Tesidolumab (LFG316) for treatment of C5-variant patients with paroxysmal nocturnal hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH) is caused by clonal expansion of hematopoietic stem cells that carry a somatic mutation in the X-linked gene *PIG-A* (phosphatidylinositol glycan anchor biosynthesis class A). The mutation leads to a deficiency of glycosylphosphatidylinositol-anchored membrane proteins.[1-3] The loss of membrane-associated complement regulatory proteins CD55 and CD59 increases susceptibility of red blood cells and platelets to complement-mediated lysis, leading to hemolytic anemia, thrombophilia and reduced life expectancy in untreated patients.[2,4]

The currently approved monoclonal antibodies targeting C5 eculizumab[5] and ravulizumab[6] significantly reduce intravascular hemolysis and transfusion dependency and improve the life expectancy of PNH patients.[7,8,9] However, there are several, mainly Japanese patients who do not respond to eculizumab,[10] due to a variant C5 protein sequence with an arginine-to-histidine change at position 885 (Arg885His) that prevents eculizumab binding.[1,10] Ravulizumab binds to the same epitope as eculizumab,[6] so it cannot bind to this C5 variant, either.

Tesidolumab (LFG316) is a fully-human IgG1/λ anti-C5 monoclonal antibody of 143 kDa (without glycosylation), with a half-life in humans of approximately 9 days. Tesidolumab blocks cleavage of C5 and prevents subsequent formation of the membrane attack complex. Crystal structure analysis of tesidolumab Fab complexed to C5 demonstrated that tesidolumab bound to a distinct epitope to that of eculizumab and ravulizumab, distant to Arg 885 (Figure 1A). Consistent with this binding mode, tesidolumab inhibited both variant and non-variant C5 activation in a functional assay (Figure 1B).

An open-label, single-arm, multicenter, proof-of-concept phase II trial was conducted at seven centers in three countries to test the efficacy of tesidolumab in patients with variant and non-variant C5. The study comprised three treatment periods, namely a 4-week treatment period 1 (days 1-29), followed by an optional 48-week treatment period 2 (days 30-365), after which an interim analysis was performed, followed by an optional treatment period 3 that allowed a maximal treatment extension up to week 312. The primary endpoint was serum lactate dehydrogenase (LDH) reduction on day 29, and secondary endpoints involved monitoring of safety, tolerability, and tesidolumab pharmacokinetics. Exploratory endpoints included the assessment of hemoglobin levels, blood transfusion requirements, free hemoglobin, reticulocyte counts, bilirubin and FACIT fatigue score and pharmacodynamics measurements including sC5b-9 and the CH50 assay (a measure of *ex vivo* serum hemolytic activity).

Adult PNH patients with a PNH clone size of ≥10% and serum LDH levels ≥1.5-fold above the upper limit of normal (ULN) were included in the study. Additional requirements were vaccination against *Neisseria meningitidis* types A, C, Y and W-135 and, if available and acceptable by local regulations, vaccination against *Neisseria meningitidis* type B at least 2 weeks prior to first dosing. Treatment with corticosteroids and/or other immunosuppressive regimens could continue if indicated for treatment of autoimmune disease (e.g., aplastic anemia).

Key exclusion criteria were history of recurrent meningitis or meningococcal meningitis despite vaccination, active infection, history of hematopoietic stem cell transplantation, positive HIV test, known or suspected hereditary complement deficiency, and severe concurrent co-morbidities (e.g., advanced cardiac disease, severe pulmonary arterial hypertension). Cytopenic patients with neutrophils $<0.5x10^9$/L or platelets $<30x10^9$/L were excluded to avoid confounding significant bone marrow failure. All centers received approval from independent ethics committees and regulatory bodies. The study was conducted in accordance with the principles of the Declaration of Helsinki. Signed informed consent was obtained from each patient before any study-related procedures were undertaken. Trial information was published on *https://ClinicalTrials.gov* before first patient first visit, clinicaltrails gov. Identifier: NCT02534909, with the investigational product tesidolumab designated as LFG316.

Nine patients (5 C5-variant, 4 C5 non-variant; 4 females, 5 males) were enrolled between Sep 2015 and March 2017 (Table 1). Five of the nine patients were transfusion-dependent (3 of these were C5-variant patients), having received an average of 16.2 (range 8-30; standard deviation [SD] 8.9) units of erythrocytes in the year prior to screening. The patients had an average PNH clone size (type III erythrocytes) at baseline of 29.5 % (SD 10.5%), mean LDH of 1,270 U/L (SD 520) and mean hemoglobin levels of 93.6 g/L (SD 25.5). Six of the patients had previously used eculizumab, but had stopped eculizumab at least 2 years before starting tesidolumab.

Patients received intravenous tesidolumab every second week at a dose of 20 mg per kg of body weight, infused over approximately 2 hours in period 1 and over 40 minutes to 2 hours thereafter. This dose was selected based on modeling of PK and PD data from a prior phase I clinical study in healthy subjects (data on file). At the time of cutoff, the nine PNH patients were successfully treated with tesidolumab for an average of 405 days (range 210-505 days), and eight of nine patients had completed treatment periods 1 and 2 (day 365).

Tesidolumab therapy rapidly decreased LDH levels and sustained the decrease over the first year of therapy in all pa-

tients (Figure 2A). The mean relative LDH reduction from baseline was 79.2% (SD 8.9%) at week 4 (n=8) and 78.8% (SD 11.4%) at week 52 (n=8) (Figure 2B). LDH decline coincided with a meaningful decrease in transfusion requirement and increase in hemoglobin levels. Up until the cutoff, only three patients (one with variant C5) required red blood cell transfusions after initiation of tesidolumab, with an average of 1.5 units/year each (Figure 2C). Concomitantly, mean hemoglobin levels increased from 93.6 g/L (SD 25.5) at baseline to 112.8 g/L (SD 24.7) at week 52, with two patients achieving hemoglobin levels of >120 g/L (Figure 2D). With the exception of reticulocytes, other markers of

Table 1. Patient characteristics and adverse events.

Patient	Age (years)	Sex	Race	C5 status	Weight (kg)	BMI (kg/m^2)	Units transfused in last year	Lactate dehydrogenase (U/L)	Hemoglobin (g/L)
A	36	Male	Caucasian	Non-variant	91.0	29.7	11	2084	87.0
B	42	Female	Caucasian	Non-variant	68.0	25.6	0	714	120.0
C	45	Male	Asian	Variant	75.8	24.8	30	1690	145.0
D	66	Female	Asian	Variant	46.8	22.3	20	938	80.0
E	35	Female	Asian	Variant	63.9	21.9	0	887	87.0
F	27	Female	Asian	Non-variant	56.5	21	8	1368	91.0
G	36	Male	Asian	Variant	67.1	24.1	12	1937	69.0
H	45	Male	Asian	Variant	99.9	31.9	0	776	63.0
I	39	Male	Caucasian	Non-variant	108.0	31.6	0	1018	100.5
Mean	41.2				75.2	25.9	9	1268	93.6

Adverse events	Tesidolumab 20 mg/kg N=9 nE, nS (%)	Preferred term	Tesidolumab 20 mg/kg. N=9, nS (%)	Preferred term	Tesidolumab 20 mg/kg. N=9, nS (%)
Total AE	73, 7 (77.8)	Any AE	7 (77.8)	Serious AE	
AE of mild intensity	59, 7 (77.8)	Headache	4 (44.4)	Enterocolitis viral	1 (11.1)
		Nasopharyngitis	4 (44.4)		
AE of moderate intensity	12, 6 (66.7)	Nausea	3 (33.3)	Infection	1 (11.1)
		Abdominal pain upper	2 (22.2)	Study drug-related AE	
AE of severe intensity	2, 1 (11.1)	Back pain	2 (22.2)	Headache	3 (33.3)
				Migraine	2 (22.2)
Study drug-related AE	17, 4 (44.4)	Blood creatine phosphokinase increased	2 (22.2)	Neutropenia	2 (22.2)
Serious AE	2, 2 (22.2)	Cystitis	2 (22.2)	Atrioventricular block first degree	1 (11.1)
AE leading to discontinuation of study treatment	0	Migraine	2 (22.2)		
		Neutropenia	2 (22.2)	Enterocolitis viral	1 (11.1)
		Oedema	2 (22.2)	Leukopenia	1 (11.1)
Study drug-related AE leading to study discontinuation	0			Migraine with aura	1 (11.1)
				Nausea	1 (11.1)
				Sinus bradycardia	1 (11.1)

nE: number of events; nS: number of subjects; AE: adverse events; BMI: body mass index.

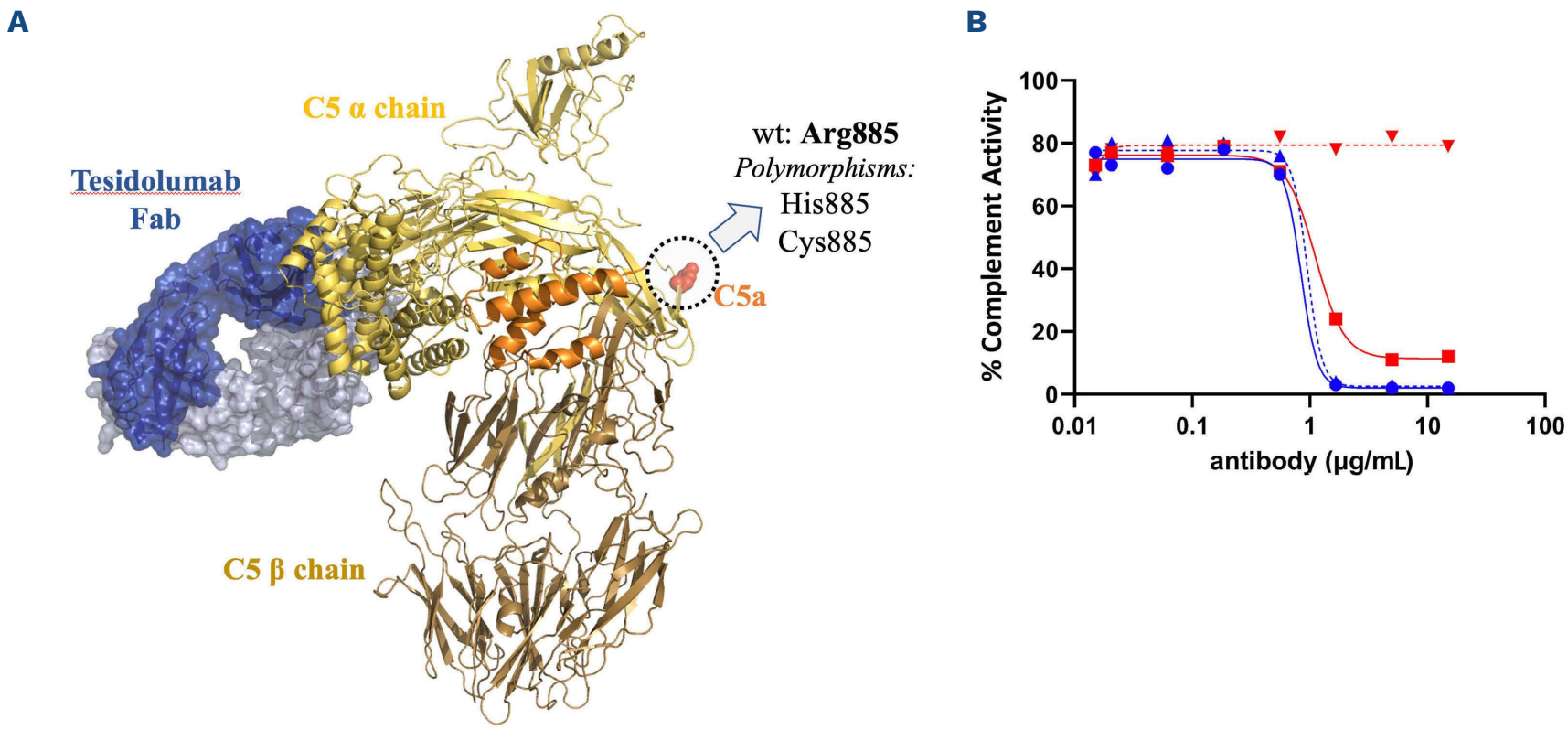

Figure 1. Crystallographic structure of the complex between complement protein 5 (C5) and tesidolumab Fab and *in vitro* activity on non-variant C5 and variant C5. (A) Human C5 (CompTech) was mixed with a 2.5-fold molar excess of tesidolumab Fab. The complex was purified by size exclusion chromatography and crystallized by vapor diffusion. Crystals diffracting to 3.5Å resolution were grown at 292 K from 0.07 M sodium acetate pH 4.6, 5.6% polyethylene glycol 4,000, 30% glycerol. A full diffraction data set was measured at the Swiss Light Source (Villigen), with a Pilatus pixel detector. The crystals were in space group $P4_3$ with one C5 complex per asymmetric unit. The structure was determined by molecular replacement, using a published C5 structure (PDB entry 3CU7) and a high-resolution structure of the tesidolumab Fab determined in house, and refined using standard crystallographic methods. Highlighted to the right is the variant location. (B) Inhibition of non-variant C5 (solid curves) and variant C5 (hatched curves) by tesidolumab (blue) or eculizumab (red). Tesidolumab blocks both C5 types, whereas variant C5 is resistant to eculizumab. The alternative pathway Wieslab Assay (EuroDiagnostica, Malmö, Sweden) was performed according to the manufacturer's instructions using human C5-depleted serum (CompTech, Tyler, TX) spiked with 7 µg/mL non-variant or variant C5 (CompTech, Tyler, TX, or Novartis in-house production). Tesidolumab or eculizumab were tested at concentrations ranging from 0.02-15 µg/mL. Percent complement activity was calculated using the formula: (sample – negative control)/(positive control – negative control) x100.

hemolysis showed similar improvements (data not shown). Free hemoglobin, which averaged 411 mg/L (SD 224 mg/L) at baseline (upper limit of detection was 450 mg/L), was reduced to 153 mg/L (SD 66 mg/L). Total bilirubin decreased from 19.4 µmol/L (SD 10.1 µmol/L) at baseline to 16.4 µmol/L (SD 9.1 µmol/L) at week 52. Type III erythrocytes increased from 29.5% (SD 10.5%) at baseline to 42.5% (SD 18.5%) at the end of year 1. Reticulocytes were at $129x10^9$/L (SD $71x10^9$/L) at baseline and remained elevated during tesidolumab therapy ($139x10^9$/L [SD $71x10^9$/L] at week 52).

Trough concentrations of tesidolumab increased slightly over time from 134 µg/mL (SD 31 µg/mL) after the first dose to 184 µg/mL (SD 32 µg/mL) at week 4 and 264 µg/mL (± 72 µg/mL) at week 52 (Figure 2E). Tesidolumab efficiently suppressed terminal complement activity. Soluble C5b-9 was reduced from 138 ng/mL at baseline (SD 62 ng/mL) to 40 ng/mL (SD 17 ng/mL) at 4 weeks and 64 ng/mL ± 9 ng/mL after 1 year of therapy (*data not shown*). *Ex vivo* CH50 assays showed a reduction of complement activity from 115 µg eq/mL (SD 35 µg eq/mL) at baseline to 1 µg eq/mL (SD 1 µg eq/mL) at week 4 and stabilized at about 2 µg eq/mL (range 1-3 µg eq/mL) at week 52 (*data not shown*).

Importantly, tesidolumab treatment improved quality of life (QoL) in all patients, as measured by the Functional Assessment of Chronic Illness Therapy (FACIT) fatigue score,[11] the mean of which increased from 38.6 (SD 10.0) at baseline to 44.0 (SD 9.1) at week 4 and to 46.9 (SD 6.5) at week 52 (Figure 2F).

Adverse events are presented in Table 1. Overall, tesidolumab was well tolerated. Seven of nine patients reported adverse events, the majority of which were mild. Two serious adverse events were reported, one acute infection of moderate severity on day 41 deemed unrelated to the study drug, the other a viral enterocolitis classified as severe and suspected to be related to the study drug. Both individuals responded well to therapy and findings resolved without complications. The most frequent study drug-related adverse events (preferred terms) were headache (n=3), migraine (n=2), and neutropenia (n=2). No thromboembolic events were observed throughout the study (*data not shown*), and D-dimer levels were reduced from 0.37 FEU/L (SD 0.29 FEU/L) at baseline to 0.20 FEU/L (SD 0.05 FEU/L) at week 52. In addition, tesidolumab had no apparent effect on platelet counts and renal function (eGFR) was stable in all patients.

In summary, tesidolumab had a favorable safety profile and

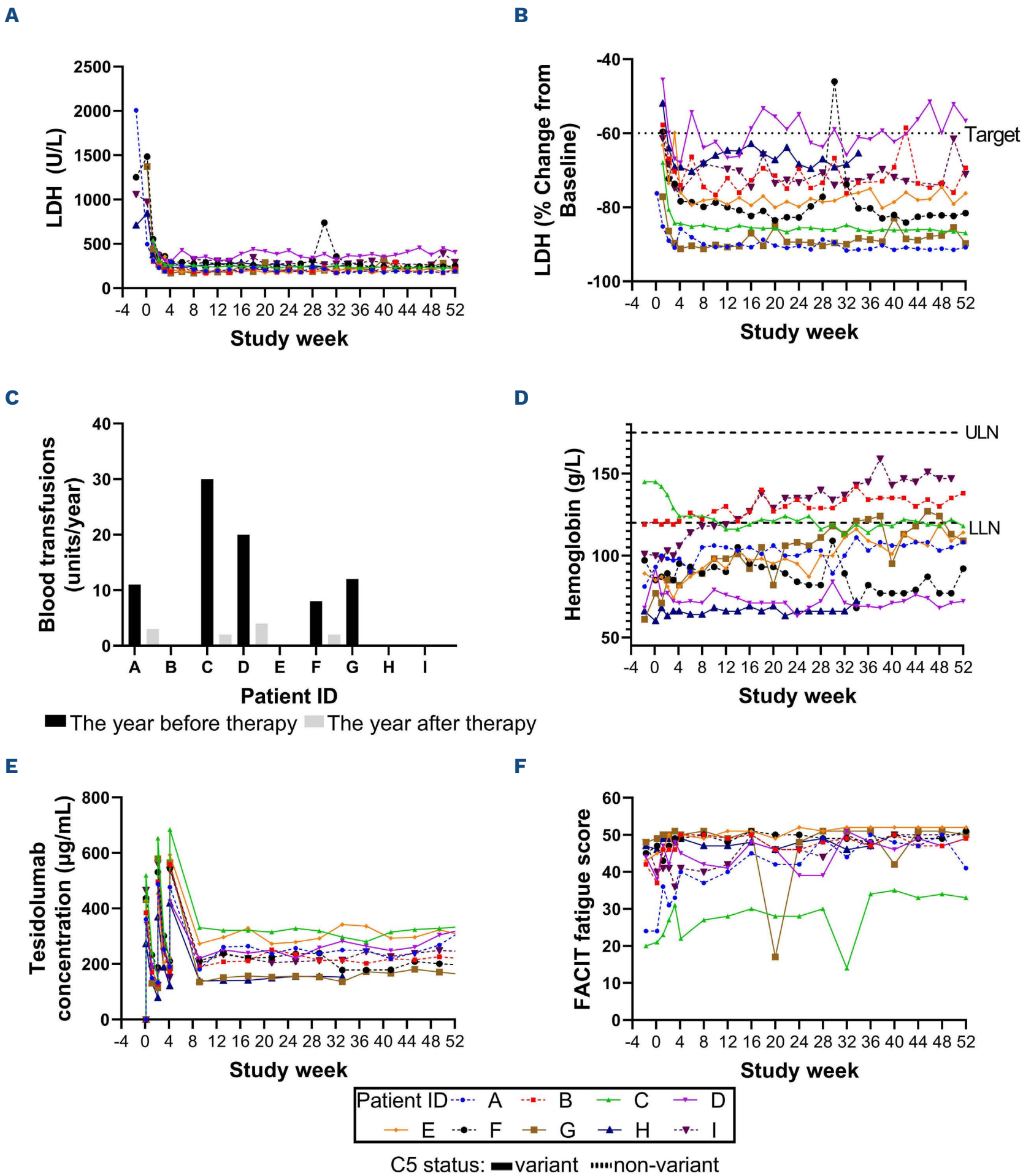

Figure 2. Key clinical endpoints. (A) Tesidolumab treatment reduced LDH in all patients. The spike in lactate dehydrogenase(LDH) after week 30 in subject F coincided with an episode of viral enterocolitis (reported as serious adverse event [SAE]) causing a 1-week delay of tesidolumab infusion. (B) Tesidolumab treatment reduced LDH levels by an average of about 75% compared to baseline. (C) Tesidolumab treatment reduced erythrocyte transfusion need compared to the year before therapy. Blood transfusions (units) were counted starting 12 months prior to the screening visit until the cutoff date and calculated as annual rate (units/year) before (■) and after (■) the start of tesidolumab treatment. Two patients given transfusions after the first dose of tesidolumab received erythrocyte units 1-2 months after initiation of therapy. The third individual was transfused 7 months after start of therapy. No further blood transfusions were given. (D) Tesidolumab treatment increased hemoglobin levels in both C5-variant and non-variant PNH patients, but only 2 patients reached hemoglobin levels observed for healthy individuals (>120 g/L). (E) Tesidolumab concentration in blood. Shown are peak and pre-dose levels for the first three treatments, followed by pre-dose levels only until the end of the study, Functional Assessment of Chronic Illness Therapy (FACIT) score (F) improved during the course of the study. LLOQ: lower level of quantification; ULN: upper limit of normal; LLN: lower limit of normal.

was efficacious for PNH patients with either variant or non-variant C5. Significant decreases of transfusion dependency and reductions of LDH concentrations to near normal levels were observed in all patients. As observed for eculizumab,[12,13] there was evidence of residual extravascular hemolysis. Reticulocyte counts and bilirubin levels remained elevated throughout treatment. Further, a proportion of type III red blood cells were coated with C3 fragments suggesting residual proximal complement pathway activation. These cells are expected to be susceptible to extravascular hemolysis as demonstrated by the lack of hemoglobin normalization in patients undergoing anti-C5 therapy. Thus, additional therapy may be necessary to achieve an optimal response in these patients.

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Disclosures

The following authors were employees at Novartis at the time of the study: CB, FM, PG, IR, AS, JMR, MR, IS, MTK, LJ, MB, YW, RD, RC and BH. Novartis has produced tesidolumab for commercial purposes for patients with complement-driven disorders. The academic authors have the following to disclose: YK received research funding by Novartis, Chugai and Alexion, is a member of advisory committees for Chugai and Alexion, speaker bureau for Alexion and consults for Chugai. JN received research funding from Novartis, Chugai and Alexion, is a member of advisory committees for Alexion and Chugai, speaker bureau for Alexion. YI is on the speaker bureau for Alexion Pharma LLC, Jansen Pharmaceutical KK and Eisai KK. LG receieved research funding by Novartis. MM, JM, HN and KA have non conflicts of interest to disclose. Novartis is committed to sharing with qualified external researchers, access to patient-level data, and supporting the clinical documents from eligible studies. These requests are reviewed and approved by an independent review panel on the basis of scientific merit. All data provided are anonymized to respect the privacy of patients who have participated in the trial in line with applicable laws and regulations. The availability of this trial data is according to the criteria and process described on www.clinicalstudydatarequest.com.

Contributions

YK and JN co-designed the study together with Novartis and were co-authors of all aspects of this paper; KA, MM, HN, YI, JM and LG were principal investigators that closely monitored their patients; IS, MR and MTK initiated the identification of tesidolumab, and progressed its development through engineering, characterization, selection, and preclinical toxicology studies; MR conceived the idea of testing tesidolumab in the C5-variant PNH patients; LJ was responsible for the generation of variant C5 reagents and in vitro analytical data comparing tesidolumab and eculizumab on variant C5; BH, CB, FM and PG co-designed the study and BH and RC were the global Novartis Medical Leads of the study; YW was the Novartis Medical Lead in Japan; IR was the Novartis Clinical Trial Lead. MB designed and analyzed the pharmacokinetic parts of the study; AS was the Novartis Research Lead for tesidolumab; JMR did the crystallographic analysis of tesidolumab; RD was the statistical expert of the study; AS, MR, RC, BH and IS wrote the manuscript and all authors reviewed it and contributed to its finalization.

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Data-sharing statement

Novartis is committed to sharing with qualified external researchers, access to patient-level data and supporting clinical documents from eligible studies. These requests are reviewed and approved by an independent review panel on the basis of scientific merit. All data provided are anonymized to respect the privacy of patients who have participated in the trial in line with applicable laws and regulations. The availability of this trial data is according to the criteria and process described on www.clinicalstudydatarequest.com.

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