LETTER TO THE EDITOR

Phase II trials of zilucoplan in paroxysmal nocturnal hemoglobinuria

Austin G. Kulasekararaj,[1] Anna-Elina Lehtinen,[2] Cecily Forsyth,[3] Shreyans Gandhi,[1] Morag Griffin,[4] Sixten Körper,[5] Gabor Mikala,[6] Petra Muus,[1,4] Ulrik Overgaard,[7] Christopher J. Patriquin,[8] Humphrey Pullon,[9] Yu-Min Shen,[10] Ruth Spearing,[11] Jeff Szer,[12] Guillemette de la Borderie,[13] Petra W. Duda,[14] Ramin Farzaneh-Far,[14] Sharan Ragunathan,[14] Camil E. Sayegh,[14] Douangsone D. Vadysirisack[14] and Hubert Schrezenmeier[5]

[1]King's College Hospital-NHS Foundation Trust, NIHR/Wellcome King's Clinical Research Facility, London, UK & King's College London, London, UK; [2]Helsinki University Hospital Comprehensive Cancer Center and University of Helsinki, Helsinki, Finland; [3]Gosford Hospital, Gosford, New South Wales, Australia; [4]St. James's University Hospital, Leeds, UK; [5]University of Ulm, Institute of Transfusion Medicine, and Institute of Transfusion Medicine and Immunogenetics Ulm, Red Cross Blood Transfusion Service Baden-Württemberg-Hessen and University Hospital Ulm, Ulm, Germany; [6]Central Hospital of Southern Pest National Institute of Hematology and Infectious Diseases, Budapest, Hungary; [7]University Hospital Copenhagen, Copenhagen, Denmark; [8]University Health Network, Toronto, Ontario, Canada; [9]Waikato Hospital, Hamilton, New Zealand; [10]UT Southwestern Medical Center, Dallas, TX, USA; [11]Christchurch Hospital, Christchurch, New Zealand; [12]Peter MacCallum Cancer Centre & The Royal Melbourne Hospital, Melbourne, Victoria, Australia; [13]UCB Pharma, Colombe, France and [14]UCB Pharma, Cambridge, MA, USA

Correspondence:
H. SCHREZENMEIER - h.schrezenmeier@blutspende.de

https://doi.org/10.3324/haematol.2022.281780

Supplementary Table S1. Key inclusion and exclusion criteria for patients in Studies 201, 202, and 203.

	Inclusion criteria	Exclusion criteria
Study 201	• ≥18 years of age • Diagnosis of PNH by flow cytometry • Females of childbearing potential must have had a negative pregnancy test at screening and 24 hours before the first dose of study drug and using effective contraception during the study • C5 inhibitor-naïve cohort: not received treatment with eculizumab before or during screening with LDH level ≥2× ULN during screening • C5 inhibitor switch cohort: treated with eculizumab for ≥6 months	• Platelet count <30,000/μL or ANC <500 cells/μL at screening • Glomerular filtration rate of <30 mL/min/1.73 m^2 • Alanine aminotransferase >2× ULN or direct bilirubin and alkaline phosphatase >2× ULN • History of meningococcal disease

Study 203	• ≥18 years of age
	• Diagnosis of PNH by flow cytometry
	• Females of childbearing potential must have been not pregnant at screening and 24 hours before the first dose of study drug and using effective contraception during the study
	• Inadequate response to eculizumab defined as treatment with eculizumab for ≥6 months plus ≥1 of the following:
	  ○ Documented LDH level ≥1.5× ULN within 90 days of screening
	  ○ Presence of known C5 mutation conferring resistance to eculizumab
		• Platelet count <30,000/μL or ANC <500 cells/μL at screening
		• Glomerular filtration rate of <60 mL/min/1.73 m^2
		• Direct bilirubin, alanine aminotransferase, or aspartate aminotransferase >1.5× ULN
		• History of meningococcal disease

ANC, absolute neutrophil count; C5, complement component 5; LDH, lactate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria; ULN, upper limit of normal.

Supplementary Table S2. Demographics and baseline characteristics.*

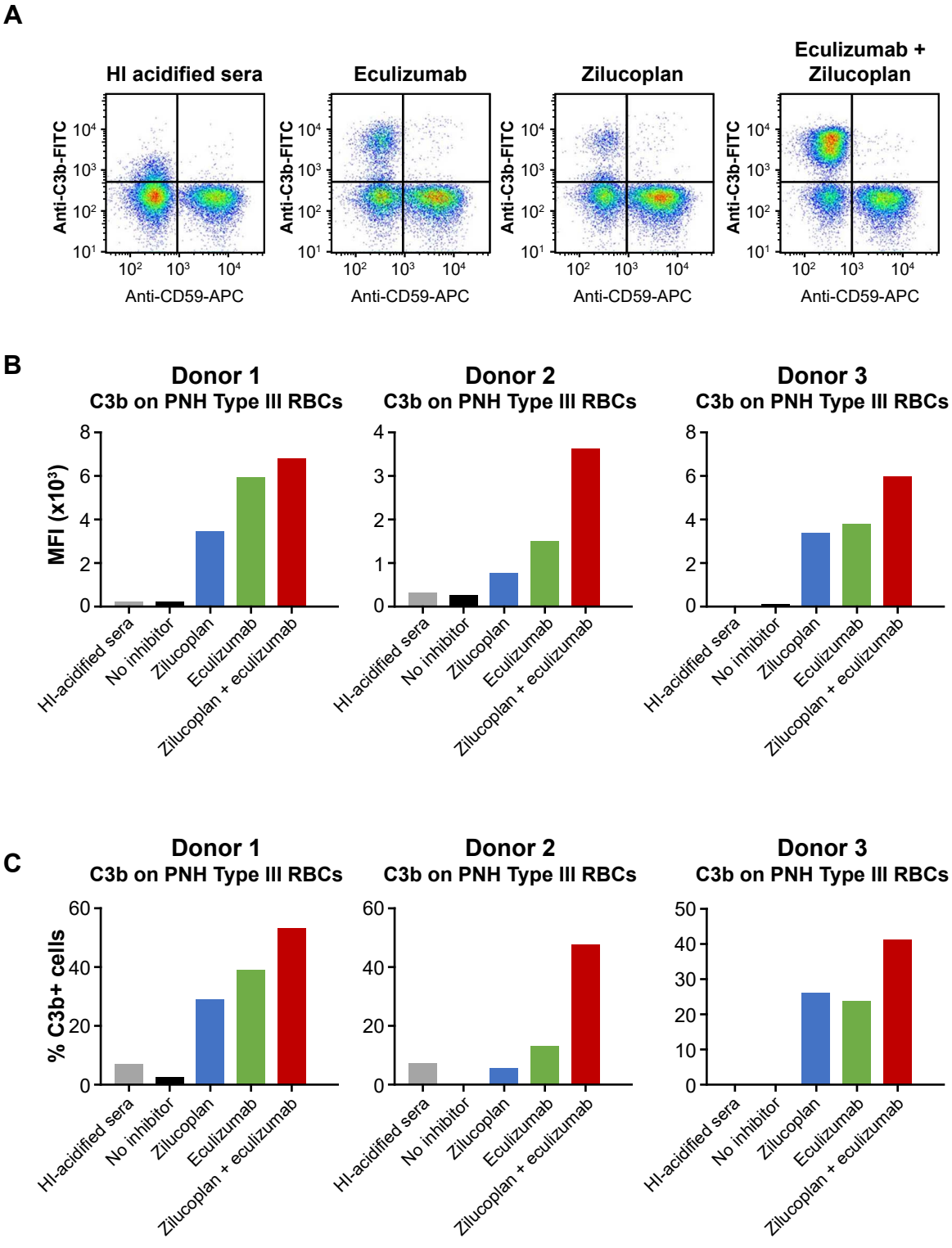
Variable	Eculizumab-naïve patients (n=10)	Eculizumab switch patients (n=19)†
Median age, y (range)	56.0 (32-81)	53.0 (21-72)
Female, n (%)	6 (60.0)	8 (42.1)
Race, n (%)		
White	10 (100.0)	15 (78.9)
Black/African American	0	3 (15.8)
Not reported	0	1 (5.3)
Mean body mass index, kg/m^2 (SD)	27.3 (6.2)	28.3 (5.6)
Median disease duration, y (range)	0.8 (0.0-12.0)	4.2 (0.5-36.0)
Median reticulocyte count, $\times 10^9$/L (range)	120 (94-331)	196 (41-382)
Median platelet count, $\times 10^9$/L (range)	132.5 (19.0-293.0)	142.0 (53.0-224.0)
Median monocyte clone size, % (range)	82.7 (45.8-99.7)	97.0 (21.5-100.0)
Median RBC clone size, % (range)	40.2 (8.3-63.3)	60.2 (5.4-99.0)
Median free hemoglobin, mg/dL (range)	7.1 (1.5-31.2)	1.8 (0.4-148.7)
Mean LDH, U/L (ULN: 234 U/L) (range; SD)	1174.1 (462-2435; 601.4)‡	288.3 (159-797; 132.4)§
Transfusion-dependent within prior 6 months, n (%)	5 (50.0)	12 (63.2)
Median duration of eculizumab treatment, y (range)	0	3.6 (0.4-12.0)
Eculizumab dose >900 mg/Q2W, n (%)	0	7 (36.8)

C5, complement component 5; LDH, lactate dehydrogenase; RBC, red blood cell; SD, standard deviation; Q2W, every 2 weeks; ULN, upper limit of normal.
*Data are representative of subgroup analyses of 2 cohorts.

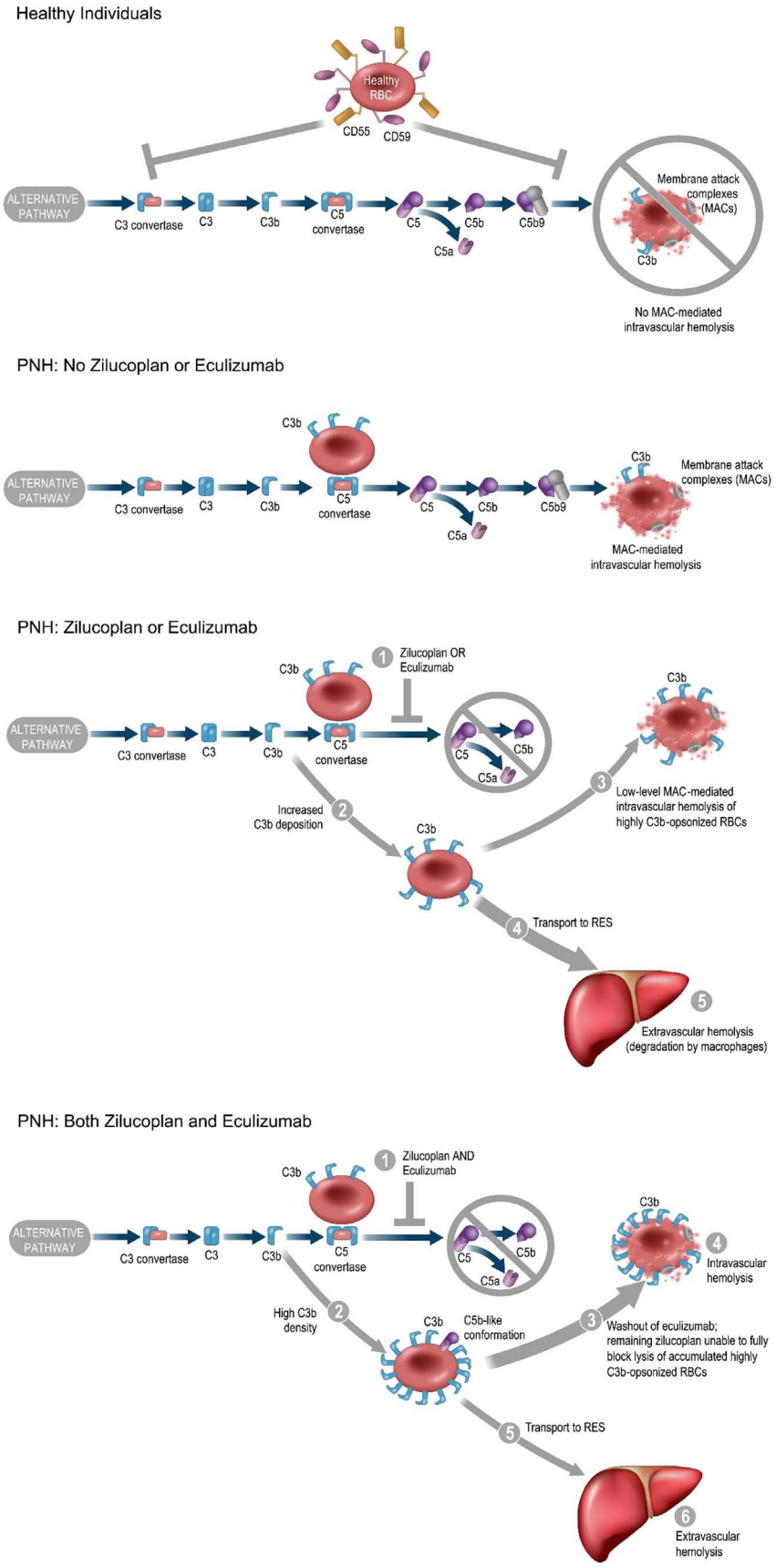
[†]Includes 16 patients from Study 201 and 3 patients from Study 203.
[‡]Average of screening and study Day 1 values.
[§]Most recent non-missing value obtained immediately before administration of first dose of zilucoplan.

Supplementary Figure S1. Effect of treatment with zilucoplan and eculizumab on C3b opsonization of PNH patient donor cells and mechanistic hypothesis for the role of C5 inhibition with zilucoplan and eculizumab in PNH. (A) Representative flow cytometric plots from a single commercially sourced PNH donor, (B) quantification of mean fluorescence intensity (MFI) from 3 commercially sourced PNH donors, (C) percentage of C3b-positive cells on Type III PNH RBCs from 3 commercially sourced PNH donors, and (D) mechanistic hypothesis for the role of C5 inhibition with zilucoplan and eculizumab in PNH. In healthy individuals, RBCs express complement regulators CD55 and CD59 on the cell surface that are protective against alternative complement pathway–mediated hemolysis via production of the MAC. In patients with PNH in the absence of treatment with zilucoplan or eculizumab, RBCs lacking CD55 and CD59 can be found at a significant proportion (type III clones) in circulation and these cells are susceptible to MAC-mediated intravascular hemolysis. In patients with PNH in the presence of either zilucoplan or eculizumab, MAC-mediated intravascular hemolysis of PNH RBCs is mostly inhibited; however, C3b opsonization of PNH RBCs can still occur, resulting in elimination of RBCs through extravascular hemolysis by macrophages in the liver. In patients with PNH in the presence of both zilucoplan and eculizumab, MAC-mediated intravascular hemolysis is effectively blocked, allowing the accumulation of highly C3b-opsonized RBCs. These highly opsonized RBCs support an elevated density of C5 convertases. Further, this high C3b loading promotes the recruitment of C5, and non-enzymatic rearrangement to a C5b-like conformation that can insert in the membrane and initiate MAC assembly. Upon eculizumab withdrawal, zilucoplan alone cannot overcome MAC-mediated intravascular hemolysis of highly C3b-opsonized RBCs. APC, allophycocyanin; C3b, complement component 3b; C5, complement component 5; CD55, cluster of differentiation 55;

CD59, cluster of differentiation 59; FITC, fluorescein isothiocyanate; HI, heat-inactivated; MAC, membrane attack complex; MFI, mean fluorescence intensity; PNH, paroxysmal nocturnal hemoglobinuria; RBC, red blood cell; RES, reticuloendothelial system.



D


Healthy Individuals
Healthy RBC
CD55
CD59
ALTERNATIVE PATHWAY
C3 convertase
C3
C3b
C5 convertase
C5
C5b
C5b9
C5a
Membrane attack complexes (MACs)
C3b
No MAC-mediated intravascular hemolysis
PNH: No Zilucoplan or Eculizumab
C3b
ALTERNATIVE PATHWAY
C3 convertase
C3
C3b
C5 convertase
C5
C5b
C5b9
C5a
C3b
Membrane attack complexes (MACs)
MAC-mediated intravascular hemolysis
PNH: Zilucoplan or Eculizumab
1 Zilucoplan OR Eculizumab
C3b
ALTERNATIVE PATHWAY
C3 convertase
C3
C3b
C5 convertase
C5
C5b
C5a
C3b
3 Low-level MAC-mediated intravascular hemolysis of highly C3b-opsonized RBCs
Increased C3b deposition
2
C3b
4 Transport to RES
5 Extravascular hemolysis (degradation by macrophages)
PNH: Both Zilucoplan and Eculizumab
1 Zilucoplan AND Eculizumab
C3b
ALTERNATIVE PATHWAY
C3 convertase
C3
C3b
C5 convertase
C5
C5b
C5a
C3b
4 Intravascular hemolysis
High C3b density
2
C3b
C5b-like conformation
3 Washout of eculizumab; remaining zilucoplan unable to fully block lysis of accumulated highly C3b-opsonized RBCs
5 Transport to RES
6 Extravascular hemolysis