Materials and Methods

Study design

The safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of intravenous (IV) infusions of CSL112 were assessed in two phase 1 studies, a single ascending dose (SAD) study (NCT01129661) and a multiple ascending dose (MAD) study (NCT01281774) conducted in healthy adult subjects at single centers in Australia.

The SAD study was a randomized, double-blind, placebo-controlled investigation performed at Royal Adelaide Hospital, Adelaide, Australia. The MAD study was a randomized, placebo-controlled, sponsor-unblinded study performed at Q-Pharm, Brisbane, Australia.

The primary objective of both studies was to evaluate the safety and tolerability of escalating doses of CSL112 after single or multiple IV infusions, together with an evaluation of the PK of apolipoprotein A-I (apoA-I) after single and multiple IV infusions of CSL112, including plasma apoA-I concentration without baseline correction, and the PD of biomarkers linked to CSL112. This manuscript focuses on the biomarker data.

Inclusion and exclusion criteria

The main inclusion criteria were: male or female healthy subjects aged between 18 and less than 55 years, weighing at least 45 kg in the SAD study (50 kg in the MAD study), capable of understanding the purposes and risks of the study, and able to provide written informed consent. Subjects were excluded from the study if they had significant medical conditions, any clinically relevant abnormal laboratory values, history of coagulopathy, hypotension, evidence of renal impairment, evidence of substance or alcohol abuse or were unable to comply with the study protocol.

Both studies were reviewed and approved by the Bellberry Human Research Ethics Committee, an Independent Ethics Committee. Subjects provided written informed consent before any study-specific assessments were performed. The studies were conducted in accordance with standards of Good Clinical Practice, as defined by the International Conference on Harmonisation, the principles outlined in the Declaration of Helsinki and all applicable national and local regulations.

Randomization

In both studies subjects were randomized to receive either CSL112 or placebo in the approximate ratio of 3:1 (active:placebo).

Demographics and baseline characteristics

The disposition of subjects in both studies is shown in Supplemental Figure I. In the SAD study, the majority of the subjects were male (63.2%) and of white race (94.7%) with a median age of 23 years, and median height and weight of 1.75 m and 78.5 kg, respectively. The majority of the subjects included in the MAD study were male (63.9%) and of white race (97.2%), with a median age of 23 years. Subject characteristics for both studies are summarized in Table 1. In the MAD study, the 3.4 g once weekly CSL112 group (n=9) were all male; the uneven distribution of males across the groups may confound data interpretation relating to dose-response relationships and gender.

Study product, dose and administration
The composition and characterization of CSL112 has been described in detail (1). Briefly, CSL112 is apoA-I purified by cold ethanol precipitation from human pooled plasma (CSL Behring) (2, 3) and reconstituted with soy PC using the sodium cholate dialysis method of Matz & Jonas (4) with modifications (1, 2). The product was subjected to sterile filtration (0.22 µm) and lyophilization and produced disc-shaped particles with high batch-to-batch consistency. The principal differences between CSL112 and a previously described formulation (CSL111) are lower levels of PC (55 moles per mole of apoA-I), lower levels of residual cholate (0.03 g per g of apoA-I) and greater particle uniformity in CSL112.

Lyophilized vials of CSL112 were dissolved in sterile water for injection and dosed based on the protein content. In both studies, the placebo comprised 0.9% sodium chloride solution for injection. In both studies CSL112 or placebo were administered intravenously via a 2 h infusion. The study product was physically masked to maintain blinding. Unblinded site personnel prepared and administered the study product.

In the SAD study subjects were stratified by body weight and received either placebo or one of six different CSL112 doses: 5, 15, 40, 70, 105 and 135 mg/kg.

In the MAD study subjects received placebo or CSL112 either once- (1W) or twice-weekly (2W) for 4 weeks: 3.4 g or 6.8 g 1W CSL112 or placebo (a total of 4 infusions); 3.4 g 2W CSL112 or placebo (a total of 8 infusions).

ApoA-I pharmacokinetic assessment

ApoA-I was assessed at a specialty lipid laboratory (Pacific Biomarkers, Seattle, WA, USA) by an immunonephelometric method run on Roche Modular P. In both studies blood samples for PK assessment were collected at time points selected to capture the peak and expected decline in apoA-I plasma concentration. The PK samples in the SAD study were collected on days 1-4, 6, 8 and 11. In the MAD study, blood samples for PK analysis were taken on days 1-4, 6, 8, 9, 15, 21-25 and 27 for 1W dosing groups and on days 1-4, 8, 11, 15, 18, 22, and 25-28 for the 2W dosing group. Validation of PK bioanalysis has been described in detail (5).

PK parameters for baseline corrected plasma concentrations of apoA-I were determined after single and multiple IV infusions of CSL112 and included area under the plasma concentration time curve from time point zero (before dosing) to the last time point above baseline (AUC0-last), maximum plasma concentration (Cmax) and half-life (t1/2).

Pharmacodynamic biomarkers

The following biomarkers were assessed in both the SAD and MAD studies; apoA-I, phosphatidylcholine (PC), ABCA1-dependent, ABCA1-independent and total cholesterol efflux capacity, HDL-VS (very small (6); also known as PreBeta1-HDL), apolipoprotein B (apoB), triglycerides (TG), total cholesterol (TC) and HDL-cholesterol (HDL-C). HDL-unesterified cholesterol (HDL-UC) and HDL-esterified cholesterol (HDL-EC) were measured in the SAD study only. Cholesterol efflux assays were performed in apoB-depleted serum samples using J774 macrophages at Vascular Strategies LLC, Plymouth Meeting, PA, USA as previously described (7). Total and ABCA1-independent cholesterol efflux, with and without cAMP induction respectively, were assayed. ABCA1-dependent efflux was calculated as the difference between total and ABCA1-independent efflux. Plate-to-plate variability was monitored with control samples and was within acceptability criteria. Efflux data were not normalized. All other PD biomarkers were assessed in plasma samples at Pacific Biomarkers, Seattle, WA, USA. HDL-VS was preserved by 21-fold dilution in 50% sucrose before freezing and measured by
enzyme-linked immunosorbent assay for PreBeta1-HDL (Sekisui Medical Co, Tokyo, Japan). HDL was separated by the polyethylene glycol (PEG) precipitation method. All cholesterol and triglycerides were measured by standard enzymatic methods.

Validation of PD assays was conducted ahead of the assessment of clinical samples to ensure accuracy of reported concentrations and activities in the presence of CSL112. Briefly, plasma samples were collected from five subjects who had a range of lipid phenotypes including two normolipidemic, one with high HDL-C, and two with low HDL-C and high TG. Samples included plasma or serum spiked with controls or CSL112 at various concentrations (v/v, 89.3/10.7). Specifically, samples were spiked with saline control, sucrose control, or 0.1, 0.8 and 2.8 mg/mL CSL112. Samples were incubated for 1 h at 37°C or in some instances at 0°C and then kept frozen at -70°C prior to analysis. Comparison of biomarker assessments in the absence and presence of CSL112 indicated no significant interference of CSL112 in the HDL-precipitation procedure by PEG or the assessments of biomarkers.

In the studies no food was provided for 8 hours before and after dosing and all data shown was obtained in fasting samples. During the SAD study, blood samples for assessing TC, HDL-UC, HDL-EC, apoB and TG were collected at 0 h [before infusion], 1, 2, 4, 8, 24, 48, 72 and 168 h. HDL-VS and cholesterol efflux capacities were assessed in blood samples taken at 0 h [before infusion], 2, 8, 24, 48, 72 and 168 h.

For the measurement of apoB, samples were taken at the same time points irrespective of 1W or 2W dosing, i.e. before dosing on days 1, 8, 15, 22 and on day 28. Samples for measurement of cholesterol efflux and HDL-VS were taken on day 1 (before dosing, at 2, 4 and 8 h), day 2, day 22 (before dosing and 2, 4 and 8 h) and day 23 in the 1W dosing groups. For the 2W dosing group these samples were collected on day 1 (before dosing and at 2, 4 and 8 h), day 2, day 25 (before dosing and 2, 4 and 8 h) and day 26.

**Pharmacodynamic Parameters**

PD parameters were calculated from biomarker concentrations and activities. The following PD parameters were assessed: area under the effect curve (AUEC) for time point zero to the time point of the last quantifiable concentration/ activity (AUEC_{0-last}), maximum biomarker concentration/activity (R_{max}), time to reach maximum concentration/activity (T_{max}).

**Statistical analysis**

The sample size was not based on formal power calculations. A sample size of nine subjects on CSL112 and three on placebo per group was chosen as the smallest number that would allow an adequate early clinical evaluation of safety and PK. In the SAD study 42 subjects received CSL112 and 15 subjects received placebo. In the MAD study, 27 subjects received CSL112 and 9 subjects received placebo.

Subjects were randomized by an individual not directly involved in the analysis of study results. A randomization block size of four (3 CSL112:1 placebo) was used to ensure the balance between dosing groups was maintained.

The data analysis involved descriptive statistics because there was no formal hypothesis to be tested during the study. The placebo group was not included in the PK analysis. In the PD analyses the placebo groups from each study were combined to form a single placebo group.

The baseline value for all analyses was the last value recorded before dosing on day 1, i.e. before administration of study medication. PK and PD baseline correction involved subtracting
the baseline value from the value obtained from each sample collected after study medication administration.

SAS Version 9.2 (SAS Institute Inc., Cary, NC) was used for the analysis of study data. Non-compartmental PK analysis was performed using model 202 (constant infusion) in WinNonlin™ Enterprise Version 5.2 (Pharsight Corporation, Montreal, Canada). Non-compartmental PD analysis of biomarkers was performed using Model 220 (baseline effect) in WinNonlin™ Enterprise Version 5.2.

References


7. de la Llera-Moya M, Drazul-Schrader D, Asztalos BF, Cuchel M, Rader DJ, Rothblat GH. The ability to promote efflux via ABCA1 determines the capacity of serum specimens with similar high-density lipoprotein cholesterol to remove cholesterol from macrophages. Arterioscler Thromb Vasc Biol. 2010;30:796-801.