Supplementary Appendix

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

Supplement to: Balwani M, Sardh E, Ventura P, et al. Phase 3 trial of RNAi therapeutic givosiran for acute intermittent porphyria. N Engl J Med 2020;382:2289-301. DOI: 10.1056/NEJMoa1913147

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ADDITIONAL METHODOLOGIC DETAILS

Details of the Primary Endpoint and Definition of Porphyria Attacks

The primary endpoint of the trial was the annualized rate of composite porphyria attacks (AAR) in patients with acute intermittent porphyria (AIP) over the 6-month intervention period. Composite porphyria attacks were those attacks requiring hospitalization, urgent healthcare visit or IV hemin administration at home. The AAR was calculated as the total number of qualifying porphyria attacks, divided by the total number of days in the intervention period, multiplied by the number of days in a year:

$$\text{AAR} = \frac{\text{Total number of qualifying porphyria attacks}}{\text{Total number of days in the intervention period}} \times 365.25$$

Mean AAR was calculated using a negative binomial regression model. The logarithm of the amount of time that each patient spends in the 6-month double-blind period was included in the model as an offset variable. With the offset variable, the negative binomial regression model essentially modeled AAR for each patient based on the length of time in the treatment period, and hence was able to account for the different lengths of follow-up time. Median AAR was calculated from individual patients' AAR.

Definition of Any Porphyria Attack

Porphyria attacks were defined as meeting all the criteria below:

- An acute episode of neurovisceral pain in the abdomen, back, chest, extremities and/or limbs.
- No other medically determined cause other than an attack related to AHP.
- Required treatment with IV glucose or hemin, carbohydrates, or analgesics (opioid or non-opioid), or other medications such as antiemetics at a dose or frequency beyond the patient's usual daily porphyria management.

The investigative site was notified of all potential AHP attacks directly by the patient or caregiver via an eDiary, telephone or email. In addition, other healthcare providers not part of the study could notify the Investigator or site about potential AHP attacks. The Investigator then confirmed whether the potential attack fulfilled the protocol definition of a porphyria attack after reviewing complete data, which could include discussions with the patient and the covering hospital physician, as well as a review of medical records and discharge summaries. Events not confirmed as porphyria attacks were reported as AEs or other with the reason provided (e.g., duplicate reporting via eDiary etc.).

The following definitions were used to identify each non-overlapping type of porphyria attack. Composite porphyria attacks, the primary and secondary endpoint of this study, were comprised of the first three porphyria attack types. All porphyria attack results presented represent composite porphyria attacks, unless otherwise noted:

1. AHP attacks requiring hospitalization: attacks that prompted a hospitalization, defined as an admission to an inpatient unit or a visit to an emergency department that results in an ≥24-hour stay.
2. AHP attacks requiring urgent healthcare visit: attacks that prompted urgent healthcare visits, defined as an urgent, unscheduled office/practice, infusion center, or an emergency department visit that did not meet the criteria for a hospitalization.
3. AHP attacks requiring IV hemin at home: attacks at home requiring IV hemin. “Home” was defined as any location that did not meet the criteria for a hospitalization or urgent healthcare visit.
4. AHP attacks at home not requiring IV hemin: attacks at home that did not require IV hemin but were treated with carbohydrates or analgesics (opioid or non-opioid) at a dose or frequency beyond the patient’s usual daily porphyria management. This type of attack was not included as part of the primary endpoint.

ALA and PBG Measurements

Delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) were measured in spot urine samples using a validated liquid chromatography/tandem mass spectrometry method at a central laboratory (Covance Inc., Salt Lake City, UT, USA). ALA and PBG levels were normalized to time-matched urine creatinine (Cr) concentrations and expressed as millimoles per mole of creatinine.

Porphyria Patient Experience Questionnaire

The Porphyria Patient Experience Questionnaire (PPEQ) is a custom questionnaire consisting of eight questions assessing quality of life measures, using a 5-point global rating of change scale. Items included in the questionnaire were not collected by other endpoints, but were known to affect patients with AHP.[1–3] Included in the PPEQ are five items that assess the change in the patients' ability to perform usual daily activities (travel, social activities, planning future events, household chores, and moderate exercise), two items that assess the patients' perceptions of their treatment (convenience and overall satisfaction), and one item that assesses the extent to which the study drug helped patients to return back to a more normal life.

Safety Assessments

Adverse events (AEs) were classified according to the *Medical Dictionary for Regulatory Activities* (MedDRA) System Organ Class and Preferred Terms. AEs were collected starting at the time the first dose of trial drug was administered (Trial Day 1) through the duration of the trial. AEs were graded based on their severity (mild, moderate, or severe) and the causal relationship to the trial drug. As porphyria attacks are recorded as an efficacy assessment of givosiran, these were not to be treated as AEs or serious AEs. However, if a patient experienced a non-porphyria AE during a porphyria attack, it was reported as an AE.

Antidrug Antibodies

Blood samples were collected to evaluate the presence of antidrug antibodies (ADA), as outlined in the protocol. A validated enzyme-linked immunosorbent assay method was used for the screening and confirmatory ADA assays. Serum samples were first analyzed with a screening assay, and those that tested positive for ADA were further evaluated in a confirmatory assay.

ADDITIONAL EFFICACY AND SAFETY RESULTS

ALA and PBG Levels at Baseline in Patients with AIP

At baseline, ALA and PBG levels were similar between givosiran and placebo groups:

- Median (interquartile range) of ALA
    - Givosiran-treated patients: 16.4 (9.4, 23.5) mmol/mol Cr
    - Placebo-treated patients: 15.7 (9.1, 24.6) mmol/mol Cr
- Median (interquartile range) of PBG
    - Givosiran-treated patients: 40.3 (33.6, 63.6) mmol/mol Cr
    - Placebo-treated patients: 42.6 (29.3, 63.8) mmol/mol Cr

These levels are markedly elevated above levels observed in normal healthy subjects:[2]

- Median (interquartile range) of ALA: 0.5 (0.2, 2.3) mmol/mol Cr
- Median (interquartile range) PBG: 0.02 (0.00, 0.22) mmol/mol Cr

Antidrug Antibodies

All patients had a baseline and ≥1 post-baseline sample for ADA evaluation. There was no treatment-induced ADA during the intervention period. One patient in the placebo group and two patients in the givosiran group tested positive for ADA at baseline. The presence of ADA at baseline had no impact on the pharmacokinetics, pharmacodynamics, or safety of givosiran during the intervention period.

Table S1. AHP Genotypes of Patients with AHP.

Mutation, n (%)	Placebo (N=46)	Givosiran (N=48)	Overall (N=94)
AIP with mutation in the *HMBS* gene	43 (93)	46 (96)	89 (95)
c.517C>T	6 (13)	6 (13)	12 (13)
c.673C>T	1 (2)	3 (6)	4 (4)
c.973C>T	1 (2)	3 (6)	4 (4)
c.445C>T	2 (4)	1 (2)	3 (3)
652-2delA	1 (2)	2 (4)	3 (3)
c.76C>T	1 (2)	2 (4)	3 (3)
c.346C>T	2 (4)	0 (0)	2 (2)
c.77G>A	1 (2)	1 (2)	2 (2)
c.839G>A	2 (4)	0 (0)	2 (2)
c.913-2A>G	1 (2)	1 (2)	2 (2)
c.982_983delCA	1 (2)	1 (2)	2 (2)
c.100C>T	0 (0)	1 (2)	1 (1)
c.1013T>C	1 (2)	0 (0)	1 (1)
c.1046_1046delA	1 (2)	0 (0)	1 (1)
c.138C>A	1 (2)	0 (0)	1 (1)
c.160+5G>A	1 (2)	0 (0)	1 (1)
c.161-1G>C	1 (2)	0 (0)	1 (1)
c.219_220delGA	1 (2)	0 (0)	1 (1)
c.323delT	0 (0)	1 (2)	1 (1)
c.331G>A	0 (0)	1 (2)	1 (1)
c.345-1G>A	1 (2)	0 (0)	1 (1)
c.361G>T	0 (0)	1 (2)	1 (1)

c.365C>A	0 (0)	1 (2)	1 (1)
c.384delA	1 (2)	0 (0)	1 (1)
c.418_419delAA	1 (2)	0 (0)	1 (1)
c.423-1G>A	1 (2)	0 (0)	1 (1)
c.457C>T	1 (2)	0 (0)	1 (1)
c.469_470delAA	0 (0)	1 (2)	1 (1)
c.499C>T	0 (0)	1 (2)	1 (1)
c.500G>A	1 (2)	0 (0)	1 (1)
c.503_504insT	0 (0)	1 (2)	1 (1)
c.518G>A; c.571G>C	0 (0)	1 (2)	1 (1)
c.530T>G	0 (0)	1 (2)	1 (1)
c.535delG	0 (0)	1 (2)	1 (1)
c.575G>A	1 (2)	0 (0)	1 (1)
c.593G>A	0 (0)	1 (2)	1 (1)
c.605dupAA	1 (2)	0 (0)	1 (1)
c.610C>T	1 (2)	0 (0)	1 (1)
c.613-2A>G	1 (2)	0 (0)	1 (1)
c.623_624delCT	1 (2)	0 (0)	1 (1)
c.652G>A	1 (2)	0 (0)	1 (1)
C.655G>C	0 (0)	1 (2)	1 (1)
c.713T>C	1 (2)	0 (0)	1 (1)
c.720_725delTCCCGA	1 (2)	0 (0)	1 (1)
c.724_743dup20	1 (2)	0 (0)	1 (1)
c.730_731delCT	0 (0)	1 (2)	1 (1)
c.771G>C	1 (2)	0 (0)	1 (1)
c.779G>A	0 (0)	1 (2)	1 (1)
c.823C>T	1 (2)	0 (0)	1 (1)

c.835_837delACTinsG	0 (0)	1 (2)	1 (1)
c.848G>A	0 (0)	1 (2)	1 (1)
c.874C>T	0 (0)	1 (2)	1 (1)
c.88-16_88-4del13insCA	0 (0)	1 (2)	1 (1)
c.88-2A>G	0 (0)	1 (2)	1 (1)
c.886C>T	0 (0)	1 (2)	1 (1)
c.905_906delCT	1 (2)	0 (0)	1 (1)
c.913-1G>A	0 (0)	1 (2)	1 (1)
c.97delA	0 (0)	1 (2)	1 (1)
c.992C>T	0 (0)	1 (2)	1 (1)
IVS4+5delG	0 (0)	1 (2)	1 (1)
IVS6-1G>C	0 (0)	1 (2)	1 (1)
Non-AIP	3 (7)	2 (4)	5 (5)
HCP with mutation in the *CPOX* gene	0 (0)	1 (2)	1 (1)
c.863T>G	0 (0)	1 (2)	1 (1)
VP with mutation in the *PPOX* gene	1 (2)	1 (2)	2 (2)
c.1330_1331delCT	0 (0)	1 (2)	1 (1)
c.309_310insT	1 (2)	0 (0)	1 (1)
AHP with a non-identified mutation	2 (4)	0 (0)	2 (2)

AHP, acute hepatic porphyria; AIP, acute intermittent porphyria; *CPOX*, coproporphyrinogen oxidase; HCP, hereditary coproporphyria; HMBS, hydroxymethylbilane synthase; *PPOX*, protoporphyrinogen oxidase; VP, variegate porphyria.

Table S2. Individual Patient Level Data for ALA and PBG levels, and AAR in Patients with AHP Types Other than AIP.

	Placebo (n=3)			Givosiran (n=2)	
	VP	**AHP***	**AHP***	**VP**	**HCP**
ALA, mmol/mol Cr					
Baseline	2.2	23.1	15.7	21.3	3.3
Month 6	2.2	12.1	17.1	0.6	0.7
PBG, mmol/mol Cr					
Baseline	1.5	42.5	15.7	35.3	0.4
Month 6	1.0	33.0	17.1	0.1	0.1
AAR during DB period	6.8	21.6	0	0	15.2

*Patients with AHP without an identified mutation; both patients were considered by the Investigator to have acute intermittent porphyria by biochemical analysis

AAR, annualized rate of composite porphyria attacks; AHP, acute hepatic porphyria; ALA, delta-aminolevulinic acid; DB, double-blind; HCP, hereditary coproporphyria; PBG, porphobilinogen; VP, variegate porphyria

Table S3. Serious Adverse Events in Patients with AHP.		
Event, n (%)	**Placebo (N=46)**	**Givosiran (N=48)**
Any serious adverse event*	4 (9)	10 (21)
Chronic kidney disease	0	2 (4)
Asthma	0	1 (2)
Device-related infection	2 (4)	1 (2)
Gastroenteritis	0	1 (2)
Hypoglycemia	0	1 (2)
Liver function test abnormal	0	1 (2)
Major depression	0	1 (2)
Pain management	0	1 (2)
Pyrexia	1 (2)	1 (2)
Escherichia urinary tract infection	1 (2)	0
Fractured sacrum	1 (2)	0
Sepsis	1 (2)	0
Septic shock	1 (2)	0

*Two serious adverse events (SAEs) in givosiran-treated patients reported as study drug related: one SAE of chronic kidney disease and one SAE of abnormal liver function test; no SAEs in placebo patients were reported as study drug related.

Table S4. Serum Aminotransferase Elevations in Patients with AHP.

N (%)	Placebo (N=46)	Givosiran (N=48)
Baseline		
ALT elevation >ULN and ≤3×ULN at baseline*	2 (4)	10 (21)
AST elevation >ULN and ≤3×ULN at baseline*	3 (7)	9 (19)
ALT or AST elevation >ULN and ≤3×ULN at baseline*	3 (7)	13 (27)
6-month double-blind period		
ALT elevation >ULN to ≤3×ULN	9 (20)	19 (40)
ALT elevation >3×ULN to ≤5×ULN	1 (2)	5 (10)
ALT elevation >5×ULN to ≤10×ULN	0	2 (4)
ALT elevation >10×ULN	0	0
ALT or AST >3×ULN with concurrent total bilirubin >2×ULN	0	0

*Defined as highest value of serum ALT or AST at screening.

AHP, acute hepatic porphyria; ALT, alanine aminotransferase; AST; aspartate aminotransferase; ULN, upper limit of normal.

Table S5. Summary of Hepatic Adverse Events in Patients with AHP.		
N (%)	**Placebo (N=46)**	**Givosiran (N=48)**
Any hepatic adverse event*	1 (2)	6 (13)
Investigations system organ class	1 (2)	6 (13)
Alanine aminotransferase increased	1 (2)	4 (8)
Aspartate aminotransferase increased	1 (2)	3 (6)
Blood bilirubin increased	1 (2)	0
Gamma-glutamyltransferase increased	0	1 (2)
Hepatic enzyme increased	0	1 (2)
Liver function test abnormal	0	1 (2)

*Hepatic adverse events include all adverse events selected according to MedDRA terms in the Drug-related hepatic disorders Standardized MedDRA Query, both narrow and broad terms.

AHP, acute hepatic porphyria.

Table S6. Summary of Renal Adverse Events in Patients with AHP.*

N (%)	Placebo (N=46)	Givosiran (N=48)
Any renal adverse event*	3 (7)	7 (15)
Investigations system organ class (SOC)	2 (4)	3 (6)
Blood creatinine increased	1 (2)	2 (4)
Blood sodium decreased	1 (2)	0
Blood urea increased	0	1 (2)
Glomerular filtration rate decreased	0	3 (6)
Metabolism and nutrition disorders SOC	1 (2)	0
Hyponatremia	1 (2)	0
Renal and urinary disorders SOC	1 (2)	5 (10)
Chronic kidney disease	0	5 (10)
Nephropathy	1 (2)	0

*Renal adverse events include all adverse events selected according to MedDRA terms in the Chronic kidney disease Standardized MedDRA Query, both narrow and broad terms.

AHP, acute hepatic porphyria; SOC, system organ class.

Table S7. eGFR and Creatinine Values for Givosiran-Treated Patients with Adverse Events of Chronic Kidney Disease

Patient	Pertinent renal medical history	Creatinine µmol/L (×ULN)				eGFR mL/min/1.73m^2			
		Screen	BL	Start of AE	Month 6	Screen	BL	Start of AE	Month 6
1	Renal impairment	118 (1.2)	128 (1.3)	168 (1.7)	117 (1.2)	46	42	31	47
2	Chronic kidney disease Hypertension	127 (1.3)	131 (1.3)	154 (1.6)	164 (1.7)	37	36	30	28
3*	Chronic kidney disease Hypertension	92 (0.9)	120 (1.2)	122 (1.2)	123 (1.2)	61	45	44	44
4*	Chronic kidney disease Hypertension Renal cyst Secondary Fanconi syndrome Nephrolithiasis	171 (1.3)	194 (1.5)	239 (1.9)	241 (1.9)	37	32	25	24
5	Hypertension	73 (0.7)	80 (0.8)	98 (1.0)	93 (0.9)	81	74	58	62

AE, adverse event; BL, baseline (prior to treatment with givosiran); eGFR, estimated glomerular filtration rate; ULN, upper limit of normal

*Patient 3 and Patient 4 underwent renal biopsies that were consistent with their underlying comorbidities.

Figure S1. Givosiran Structure.

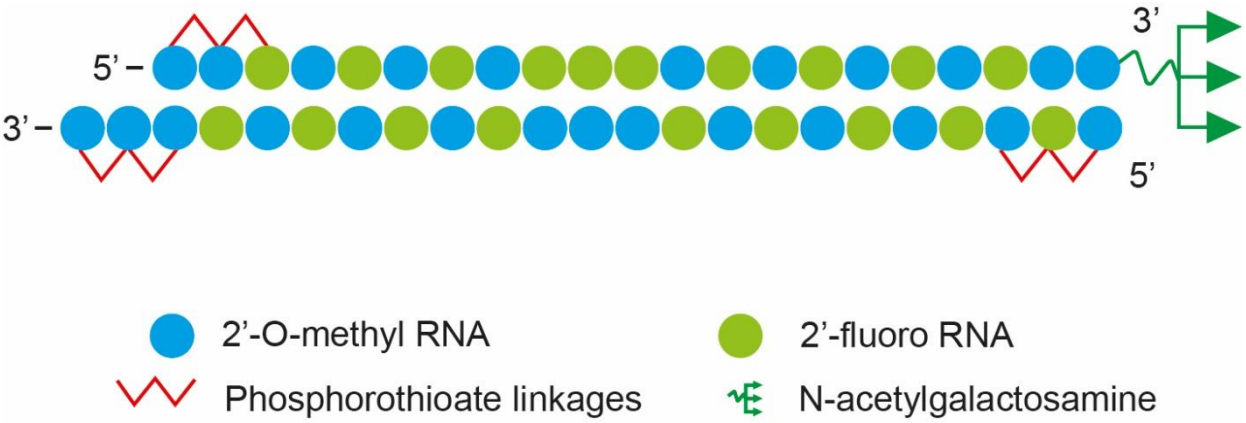


Figure S2. Patient Disposition of Patients with AHP.

*Patients with other AHP includes patients with hereditary coproporphyria, variegate porphyria, or without an identified AHP mutation.

AE, adverse event; AHP, acute hepatic porphyria; AIP, acute intermittent porphyria; LFT, liver function test.

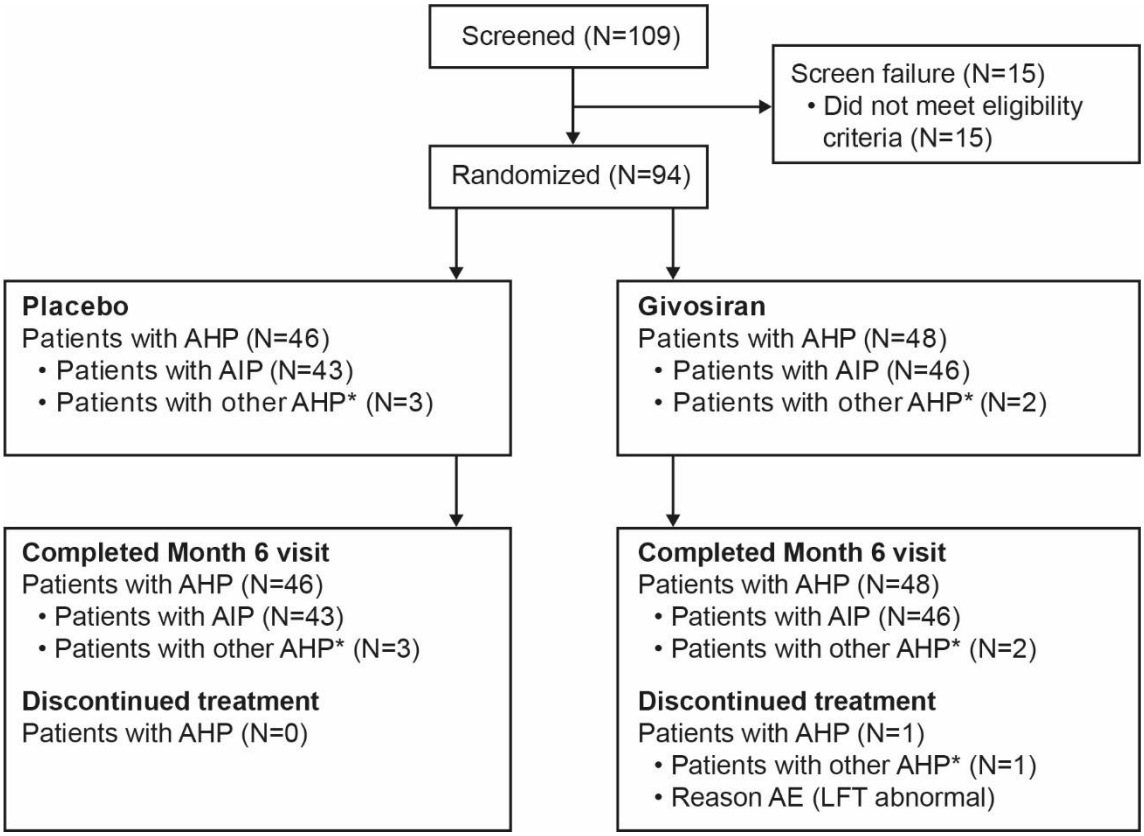


Figure S3. Mean Number of Attacks per Month in Patients with AIP.

The estimate at Month 0 represents the period between randomization and 6 months previous.

One month = 28 days.

AIP, acute intermittent porphyria.

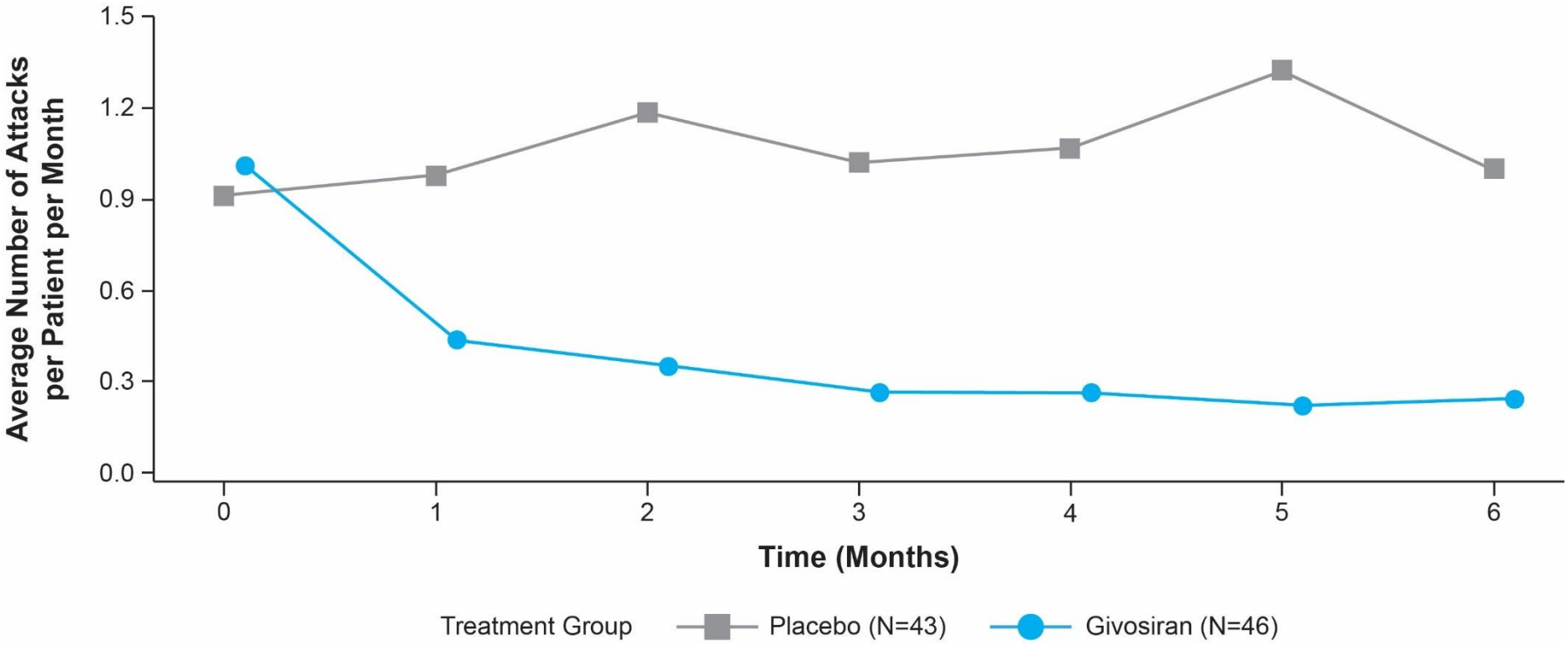


Figure S4. AAR in Individual Patients During 6-Month Double-Blind Period in Patients with AHP.

AAR, annualized rate of composite porphyria attacks; AHP, acute hepatic porphyria.

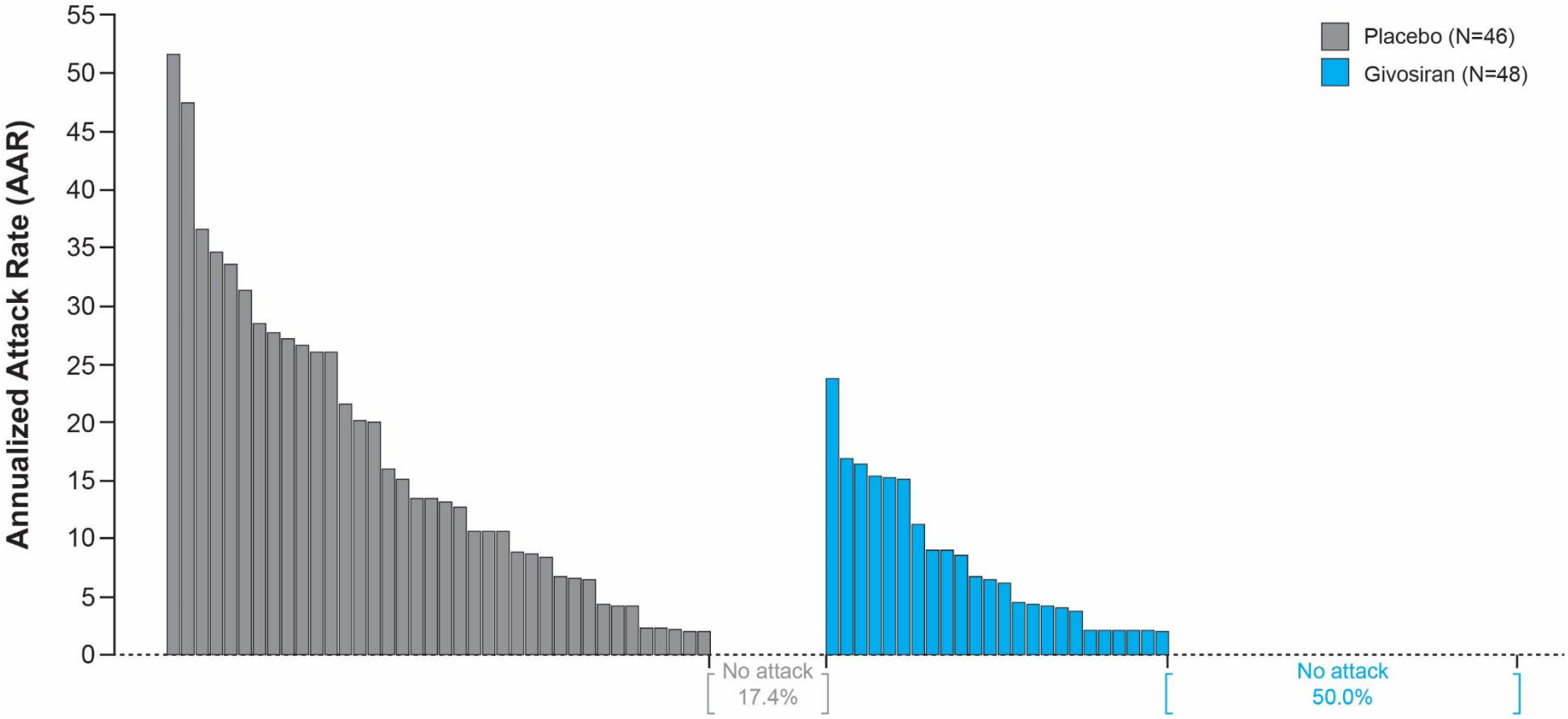


Figure S5. Pre-specified Subgroup Analysis of AAR in Patients with AIP.

AAR, annualized rate of composite porphyria attacks; AIP, acute intermittent porphyria; CI, confidence interval.

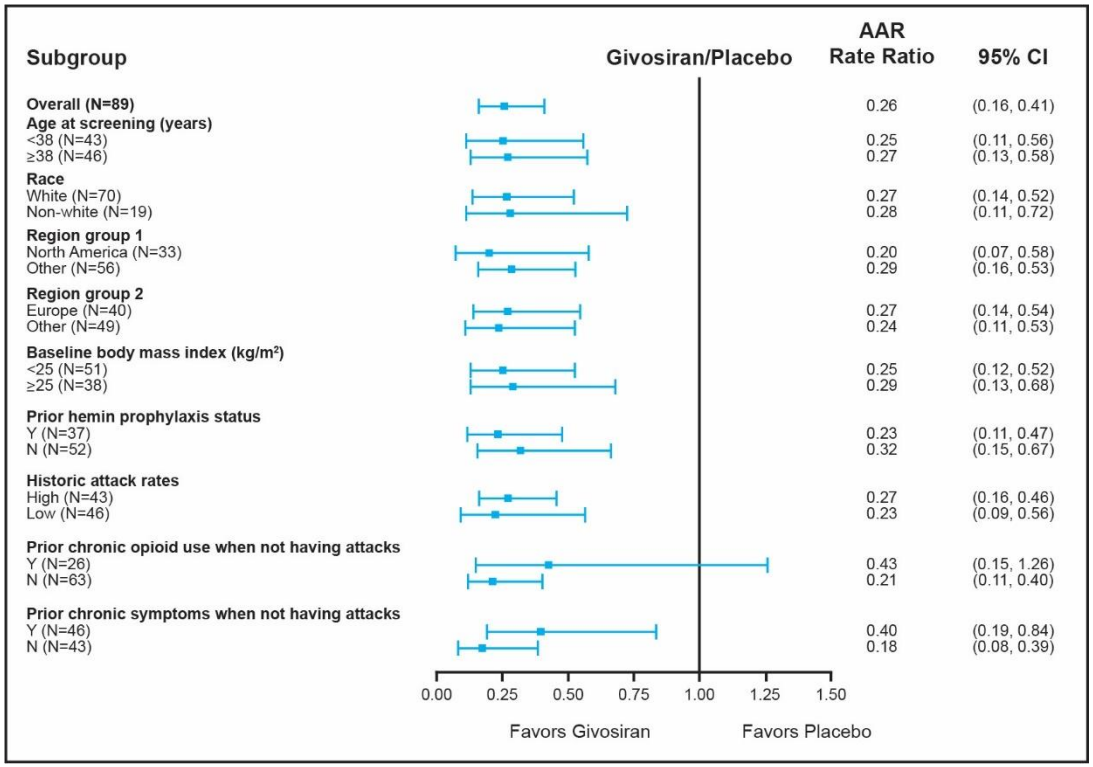


Figure S6. Median of Change from Baseline in Weekly Mean Score of Daily Worst Pain in Patients with AIP.

AIP, acute intermittent porphyria.

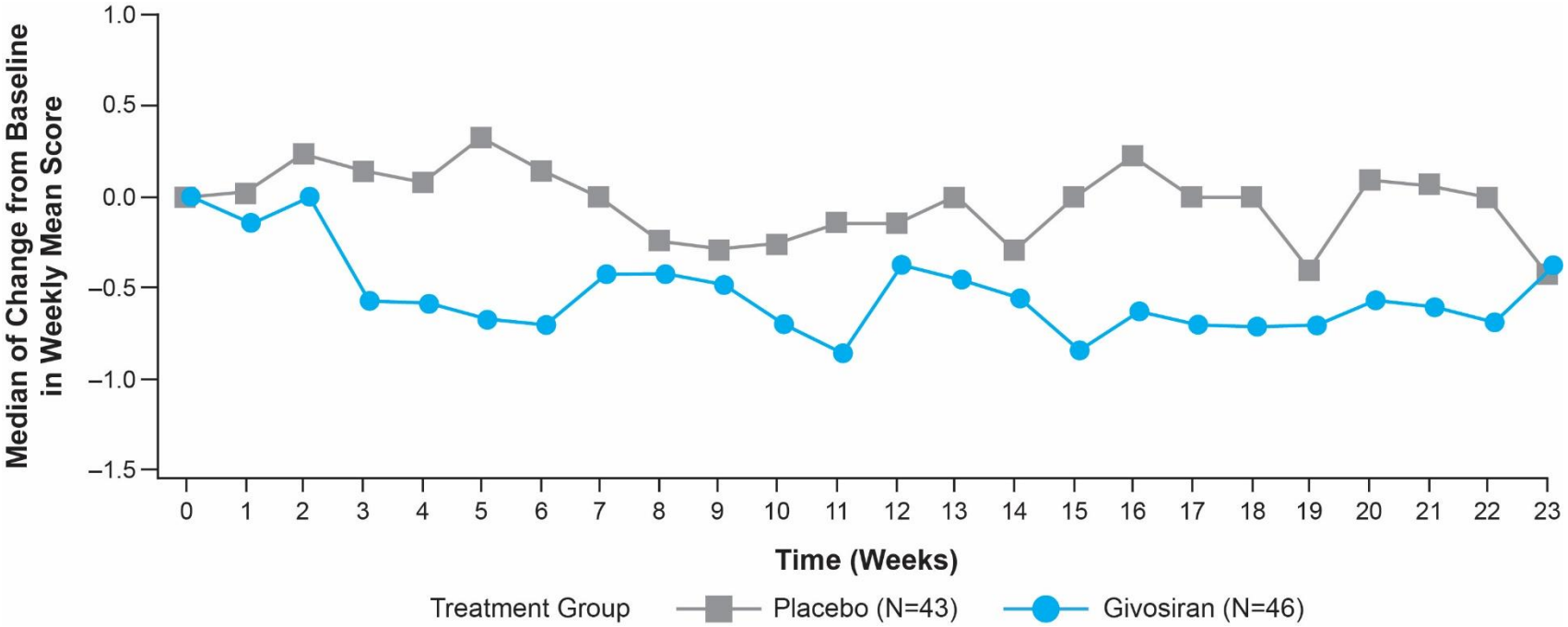


Figure S7. LS Mean Difference in the Change from Baseline to Month 6 in Short Form-12 Health Survey Domain Scores in Patients with AIP.

AIP, acute intermittent porphyria; CI, confidence interval; LS, least square.

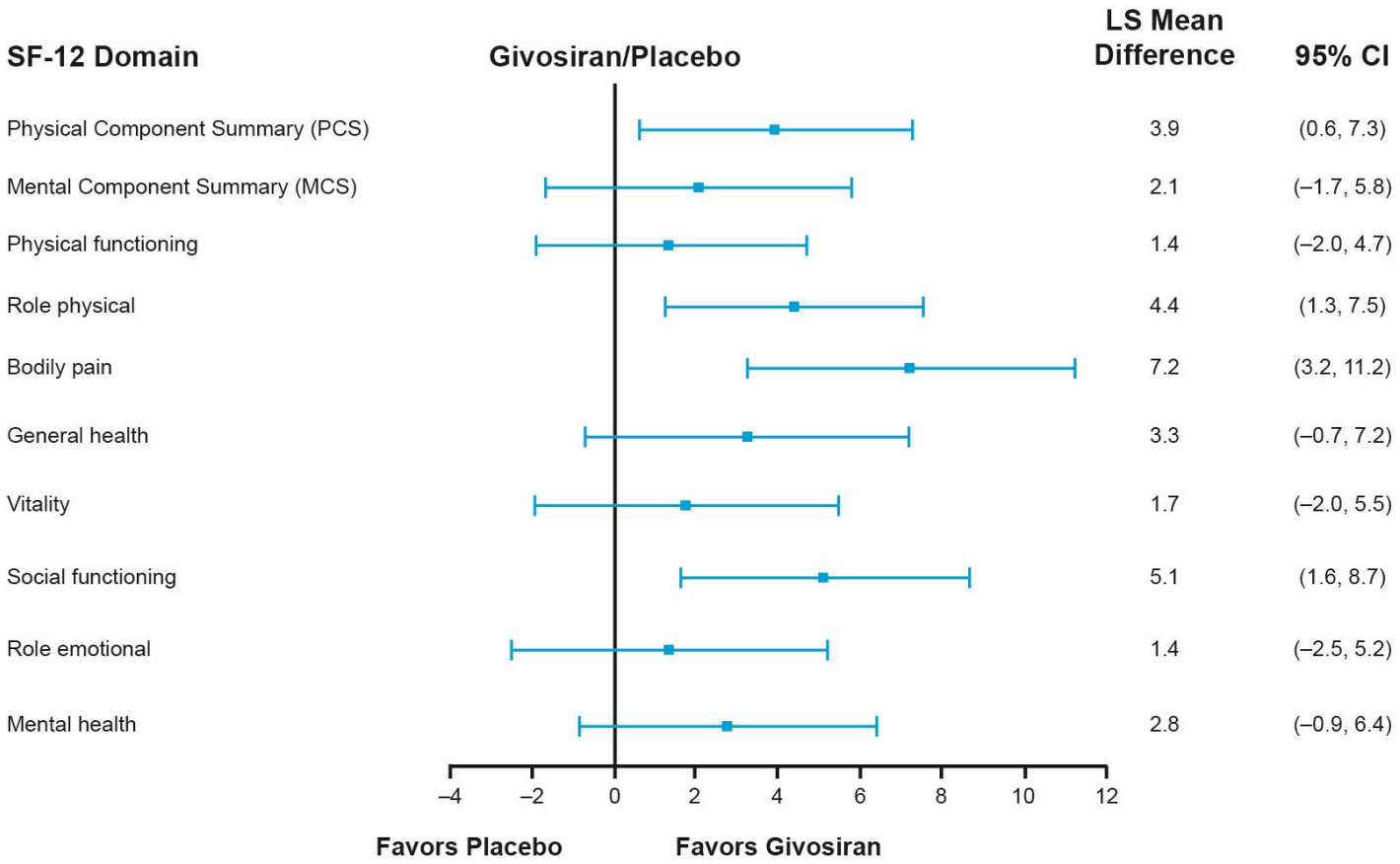


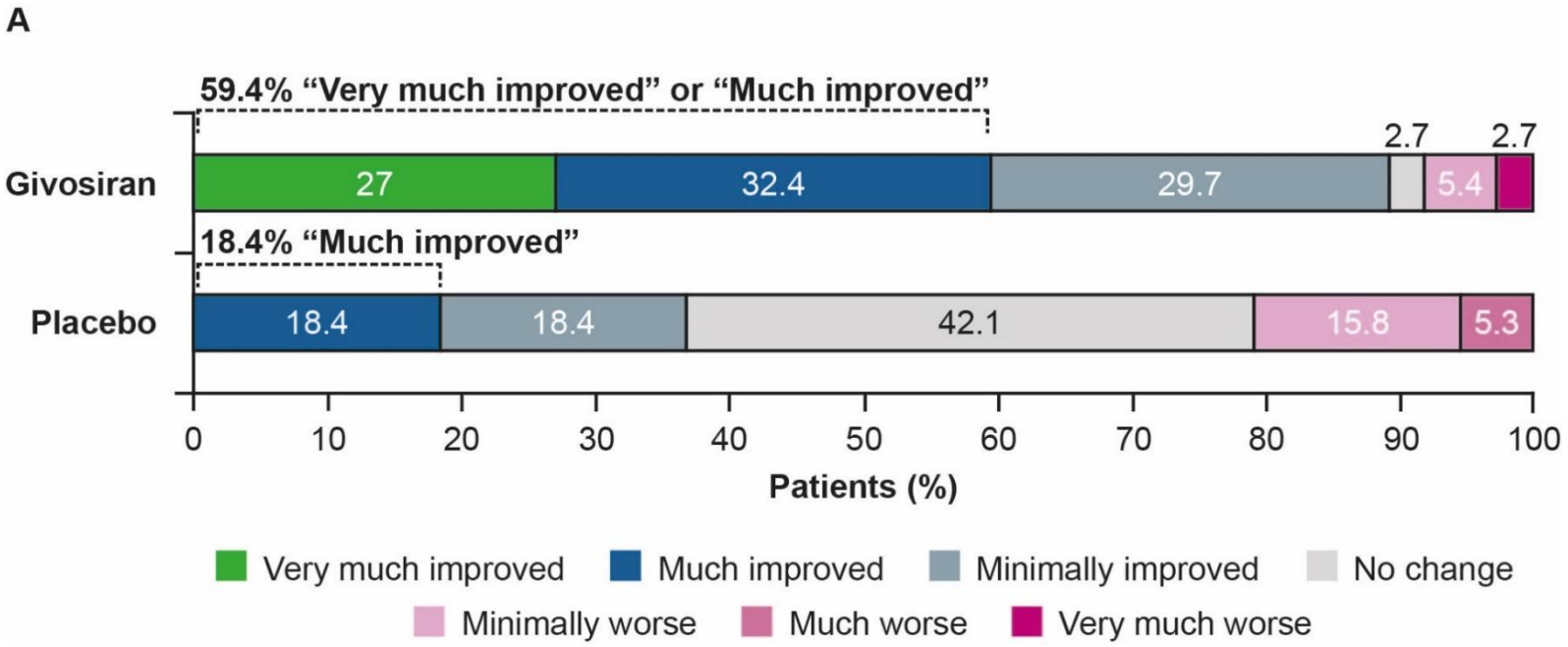
Figure S8. Patient Experience Questionnaires.

Patient Global Impression of Change (PGIC) questionnaire in patients with AHP at Month 6.

PGIC measures the patient's belief about the efficacy of treatment on a single item using a 7-point global rating of change scale which is anchored to "since the start of the study".

Results displayed are among those who responded (38 out of the 46 placebo patients and 37 out of the 48 givosiran patients); only one patient with AHP types other than AIP responded to the question.

AHP, acute hepatic porphyria.



Porphyria Patient Experience Questionnaire (PPEQ) in Patients with AHP at Month 6.

PPEQ is a custom-designed questionnaire assessing quality of life, activities of daily living, and treatment satisfaction.

Results are shown for patients who responded (37 of 46 placebo patients and 36 of 48 givosiran patients).

Presented are the percentage of patients with response “Much Better” (other options were “Minimally Better”, “No Change”, “Minimally Worse”, and “Much Worse”), except for "Study Drug Helping More Normal Life" category, which presents the percentage of patients with response “Always” or “Most of the time” (other options were “Sometimes”, “Rarely”, and “Never”).

AHP, acute hepatic porphyria.

B

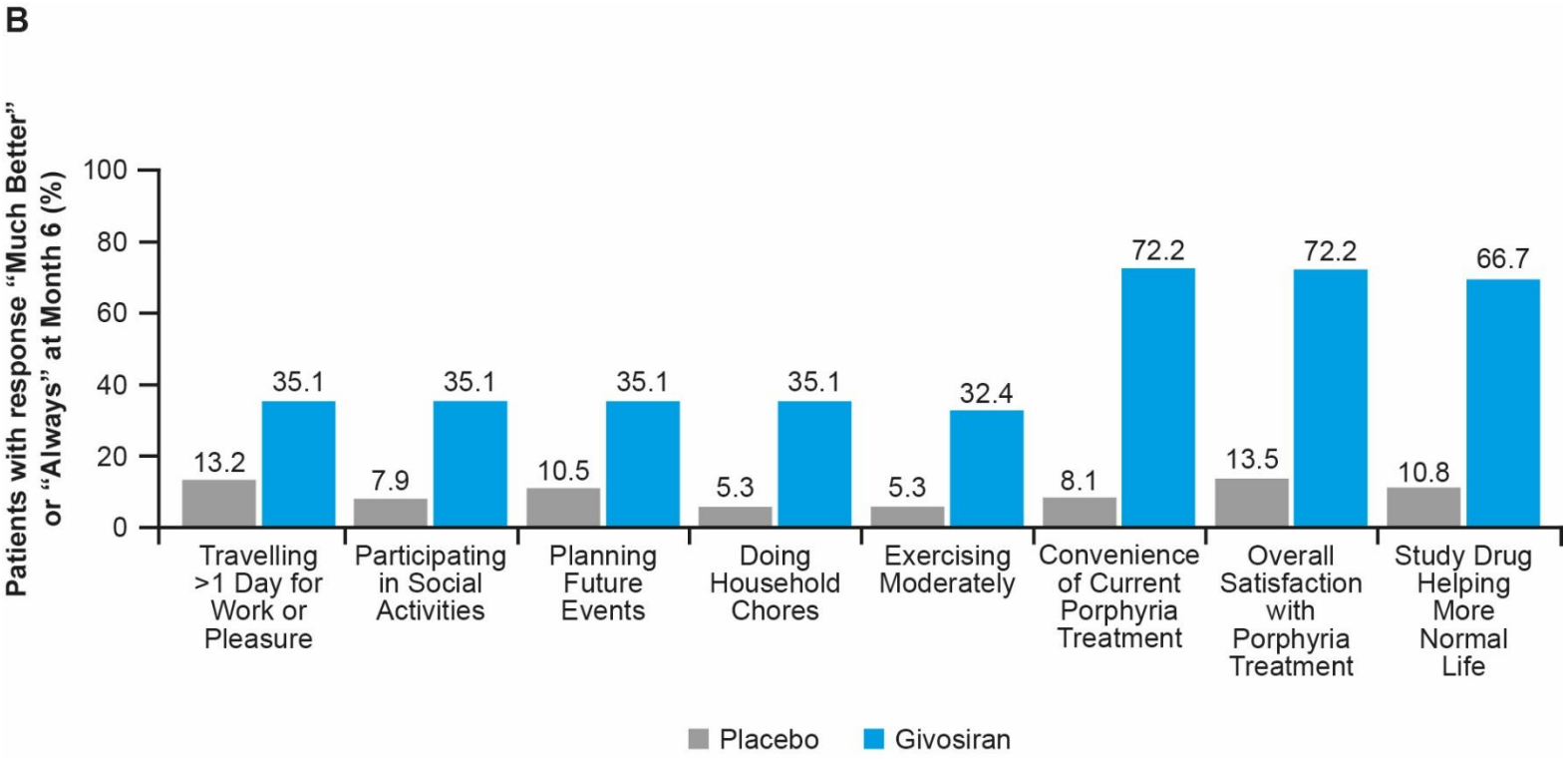
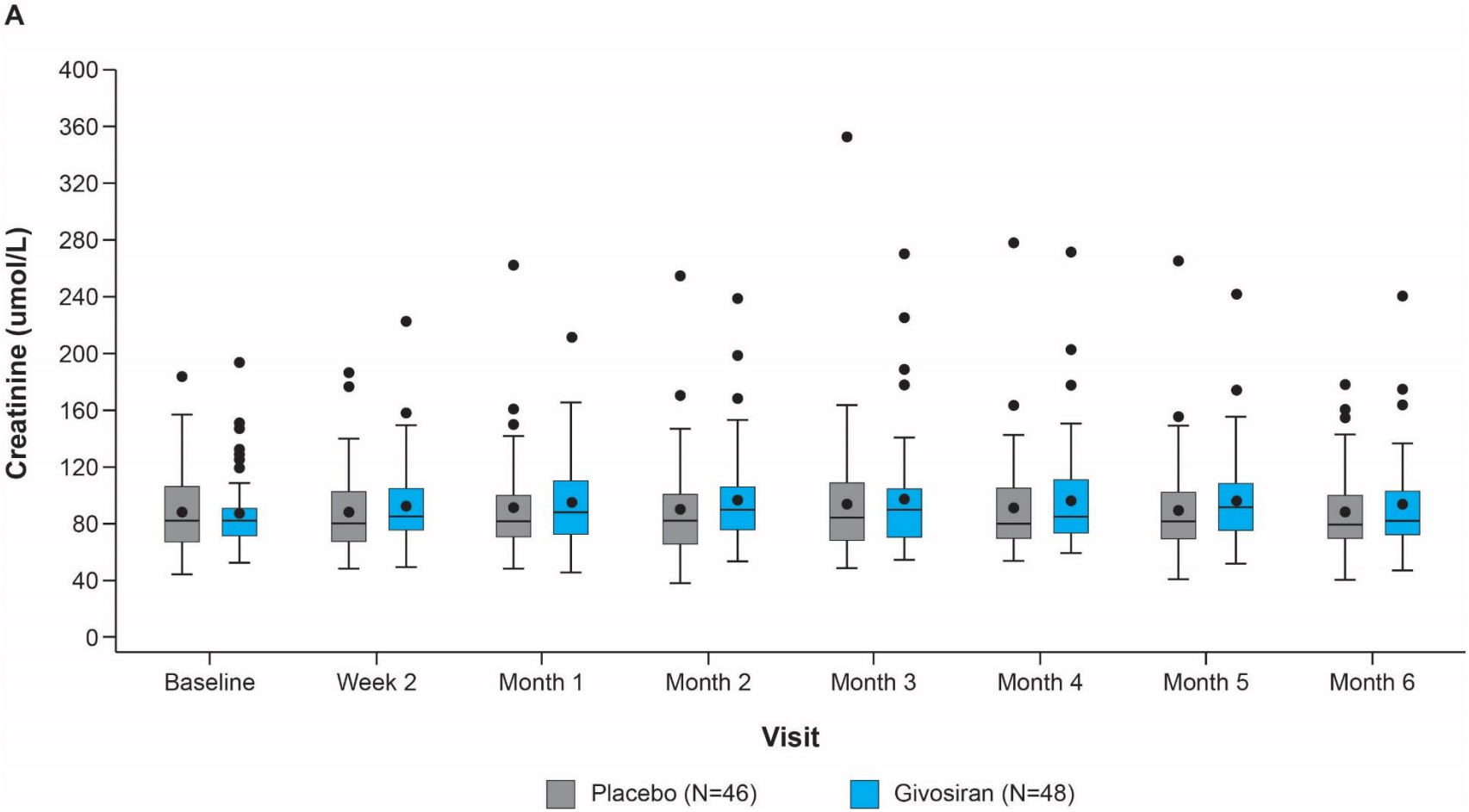

Patients with response "Much Better" or "Always" at Month 6 (%)
100
80
60
40
20
0
13.2
35.1
7.9
35.1
10.5
35.1
5.3
35.1
5.3
32.4
8.1
72.2
13.5
72.2
10.8
66.7
Travelling >1 Day for Work or Pleasure
Participating in Social Activities
Planning Future Events
Doing Household Chores
Exercising Moderately
Convenience of Current Porphyria Treatment
Overall Satisfaction with Porphyria Treatment
Study Drug Helping More Normal Life
Placebo
Givosiran

Figure S9. Renal Function in Patients with AHP.

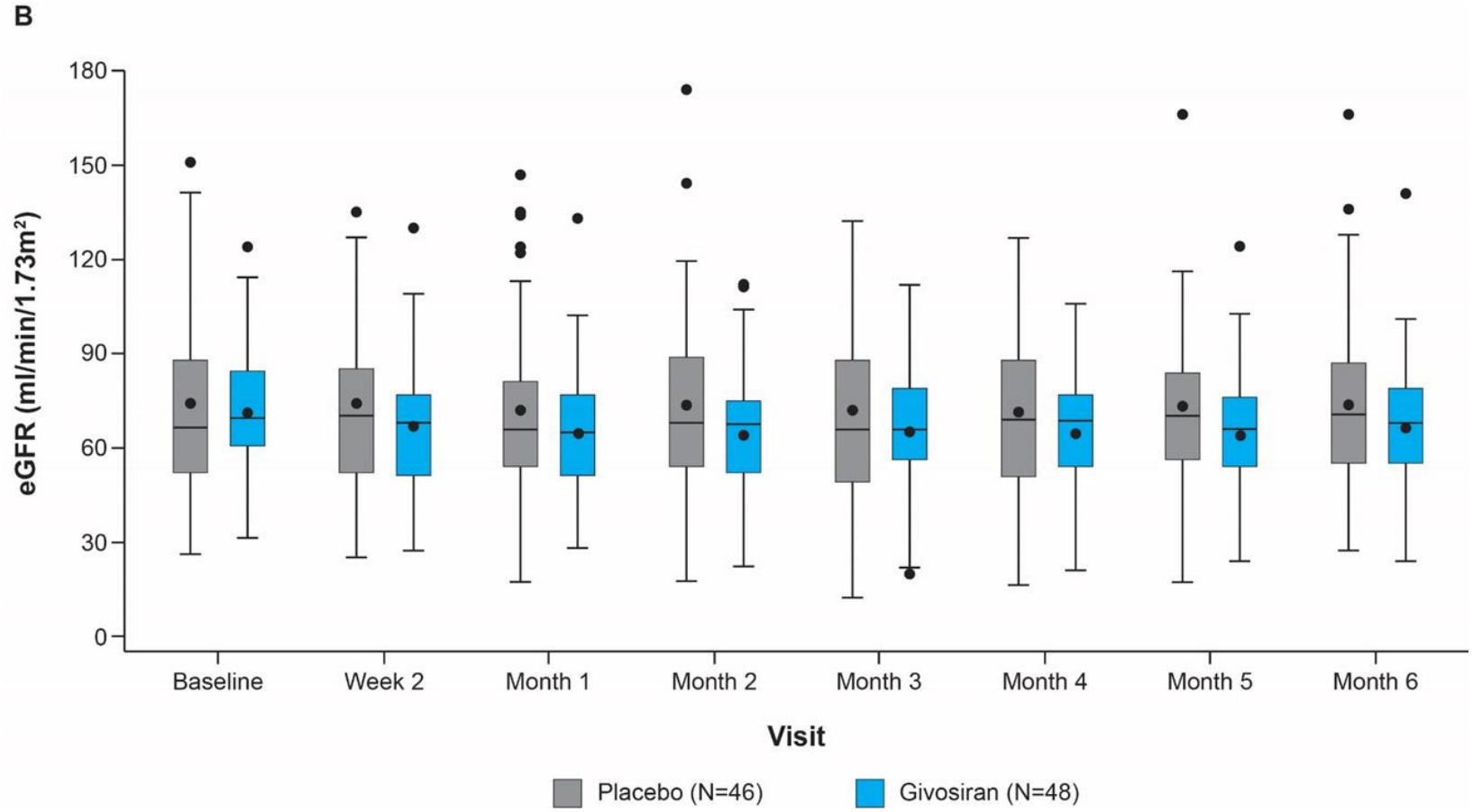
Creatinine (umol/L) Values Relative to ULN in Patients with AHP.

AHP, acute hepatic porphyria; ULN, upper limit of normal.



eGFR in Patients with AHP.

AHP, acute hepatic porphyria; eGFR, estimated glomerular filtration rate.



References

1. American Porphyria Foundation. The voice of the patient patient-focused drug development meeting acute porphyrias. 2017. Available from: https://www.fda.gov/media/130386/download. Accessed December 6, 2019.

2. Gouya L, Ventura P, Balwani M, et al. EXPLORE: a prospective, multinational, natural history study of patients with acute hepatic porphyria with recurrent attacks. Hepatology 2019;doi: 10.1002/hep.30936. [Epub ahead of print].

3. Simon A, Pompilus F, Querbes W, et al. Patient perspective on acute intermittent porphyria with frequent attacks: a disease with intermittent and chronic manifestations. Patient 2018;11:527-37.