7	-86.2	246.3	-1836.7	-85.9
	90% CI	(165.4, 312.3)	(-2614.2, -1029.1)	(-93.5, -78.9)	(158.3, 334.2)	(-2907.2, -766.3)	(-96.5, -75.3)
	P-value	0.0012	0.0057	<.0001	0.0071	0.0273	0.0003
Hemoglobin (g/L)							
Baseline	n	7			5		
	Mean	88.5			76.9		
	90% CI	(78.6, 98.5)			(64.9, 88.9)		
Week 2	n	7	7	7	4	4	4
	Mean	95.4	6.9	8.1	104.3	31.8	45.5
	90% CI	(85.4, 105.4)	(2.6, 11.1)	(2.9, 13.2)	(97.4, 111.1)	(19.6, 44.0)	(24.1, 66.8)
	P-value	<.0001	0.0203	0.0229	<.0001	0.0087	0.0153
Week 4	n	6	6	6	4	4	4
	Mean	95.5	9.6	11.6	109.0	36.6	51.8
	90% CI	(82.6, 108.4)	(-0.1, 19.4)	(-0.1, 23.4)	(89.6, 128.4)	(15.6, 57.6)	(19.6, 84.1)
	P-value	<.0001	0.1038	0.1022	0.0009	0.0264	0.0323
Week 8	n	7	7	7	4	4	4
	Mean	103.6	15.0	18.1	110.5	38.1	53.0
	90% CI	(95.3, 111.9)	(8.3, 21.7)	(9.5, 26.6)	(78.3, 142.7)	(7.5, 68.6)	(8.0, 98.1)
	P-value	<.0001	0.0049	0.0065	0.0040	0.0608	0.0696
Week 12	n	6	6	6	3	3	3
	Mean	115.2	23.4	26.7	109.0	37.1	51.8
	90% CI	(105.6, 124.7)	(11.5, 35.2)	(12.8, 40.6)	(58.9, 159.1)	(-7.0, 81.2)	(-14.3, 117.9)

	P-value	<.0001	0.0105	0.0117	0.0239	0.1334	0.1495
Bilirubin (µmol/L)							
Baseline	n	7			6		
	Mean	32.4			35.3		
	90% CI	(27.2, 37.6)			(23.3, 47.3)		
Week 2	n	7	7	7	5	5	5
	Mean	12.6	-19.8	-60.8	12.6	-23.9	-64.2
	90% CI	(8.0, 17.2)	(-25.6, -14.0)	(-74.2, -47.3)	(3.3, 21.9)	(-39.9, -8.0)	(-87.2, -41.1)
	P-value	0.0018	0.0005	0.0001	0.0441	0.0330	0.0040
Week 4	n	7	7	7	5	5	5
	Mean	12.4	-20.0	-58.8	10.6	-25.9	-70.0
	90% CI	(8.5, 16.3)	(-27.8, -12.2)	(-75.0, -42.6)	(2.7, 18.5)	(-41.2, -10.7)	(-88.9, -51.1)
	P-value	0.0008	0.0025	0.0004	0.0465	0.0222	0.0014
Week 8	n	7	7	7	5	5	5
	Mean	12.1	-20.2	-59.7	11.0	-25.5	-68.2
	90% CI	(5.6, 18.6)	(-29.5, -11.0)	(-86.3, -33.0)	(3.8, 18.2)	(-41.2, -9.8)	(-86.1, -50.3)
	P-value	0.0110	0.0054	0.0048	0.0316	0.0257	0.0012
Week 12	n	6	6	6	4	4	4
	Mean	11.7	-21.6	-64.4	14.8	-23.2	-57.5
	90% CI	(8.9, 14.4)	(-26.5, -16.7)	(-72.3, -56.5)	(6.2, 23.3)	(-44.6, -1.8)	(-80.1, -34.9)
	P-value	0.0004	0.0003	<.0001	0.0270	0.0842	0.0093
Reticulocyte (10E9/L)							
Baseline	n	7			6		
	Mean	217.6			211.1		
	90% CI	(168.4, 266.7)			(132.3, 289.8)		
Week 2	n	7	7	7	5	5	5
	Mean	134.6	-83.0	-34.0	106.2	-94.2	-19.0
	90% CI	(98.1, 171.1)	(-140.8, -25.2)	(-53.7, -14.2)	(74.6, 137.8)	(-201.6, 13.2)	(-92.6, 54.5)
	P-value	0.0004	0.0316	0.0155	0.0020	0.1347	0.6105
Week 4	n	6	6	6	5	5	5
	Mean	110.7	-118.4	-47.3	67.8	-132.6	-56.4
	90% CI	(87.8, 133.5)	(-181.6, -55.3)	(-65.1, -29.5)	(39.8, 95.8)	(-219.5, -45.8)	(-83.3, -29.5)
	P-value	0.0002	0.0129	0.0031	0.0067	0.0312	0.0111
Week 8	n	7	7	7	5	5	5
	Mean	103.9	-113.7	-49.5	81.0	-119.4	-33.3
	90% CI	(72.9, 134.8)	(-166.8, -60.6)	(-66.3, -32.6)	(59.9, 102.1)	(-231.0, -7.8)	(-105.1, 38.6)
	P-value	0.0006	0.0059	0.0013	0.0012	0.0846	0.3799
Week 12	n	6	6	6	4	4	4
	Mean	130.8	-93.0	-35.4	104.8	-84.9	-8.7
	90% CI	(92.5, 169.2)	(-169.9, -16.1)	(-59.0, -11.7)	(65.4, 144.1)	(-230.1, 60.3)	(-125.4, 108.1)
	P-value	0.0010	0.0588	0.0295	0.0082	0.2625	0.8722
Haptoglobin (g/L)							
Baseline	n	7			6		
	Mean	0.1			0.1		
	90% CI	(,)			(,)		
Week 2	n	7	7	7	5	5	5
	Mean	0.3	0.2	445.7	0.4	0.4	716.0
	90% CI	(0.1, 0.5)	(0.0, 0.4)	(77.3, 814.1)	(-0.1, 0.9)	(-0.1, 0.8)	(-236.3, 1668.3)
	P-value	0.0281	0.0570	0.0570	0.1418	0.1842	0.1842
Week 4	n	7	7	7	5	5	5
	Mean	0.2	0.2	374.3	0.5	0.4	876.0
	90% CI	(0.0, 0.4)	(-0.0, 0.4)	(-0.3, 748.9)	(0.0, 1.0)	(-0.0, 0.9)	(-58.3, 1810.3)
	P-value	0.0491	0.1002	0.1002	0.0899	0.1163	0.1163
Week 8	n	7	7	7	5	5	5
	Mean	0.4	0.3	654.3	0.4	0.4	728.0
	90% CI	(0.0, 0.7)	(-0.0, 0.7)	(-16.1, 1324.6)	(0.1, 0.8)	(0.0, 0.7)	(15.5, 1440.5)

	P-value	0.0714	0.1067	0.1067	0.0684	0.0949	0.0949
Week 12	n	6	6	6	5	5	5
	Mean	0.2	0.2	396.7	0.2	0.1	260.0
	90% CI	(-0.0, 0.5)	(-0.1, 0.5)	(-189.8, 983.1)	(-0.0, 0.4)	(-0.1, 0.3)	(-169.3, 689.3)
	P-value	0.1486	0.2311	0.2311	0.1484	0.2663	0.2663
Erythrocytes (10E12/L)							
Baseline	n	7			6		
	Mean	3.1			2.5		
	90% CI	(2.7, 3.4)			(2.1, 2.9)		
Week 2	n	7	7	7	5	5	5
	Mean	3.5	0.5	16.2	3.4	1.0	45.3
	90% CI	(3.2, 3.9)	(0.3, 0.6)	(11.4, 21.0)	(2.9, 3.8)	(0.6, 1.4)	(23.4, 67.2)
	P-value	<.0001	0.0006	0.0006	<.0001	0.0050	0.0116
Week 4	n	6	6	6	5	5	5
	Mean	3.8	0.7	24.4	3.6	1.3	55.7
	90% CI	(3.2, 4.4)	(0.4, 1.1)	(13.7, 35.1)	(3.0, 4.3)	(0.7, 1.8)	(23.7, 87.6)
	P-value	<.0001	0.0063	0.0058	0.0003	0.0105	0.0206
Week 8	n	7	7	7	5	5	5
	Mean	4.2	1.2	39.5	3.7	1.4	60.9
	90% CI	(3.8, 4.6)	(0.9, 1.4)	(26.8, 52.2)	(2.9, 4.6)	(0.6, 2.1)	(21.5, 100.4)
	P-value	<.0001	0.0002	0.0009	0.0006	0.0181	0.0301
Week 12	n	6	6	6	4	4	4
	Mean	4.6	1.4	44.7	3.8	1.4	60.1
	90% CI	(4.1, 5.0)	(0.9, 1.8)	(28.1, 61.3)	(2.7, 4.9)	(0.5, 2.3)	(12.8, 107.4)
	P-value	<.0001	0.0014	0.0029	0.0041	0.0370	0.0581

1 Note: Change refers to change from baseline.

2 CI, confidence interval; SD, standard deviation

Supplementary Table 2. Absolute and percent change from baseline in C3d+type-II, type-III erythrocytes and PNH clone size at weeks 2, 4, 8 and 12 (pharmacodynamic analysis set)

		Iptacopan 25 mg/100 mg n=7			Iptacopan 50 mg/200 mg n=6		
		Raw	Absolute change	% Change	Raw	Absolute change	% Change
C3d+type-III erythrocytes (%)							
Baseline	n	7			6		
	Mean	1.5			1.6		
	90% CI	(0.8, 2.3)			(-0.2, 3.4)		
Week 2	n	7	7	7	5	5	5
	Mean	0.8	-0.7	-53.5	1.0	-0.9	-73.2
	90% CI	(0.1, 1.4)	(-1.4, -0.0)	(-82.5, -24.5)	(-0.9, 2.9)	(-2.0, 0.3)	(-106.8, -39.6)
	P-value	0.0575	0.0916	0.0116	0.3138	0.1958	0.0097
Week 4	n	7	7	7	5	5	5
	Mean	0.6	-0.9	-52.5	0.2	-1.6	-83.0
	90% CI	(0.3, 1.0)	(-1.7, -0.1)	(-73.2, -31.8)	(-0.0, 0.5)	(-3.6, 0.3)	(-87.7, -78.2)
	P-value	0.0158	0.0722	0.0026	0.1114	0.1460	<.0001
Week 8	n	7	7	7	5	5	5
	Mean	0.2	-1.3	-84.0	0.1	-1.7	-94.6
	90% CI	(0.1, 0.4)	(-2.0, -0.6)	(-92.8, -75.3)	(-0.1, 0.3)	(-3.7, 0.3)	(-96.8, -92.4)
	P-value	0.0259	0.0134	<.0001	0.2091	0.1381	<.0001
Week 12	n	6	6	6	4	4	4
	Mean	0.2	-1.0	-87.7	0.2	-2.0	-84.6
	90% CI	(0.1, 0.3)	(-1.5, -0.6)	(-93.5, -81.9)	(-0.2, 0.6)	(-4.6, 0.5)	(-96.7, -72.5)
	P-value	0.0225	0.0054	<.0001	0.2658	0.1539	0.0005
Clone size (PNH red blood cells) (%)							
Baseline	n	6			6		
	Mean	33.6			49.1		
	90% CI	(18.7, 48.6)			(24.7, 73.6)		
Week 2	n	7	6	6	5	5	5
	Mean	54.9	20.9	88.8	65.6	20.5	146.3
	90% CI	(45.4, 64.5)	(11.2, 30.6)	(28.0, 149.7)	(37.7, 93.6)	(13.6, 27.3)	(-63.9, 356.5)
	P-value	<.0001	0.0075	0.0322	0.0074	0.0032	0.2120
Week 4	n	7	6	6	5	5	5
	Mean	65.9	30.4	130.5	73.7	28.5	243.0
	90% CI	(56.4, 75.4)	(17.0, 43.8)	(42.6, 218.4)	(50.3, 97.2)	(18.7, 38.4)	(-137.4, 623.5)
	P-value	<.0001	0.0060	0.0304	0.0026	0.0035	0.2449
Week 8	n	7	6	6	5	5	5
	Mean	79.6	43.9	186.9	85.1	39.9	395.9
	90% CI	(69.1, 90.1)	(26.3, 61.5)	(65.7, 308.2)	(69.4, 100.7)	(23.4, 56.3)	(-261.9, 1053.6)
	P-value	<.0001	0.0040	0.0266	0.0003	0.0066	0.2688
Week 12	n	6	5	5	4	4	4
	Mean	82.9	43.4	167.5	91.1	46.8	642.7
	90% CI	(73.1, 92.7)	(26.3, 60.6)	(41.4, 293.7)	(81.8, 100.3)	(13.0, 80.5)	(-639.4, 1924.8)
	P-value	<.0001	0.0057	0.0473	0.0002	0.0471	0.3231

Note: Change refers to change from baseline.

CI, confidence interval; SD, standard deviation; PNH, paroxysmal nocturnal hemoglobinuria

Supplementary Table 3. Incidence of AEs by preferred term (full analysis set)

	Treatment period 1 Iptacopan		Treatment period 2 Iptacopan		Extension phase Iptacopan	
	25 mg n=7	50 mg n=6	100 mg n=7	200 mg n=5	100 mg n=5	200 mg n=3
Patients with at least one AE, n (%)	2 (28.6)	4 (66.7)	3 (42.9)	1 (20.0)	2 (40.0)	2 (66.7)
Headache	0	3 (50.0)	0	0	1 (20.0)	0
Upper respiratory tract infection	0	0	2 (28.6)	0	0	0
Cough	2 (28.6)	0	1 (14.3)	0	1 (20.0)	1 (33.3)
Abdominal discomfort	0	0	0	0	2 (40.0)	0
Abdominal pain	0	0	0	0	1 (20.0)	0
Musculoskeletal chest pain	0	0	1 (14.3)	0	0	0
Dry throat	0	0	1 (14.3)	0	0	0
Abdominal distension	1 (14.3)	0	0	0	0	0
Flatulence	0	0	1 (14.3)	0	0	0
Impaired fasting glucose	0	0	1 (14.3)	0	0	0
Oropharyngeal pain	1 (14.3)	0	0	0	1 (20.0)	0
Nasopharyngitis	0	0	0	1 (20.0)	0	0
Back pain	0	0	0	0	0	1 (3.3)
Hematuria	0	0	0	0	1 (20.0)	0
Dyspepsia	0	0	0	0	1 (20.0)	0
Joint stiffness	0	0	0	0	1 (20.0)	0
Pyrexia	1 (14.3)	0	0	0	1 (20.0)	0
Blood alkaline phosphatase increase	0	1 (16.7)	0	0	1 (20.0)	0
Reverse tri-Iodothyronine increase	0	0	1 (14.3)	0	0	0
Thyroxine-free increase	0	0	1 (14.3)	0	0	0
Blood luteinizing hormone increase	0	0	0	0	1 (20.0)	0
Gamma-glutamyl transferase increase	0	0	0	0	1 (20.0)	0

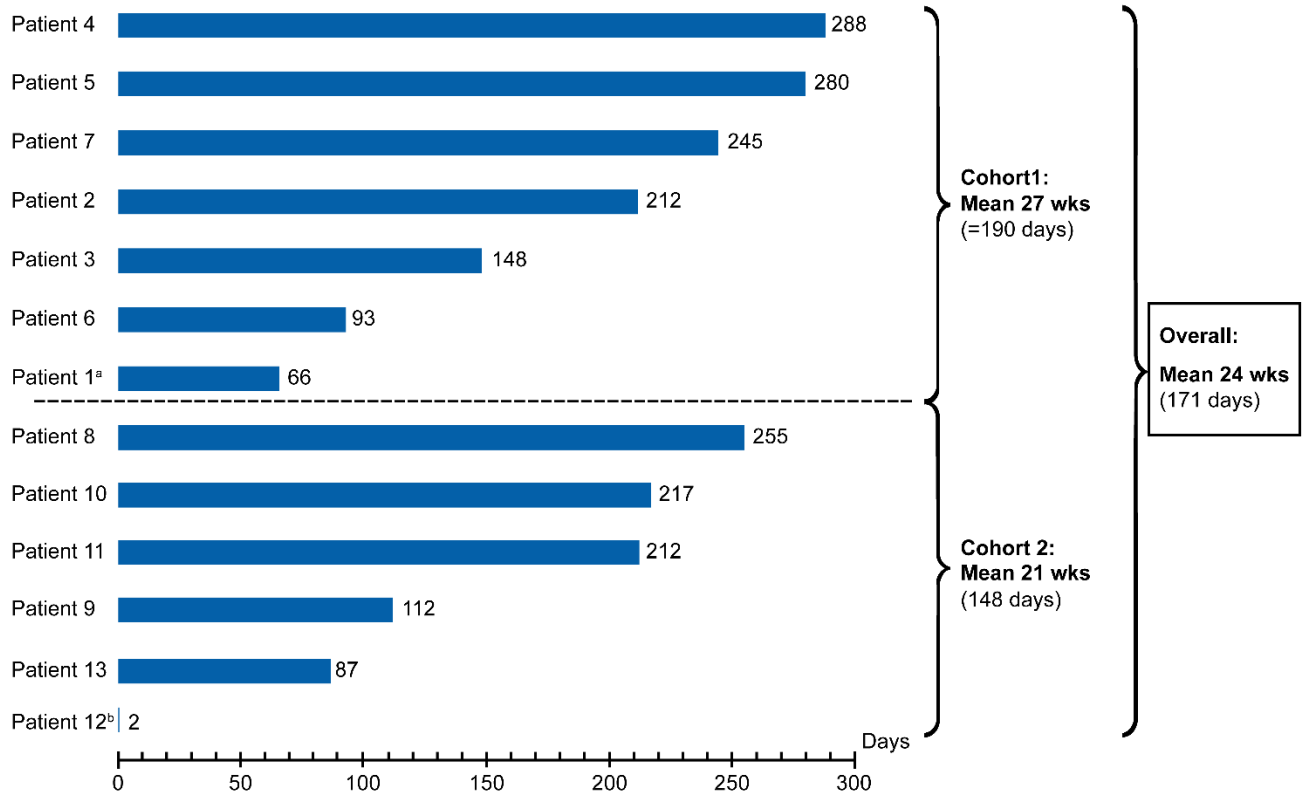
AE, adverse events

Supplementary Table 4. Pharmacokinetic parameters of iptacopan 25, 50, 100 and 200 mg doses on day 29 and 57 (pharmacokinetic analysis)

	Day 29		Day 57	
Pharmacokinetic parameter (Unit)	**Iptacopan 25 mg bid n=7**	**Iptacopan 50 mg bid n=5**	**Iptacopan 100 mg bid n=6**	**Iptacopan 200 mg bid n=5**
AUC_{last} (hr*ng/mL)	6290 (4000 - 9860)	8570 (6490 - 9920)	11900 (8940 - 14900)	19900 (12400 - 27400)
C_{max} (ng/mL)	1450 (830 - 2700)	1800 (1270 - 2140)	2640 (2040 - 3650)	4520 (2980 - 6730)
T_{max} (hr), Median (min – max)	2.00 (1.00 - 4.00)	2.00 (1.00 - 2.00)	1.53 (0.833 - 2.00)	2.00 (1.00 - 4.13)

Data represented as mean (minimum – maximum) unless specified

Supplementary Figure 1. Treatment duration of iptacopan (full analysis set)

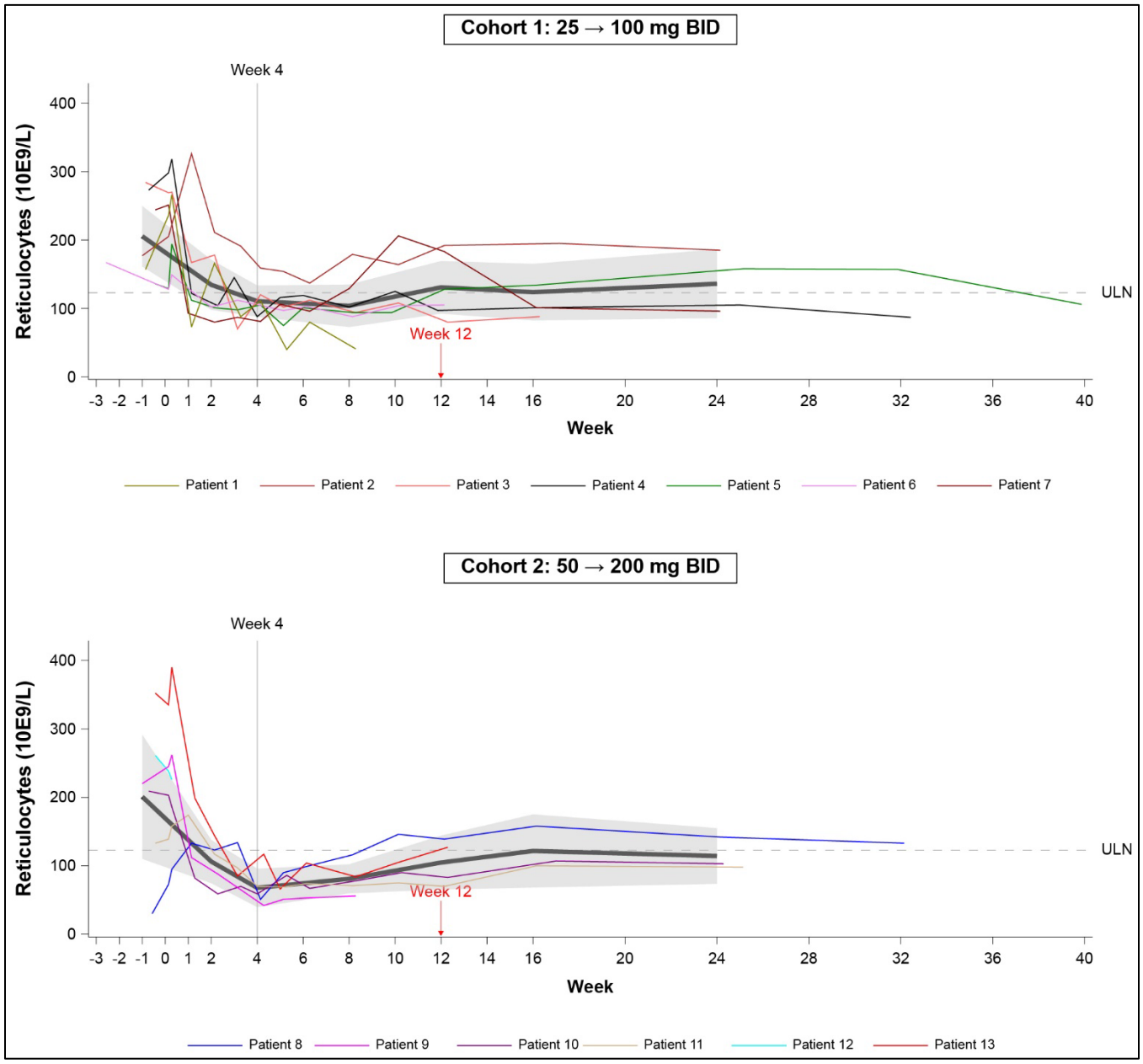


[a]One patient in cohort 1 started study drug taper prior to cutoff and discontinued after cutoff by patient preference and upon physician decision

[b]One patient in cohort 2 discontinued after 2 days of dosing due to non-severe AE of headache

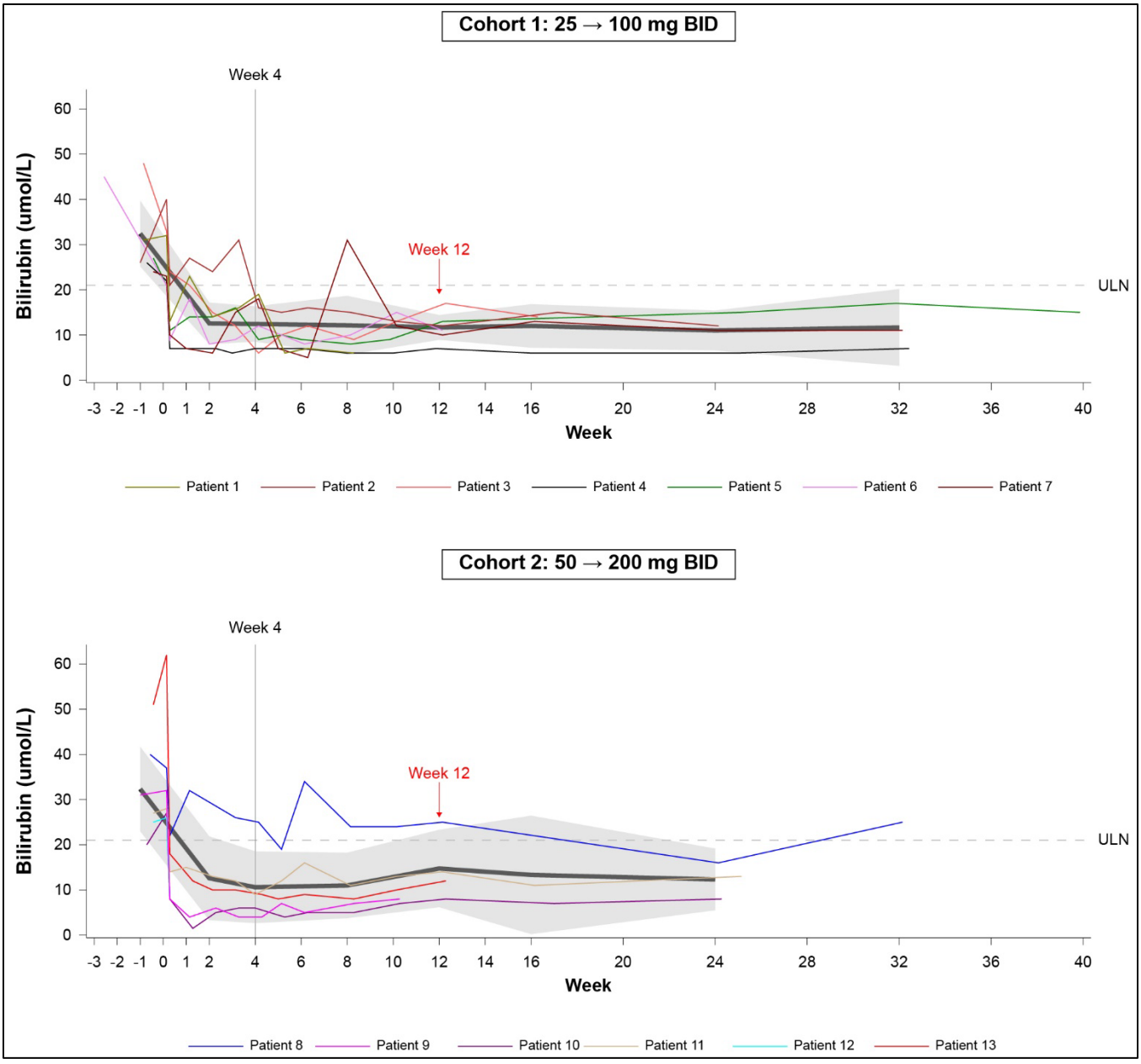
Supplementary Figure 2. Change in markers of hemolysis over time for iptacopan 25 / 100 mg and 50 / 200 mg cohorts (pharmacodynamic analysis set)

Reticulocytes



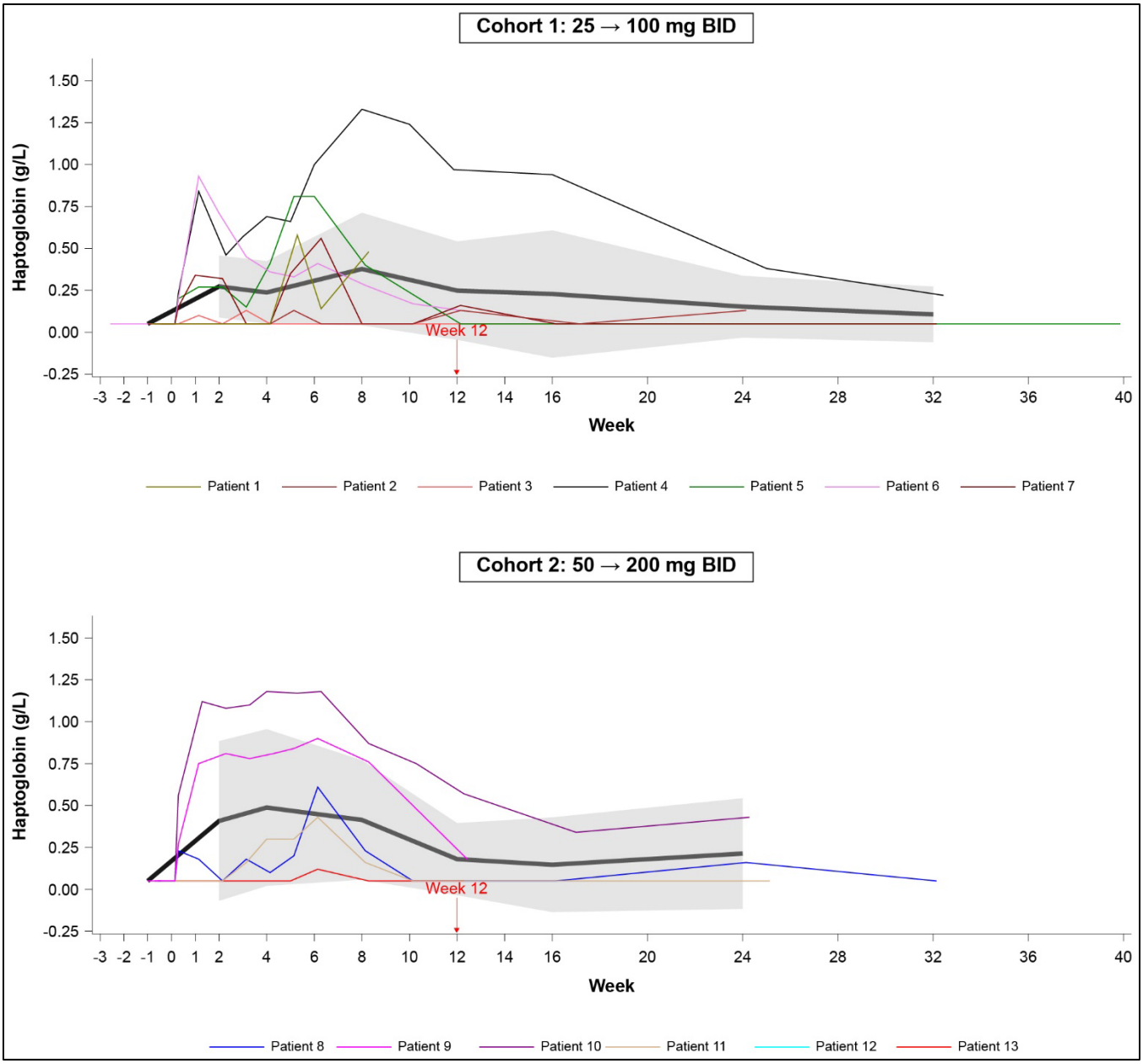
BID, twice daily

Bilirubin



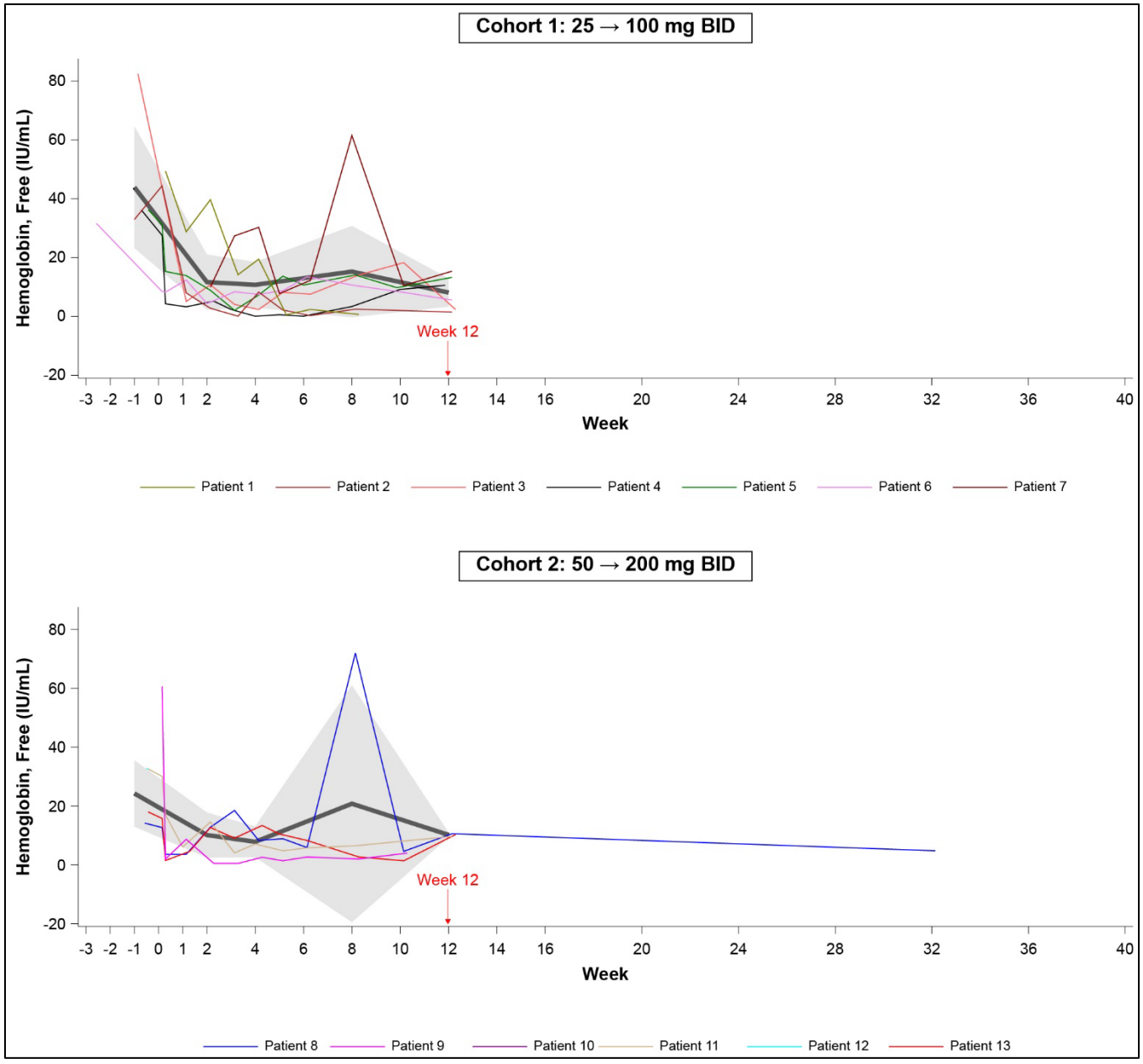
BID, twice daily

Haptoglobin



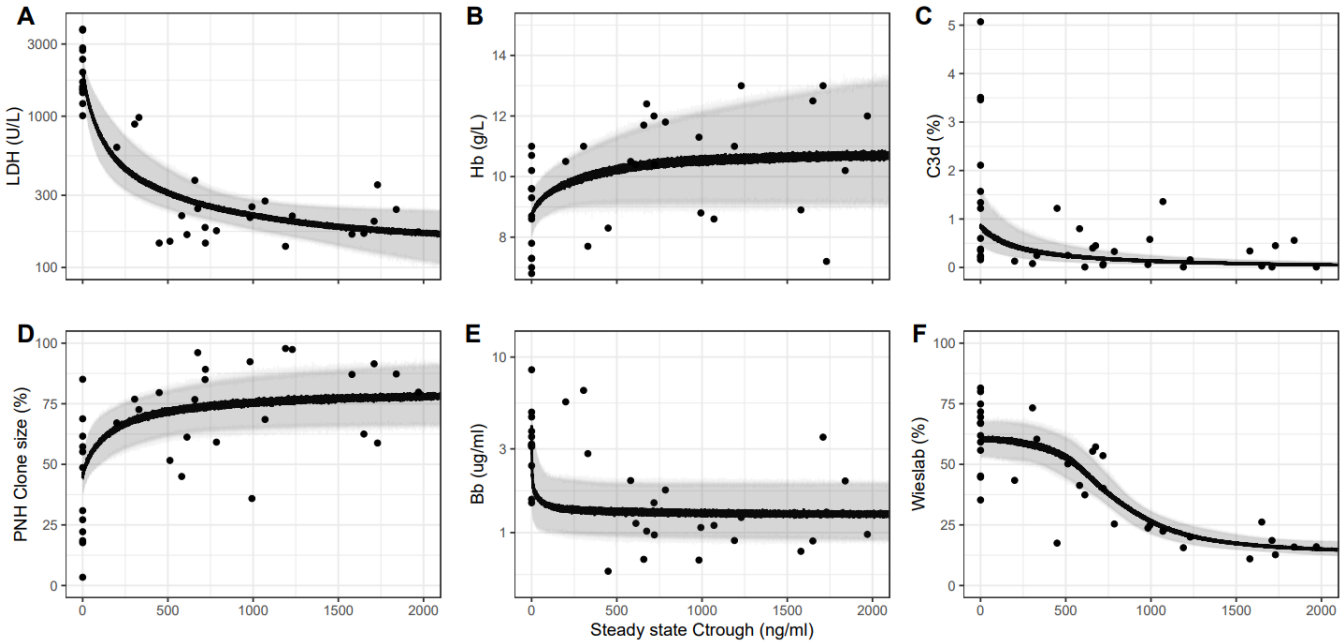
BID, twice daily

Free Hemoglobin



BID, twice daily

Supplementary Figure 3. Exposure-response of blood lactate dehydrogenase levels (A), hemoglobin (B), C3 deposition (C), PNH clone size (D), Bb plasma concentrations (E) and inhibition of complement by the Wieslab assay (F)



Hb, hemoglobin; LDH, lactate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria

Exposure-response data at baseline, days 29 and 57 was used to fit sigmoid Emax functions for each variable using nonlinear mixed effect modeling. Black line: model prediction including residual variability. Grey shaded area: 90% confidence interval of the model.

Supplementary text

Method description for Wieslab Complement assay (alternative pathway)

Assessment of the effect of iptacopan on the complement alternative pathway was performed using a commercially available assay (Wieslab® Complement system (alternative pathway), Euro Diagnostica, COMPL AP330). In brief, the wells of the microtiter strips were pre-coated with specific activators of the alternative pathway (lipopolysaccharide). The calibration standards (STDs), the quality controls (QCs) and the unknown serum samples were diluted 1:18 in Diluent AP, containing a specific blocker to ensure that only the alternative pathway is activated. 100 µl of diluted STDs, QCs and unknown serum samples were added to the wells and incubated for 1 h at 37°C. After a washing step, 100 µl of alkaline phosphatase-conjugated antihuman C5b-9 was added to each well. The plate was incubated for 30 min at room temperature (RT), following by an additional washing step. 100 µl substrate solution was added to each well, followed by incubation for 30 min at RT. Absorbance values were read at 405 nm on a microplate reader. Samples were measured in duplicates.

Calibration standards (STDs) and quality control (QC) samples were used to define acceptance criteria for each analytical run. Human serum obtained from healthy volunteers was used as blank matrix for the preparation of QC samples. Human, heat-inactivated serum from male AB plasma (Sigma Aldrich Chime SARL Ref. H3667) was used as negative control and for the preparation of calibration standards and for the dilution of QC samples. No minimum dilution of unknown serum was required.

The data was analyzed using SoftMax® Pro, version GxP 6.5 (Molecular Devices, Sunnyvale, U.S.A) and Watson® LIMS, Version 7.4.2 SP1 (Thermo Electron Corporation, Philadelphia, PA,

U.S.A). The complement alternative pathway activity was calculated as mean from the duplicates and reported as percentage (%).