CSL112 Enhances Cholesterol Efflux Equally in Patients with High and Low HDL Functionality

Andreas Gille[1], Gunter Hartel[1], Denise D'Andrea[2], Samuel D Wright[2]
[1]CSL Limited, Parkville, VIC, Australia, [2]CSL Behring, King Of Prussia, PA, USA

Introduction

- The ability of high-density lipoprotein (HDL) to remove cholesterol from macrophages in atherosclerotic plaque is thought to underlie its inverse correlation with cardiovascular risk.
- CSL112 is a novel formulation of apolipoprotein A-I (apoA-I) purified from human plasma and reconstituted to form HDL particles suitable for infusion, which is currently in development for acute coronary syndrome (ACS).
- Two Phase 1 studies in healthy subjects and one Phase 2a study in patients with stable atherosclerotic disease have demonstrated favorable safety and pharmacokinetic (PK) behavior and strong elevation of cholesterol efflux capacity upon either single or multiple infusions of CSL112.[1–4]
- **The aim of the current study was to assess the influence of subject-specific HDL functionality, sometimes described as HDL dysfunction, on the pharmacodynamic effects of CSL112.**

Cholesterol removal from atherosclerotic plaque and its proposed removal by CSL112

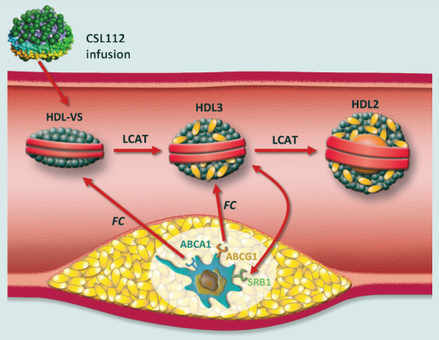


Figure 1

- In reverse cholesterol transport, free cholesterol (FC) is transferred from cells to HDL-VS (pre-β1 HDL) via the ABCA1 transporter, which is abundant in plaque macrophages in atherosclerotic lesions. FC in the HDL particle is then esterified by lecithin:cholesterol acyltransferase (LCAT) forming larger HDL particles (HDL3 and HDL2). FC is also transferred to mature HDL via the ABCG1 and SRB1 transporters. Esterified HDL cholesterol is then transferred to the liver.
- Infusion of CSL112 increases both formation of HDL-VS (pre-β1 HDL) and cholesterol efflux capacity, predominantly via the ABCA1 transporter.[2, 4]

Methods

Study design

- Two Phase 1 studies of CSL112 have been conducted; single (SAD) and multiple ascending dose (MAD) studies.
  - In the SAD study (NCT01129661), healthy subjects were randomized to receive a single infusion of placebo or 5, 15, 40, 70, 105, 135 mg/kg CSL112.
  - In the MAD study (NCT01281774), healthy subjects were randomized to receive: placebo, 3.4 or 6.8 g CSL112 once weekly (1W; 4 infusions); or placebo or 3.4 g CSL112 twice weekly (2W; 8 infusions) for 4 weeks.
- In the Phase 2a study (NCT01499420), patients with stable atherosclerotic disease (defined as a history of atherosclerotic coronary artery disease (CAD), surgical revascularization or peripheral vascular disease) were randomized to receive a single infusion of placebo or 1.7, 3.4 or 6.8 g of CSL112.
- Plasma and serum samples were obtained at multiple time-points allowing assessment of apoA-I, lipid profile and cholesterol efflux capacity in all three studies.

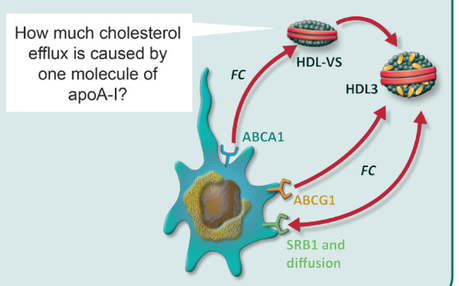
PK and biomarker assessments

- Plasma apoA-I and apolipoprotein B were measured by an immunoturbidimetric method.
- Serum total cholesterol efflux capacity was measured using the method described by de la Llera-Moya *et al.*[5]
- HDL-VS was assessed by ELISA using an anti-pre-β1 HDL antibody (Sekisui, Lexington, USA).
- Other lipid parameters were assessed by standard enzymatic methods.

Statistical analysis

- The specific cholesterol efflux activity of apoA-I (HDL function), was calculated in all subjects at baseline as follows:

$$\text{Specific apoA-I activity} = \frac{\text{cholesterol efflux capacity}}{\text{apoA-I concentration}} \left[\frac{L\%}{g}\right]$$



- An unpaired t-test was used to compare baseline levels of apoA-I, HDL-C, cholesterol efflux and HDL function between healthy subjects (Phase 1 studies) and patients (Phase 2a) post-hoc.
- Change in total cholesterol efflux (baseline-corrected area under the effect curve, $AUEC_{0\text{-}24h}$) was plotted against change in apoA-I (baseline-corrected area under the curve, $AUC_{0\text{-}24h}$) and linear regression analysis used to compare the relationship in healthy subjects (Phase 1 SAD and MAD combined) and patients (Phase 2a).
- Subjects were pooled from all three studies and stratified into tertiles by baseline HDL function. Linear regression analysis was used to determine the effect of baseline HDL function cholesterol efflux for each tertile.

Limitations

- Study limitations which may potentially introduce bias include healthy subjects in the Phase 1 SAD study who were overweight or obese; patients receiving concomitant medication and the post-hoc nature of the analysis.

Results

- In total 93 healthy subjects (Phase 1) and 44 patients (Phase 2a) were treated with CSL112 or placebo. Baseline demographics and lipid profiles of the study populations are shown in Table 1.

Table 1. Baseline demographics and lipid profile

	Phase 1 SAD (n=57)	Phase 1 MAD (n=36)	Phase 2a (n=44)
Demographics			
Age, years	26.0 (8.2)	25.3 (6.9)	59.1 (9.8)
Sex, % male	63.2	63.9	72.7
BMI, kg/m²	27.2 (5.9)	24.1 (3.7)	31.1 (7.2)
Race, % white	94.7	97.2	79.5
Lipid Profile			
ApoA-I, mg/dL	119.8 (16.6)	122.5 (23.3)	128.3 (19.8)
Cholesterol efflux capacity, % efflux/4h	10.5 (2.3)	10.6 (2.5)	9.4 (2.7)
Pre-β1 HDL, µg/mL	15.4 (7.4)	17.4 (7.6)	19.5 (8.0)
Total cholesterol, mg/dL	172.1 (35.0)	167.8 (32.1)	166.5 (43.4)
HDL cholesterol, mg/dL	54.9 (11.6)	54.9 (15.5)	44.1 (10.7)
Non-HDL total cholesterol, mg/dL	117.1 (36.9)	112.9 (28.1)	122.4 (43.0)
Apolipoprotein B, mg/dL	70.0 (19.8)	70.0 (16.3)	87.4 (28.0)
Triglycerides, mg/dL	88.3 (52.5)	84.3 (35.7)	169.7 (87.8)

Shown are mean and (standard deviation) unless otherwise stated

Comparison of functional and concentration measures of HDL

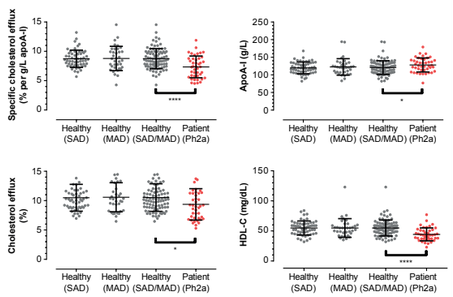


Figure 2

- Atherosclerotic patients had lower HDL function than healthy subjects (p<0.0001), consistent with prior work showing impaired HDL function in CAD patients.[6]
- Specific cholesterol efflux activity of apoA-I (HDL function) is a better discriminator than apoA-I or cholesterol efflux alone.

**** p<0.0001, * p<0.05
Individual values are shown alongside the mean and standard deviation

Infusion of CSL112 causes an immediate, profound and sustained increased in apoA-I and a dramatic increase in cholesterol efflux capacity

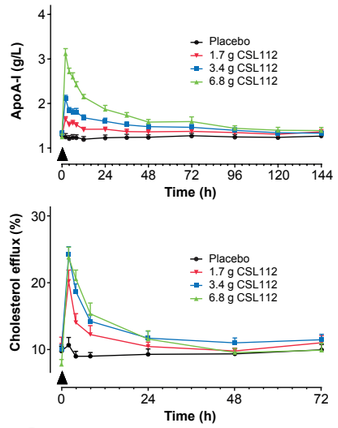


Figure 3

- Infusion of CSL112 induced a rapid increase in apoA-I, which peaked at the end of infusion (2 h) at 2- to 3-fold above baseline and remained elevated for up to 72 hours.
- Cholesterol efflux capacity peaked at the end of infusion (2 h) with CSL112 and was elevated 2- to 3-fold above baseline at high doses of CSL112.
- Data shown are taken from the Phase 2a study; effects were similar in healthy subjects (Phase 1).

▲ = infusion of CSL112

CSL112 enhances cholesterol efflux to a similar extent in healthy subjects and in patients with stable atherosclerotic disease

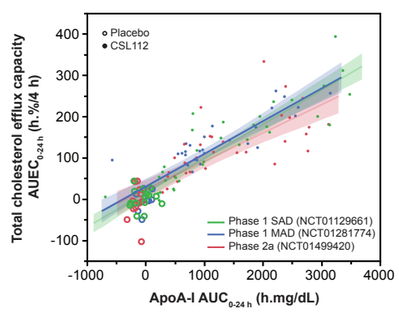


Figure 4

- In all three study populations a linear relationship was observed between individual baseline-corrected apoA-I exposure and baseline-corrected total cholesterol efflux.
- Coincident lines indicate equal elevation in efflux between healthy subjects and patients (p=0.111).

The ability of CSL112 to enhance cholesterol efflux capacity is not influenced by baseline HDL function

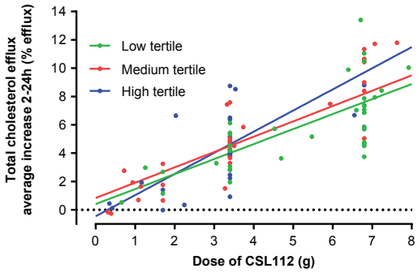


Figure 5

- Cholesterol efflux capacity post infusion (2 h) to 24 hours increased in a dose-dependent manner following infusion of CSL112. Cholesterol efflux was similarly elevated between subjects in the highest and lowest tertiles of HDL functionality.

Conclusions

- "Specific cholesterol efflux activity" of apoA-I, a biomarker of HDL function, differed more significantly between healthy and atherosclerotic populations than either apoA-I or cholesterol efflux alone.
- CSL112 caused strong and quantitatively similar elevation in cholesterol efflux regardless of baseline specific cholesterol efflux activity.
- These data suggest that CSL112 will effectively elevate cholesterol efflux in patients with impaired HDL functionality.
- Prospective studies are needed to confirm the utility of specific cholesterol efflux activity of apoA-I as a biomarker of CAD risk or a biomarker of therapeutic efficacy.

CSL112 may offer a novel means to rapidly remove cholesterol from plaque following an ACS event and rapidly reduce the recurrence of cardiovascular events.

References

1. Easton R *et al. J Clin Pharmacol* 2014; 54: 301–10.
2. Gille A *et al. Arterioscler Thromb Vasc Biol* 2014; 34: 2106–14.
3. Tricoci P *et al. Circulation* 2013; 128: A18589.
4. Gille A *et al. Circulation* 2013; 128: A15780.
5. de la Llera-Moya M *et al. Arterioscler Thromb Vasc Biol* 2010; 30: 796-801.
6. Khera AV *et al. N Engl J Med* 2011:364:127-35.

Disclosures

- Funding of the clinical trials (NCT01129661, NCT01281774, NCT01499420) by CSL Limited.
- All authors are employees of CSL Limited or CSL Behring.
- Presentation includes investigational uses of drugs.

Acknowledgements

Editorial assistance was provided by Meridian HealthComms on behalf of CSL Behring.