Phase II trials of zilucoplan in paroxysmal nocturnal hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, chronic, clonal hematopoietic stem cell disorder. Uncontrolled complement activation is central in the pathogenesis of PNH.[1-3] Approved first-line treatments of PNH are eculizumab and ravulizumab, which inhibit the activity of complement component 5 (C5).[1,4-6]

Zilucoplan, a novel C5 inhibitor, is a small (3.5 kDa), 15-amino acid macrocyclic peptide that binds to C5 with high affinity and specificity.[7] Zilucoplan inhibits complement via a dual mechanism in that it prevents cleavage of C5 into C5a and C5b and binds to the domain of C5 corresponding to C5b, thereby blocking the binding of C5b to C6.[7] Zilucoplan prevents activation of the terminal complement pathway and assembly of the membrane attack complex that results in lysis of glycosylphosphatidylinositol-anchored protein-deficient red blood cells (RBC) in PNH.[7,8]

The efficacy, pharmacodynamics, safety, and tolerability of zilucoplan were evaluated in adult patients with PNH in two phase II 12-week studies (Study 201, NCT03078582; Study 203, NCT03030183) and a long-term extension study (NCT03225287) (Figure 1A, B). Eligibility criteria are summarized in *Online Supplementary Table S1*. The primary endpoint of the 12-week studies was change from baseline in serum lactate dehydrogenase (LDH) levels. This analysis included ten eculizumab-naïve patients and 19 who had received prior eculizumab treatment (eculizumab-switch cohort) (Figure 1B). All ten eculizumab-naïve patients entered the extension study, two (20.0%) of whom discontinued. In the switch cohort, eight of 19 (42.1%) patients discontinued and 11 (57.9%) entered the extension study; two (10.5%) patients discontinued and nine (47.4%) were still receiving zilucoplan treatment at data cutoff (November 2020).

The patients' demographics and baseline characteristics are provided in *Online Supplementary Table S2*. As expected, the eculizumab-naïve cohort had higher baseline LDH and median-free hemoglobin than the switch cohort.

In the eculizumab-naïve cohort, treatment with zilucoplan resulted in consistent, complete, and sustained inhibition of both the classical and alternative complement pathways (Figure 2A), leading to rapid, substantial, and sustained LDH decreases from baseline (median LDH, 378.0 U/L [1.6× upper limit of normal, ULN, of 234 U/L]) (Figure 2B). Of the five patients who required one or more transfusions (irrespective of the number of units) in the 6 months before the start of the study, two (40.0%) became transfusion-independent after zilucoplan treatment initiation (Figure 2C). Zilucoplan treatment led to a consistent decrease of median free hemoglobin (baseline 7.10 mg/dL) at all post-baseline time points (range of the median change, –3.90 to –5.95 mg/dL). Mean changes from baseline to each post-baseline time point in all other secondary endpoints, including total bilirubin, total hemoglobin, haptoglobin, reticulocytes, and hemoglobinuria were generally small or variable, displaying no clear trend for the naïve cohort (*data not shown*).

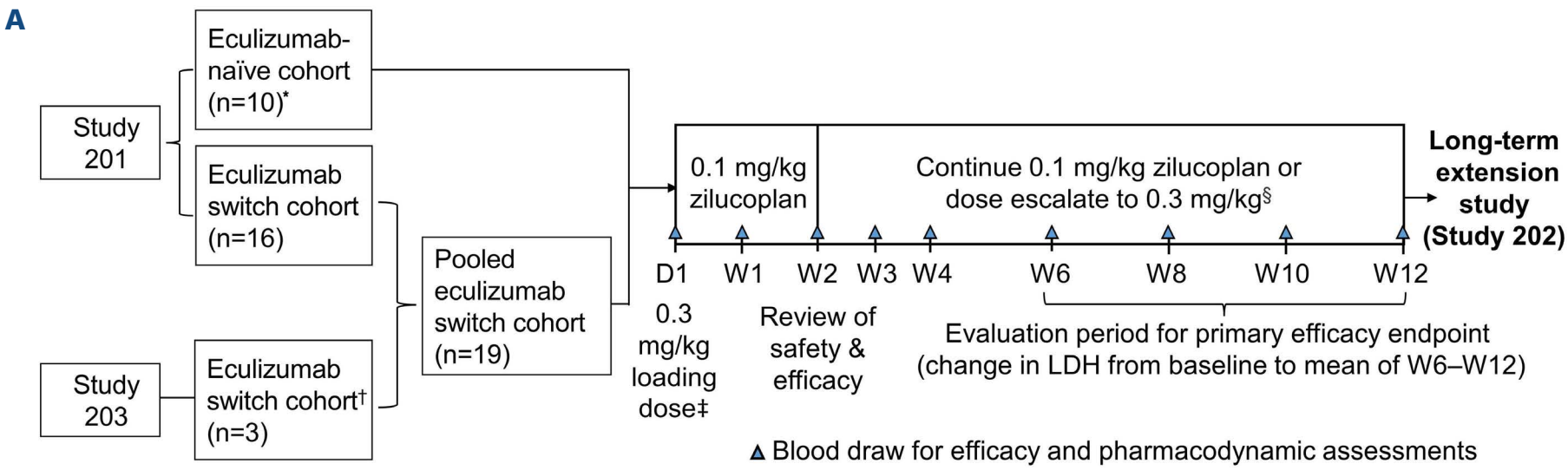
Zilucoplan treatment in the switch cohort led to complete and sustained inhibition of both the classical and alternative complement pathways (Figure 3A). Patients treated in the switch cohort had a median LDH increase of 230.3 U/L from baseline during the primary evaluation period (Figure 3B). In the seven (36.8%) patients who were transfusion-independent, the mean (standard deviation) baseline LDH value was 232.6 (22.6) U/L. After an initial increase in mean LDH that peaked at week 6, values remained consistent at approximately 1.5×ULN in transfusion-independent patients in the switch cohort (Figure 3B).

In transfusion-dependent patients in the switch cohort, the mean baseline LDH value was significantly higher than in the transfusion-independent group. Despite zilucoplan treatment, transfusion-dependent patients in the switch cohort experienced increased mean LDH values that reached their highest levels at week 20 (924.7 U/L) (Figure 3B). Based on the investigators' medical evaluation, patients with evidence of increased hemolysis discontinued zilucoplan and resumed eculizumab treatment, resulting in stabilization of LDH. Among the 12 transfusion-dependent patients in the switch cohort, including some who had received treatment for less than 6 months, four became transfusion-independent after initiation of zilucoplan (Figure 3C).

Within the switch cohort, patients who discontinued and were considered to have had switch failure had higher reticulocyte counts at baseline than those who were considered switch successes (Figure 3D). At baseline, the switch cohort had a median free hemoglobin of 1.80 mg/dL; variable changes with a median range of 0.00-1.70 mg/dL were observed across all post-baseline time points. Mean changes from baseline to each post-baseline time point in all other secondary endpoints were generally small or variable with no clear trends.

Zilucoplan, which can be administered at home as a subcutaneous, small-volume (<1 mL) injection with a thin (29G) needle, was well tolerated, with >18.6 patient-years of exposure and a mean duration of exposure of 36.4 weeks. In the initial 12-week study period, all patients (n=29) experienced adverse events, of whom 11 (37.9%) had treatment-related adverse events (most common [occurring in >1 patient]: headache [n=4], hemolysis [n=4], dizziness [n=2], fatigue [n=2], and injection site bruising [n=2]). No thrombotic events were observed. During the 12-week study period, treatment-related adverse events occurred in fewer patients in the eculizumab-naïve cohort

(20.0%, n=2/10) than in the switch cohort (47.4%, n=9/19). Four (13.8%) patients experienced serious adverse events (pyrexia and febrile non-hemolytic transfusion reaction [naïve cohort; n=1], urinary tract infection, gastroenteritis, and pyrexia [switch cohort; n=1 each]); none was considered treatment-related. In the long-term extension study (n=19), all patients experienced adverse events, of whom four (21.1%) had treatment-related adverse events (most common [occurring in >1 patient]: headache [n=2], injection site bruising [n=2]). Treatment-related adverse events occurred at similar frequencies in the naïve (20.0% [n=2/10]) and switch (22.2% [n=2/9]) cohorts. Six (31.6%) patients experienced serious adverse events (anemia [n=2]; deep vein thrombosis [n=1]; headache, nausea, osteoarthritis, and rotator cuff syndrome [n=1]; infectious enterocolitis



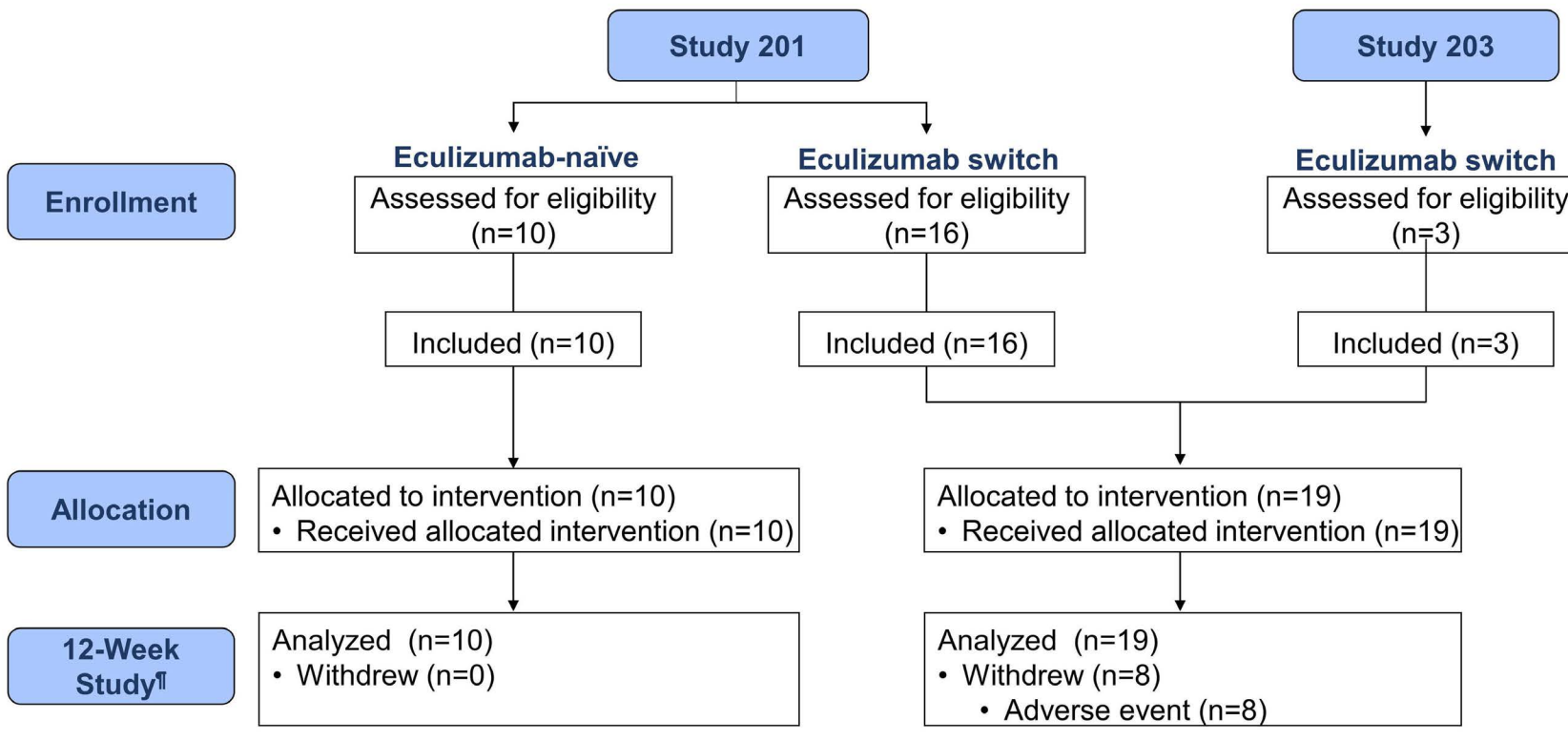
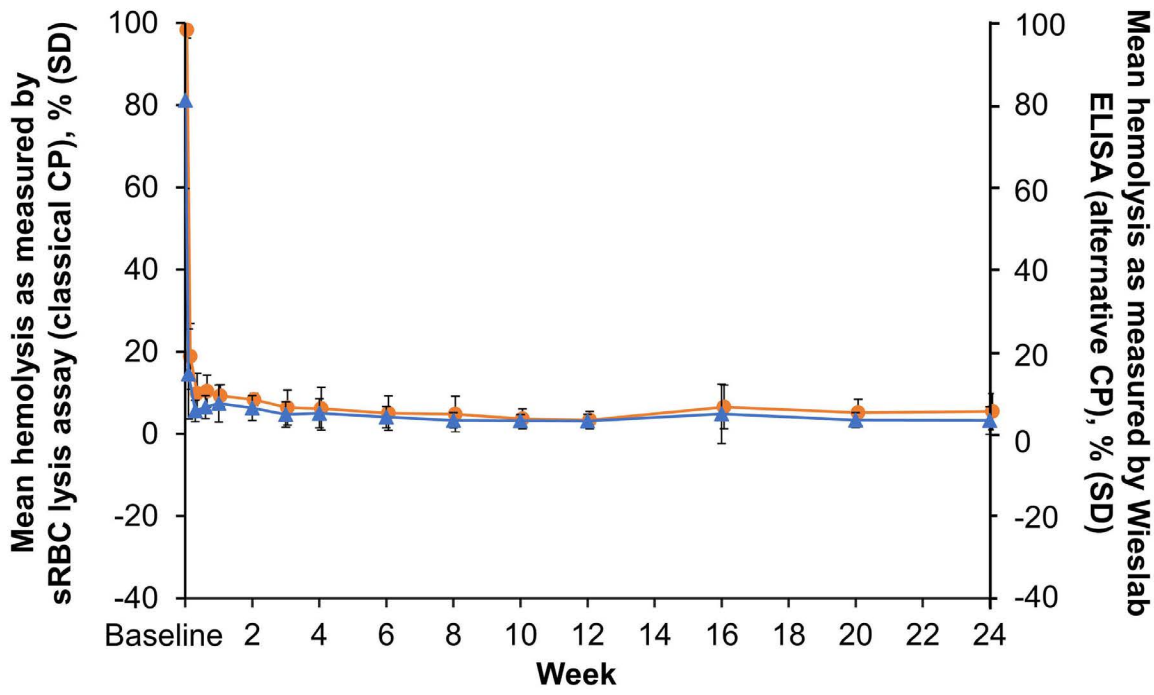


Figure 1. (A) Study designs for Studies 201 and 203 and (B) patients' flow, illustrated in a CONSORT diagram. The two 12-week, single-arm studies (Study 201; conducted from April 2017 to January 2018, and Study 203; conducted from September 2017 to February 2018) enrolled 26 and three patients, respectively. The analysis includes ten patients from Study 201 who were eculizumab-naïve and 19 who had previously been treated with eculizumab (eculizumab switch cohort; 16 patients from Study 201 and 3 patients from Study 203). All ten patients in the eculizumab-naïve cohort and 11/19 patients in the switch cohort entered the open-label extension study. *The eculizumab-naïve cohort consisted of patients with no prior exposure to eculizumab. †The switch cohort included patients with prior exposure to eculizumab for ≥6 months before screening. On day 1, a single loading dose of 0.3 mg/kg zilucoplan was administered subcutaneously. Thereafter, patients self-injected subcutaneous zilucoplan at home daily for the subsequent 12 weeks. Dose escalation to 0.3 mg/kg daily could be initiated at week 2 if a lactate dehydrogenase level of <1.5 times the upper limit of normal was not achieved or an overt breakthrough episode of hemolysis occurred (assessed via investigator judgement). A dose increase to 0.3 mg/kg was made in ten patients in the eculizumab-naïve cohort and 16 patients in the switch cohort after a median time of 19 days (range, 15-669 days) and 19.5 days (range, 1-57 days), respectively. ‡Blood samples for pharmacodynamics were collected within 1 hour of the administration of the first dose and at 1, 3, and 6 hours after the dose on day 1. §For patients who had a zilucoplan dose increase to 0.3 mg/kg, samples for pharmacodynamics were collected before the new dose, on day 1 of the new dose, and thereafter at scheduled visits. ¶No patients from either study were lost to follow-up. D: day; W: week; LDH: lactate dehydrogenase.

A



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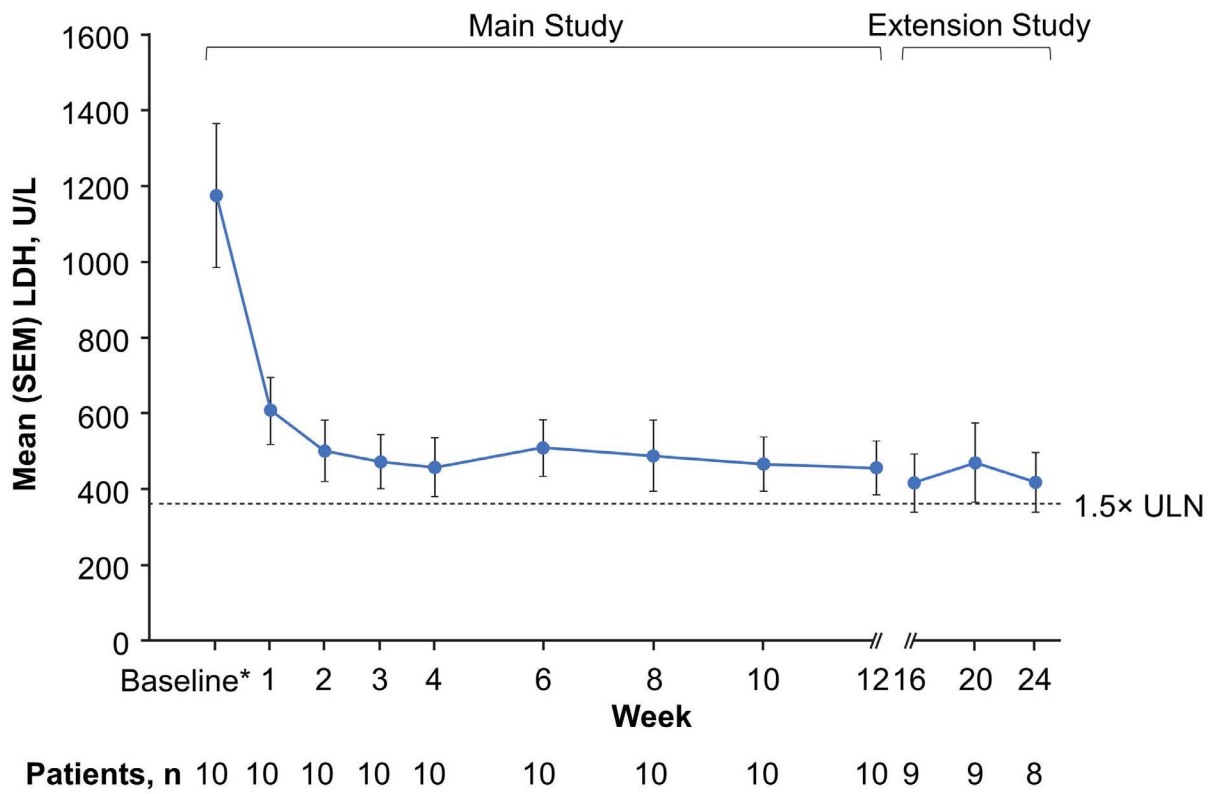


C

Pre-Study							Main Study			Extension Study			Pre-Dose Transfusion Rate (per week)	Post-Dose Transfusion Rate (per week)
Patient ID	–24W to –20W	–20W to –16W	–16W to –12W	–12W to –8W	–8W to –4W	–4W to 0W	0W to 4W	4W to 8W	8W to 12W	12W to 16W	16W to 20W	20W to 24W		
201-007-002	–	–	–	–	1	2	1	1	1	–	1	1	0.12	0.19
201-010-001	2	–	1	–	1	–	1	–	1	1	–	1	0.16	0.16
201-012-002	1	–	1	–	1	–	1	–	–	–	–	–	0.12	0.05
201-013-002	1	1	1	1	1	–	–	–	–	–	–	–	0.19	0.00
201-014-001	–	–	–	1	–	–	–	–	–	–	–	–	0.04	0.00
201-007-001	–	–	–	–	–	–	–	–	–	–	–	–	0.00	0.00
201-009-001	–	–	–	–	–	–	–	–	–	–	–	–	0.00	0.00
201-013-001	–	–	–	–	–	–	–	–	–	–	–	–	0.00	0.00
201-013-003	–	–	–	–	–	–	–	–	–	–	–	–	0.00	0.00
201-017-002	–	–	–	–	–	–	1	–	–	–			0.00	0.06
Total													**0.06**	**0.05**

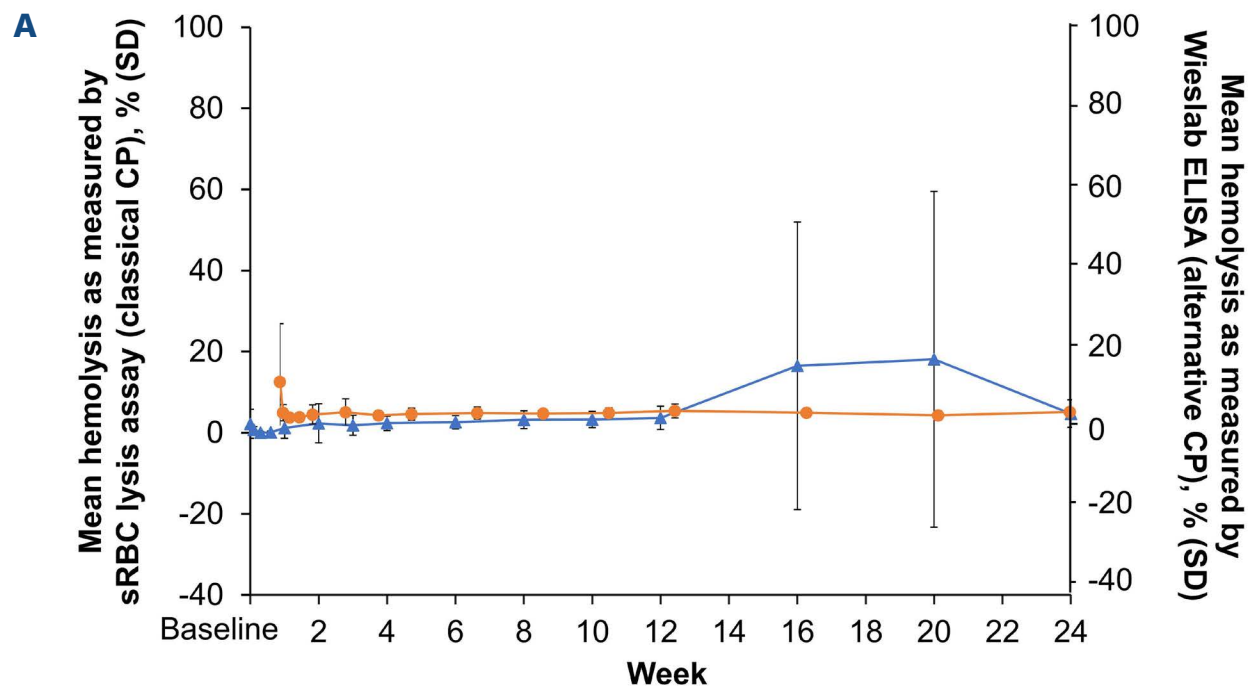
Number of transfusions during a 4W period Discontinued

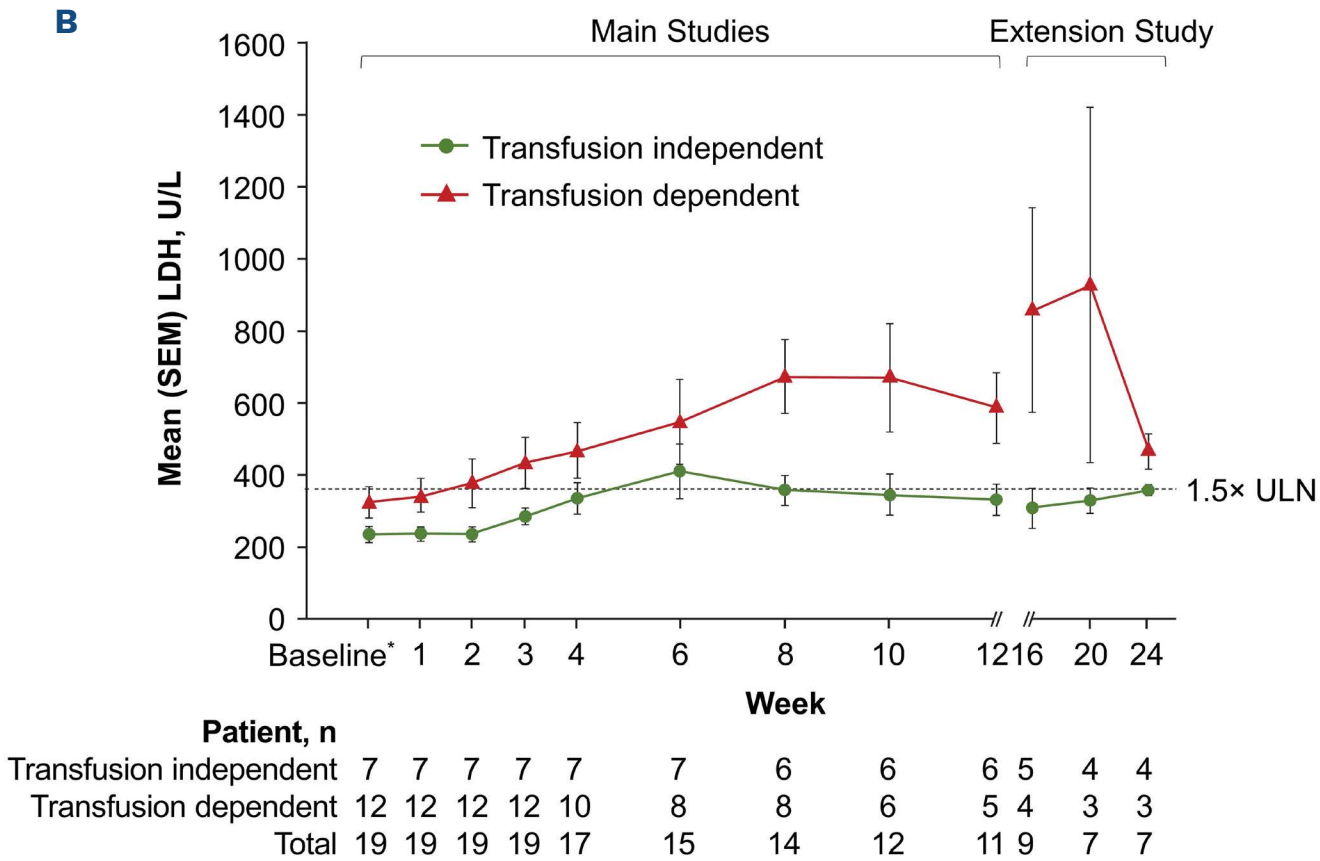
Figure 2. Effect of zilucoplan on patients with paroxysmal nocturnal hemoglobinuria in the eculizumab-naïve cohort. (A-C) Mean complement activity as measured by sheep red blood cell (sRBC) assay (classical complement pathway; left y-axis, orange circles)[15] and Wieslab enzyme-linked immunosorbent assay (ELISA) (alternative complement pathway; right y-axis, blue triangles) (A),[16] mean lactate dehydrogenase (LDH) levels (B), and transfusion requirements (C) before and after initiation of zilucoplan. Change in serum LDH, sRBC lysis, and Wieslab ELISA at each time point were analyzed using the two-sided Wilcoxon signed-rank test in each cohort. Missing data were not imputed. *Baseline is the average of the screening and day 1 LDH values per patient. sRBC: sheep red blood cell; CP: complement pathway; SD: standard deviation; ELISA: enzyme-linked immunosorbent assay; SEM: standard error of the mean; LDH: lactate dehydrogenase; ULN: upper limit of normal; ID: identity; W: week.

and tongue hematoma [n=1]; encephalopathy, pneumococcal pneumonia, and suicide attempt [n=1]), of which headache and nausea experienced by one (5.3%) patient were deemed treatment-related.

Twenty-one injection site reactions occurred in 12 (41.4%) patients across the 12-week and extension study periods; all were mild except for one event of moderate severity. Headache was the most common adverse event across all study periods, occurring in 12 (41.4%) patients. No deaths or meningococcal infections occurred during the studies.

To understand the mechanism for switch failure, an experimental analysis was performed in which the impact of treatment on hemolytic protection of commercially sourced type III PNH RBC after complement activation and C3b opsonization was studied by flow cytometry (*Online Supplementary Figure S1*). In the absence of complement activation (heat-inactivated sera condition), type III RBC (absence of CD59 expression) accounted for 60% of the total RBC pool in the analyzed PNH donor, while the type II RBC population (partial/reduced CD59 levels) was small in this donor and consequently excluded from further analysis. Low levels of C3b were detected on type III but not type I (high CD59 expression) RBC. Acidification of complement-competent serum resulted in alternative pathway activation and lysis of type III RBC in the absence of C5 inhibition (*data not shown*). Blocking C5 activation with either eculizumab or zilucoplan resulted in partial protection of type III RBC from lysis and deposition of C3b on the type III RBC. In the presence of both eculizumab and zilucoplan,





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C

Patient ID	Pre-Study –24W to –20W	Pre-Study –20W to –16W	Pre-Study –16W to –12W	Pre-Study –12W to –8W	Pre-Study –8W to –4W	Pre-Study –4W to 0W	Main Studies 0W to 4W	Main Studies 4W to 8W	Main Studies 8W to 12W	Extension Study 12W to 16W	Extension Study 16W to 20W	Extension Study 20W to 24W	Pre-Dose Transfusion Rate (per week)	Post-Dose Transfusion Rate (per week)
201-005-001	–	1	2	–	–	–	2	1					0.12	0.70
201-005-002	3	1	1	1	2	1	1	2	–	–	–		0.35	0.16
201-007-003	–	–	–	–	1	–	–	–					0.04	0.00
201-007-004	1	–	–	1	–	–	1	–					0.08	0.19
201-009-003	1	1	–	–	1	–	1	1	–				0.12	0.20
201-009-004	1	1	1	2	1	1	1	1	1				0.27	0.25
201-009-005	–	–	–	–	1	–	–	1	–	–	1	–	0.04	0.08
201-014-002	–	1	–	–	–	–	–	–					0.04	0.00
201-014-003	–	–	–	1	–	–	–	–	–				0.04	0.00
201-016-002	1	–	–	–	–	–	–	–	–				0.04	0.00
201-017-001	2	1	2	1	1	3	2	2	1	2	2	2	0.39	0.43
203-019-002	–	–	–	1	2	3	–	2	–	2	–	1	0.23	0.19
201-003-001	–	–	–	–	–	–	–	–	–				0.00	0.00
201-003-002	–	–	–	–	–	–	–	–	–	–	–	–	0.00	0.00
201-003-003	–	–	–	–	–	–	–	–	–	–	–	–	0.00	0.00
201-006-001	–	–	–	–	–	–	–	–	–	–	–	–	0.00	0.00
201-009-006	–	–	–	–	–	–	–	–					0.00	0.00
203-004-001	–	–	–	–	–	–	–	–	–	–	–		0.00	0.00
203-019-001	–	–	–	–	–	–	–	–	–	–	–	–	0.00	0.00
Total													**0.09**	**0.12**

Number of transfusions during a 4W period Discontinued

D

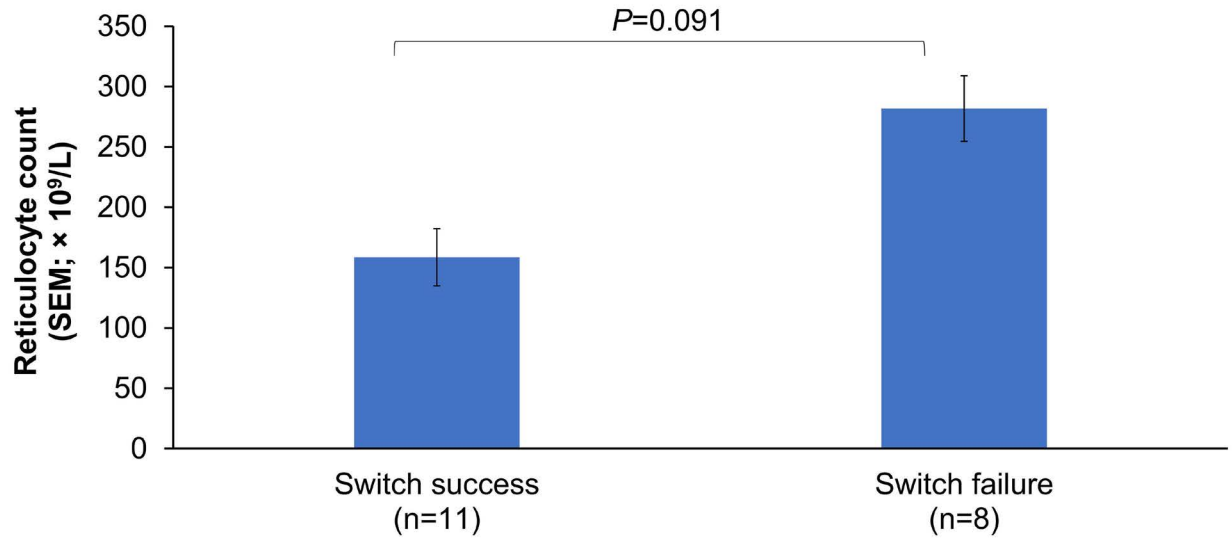

Figure 3. Effect of zilucoplan on patients with paroxysmal nocturnal hemoglobinuria in the eculizumab switch cohort. (A-C) Mean complement activity as measured by sheep red blood cell (sRBC) assay (classical complement pathway; left y-axis, orange circles)[15] and Wieslab enzyme-linked immunosorbent assay (ELISA) (alternative complement pathway; right y-axis, blue triangles) (A),[16] mean lactate dehydrogenase (LDH) reductions (B), transfusion requirements before and after initiation of zilucoplan (C), and mean counts at baseline in the switch cohort, stratified by switch success (N=8) *versus* switch failure (N=11; 282×10⁹/L *vs*. 159×10⁹/L; male upper limit of normal: 130×10⁹/L; female upper limit of normal: 120×10⁹/L) (D). Switch failure was defined as zilucoplan discontinuation during the first 12 weeks; patients could resume eculizumab treatment per individual investigator's procedures. Changes in serum LDH, sRBC lysis, and Wieslab ELISA at each time point were analyzed using the two-sided Wilcoxon signed-rank test in each cohort. Missing data were not imputed. *Most recent non-missing value obtained immediately before administration of the first dose of zilucoplan; mean (standard deviation) baseline LDH values: transfusion-independent (N=7), 232.6 (22.6) U/L; transfusion-dependent (N=12), 320.8 (44.4) U/L (*P*=0.0296). sRBC: sheep red blood cell; CP: complement pathway; SD: standard deviation; ELISA: enzyme-linked immunosorbent assay; SEM: standard error of the mean; ULN: upper limit of normal; LDH: lactate dehydrogenase; ID: identity; W: week.

type III RBC were protected from lysis and present at levels similar to those in controls (heat-inactivated serum) (*Online Supplementary Figure S1A*), and a larger proportion of highly C3b-opsonized type III RBC was generated compared with the proportions following treatment with eculizumab or zilucoplan alone (*Online Supplementary Figure S1B, C*). During the switch protocol, eculizumab and zilucoplan are both circulating in the blood at therapeutic concentrations for several days to over a week.[9] The combination of eculizumab and zilucoplan enabled the accumulation of high densities of C3b on PNH

type III RBC, an effect not observed in type I RBC. High concentrations of C3b may enable a non-enzymatic cleavage of C5 on the surface of RBC that cannot be inhibited by a C5 inhibitor, including zilucoplan. Prior studies have suggested that a high density of membrane-bound C3b can directly activate C5, leading to membrane attack complex formation without proteolytic cleavage of C5 into C5a and C5b.[10,11] These prior analyses demonstrated conformational activation of C5 in the absence of convertases or other enzymes that cannot be inhibited by different individual C5 inhibitors alone.[10,11] We hypothesize that after eculizumab washout, densely C3-opsonized RBC bind C5, which then adopts a C5b-like conformation[11] that cannot be efficiently inhibited by zilucoplan, resulting in intravascular hemolysis of this cell population (*Online Supplementary Figure S1D*).

The management of patients with PNH should seek to achieve complete and sustained inhibition of terminal complement. Residual free C5 was associated with an increased risk of breakthrough intravascular hemolysis in patients on other C5 inhibitors.[2,3,12,13] Free C5 was not assessed in our trial, but complete complement inhibition was seen using functional assays in the current studies (Figures 2A and 3A) and in other populations (ie., patients with generalized myasthenia gravis).[7]

In conclusion, in eculizumab-naïve patients with PNH treated with zilucoplan, LDH reductions were similar to those previously reported with eculizumab,[4] which agrees with the pharmacodynamic effects of zilucoplan. Despite confirmed complete complement inhibition, transfusion-dependent patients in the switch cohort with high reticulocyte counts failed to respond sufficiently to zilucoplan.

We expand upon the findings of other research groups to provide a rationale for increased intravascular hemolysis in patients who switched from eculizumab to zilucoplan.[10,11] This phenomenon is thought to be PNH-specific as a result of the disease-induced absence of glycosylphosphatidylinositol-anchored membrane proteins, including inhibitors of the complement cascade, on RBC. Overall, zilucoplan therapy was safe and well tolerated in patients with PNH.

Despite the cessation of clinical development of zilucoplan in PNH, the efficacy and safety profile of this novel C5 inhibitor in generalized myasthenia gravis,[7] along with the flexibility of once-daily, at-home, subcutaneous injections, has established zilucoplan as another potential option in the growing armamentarium of C5 inhibitors.[3] It has been suggested that combined treatment targeting different components of the complement cascade might overcome the residual hemolysis seen in a proportion of patients with PNH treated with a C5 inhibitor.[2,14]

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

Received: August 5, 2022.
Accepted: July 27, 2023.
Early view: August 3, 2023.

Disclosures

AGK has received speaker bureau, consultancy, and advisory board honoraria and travel expenses from Akari, Alexion Pharmaceuticals, Amgen, Apellis, BioCryst Pharmaceuticals, Celgene/Bristol Myers Squibb, Novartis, Ra Pharmaceuticals/UCB Pharma, Roche, and Sobi. A-EL has received speaking and/or consultant/advisory board honoraria from Alexion Pharmaceuticals. CF has received speaking and advisory board honoraria from Alexion Pharmaceuticals. MG has received speaking and/or advisory board honoraria from Alexion Pharmaceuticals and Sobi, consulted for and participated in an advisory board for BioCryst Pharmaceuticals, Novartis, and Regeneron Pharmaceuticals, and participated in a Medscape educational program funded by an educational grant from Apellis Pharmaceuticals. SK has received speaking and advisory board honoraria from Alexion Pharmaceuticals. GM has received research grant funding from AbbVie and consulting fees, speaking honoraria, and/or travel support from Amgen, Bristol Myers Squibb/Celgene, Janssen-Cilag, Novartis, Sanofi, and Takeda. PM has received lecture fees and travel support from Sobi and participated in an advisory board for Novartis. UO has received speaking and/or advisory board honoraria from Alexion Pharmaceuticals and Sobi. CJP has received speaking and/or advisory board honoraria from Alexion Pharmaceuticals, Apellis Pharmaceuticals, BioCryst Pharmaceuticals, and Sanofi. JS has received speaking and/or advisory board honoraria

from Alexion Pharmaceuticals, Apellis Pharmaceuticals, BioCryst Pharmaceuticals, Novartis, Pfizer, Prevail Therapeutics, and Sanofi-Genzyme. GdlB, PWD, and SR, are employees of UCB Pharma and own stock and/or hold stock options in the company. CES and DDV are former employees of UCB Pharma and Ra Pharmaceuticals. RF-F is a former employee of Ra Pharmaceuticals. HS has received research funding and honoraria as a speaker in symposia or for service on advisory boards from Alexion Pharmaceuticals, Apellis Pharmaceuticals, Novartis, Ra Pharmaceuticals, Roche, and Sanofi (all to the institution of HS). HP, Y-MS, RS, and SG have no conflicts of interest to disclose.

Contributions

AGK, PM, and HS contributed to the concept and design of the research, were study investigators, and contributed to the analysis of data. A-EL, CF, SG, MG, SK, GM, UO, CJP, HP, Y-MS, and JS were study investigators. RS was a study investigator and contributed to analysis of data. SR performed the mechanistic studies and contributed to the interpretation of data. GdlB, PWD, and RF-F contributed to the interpretation of data. CES and DDV contributed to the design of mechanistic studies and interpretation of data. All authors reviewed and approved the manuscript for submission.

Acknowledgments

The authors would like to acknowledge the following contributors: Stephen Babcock, MS, of UCB Pharma, for support as Clinical Project Manager for the studies; Anita Hill, MD, PhD, formerly of the National Health Service, UK, for work as a study investigator; and Veronica Porkess, PhD, CMPP, of UCB Pharma, for publication and editorial support.

Funding

Studies 201, 202, and 203 were funded by Ra Pharmaceuticals (Cambridge, MA, USA; now a part of UCB Pharma [Brussels, Belgium]). Medical writing and editorial assistance were provided by Jessica Deckman, PhD, CMPP, of The Lockwood Group (Stamford, CT, USA), and funded by UCB Pharma, in accordance with Good Publications Practice (GPP) 2022 guidelines (*https://www.ismpp.org/gpp-2022*).

Data-sharing statement

Underlying data from this manuscript may be requested by qualified researchers 6 months after product approval in the USA and/or Europe, or global development is discontinued, and 18 months after study completion. Investigators may request access to anonymized individual patient-level data and redacted study documents, which may include: analysis-ready datasets, study protocols, annotated case report forms, statistical analysis plans, dataset specifications, and clinical study reports. Prior to use of the data, proposals need to be approved by an independent review panel at *www.Vivli.org* and a signed data-sharing agreement will need to be executed. All documents are available in English only, for a prespecified time, typically 12 months, on a password-protected portal.

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