

# PHARMACODYNAMICS

## The effect of LCZ696 (sacubitril/valsartan) on amyloid- $\beta$ concentrations in cerebrospinal fluid in healthy subjects

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### AIMS

LCZ696 (angiotensin receptor neprilysin inhibitor) is a novel drug developed for the treatment of heart failure with reduced ejection fraction. Neprilysin is one of multiple enzymes degrading amyloid- $\beta$  (A $\beta$ ). Its inhibition may increase A $\beta$  levels. The potential exists that treatment of LCZ696, through the inhibition of neprilysin by LBQ657 (an LCZ696 metabolite), may result in accumulation of A $\beta$ . The aim of this study was to assess the blood–brain-barrier penetration of LBQ657 and the potential effects of LCZ696 on cerebrospinal fluid (CSF) concentrations of A $\beta$  isoforms in healthy human volunteers.

### METHODS

In a double-blind, randomized, parallel group, placebo-controlled study, healthy subjects received once daily LCZ696 (400 mg,  $n = 21$ ) or placebo ( $n = 22$ ) for 14 days.

### RESULTS

LCZ696 had no significant effect on CSF AUUC(0,36 h) of the aggregable A $\beta$  species 1–42 or 1–40 compared with placebo (estimated treatment ratios 0.98 [95% CI 0.73, 1.34;  $P = 0.919$ ] and 1.05 [95% CI 0.82, 1.34;  $P = 0.702$ ], respectively). A 42% increase in CSF AUUC(0,36 h) of soluble A $\beta$  1–38 was observed (estimated treatment ratio 1.42 [95% CI 1.05, 1.91;  $P = 0.023$ ]). CSF levels of LBQ657 and CSF A $\beta$  1–42, 1–40, and 1–38 concentrations were not related ( $r^2$  values 0.022, 0.010, and 0.008, respectively).

### CONCLUSIONS

LCZ696 did not cause changes in CSF levels of aggregable A $\beta$  isoforms (1–42 and 1–40) compared with placebo, despite achieving CSF concentrations of LBQ657 sufficient to inhibit neprilysin. The clinical relevance of the increase in soluble CSF A $\beta$  1–38 is currently unknown.

## WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Neprilysin is one of multiple enzymes able to degrade amyloid- $\beta$  (A $\beta$ ); its inhibition may increase A $\beta$  levels.
- Aggregable A $\beta$  isoforms are known to accumulate in Alzheimer's disease.
- A theoretical and unproven potential exists that treatment with LCZ696 (angiotensin receptor neprilysin inhibitor) may result in the accumulation of A $\beta$  isoforms.

## WHAT THIS STUDY ADDS

- Once daily LCZ696 (400 mg) for 14 days does not cause changes in CSF levels of aggregable A $\beta$  isoforms 1–42 and 1–40 compared with placebo, despite achieving CSF concentrations sufficient to inhibit neprilysin. The clinical relevance of the increase in CSF A $\beta$  1–38 is unknown.

## Introduction

LCZ696 (sacubitril/valsartan) is the first-in-class angiotensin receptor neprilysin inhibitor (ARNI) developed for the treatment of heart failure (HF) with reduced ejection fraction. Oral administration of LCZ696 delivers systemic exposure to sacubitril (AHU377), which is further metabolized to LBQ657, and valsartan, providing simultaneous inhibition of neprilysin (by LBQ657) and blockade of the angiotensin II type 1 (AT<sub>1</sub>) receptor (by valsartan) [1]. The efficacy and safety of LCZ696 200 mg twice daily ( $n = 4187$ ) compared with enalapril 10 mg twice daily ( $n = 4212$ ) on mortality and morbidity in patients with HF with reduced ejection fraction was assessed in the PARADIGM-HF trial [2]. In this trial, LCZ696 200 mg twice daily significantly reduced the risk of cardiovascular (CV) death or hospitalization for HF compared with enalapril 10 mg twice daily (21.8% vs. 26.5%, respectively, hazard ratio [HR] 0.80, 95% confidence interval [CI] 0.73, 0.87;  $P = 0.0000002$ ), was superior to enalapril in reducing the risk of death from any cause and decreased HF symptoms and physical limitations [2]. The benefits of LCZ696 in patients with HF are thought to result from the enhanced activity of protective endogenous neprilysin substrates, such as natriuretic peptides, and the simultaneous inhibition of organ injury driven by sustained activation of the renin-angiotensin-aldosterone system [3].

Amyloid- $\beta$  (A $\beta$ ) is generated in the brain through sequential cleavage of amyloid precursor protein (APP) by  $\beta$ - and  $\gamma$ -secretases [4]. A $\beta$  is removed from the brain by multiple processes, including transport into cerebrospinal fluid (CSF) and the bloodstream, and enzymatic degradation [5]. *In vitro* and non-clinical studies suggest that neprilysin is one of multiple enzymes involved in the proteolytic degradation of A $\beta$  [6–8]. Other proteases with A $\beta$ -degrading properties include insulin degrading enzyme, endothelin converting enzyme, angiotensin converting enzyme, thimet oligopeptidase and plasmin [9–11]. The relative contribution of individual enzymes to the proteolytic degradation of A $\beta$  remains unknown. The potential exists that treatment with LCZ696, through inhibition of neprilysin by LBQ657, may result in accumulation of A $\beta$  species such as A $\beta$  1–42, 1–40 and 1–38. Senile plaques composed of aggregation-prone A $\beta$  subtypes (e.g. A $\beta$  1–42 and A $\beta$  1–40) are found in the brain of patients with Alzheimer's disease (AD) [12–14]. However the role of A $\beta$  in the pathophysiology of AD is not conclusively defined [15].

The blood–brain-barrier (BBB) penetration of LBQ657 and the potential effects of LCZ696 on CSF concentrations of A $\beta$  isoforms were assessed in the present clinical study.

## Methods

### Study participants

The study enrolled healthy male and female volunteers aged 18–55 years (excluding women of child bearing potential),  $\geq 50$  kg in weight, with a body mass index (BMI) within the range of 18–30 kg m<sup>-2</sup>. Key exclusion criteria included use of prescription drugs, herbal supplements (within 2 weeks prior to baseline) or over-the-counter drugs and dietary supplements (within 4 weeks prior to baseline), and a known history of angioedema. All study participants were enrolled at a single centre (PAREXEL International, California, USA).

### Study design

This was a double-blind, randomized, parallel group, placebo-controlled study designed to investigate the effect of multiple doses of LCZ696 on CSF A $\beta$  isoform concentrations in healthy human volunteers. The study protocol was reviewed by an independent Institutional Review Board (Aspire IRB, LLC; centre number 1001). The study was conducted in accordance with ICH-Good Clinical Practice guidelines and the Declaration of Helsinki. All participants provided written, informed consent prior to randomization.

The study consisted of an initial screening period (day –21 to day –4), a safety baseline (day –3), a pharmacodynamics (PD) baseline (day –2 to day –1), and a 2 week treatment period (days 1–14) (Supplementary Material S1). Post-treatment PD/pharmacokinetic (PK) assessments were performed on days 14 and 15 and the study concluded with an end of study visit (day 19). Subjects were domiciled for 18 nights at the study centre and randomized 1 : 1 to either LCZ696 400 mg once daily for 14 days or matching placebo once daily for 14 days. LCZ696 was taken with water in the morning for 14 days, after an overnight fasting period (~10 h) with no food intake permitted until 1 h post-dose.

### PD assessments

The primary end point was the change from baseline 36 h area under the effect curve (AUEC(0,36 h)) of A $\beta$  1–40 CSF concentration, with LCZ696 compared with placebo. Secondary

end points included the change from baseline of AUEC(0,24 h) for CSF A $\beta$  1–40, and AUEC(0,36 h) and AUEC(0,24 h) for CSF A $\beta$  1–42 and 1–38, with LCZ696 compared with placebo. Change from baseline AUEC(0,36 h) and AUEC(0,24 h) of A $\beta$  1–40 plasma concentrations were measured as an exploratory assessment.

Serial CSF samples were taken from day –2 to day –1 (PD baseline) and from day 14 to day 15 from an indwelling spinal catheter inserted into the lower spinal canal by trained personnel using a standard operating procedure at time points that matched 30 min pre-dose, and 1, 2, 4, 8, 12, 24 and 36 h post-dose. In each case, up to 2 ml of CSF was required to flush the tubing connected to the indwelling catheter, followed by collection of a total of 6 ml used for analysis. CSF aliquots were supplemented with 0.2% (v/v) Tween-20 prior to storage at –70°C. CSF sample collection itself may result in an increase in CSF A $\beta$  concentrations (placebo-drift). As such, the study was baseline and placebo controlled and these measurements served as reference points to distinguish study drug and procedural-related effects. In addition, a parallel group (rather than crossover) design was employed.

Blood samples were obtained by direct venipuncture or indwelling cannula inserted in a forearm vein. In total, ~4 ml of blood was collected into EDTA monovettes or EDTA vacutainers to obtain a final volume of 1.3 ml plasma. Validated, sandwich-based multiplexed immunoassays were used to determine separately A $\beta$  isoforms in CSF with lower limits of quantification (LLOQ) for A $\beta$  1–40, 1–42 and 1–38 isoforms of 126.2 pg ml<sup>-1</sup>, 46.6 pg ml<sup>-1</sup> and 70.0 pg ml<sup>-1</sup>, respectively. A validated immunoassay was used to determine A $\beta$  1–40 in plasma (LLOQ of 5.04 pmol l<sup>-1</sup>). Validation included the assessment of parallelism, selectivity, reproducibility and stability. Acceptance criteria (accuracy, precision) were defined for each of these assessments. The sensitivity of each method was based on the lowest concentration of the analyte in a biological sample that can be quantitatively determined with acceptable precision and accuracy.

### PK assessments

Steady-state PK assessments of LCZ696 analytes (sacubitril, LBQ657 and valsartan) in plasma and of LBQ657 in CSF were carried out using a validated LC-MS/MS method. Plasma and CSF samples were collected on day 14 at 30 min pre-dose and 1, 2, 4, 8, 12, 24 and 36 h post-dose. Blood samples (3 ml) were obtained by direct venipuncture or indwelling cannula inserted into a forearm vein and collected in K<sub>2</sub>EDTA-containing polyethylene sample tubes. Tubes were immediately inverted gently and stored on ice prior to centrifugation. Centrifugation was carried out within 30 min of collection, between 2 and 8°C for 10 min at ~1500 g. Immediately thereafter, plasma was transferred to a 2 ml polypropylene sample tube and stored on dry ice. Tubes were maintained in storage conditions of  $\leq -20^{\circ}\text{C}$  prior to analysis.

CSF samples (2 ml, as described above) were transferred into two polypropylene screw cap tubes (1 ml aliquots) without additives and placed immediately on dry ice and maintained in storage conditions of  $\leq -20^{\circ}\text{C}$  prior to analysis. Samples were labelled with the exact times of dosing and collection. Validated, specific LC-MS/MS methods were used to quantify sacubitril, LBQ657 and valsartan in plasma

with LLOQ of 1.00 ng ml<sup>-1</sup>, 20.0 ng ml<sup>-1</sup> and 10.0 ng ml<sup>-1</sup>, respectively. The LLOQ for quantification of LBQ657 in CSF using LC-MS/MS was 0.2 ng ml<sup>-1</sup>.

### Safety assessments

Assessments included monitoring and recording adverse events (AEs), monitoring of haematology, blood chemistry and urine, vital signs, electrocardiograms, physical condition, body weight and food intake, and physical examination at safety baseline, day 14 and day 19, including neurological examination and fundoscopy.

### Statistical analyses

Forty subjects were required to ensure that 34 subjects ( $n = 17$  per group) completed the study. The sample size was chosen to obtain less than 1 x standard deviation (SD) half-width for the 95% CI of difference to placebo in change from baseline of AUEC(0,36 h) for CSF concentrations of A $\beta$  1–40.

All PD analyses were performed on subjects with more than one post-baseline PD assessment without any significant protocol deviation (PD population). All PK analyses were performed on subjects with more than one valid PK concentration measurement, and who received study drug without any significant protocol deviation (PK population). Samples obtained from the placebo treatment group for PK analysis were assessed in order to exclude treatment mis-randomizations but were not included in this data set. Safety analyses were performed on all subjects who received any study drug (safety population).

The primary end point was analyzed using a linear model with treatment as fixed effect and baseline AUEC as covariate, with 95% CIs presented for the treatment difference. In addition, the change from baseline in log-scale was analyzed with treatment as a fixed effect and log-transformed baseline value as a covariate. The 95% CI for the treatment ratio (LCZ696 400 mg vs. placebo) was computed and presented for the ratio to baseline in AUEC. Study subjects with missing post-dose measurements for all time points on day 14 were excluded from the primary analysis. Subjects with missing post-dose measurements for some time points on day 14 were assessed regarding the number of completed measurements for inclusion in the primary analysis. Additional supportive analyses included assessment of 24 h AUEC change from baseline in linear and log scale. Concentration–time profiles and individual AUEC (24 h and 36 h) and change from baseline data were explored graphically. Similar analyses were performed for the secondary variables (24 h and 36 h AUEC change from baseline in linear and log scale for A $\beta$  1–42 and 1–38 isoform concentrations in CSF) and for the exploratory assessment of A $\beta$  1–40 concentrations in plasma.

PK parameters of AUC(0,  $\tau_{ss}$ ),  $C_{max}$ ,  $C_{trough}$  and  $t_{max}$  were determined for LCZ696 analytes sacubitril, LBQ657 and valsartan in plasma and LBQ657 in CSF from concentration–time profiles using actual recorded sampling times and non-compartmental methods (Phoenix, v6.2 or higher). PK parameters were evaluated with summary statistics. Concentrations of analytes below LLOQ were treated as zero for all PK calculations, including summary statistics, and a geometric mean was not reported if the dataset included zero values. The BBB

penetration of LBQ657 was calculated by estimating the CSF : plasma exposure ratio.

For PD/PK analyses, plasma and CSF LBQ657 concentrations were plotted against plasma and CSF A $\beta$  concentrations.

## Results

### Participant disposition and characteristics

Forty-three subjects were randomized to study treatment (LCZ696,  $n = 21$ ; placebo,  $n = 22$ ) and 39 subjects completed the study (LCZ696,  $n = 20$ ; placebo,  $n = 19$ ). Four subjects discontinued due to an AE ( $n = 1$ ) or protocol deviations ( $n = 3$ ). A further four subjects were excluded from the PD evaluation population, two due to missing blood or CSF samples and two due to the use of medications not allowed by the study protocol. All randomized subjects were included in the safety evaluation ( $n = 43$ ), 35 subjects in the PD evaluation (LCZ696,  $n = 17$ ; placebo,  $n = 18$ ) and 19 subjects in the PK evaluation (LCZ696,  $n = 19$ ; placebo,  $n = 0$ ). Baseline characteristics were generally well balanced between groups (Table 1). All subjects were male.

**Table 1**

Baseline characteristics (all randomized patients)

	LCZ696 $n = 21$	Placebo $n = 22$	Total $n = 43$
<b>Age, years</b>			
<b>Mean (SD)</b>	36.4 (11.3)	39.7 (9.7)	38.1 (10.5)
<b>Median</b>	37.0	42.0	38.0
<b>Range</b>	21–55	21–54	21–55
<b>Male, <math>n</math> (%)</b>	21 (100)	22 (100)	43 (100)
<b>Predominant race, <math>n</math> (%)</b>			
<b>Caucasian</b>	1 (5)	16 (73)	27 (63)
<b>Black</b>	8 (38)	4 (18)	12 (28)
<b>Asian</b>	1 (5)	1 (5)	2 (5)
<b>Other</b>	1 (5)	0	1 (2)
<b>Native American</b>	0	1 (5)	1 (2)
<b>Ethnicity, <math>n</math> (%)</b>			
Other	17 (81)	17 (77)	34 (79)
Hispanic/Latino	4 (19)	5 (23)	9 (21)
<b>Height, cm</b>			
<b>Mean (SD)</b>	178.0 (8.3)	175.7 (6.9)	176.9 (7.6)
<b>Median</b>	178.0	177.0	177.0
<b>Range</b>	154–189	164–190	154–190
<b>Weight, kg</b>			
<b>Mean (SD)</b>	85.2 (9.5)	79.7 (11.0)	82.4 (10.5)
<b>Median</b>	85.1	77.8	82.5
<b>Range</b>	68–103	60–94	60–103
<b>BMI, <math>\text{kg m}^{-2}</math></b>			
<b>Mean (SD)</b>	26.9 (2.4)	25.8 (3.4)	26.3 (2.9)
<b>Median</b>	27.3	27.3	27.3
<b>Range</b>	22–30	18–30	18–30

BMI, body mass index; SD, standard deviation

### Amyloid- $\beta$ in CSF

Compared with placebo, LCZ696 treatment was not associated with a change from baseline to day 14 in CSF A $\beta$ 1–42 AUEC(0,36 h), when assessed by treatment comparison or visual inspection of the concentration time–profile (Table 2, Figure 1A). Similarly, there was no change from baseline in CSF A $\beta$ 1–40 AUEC(0,36 h) with LCZ696 compared with placebo (Table 2, Figure 1B).

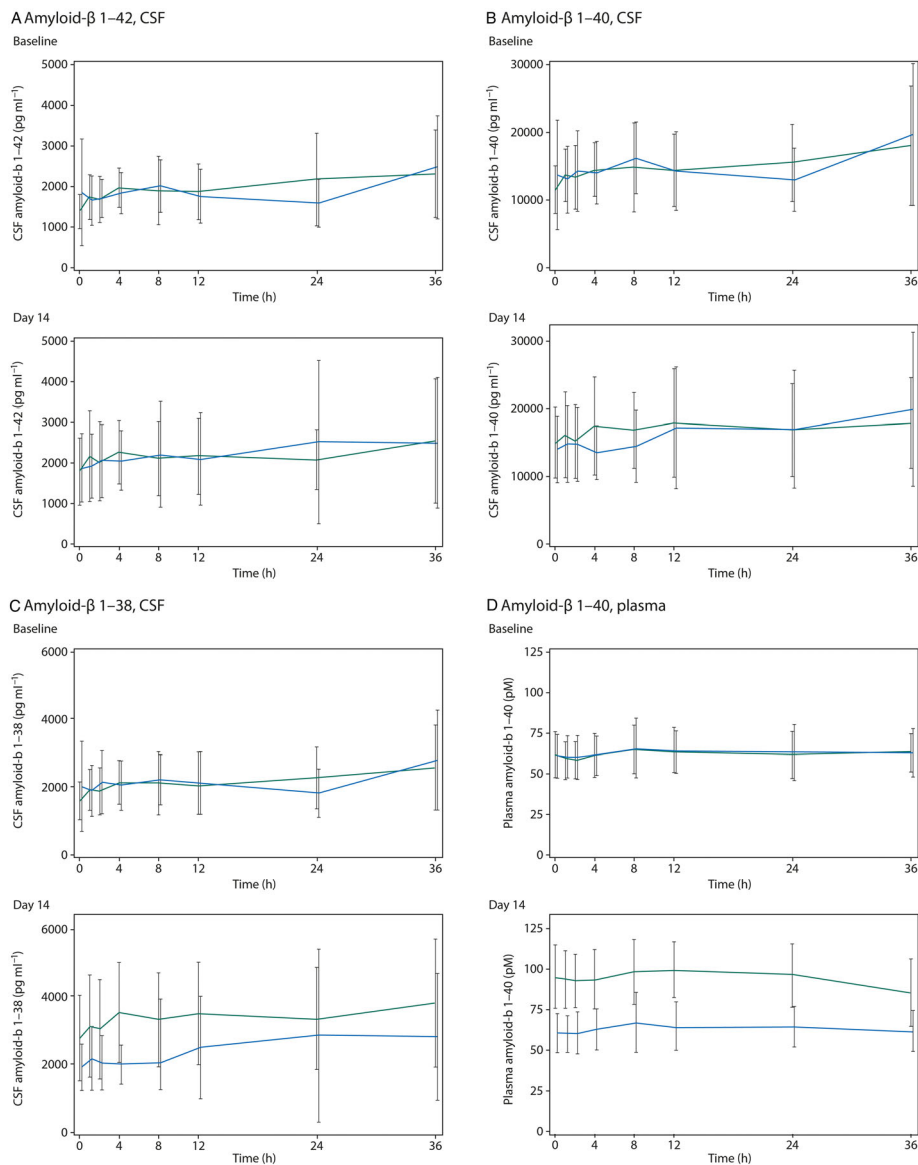
An increase was observed in CSF A $\beta$  1–38 concentrations in the LCZ696 group compared with placebo at day 14, and was most apparent at time points between 0 and 8 h (Figure 1C). There was a 42% increase in CSF A $\beta$  1–38 AUEC (0,36 h) with LCZ696 compared with placebo as assessed by the treatment ratio, a difference which reached statistical significance ( $P = 0.023$ ). However, the absolute treatment difference in CSF A $\beta$  1–38 AUEC(0,36 h) between LCZ696 and placebo for change from baseline to day 14 was not statistically significant ( $P = 0.058$ ) (Table 2).

Treatment comparisons of change from baseline of CSF A $\beta$  isoform AUEC(0,24 h) (24 h area under the effect curve) were also analyzed (Table 3). Compared with placebo, LCZ696 was not associated with an increase from baseline to day 14 in CSF AUEC(0,24 h) for A $\beta$  1–42 and 1–40 isoforms. However,

**Table 2** Change from baseline of cerebrospinal fluid and plasma amyloid- $\beta$  isoforms AUEC(0,36 h) (pg ml<sup>-1</sup> h) on day 14 (PD analysis set)

	Absolute AUEC(0,36 h) Baseline	Day 14	Adjusted mean change from baseline* AUEC(0,36 h)	Estimated treatment difference* (95% CI)	P value*	Adjusted geometric mean†	Estimated treatment ratio† (LCZ696 : placebo)	95% CI of ratio† (LCZ696 : placebo)	P value†
<b>CSF</b>									
<b>Amyloid-<math>\beta</math> 1–42</b>									
<b>LCZ696, n = 17</b>	73167.4	81043.7	9703.18	-5754.79 (-32122.78, 20613.20)	0.660	1.12	0.98	0.73, 1.34	0.919
<b>Placebo, n = 18</b>	66702.1	83885.4	15457.97			1.14			
<b>Amyloid-<math>\beta</math> 1–40</b>									
<b>LCZ696, n = 17</b>	551061.5	630561.3	82414.33	16332.96 (-135541.51, 168207.42)	0.828	1.14	1.05	0.82, 1.34	0.702
<b>Placebo, n = 18</b>	536543.5	605377.5	66081.37			1.09			
<b>Amyloid-<math>\beta</math> 1–38</b>									
<b>LCZ696, n = 17</b>	79256.6	126201.7	47183.26	31549.18 (-1100.04, 64198.40)	0.058	1.58	1.42	1.05, 1.91	0.023
<b>Placebo, n = 18</b>	76621.5	92480.6	15634.09			1.11			
<b>Plasma</b>									
<b>Amyloid-<math>\beta</math> 1–40</b>									
<b>LCZ696, n = 17</b>	2287.0	3430.9	1143.76	1144.05 (946.70, 1341.40)	<0.001	1.50	1.50	1.41, 1.59	<0.001
<b>Placebo, n = 18</b>	2296.9	2296.4	-0.29			1.00			

\*Adjusted means (SE), 95% CIs for mean difference and P values are determined from a linear model on change from baseline AUEC with treatment as fixed effect and baseline AUEC as a continuous covariate. †The change from baseline AUEC in log scale was analyzed using a fixed effect model with treatment as fixed effect and log transformed baseline AUEC as continuous covariate. AUEC, area under the effect curve; CI, confidence interval; CSF, cerebrospinal fluid; PD, pharmacodynamic; SE, standard error



**Figure 1**

Concentration of amyloid- $\beta$  isoforms by timepoint at baseline and on day 14 for LCZ696 (green lines) and placebo (blue lines) groups, (A) amyloid- $\beta$  1–42 in cerebral spinal fluid, (B) amyloid- $\beta$  1–40 in cerebral spinal fluid, (C) amyloid- $\beta$  1–38 in cerebral spinal fluid and (D) amyloid- $\beta$  1–40 in plasma

LCZ696 compared with placebo was associated with a statistically significant increase from baseline to day 14 in CSF AUEC (0,24 h) for A $\beta$  1–38 as assessed by the treatment ratio ( $P = 0.010$ ) and by the absolute treatment difference between LCZ696 and placebo ( $P = 0.026$ ).

To assess trends within treatment groups, individual CSF A $\beta$  isoform AUEC(0,36 h) and AUEC(0,24 h) values at baseline and day 14 were plotted (Figure 2 for AUEC(0,36 h)). Visual inspection did not reveal any apparent differences or unidirectional trends in A $\beta$  1–42 and 1–40 AUEC(0,36 h) values or group imbalances in either treatment group. The individual exhibiting the largest increase in, and highest post-treatment value of, CSF A $\beta$  1–42 AUEC(0,36 h) received placebo (Figure 2A, B). Subjects in the LCZ696 group appeared to have an increase in CSF A $\beta$  1–38 AUEC(0,36 h)

values, compared with placebo. As above, the individual exhibiting the largest increase in, and highest post-treatment value of, CSF A $\beta$  1–38 AUEC(0,36 h) received placebo (Figure 2C). Individual CSF A $\beta$  isoform AUEC(0,24 h) analyses were comparable and supportive of AUEC(0,36 h) data outlined above (data not shown).

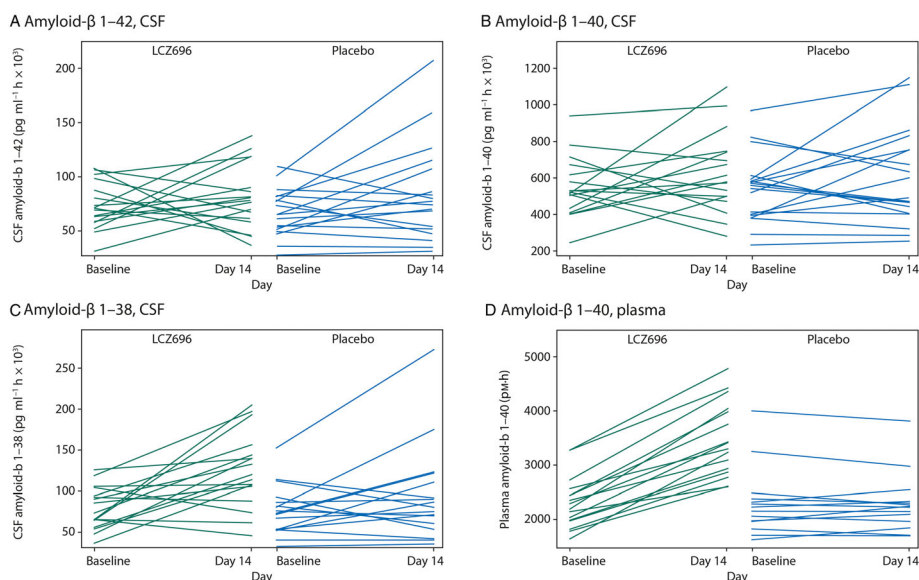
### Amyloid- $\beta$ in plasma

The effects of LCZ696 on plasma A $\beta$  1–40 levels were also explored. A $\beta$  1–40 was selected as plasma biomarker because A $\beta$  1–40 plasma concentrations were expected to be higher and associated with a lower variability relative to plasma concentrations of A $\beta$  1–42 and A $\beta$  1–38. At day 14, plasma A $\beta$  1–40 levels were higher in the

**Table 3** Change from baseline of cerebrospinal fluid and plasma amyloid- $\beta$  isoforms AUEC(0,24 h) (pg ml<sup>-1</sup> h) on day 14 (PD analysis set)

	Absolute AUEC (0,24 h) Baseline	Day 14	Adjusted mean change from baseline* AUEC(0,24 h)	Estimated treatment difference* (95% CI)	P value*	Adjusted geometric mean†	Estimated treatment ratio†(LCZ696 : Placebo)	95% CI of ratio† (LCZ696 : placebo)	P value†
<b>CSF</b>									
<b>Amyloid-<math>\beta</math> 1–42</b>									
LCZ696, n = 17	47289.5	52090.0	5709.89	-4374.08 (-19372.94, 10624.79)	0.557	1.11	0.96	0.73, 1.26	0.767
Placebo, n = 18	42604.7	53547.5	10083.97			1.16			
<b>Amyloid-<math>\beta</math> 1–40</b>									
LCZ696, n = 17	356264.3	412403.2	58593.53	20441.88 (-71901.30, 112785.06)	0.655	1.16	1.07	0.86, 1.34	0.546
Placebo, n = 18	342756.1	383226.1	38151.65			1.08			
<b>Amyloid-<math>\beta</math> 1–38</b>									
LCZ696, n = 17	51304.9	81642.5	30492.92	21916.37 (-2767.16, 41065.58)	0.026	1.58	1.43	1.10, 1.86	0.010
Placebo, n = 18	49484.2	58207.5	8576.55			1.11			
<b>Plasma</b>									
<b>Amyloid-<math>\beta</math> 1–40</b>									
LCZ696, n = 17	1528.9	2335.9	806.96	801.09 (676.15, 926.03)	<0.001	1.53	1.52	1.43, 1.62	<0.001
Placebo, n = 18	1533.4	1539.2	5.87			1.01			

\*Adjusted means (SE), 95% CIs for mean difference and P values are determined from a linear model on change from baseline AUEC with treatment as fixed effect and baseline AUEC as a continuous covariate. †The change from baseline AUEC in log scale was analyzed using a fixed effect model with treatment as fixed effect and log transformed baseline AUEC as continuous covariate. AUEC, area under the effect curve; CI, confidence interval; CSF, cerebrospinal fluid; PD, pharmacodynamic; SE, standard error



**Figure 2**

Individual subject ping-pong plots of amyloid- $\beta$  isoform AUEC(0,36 h) at baseline and at day 14 for LCZ696 (left-hand graph of each panel) and placebo (right-hand graph of each panel) groups, (A) amyloid- $\beta$  1–42 in cerebral spinal fluid, (B) amyloid- $\beta$  1–40 in cerebral spinal fluid, (C) amyloid- $\beta$  1–38 in cerebral spinal fluid and (D) amyloid- $\beta$  1–40 in plasma

LCZ696 group compared with the placebo group at all time points (Figure 1D). Overall, there was a significant increase of 50% ( $P < 0.001$ ) from baseline to day 14 in

plasma A $\beta$  1–40 AUEC(0,36 h) with LCZ696 compared with placebo (Table 2). All subjects receiving LCZ696 had increases in plasma A $\beta$  1–40 AUEC(0,36 h) values from

**Table 4**

Summary statistics for pharmacokinetic (PK) parameters in plasma and cerebrospinal fluid (CSF) for LCZ696 analytes (sacubitril, LBQ657 and valsartan) (PK analysis set)

	AUC(0, $\tau$ , <sub>ss</sub> )(ng ml <sup>-1</sup> h)	C <sub>max</sub> (ng ml <sup>-1</sup> )	C <sub>trough</sub> <sup>*</sup> (ng ml <sup>-1</sup> )	t <sub>max</sub> (h)
<b>Plasma, n</b>	19	19	19	19
<b>Sacubitril</b>	3220 (1530)	1710 (682)	0.412 (0.787)	NA
<b>Mean (SD)</b>	47.5	39.9	191.0	NA
<b>CV% Mean</b>	3010	1740	0	1.00
<b>Median</b>	1030–7830	553–3150	0–2.83	1.00–4.00
<b>Min–max</b>				
<b>LBQ657</b>				
<b>Mean (SD)</b>	137 000 (39 400)	14 100 (3600)	1840 (907)	NA
<b>CV% Mean</b>	28.7	25.5	49.3	NA
<b>Median</b>	131 000	14 200	1710	2.00
<b>Min–max</b>	66 800–218 000	7790–19 600	455–3720	1.00–4.00
<b>Valsartan</b>				
<b>Mean (SD)</b>	21 300 (11 200)	3910 (2100)	180 (113)	NA
<b>CV% Mean</b>	52.8	53.7	62.9	NA
<b>Median</b>	19 400	3420	145	1.03
<b>Min–max</b>	7940–49 500	1030–9380	57.7–532	1.00–2.05
<b>CSF, n</b>	16†	17‡	16†	17‡
<b>LBQ657</b>				
<b>Mean (SD)</b>	387 (261)	19.2 (11.3)	13.2 (12.4)	NA
<b>CV% Mean</b>	67.4	58.9	94.0	NA
<b>Median</b>	338	17.9	10.2	8.00
<b>Min–max</b>	154–1290	9.09–58.8	3.93–56.6	3.98–12.0

\*For all analytes in both plasma and CSF, C<sub>trough</sub> was observed at 24 h post-dose. †Data from three subjects were excluded due to insufficient concentration data for estimation of AUC(0, $\tau$ ) and C<sub>trough</sub>. ‡All PK parameters from two subjects were excluded from summary statistics due to insufficient concentration data. AUC, area under the curve; C<sub>max</sub>, maximum plasma concentration; CSF, cerebrospinal fluid; C<sub>trough</sub>, trough plasma concentration; CV, coefficient of variation; NA, not available; PK, pharmacokinetic; SD, standard deviation; t<sub>max</sub>, time to maximum concentration



baseline to day 14 (Figure 2D). A similar observation was made for AUEC(0,24 h) (Table 3).

### LCZ696 plasma and CSF PK

Following oral administration of LCZ696, peak concentrations ( $C_{max}$ ) of sacubitril, LBQ657 and valsartan were reached in plasma at median  $t_{max}$  times of 1 h, 2 h and 1 h, respectively (Table 4). In contrast to steady-state PK of LCZ696 in plasma, the concentration of LBQ657 in CSF increased slowly, reaching  $C_{max}$  in a median  $t_{max}$  time of 8 h (Table 4, Figure 3). At steady-state, mean  $C_{max}$  and trough CSF concentrations ( $C_{trough}$ ) of LBQ657 were 19.2 ng ml<sup>-1</sup> and 13.2 ng ml<sup>-1</sup>, respectively. The CSF : plasma ratio of LBQ657 exposure (AUC(0, $\tau_{ss}$ )) was estimated to be 0.002825. Since sacubitril and valsartan do not inhibit neprilysin, CSF concentrations of these LCZ696 analytes were not measured.

### PD/PK assessment of an individual outlier

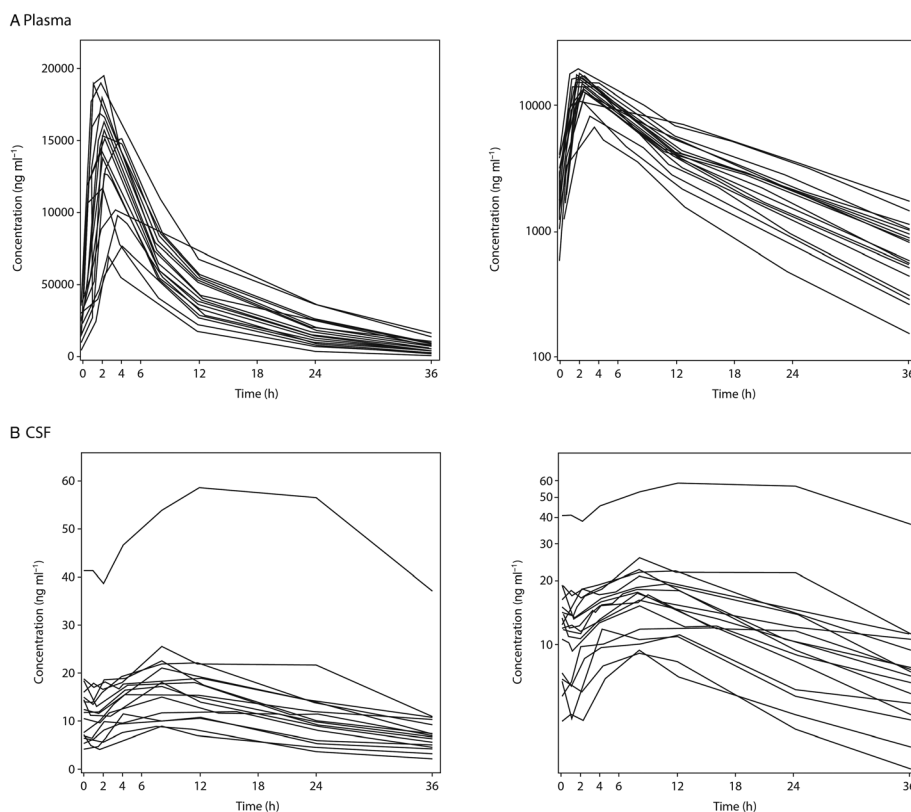
Upon assessment of steady-state PK data, it was apparent that one subject exhibited >2-fold higher peak CSF LBQ657 concentrations compared with all other subjects (Figure 3B). The CSF  $C_{max}$  and  $C_{trough}$  values for this individual were reported as 58.8 ng ml<sup>-1</sup> and 56.6 ng ml<sup>-1</sup> in comparison with the treatment group mean values of 19.2 ng ml<sup>-1</sup> and 13.2 ng ml<sup>-1</sup>, respectively. However, plasma  $C_{max}$  of LBQ657

in this subject (19 000 ng ml<sup>-1</sup>) was within the observed variability of plasma LBQ657  $C_{max}$  values in the LCZ696 treatment group (Table 4). Assessment of individual A $\beta$  isoform AUEC(0,36 h) and AUEC(0,24 h) data indicated that levels of A $\beta$  isoforms in this subject did not increase at day 14 relative to baseline (baseline AUEC(0,36 h) (pg ml<sup>-1</sup> h); A $\beta$  1–42, 107549; A $\beta$  1–40, 517338; A $\beta$  1–38, 67382; day 14 AUEC(0,36 h) (pg ml<sup>-1</sup> h); A $\beta$  1–42, 36572; A $\beta$  1–40, 288260; A $\beta$  1–38 46409).

### Relationship of LBQ657 and A $\beta$ isoform concentrations

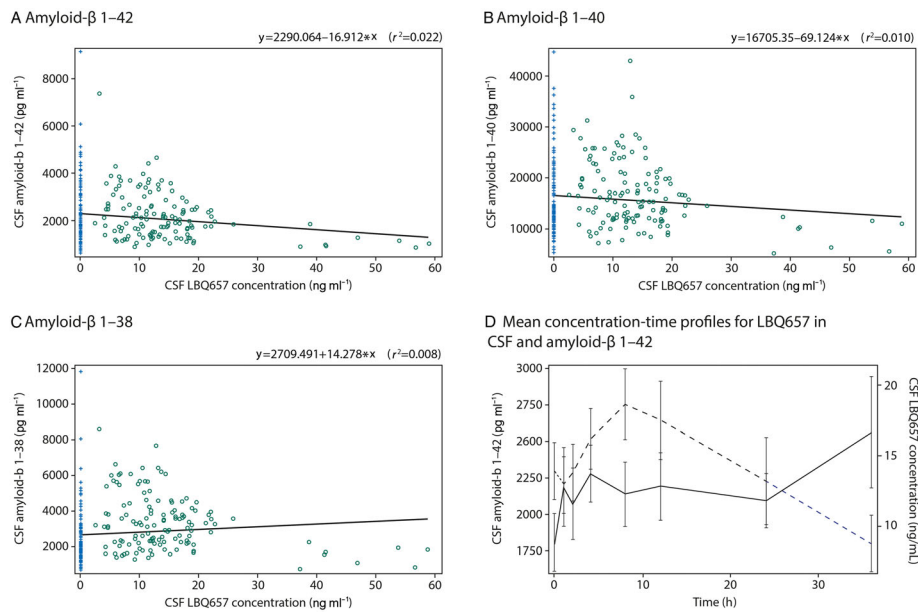
The relationship between LBQ657 levels and A $\beta$  isoform levels was explored through analysis of scatter plots (Figure 4). The R-square values for LBQ657 CSF concentration and CSF A $\beta$  1–42, 1–40, and 1–38 were 0.022, 0.010 and 0.008, respectively (Figure 4A–C). Similar results were obtained through analysis of LBQ657 CSF area under the concentration–time curve at steady-state (AUC(0, $\tau_{ss}$ )) and CSF A $\beta$  isoform levels (data not shown). Mean concentration–time profiles of CSF LBQ657 and A $\beta$  1–42 (Figure 4D) also support that there was no relationship between LBQ657 and A $\beta$  CSF concentrations.

Additional analyses of LBQ657 and plasma A $\beta$  1–40 indicated that there was a weak relationship between LBQ657 and A $\beta$  1–40 plasma levels. R-square values for the relationships between plasma LBQ657 and A $\beta$  1–40 concentrations



**Figure 3**

Individual subject LBQ657 concentrations vs. time on day 14 following oral administration of LCZ696 at 400 mg once daily, (A) plasma and (B) cerebral spinal fluid. Left-hand graph of each panel is a linear plot with an expanded time scale, right-hand graph of each panel is a semi-logarithmic plot



**Figure 4**

Panels (A)–(C) show individual scatter plots of cerebral spinal fluid concentrations of amyloid- $\beta$  isoforms vs. LQ657 concentrations on day 14 following oral administration of LCZ696 at 400 mg once daily (open circles) or placebo (plus signs), (A) amyloid- $\beta$  1-42, (B) amyloid- $\beta$  1-40 and (C) amyloid- $\beta$  1-38. The solid line represents the regression ( $r^2$ ). Panel (D) shows mean amyloid- $\beta$  1-42 concentrations in cerebral spinal fluid (solid line, left y-axis) and LQ657 concentrations in cerebral spinal fluid (dashed lines, right y-axis) vs. time on day 14 following oral administration of LCZ696 at 400 mg once daily

and plasma LQ657 AUC(0,  $\tau_{ss}$ ) and A $\beta$  1-40 AUEC(0,36 h) were 0.293 and 0.617, respectively.

### Safety and tolerability

More subjects in the LCZ696 group ( $n = 19$ ) compared with the placebo group ( $n = 14$ ) reported AEs related to the procedure of CSF collection (Supplementary Material S2). All AEs were of mild or moderate intensity and resolved by the end of the study (data not shown). Seven subjects reported AEs considered to be related to study treatment, two subjects receiving LCZ696 and five subjects receiving placebo. In the LCZ696 group, these were mild lightheadedness and temporomandibular joint pain. Both AEs were resolved by the study end. Two serious AEs (SAEs) were reported, one in each treatment group (post-dural puncture headache [LCZ696] and mild sacral pain [placebo], which represented the only study discontinuations secondary to an AE).

### Discussion

The key finding of this study in healthy subjects is that LCZ696 400 mg once daily did not result in changes in CSF concentrations of the aggregable A $\beta$  isoforms 1-40 and 1-42. The lack of effect of LCZ696 was evidenced by unchanged A $\beta$  1-40 and 1-42 AUEC(0,36 h) and AUEC(0,24 h), and confirmed by unchanged concentration-time profiles of A $\beta$  1-42 and 1-40 in CSF. Individual subject AUECs at baseline and on day 14 did not reveal any obvious changes with LCZ696 compared with placebo, and there was no apparent relationship between CSF LQ657 and CSF A $\beta$  1-42 and 1-40

concentrations. Despite very low BBB penetration and considering low protein binding in CSF compared with plasma, observed CSF concentrations of the neprilysin inhibitor LQ657 were sufficient to inhibit neprilysin.

In contrast to unchanged CSF A $\beta$  1-42 and 1-40 levels, an increase from baseline in CSF A $\beta$  1-38 AUEC(0,36 h) and AUEC(0,24 h) with LCZ696 compared with placebo was observed. Concentration-time profiles of CSF A $\beta$  1-38 concentrations and individual subject AUECs at baseline and on day 14 were associated with considerable variability. However, there was no apparent relationship between CSF A $\beta$  1-38 concentrations and LQ657 plasma and CSF concentrations. A $\beta$  1-38 is soluble [16], more readily transported within the brain interstitial space and into the CSF, and may be more susceptible to increase with neprilysin inhibition compared with the more hydrophobic and aggregation-prone isoform A $\beta$  1-42 [17, 18]. In addition, neprilysin degraded monomeric A $\beta$  1-40 *in vitro* while no significant proteolysis of aggregated A $\beta$  1-40 was observed [19], providing support for a potential differential effect of neprilysin inhibition on aggregable vs. soluble A $\beta$  isoforms.

To the best of our knowledge, there is no obvious or conclusive evidence in the literature showing that an isolated increase of CSF A $\beta$  1-38 concentrations results in or facilitates A $\beta$  plaque formation in the brain or cognitive decline. While *in vitro* evidence suggests that A $\beta$  plaque formation involves conformational conversion of A $\beta$  oligomers [20–22], it remains unknown whether an isolated increase in CSF A $\beta$  1-38 may alter the propensity of other A $\beta$  isoforms to form oligomers *in vivo*. However, the pattern of change in CSF concentrations of A $\beta$  isoforms observed with LCZ696 (isolated increase in CSF A $\beta$  1-38) is substantially different from that observed

in patients with prodromal AD and AD (decrease in CSF A $\beta$  1–42 [23–25]) or in children with Down's syndrome (increase in CSF A $\beta$  1–42, 1–40 and 1–38 at 54 months [26]). Furthermore, A $\beta$  1–38 has been shown to accumulate only in brain plaques in patients with familial AD due to *APP* mutations within the A $\beta$  coding region, a finding that is unrelated to neprilysin inhibition [27]. Unlike A $\beta$  1–42 and 1–40, A $\beta$  1–38 was absent in parenchymal A $\beta$  deposits in patients with sporadic AD, patients with presenilin mutations and in individuals with Down's syndrome [27], supporting that an isolated increase in CSF A $\beta$  1–38 is unlikely to be clinically meaningful with regards to parenchymal A $\beta$  deposits in the brain. Furthermore, CSF A $\beta$  1–38 is not recommended by the Alzheimer's Biomarker Standardization Initiative as a biomarker that is neurochemically compatible with AD [28]. Other neurodegenerative diseases such as dementia with Lewy bodies and Niemann–Pick disease type C, in which CSF concentrations of A $\beta$  1–38 are decreased or increased, respectively, are also associated with a complex change of multiple A $\beta$  isoforms [29, 30], again suggesting that an isolated increase in CSF A $\beta$  1–38 without concomitant changes in other CSF A $\beta$  isoforms is unlikely to be clinically important.

Administration of LCZ696 was associated with an increase in plasma A $\beta$  1–40 concentrations. While more than 30 structurally unrelated precursor proteins with a propensity to form amyloid fibrils were identified in various forms of amyloidosis, plasma A $\beta$  has not been implicated in the pathophysiology of diseases involving systemic or organ-specific amyloidosis outside the CNS [31, 32], and results from studies investigating the utility of plasma A $\beta$  levels to predict cognitive decline are inconsistent. While baseline plasma A $\beta$  1–42 levels were decreased in patients who transitioned to cognitive decline [33], baseline plasma A $\beta$  levels were not related to cognitive decline in studies in patients with mild cognitive impairment and AD [34]. Notably, there was a decrease in plasma A $\beta$  in apolipoprotein E epsilon 4 carriers with mild cognitive impairment, which is considered a risk factor for cognitive decline, and an increase in plasma A $\beta$  in patients with AD following treatment with simvastatin. While non-clinical and clinical results were inconsistent with regards to AD prevention by statins, there was no evidence for statins facilitating cognitive decline [35]. Therefore, the observed increase in plasma A $\beta$  1–40 with LCZ696 is not considered to be clinically relevant but to reflect a PD change related to neprilysin inhibition.

The results of this study suggest that disposition pathways or enzymes other than neprilysin may be more important in the clearance of CSF A $\beta$  in humans [10]. This conclusion is supported by the observation that multiple other enzymes are implicated in A $\beta$  degradation [10]. Since the role of A $\beta$  in the pathophysiology of AD is still not well defined [15], CSF and plasma A $\beta$  measured in this study are considered to be a biomarker of target engagement reflecting neprilysin inhibition and not a surrogate biomarker to predict the development of A $\beta$  plaques in the brain, cognitive decline or AD. However, human genetic data support the lack of a relationship between neprilysin and AD. A large meta-analysis of human genome-wide association studies in 74046 individuals did not reveal any association between variations in the neprilysin gene (membrane metallo-endopeptidase [*MME*]) and AD [36]. The lack of any such association supports the

conclusion that common *MME* genetic variations are unlikely to be a clinically meaningful risk factor for AD in humans. Moreover, no obvious neurocognitive deficit has been reported for human carriers of *MME* loss of function mutations [37]. This is consistent with the finding of this study demonstrating that LCZ696 did not affect CSF concentrations of the A $\beta$  isoforms 1–42 and 1–40, which are poorly soluble, rapidly aggregate and the main component of A $\beta$  plaques in the brain and therefore considered to have the greatest amyloidogenic properties [15, 38, 39].

Whilst the results of this study are reassuring, it should be noted that healthy subjects were enrolled rather than patients with HF, the target patient population of LCZ696, due to the need for serial CSF collections and to reduce confounding factors related to concomitant diseases and medications that may have impacted study results. However, LBQ657 CSF concentrations achieved in healthy subjects were sufficient to inhibit neprilysin, enabling the study of clinically relevant doses of LCZ696 on A $\beta$  levels. It cannot be excluded that the turnover of CSF A $\beta$  in patients with prodromal or manifest AD is different from the turnover of CSF A $\beta$  in healthy subjects. However pre-existing reductions in A $\beta$  1–42 and 1–40 CSF levels would have been likely to confound the interpretation of study results in this specific patient population. Therefore, the selection of healthy subjects to investigate the PDeffect of neprilysin inhibition on CSF A $\beta$  levels is considered appropriate.

The treatment duration (2 weeks) was considered sufficient to ensure plasma PK steady-state, allowing for equilibrium between CSF and plasma LBQ657 concentrations and providing a sufficient time window between CSF collection periods to avoid procedure-related increases in CSF A $\beta$  (placebo-drift). Extrapolation of the study results following 2 weeks of dosing to long term administration of LCZ696 is relevant because of the intended chronic use of LCZ696. It could be hypothesized that alternative proteolytic pathways are activated with continued dosing of LCZ696 that compensate for neprilysin inhibition. It is therefore proposed that 2 weeks of dosing with LCZ696 may reflect or overestimate changes in CSF A $\beta$  following chronic dosing. However, this has yet to be elucidated.

The results from the present study demonstrate that administration of LCZ696 400 mg once daily for 14 days in healthy subjects does not cause changes in CSF A $\beta$  1–42 and 1–40 concentrations. The clinical relevance of the associated increase in CSF A $\beta$  1–38 is unknown.

## Competing Interests

All authors have completed the Unified Competing Interest form at [http://www.icmje.org/coi\\_disclosure.pdf](http://www.icmje.org/coi_disclosure.pdf) (available on request from the corresponding author). SJ reports grants from Novartis Pharma AG, during the conduct of the study, HG and SJ were employees of PAREXEL International at the time the study was conducted and hold or were eligible to receive PAREXEL stocks. THL, CT, SA, PP, MAV, MH and IR were Novartis employees at the time the study was conducted and hold or were eligible to receive Novartis stocks.

## Author contributions

THL and IR conceptualized this study. All authors contributed to the development of study design and clinical study protocol. THL, CT, HG and SJ contributed to the conduct of the study; HG was the study investigator. MAV led the analysis of A $\beta$  in CSF and plasma samples. SA conducted the PK analysis. PP conducted all statistical analysis. All authors contributed to the reporting of study results and writing of the manuscript.

*The study was conducted at PAREXEL International, Glendale, CA, USA. CSF A $\beta$  samples were analyzed at SGS Cephac, France. CSF and plasma PK samples were analyzed at Wuxi, China. The authors were assisted in the preparation of the manuscript by Derek Lavery, a professional medical writer contracted to CircleScience, an Ashfield company (part of UDG Healthcare plc). Writing support was funded by Novartis Pharma AG, Basel, Switzerland.*

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

<http://onlinelibrary.wiley.com/doi/10.1111/bcp.12861/supinfo>.

**Figure S1** Design of a double-blind, randomized, parallel group, placebo-controlled study to investigate the effect of multiple doses of LCZ696 on cerebral spinal fluid amyloid- $\beta$  isoform concentrations in healthy human subjects. Male subjects received either LCZ696 at 400 mg once daily ( $n = 21$ ) or placebo ( $n = 22$ ) for 14 days. PD, pharmacodynamic (cerebral spinal fluid concentrations of amyloid- $\beta$  1–42, 1–40 and 1–38 and plasma concentrations of amyloid- $\beta$  1–40). PK, pharmacokinetic (assessment of LCZ696 analytes [sacubitril, LBQ657 and valsartan] in plasma and of LBQ657 in cerebral spinal fluid). EOS, end of study.

**Table S1** Adverse events, serious adverse events, discontinuations and deaths (safety population).