DRUG DEVELOPMENT

Blocking endothelial lipase with monoclonal antibody MEDI5884 durably increases high density lipoprotein in nonhuman primates and in a phase 1 trial

John E. Le Lay[1†], Qun Du[2], Minal B. Mehta[1], Nicholas Bhagroo[1], B. Timothy Hummer[3], Judith Falloon[4], Glenn Carlson[5], Anton I. Rosenbaum[6], ChaoYu Jin[7], Holly Kimko[8], Lan-Feng Tsai[9], Steven Novick[10], Bill Cook[4], David Han[11], Chang Yeop Han[12], Tomas Vaisar[12], Alan Chait[12], Sotirios K. Karathanasis[13], Christopher J. Rhodes[13], Boaz Hirshberg[4], Melissa M. Damschroder[2], Judith Hsia[4], Joseph S. Grimsby[13]*

Cardiovascular disease (CVD) is the leading global cause of death, and treatments that further reduce CV risk remain an unmet medical need. Epidemiological studies have consistently identified low high-density lipoprotein cholesterol (HDL-C) as an independent risk factor for CVD, making HDL elevation a potential clinical target for improved CVD resolution. Endothelial lipase (EL) is a circulating enzyme that regulates HDL turnover by hydrolyzing HDL phospholipids and driving HDL particle clearance. Using MEDI5884, a first-in-class, EL-neutralizing, monoclonal antibody, we tested the hypothesis that pharmacological inhibition of EL would increase HDL-C by enhancing HDL stability. In nonhuman primates, MEDI5884 treatment resulted in lasting, dose-dependent elevations in HDL-C and circulating phospholipids, confirming the mechanism of EL action. We then showed that a favorable lipoprotein profile of elevated HDL-C and reduced low-density lipoprotein cholesterol (LDL-C) could be achieved by combining MEDI5884 with a PCSK9 inhibitor. Last, when tested in healthy human volunteers, MEDI5884 not only raised HDL-C but also increased HDL particle numbers and average HDL size while enhancing HDL functionality, reinforcing EL neutralization as a viable clinical approach aimed at reducing CV risk.

INTRODUCTION

Coronary heart disease (CHD) is one of the main causes of morbidity and mortality worldwide. Coronary atherosclerosis, the underlying condition responsible for CHD, is the accumulation of cholesterol-rich plaques in the walls of coronary arteries that induce inflammation, decrease endothelium-dependent vasorelaxation, and can ultimately lead to major adverse cardiovascular (CV) events such as myocardial infarction. Despite the effectiveness of current therapies focused on regulating low-density lipoprotein cholesterol (LDL-C), there remains a substantial residual risk in patients with atherosclerotic CV disease (CVD) highlighting the need for novel approaches that target additional risk factors (*1*). Low high-density lipoprotein cholesterol (HDL-C) concentrations are commonly associated with increased risk of CVD in epidemiological studies (*2*). The atheroprotective effect of HDL is classically attributed to its central role in promoting the initial step in reverse cholesterol transport (RCT) as first postulated more than 50 years ago (*3*). A correlation between positive CV outcomes with increased HDL-C has yet to be established through clinical testing, but it is now generally accepted that HDL-mediated cholesterol efflux capacity is a better surrogate measure for CVD risk prediction than HDL-C itself (*4*, *5*). Although the capacity to facilitate cholesterol efflux is its most well-validated activity, other beneficial functions have also been assigned to HDL including anti-inflammatory, antioxidant, and antifibrotic properties, as well as the ability to impart endothelial protection (*6*). Because comparative studies in the clinical setting do not exist to establish the superiority of any of these activities over another, interventions that raise HDL-C and result in improved HDL functionality across multiple parameters could be an attractive approach toward achieving CV risk reduction.

Endothelial lipase (EL) is a circulating phospholipase A1 enzyme and member of the triglyceride (TG) lipase family that plays a key role in regulating plasma HDL metabolism. Within the TG lipase family, which includes hepatic lipase and lipoprotein lipase, EL has the highest degree of specificity and substrate preference for HDL phospholipid (HDL-PL) (*7*). Through hydrolysis of HDL-PL, it promotes lipid depletion, destabilization, and renal clearance of HDL particles (*7*, *8*). In preclinical models, overexpression of EL has been shown to reduce HDL-C, whereas germline deletion of the gene encoding EL (*LIPG*) in mice leads to increased HDL-C (*9*–*12*). Furthermore, human genetics studies (*13*–*15*), including the identification of loss-of-function variants (*9*, *16*–*18*), have confirmed the

[1]Bioscience Metabolism, Research and Early Development, Cardiovascular, Renal, and Metabolism (CVRM), BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD 20878, USA. [2]Biologic Therapeutics, Antibody Discovery and Protein Engineering, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD 20878, USA. [3]CVRM Safety, Clinical Pharmacology and Safety Sciences, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD 20878, USA. [4]Clinical Development, Research and Early Development, CVRM, BioPharmaceuticals Medical, AstraZeneca, Gaithersburg, MD 20878, USA. [5]Clinical CV, Late Stage Development, CVRM, BioPharmaceuticals Medical, AstraZeneca, Gaithersburg, MD 20878, USA. [6]Integrated Bioanalysis, Clinical Pharmacology and Quantitative Pharmacology, Clinical Pharmacology and Safety Sciences, R&D, AstraZeneca, South San Francisco, CA 94080, USA. [7]Clinical Immunology and Bioanalysis, Clinical Pharmacology and Safety Sciences, BioPharmaceuticals R&D, AstraZeneca, South San Francisco, CA 94080, USA. [8]Clinical Pharmacology and DMPK, Clinical Pharmacology and Safety Sciences, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD 20878, USA. [9]CVRM Biometrics, Data Sciences and AI, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD 20878, USA. [10]Data Sciences and Quantitative Biology, Discovery Sciences, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD 20878, USA. [11]Parexel International, Glendale, CA 91206, USA. [12]Division of Metabolism, Endocrinology and Nutrition, Department of Medicine, University of Washington, Seattle, WA 98915, USA. [13]Research and Early Development, Cardiovascular, Renal, and Metabolism, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD 20878, USA.
*Corresponding author. Email: joseph.grimsby@astrazeneca.com
†Present address: Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46225, USA.

relationship between plasma EL abundance and HDL metabolism in humans with EL elevation observed in multiple pathophysiological conditions including metabolic syndrome, obesity, and coronary artery disease (*19*–*24*). Collectively, these data indicate that inhibition of EL enzymatic activity could be an effective means toward increases in HDL-C and RCT flux that may contribute to reduced incidence of CVD events.

Here, we describe the development of MEDI5884, a potent, humanized monoclonal antibody (mAb) that specifically and effectively neutralizes EL. In vitro, we established that MEDI5884 is highly selective for EL, with no detectable binding to other related TG lipase family members. In nonhuman primate (NHP) studies, we showed that neutralization of EL with MEDI5884 results in dose-dependent elevations in HDL-C and HDL-PL, consistent with the mechanism of action. Last, we demonstrated the safety and clinical efficacy of MEDI5884 in a phase 1 trial in healthy human volunteers (NCT03001297), establishing that EL neutralization is a viable HDL-targeting therapeutic intervention with the potential to address residual CVD risk.

RESULTS

MEDI5884 is a potent, EL-neutralizing mAb

MEDI5884 is a humanized and affinity-matured immunoglobulin G (IgG) 4Pκ originally generated by mouse hybridoma technology. MEDI5884's affinity for human and cynomolgus monkey (cyno) EL, as determined by surface plasmon resonance, was 4.1 and 1.6 nM, respectively. Species specificity of MEDI5884 was demonstrated in Octet binding assays, which confirmed binding to human and cyno EL, but not to mouse, rat, or rabbit EL (Fig. 1A and fig. S1A). To assess its inhibitory potential, MEDI5884 was tested in an assay of EL activity in which HDL is the substrate for phospholipid hydrolysis. Consistent with the binding results, MEDI5884 dose dependently neutralized both human and cyno EL with half-maximal inhibitory concentrations (IC_{50}) of 1.3 ± 0.53 and 1.7 ± 0.55 nM, respectively but had no impact on mouse or rat EL activity (Fig. 1B and fig. S1B). Because EL shares sequence homology with other TG lipases, similar activity assays using very low-density lipoprotein (VLDL) as the substrate were developed for both hepatic lipase and lipoprotein lipase. Even at the highest concentration tested (1000 nM), MEDI5884 showed no evidence of inhibiting VLDL lipolysis in these assays (table S1). To characterize the ability of MEDI5884 to neutralize EL in vivo, we administered MEDI5884 to mice after adenoviral expression of human EL (hEL). In this system, overexpression of hEL markedly and consistently reduced endogenous HDL-C within 4 days (Fig. 1C). Subsequent administration of an isotype control antibody failed to affect EL activity as evidenced by a progressive decline in HDL-C over the next 24 hours. In contrast, MEDI5884 prevented further reduction in HDL-C during the course of treatment and resulted in restoration of HDL-C nearly reaching baseline concentrations (Fig. 1C), demonstrating that MEDI5884 can inhibit hEL in vivo. Collectively, these results confirmed that MEDI5884 is a potent, highly selective, EL-binding antibody capable of neutralizing both human and cyno EL.

Effect of MEDI5884 on lipoprotein metabolism in NHPs

To assess the effect of endogenous EL neutralization on lipoprotein metabolism, a pharmacokinetic (PK) and pharmacodynamic (PD) study was conducted in cynomolgus monkeys in which single doses

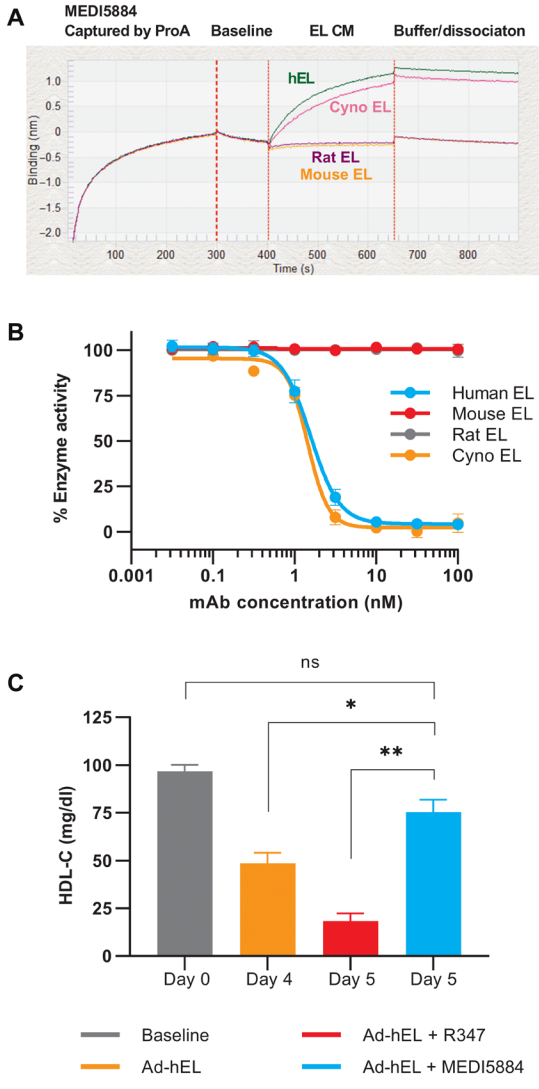

Fig. 1. MEDI5884 binds and neutralizes human EL in vitro and in vivo. (**A**) Species-specific binding of MEDI5884 was demonstrated using conditioned media (CM) containing human EL (hEL), cynomolgus monkey EL (cyno EL), mouse EL, or rat EL ($n = 3$). (**B**) EL activity was measured in CM containing hEL, cyno EL, mouse EL, or rat EL in the presence of MEDI5884 at the indicated concentrations ($n = 3$ or 4). Data are presented as means ± SD. (**C**) Four days after delivery of adenovirus to overexpress hEL (Ad-hEL) ($n = 12$), mice were administered a 30 mg/kg dose of MEDI5884 ($n = 5$) or isotype control antibody R347 ($n = 7$). HDL-C was measured in plasma collected before Ad-hEL delivery (day 0), before antibody administration (day 4), and 1 day after antibody treatment (day 5). Data are presented as means ± SEM. *$P < 0.01$ and **$P < 0.0001$, determined by analysis of variance (ANOVA). ns, not significant.

of MEDI5884 (0.5, 6, or 30 mg/kg) were administered via subcutaneous injection. Consistent with its role in HDL metabolism, neutralization of EL with MEDI5884 increased plasma HDL-C in a dose-dependent manner (Fig. 2A). For the 30 mg/kg dose, an approximate twofold increase in HDL-C was achieved within 2 weeks, which persisted for the duration of the 2-month study (Fig. 2A). The 6 mg/kg dose had a similar maximum effect, but HDL-C returned to baseline concentrations by the end of the study, whereas the 0.5 mg/kg dose resulted in a smaller magnitude of HDL-C increase and a return to baseline within 3 weeks. ApoA1, the major

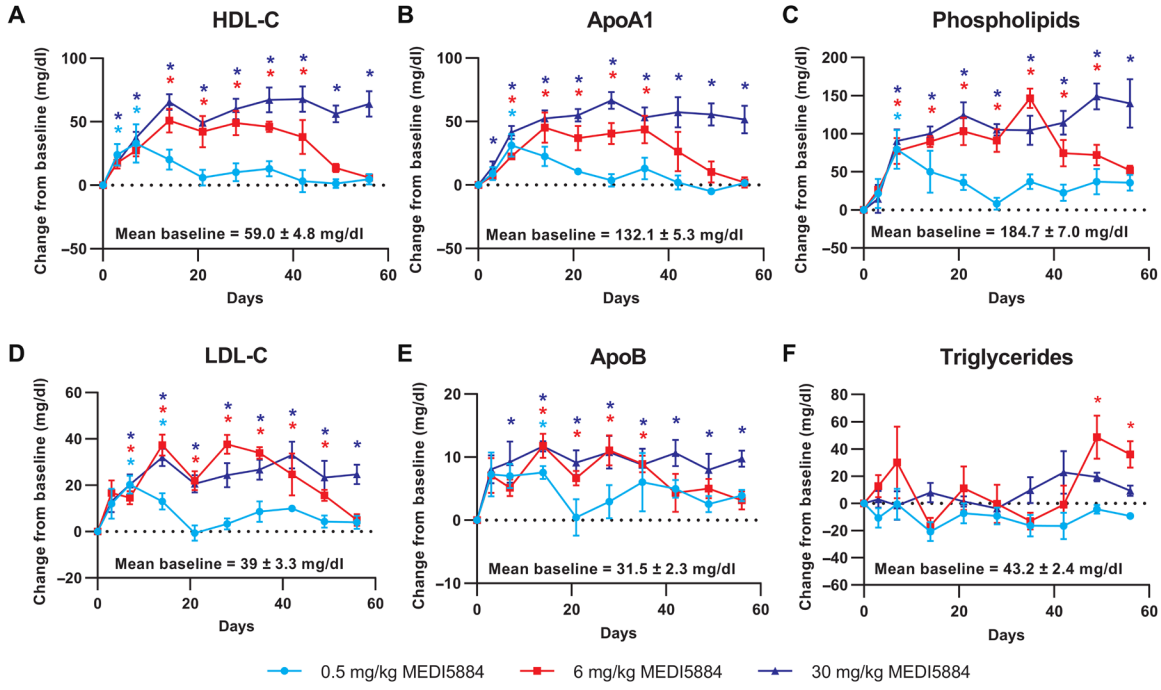

Fig. 2. Effect of MEDI5884 on lipoprotein metabolism in NHPs. Healthy cynomolgus monkeys were administered a single subcutaneous dose of MEDI5884 at either 0.5, 6, or 30 mg/kg ($n = 3$ for each dose group). HDL-C (**A**), ApoA1 (**B**), phospholipids (**C**), LDL-C (**D**), ApoB (**E**), and triglycerides (**F**) were measured in plasma samples collected at the indicated time points. Data are presented as average change from baseline measurements ± SEM. The mean baseline value ($n = 9$) is indicated in each plot. *$P < 0.05$ relative to baseline, determined by ANOVA.

apolipoprotein constituent of HDL particles, was also increased in a dose-dependent manner (Fig. 2B), and a commensurate increase in plasma phospholipids indicated that MEDI5884 inhibited the ability of EL to hydrolyze its substrate (Fig. 2C). In line with previously published reports in rodent models (*25*, *26*), we also observed clear increases in LDL-C and ApoB, the distinguishing apolipoprotein component of LDL and VLDL, that paralleled the elevation in HDL-C, but these changes were smaller in magnitude (Fig. 2, D and E). No clear treatment-related effect on plasma TGs was observed (Fig. 2F), whereas increases in total cholesterol (total-C) and non–HDL-C reflected the changes in HDL-C and LDL-C (fig. S2, A and B). All PD effects were consistent with MEDI5884 exposure, which was nonlinear in this dose range as shown by greater than dose-proportional increases in observed maximum concentration (C_{max}) and area under the curve (AUC) (fig. S3 and table S2). The nonlinear PK is likely a consequence of target-mediated disposition observed in the wide range of doses used in this study. These results demonstrate that MEDI5884, by neutralizing endogenous EL and inhibiting phospholipid hydrolysis, is capable of considerable and sustained HDL-C raising in NHPs.

PCSK9 inhibition prevents MEDI5884-dependent LDL-C raising

In light of the increased LDL-C that was observed after EL neutralization in cynomolgus monkeys, we designed a combination pharmacology study to assess the impact of PCSK9 inhibition on lipoprotein metabolism after MEDI5884 treatment. To better mimic a hypothetical patient population already on LDL-C–lowering medication, monkeys were pretreated with a PCSK9-neutralizing antibody for 4 weeks to establish a baseline of low LDL-C. Compared to vehicle-treated animals, PCSK9 inhibition reduced LDL-C by about 70% (Fig. 3A) and maintained that reduction for the duration of the 4-week lead-in period, with no appreciable effect on HDL-C (Fig. 3B). In animals treated with vehicle during the lead-in period, subsequent administration of MEDI5884 led to the expected increases in both HDL-C and LDL-C. However, when administered on top of the PCSK9 inhibitor during the second 4-week period, MEDI5884 maintained the ability to raise HDL-C to a similar degree, whereas the magnitude of LDL-C increase was blunted, indicating that the LDL particles continued to be taken up by the LDL receptor. The patterns of LDL-C and HDL-C were matched by respective changes in ApoB and ApoA1 (fig. S4, A and B). This result indicates that the increase in LDL-C observed with MEDI5884 treatment in monkeys can be mitigated by mechanisms that up-regulate the LDL receptor, such as PCSK9 neutralization.

Effect of MEDI5884 on lipoprotein metabolism in man

On the basis of its ability to induce sustained HDL-C increases in NHPs, a first-in-human, phase 1, randomized, blinded, placebo-controlled study (NCT03001297) was conducted to evaluate the safety, tolerability, and efficacy of MEDI5884 in healthy human volunteers (table S3). Participants were randomized to receive a single subcutaneous dose of 30, 100, 300, or 600 mg of MEDI5884 or placebo with follow-up duration dependent on the cohort and PK time course profile (fig. S5). MEDI5884 was generally well-tolerated as there were no treatment emergent adverse events that led to withdrawal from the study, with injection site reactions being most commonly reported (table S4). As was seen in NHPs, dose-dependent increases in HDL-C, ApoA1, and HDL-PL were observed in this study (Fig. 4, A to C); however, the magnitudes of these increases

were markedly less, with maximum effects on HDL-C reaching about 50% over baseline. Nevertheless, near-peak effects on HDL-C were observable 28 days after treatment in all but the lowest dose group, indicating that a once-monthly dosing regimen of MEDI5884 could be sufficient to sustain the elevation. Although the HDL-C raising elicited by MEDI5884 in NHPs was paralleled by an increase in LDL-C, this effect was not as profound in humans. Rather, changes in LDL-C and ApoB-100 were mostly negligible and not consistent with dose, only emerging in the 600 mg group at the final time point (Fig. 4, D and E). There was no clear dose-related effect on TGs, which were variable and did not consistently change from baseline measurements (Fig. 4F). Total-C and non–HDL-C changed in accordance with HDL-C and LDL-C values (fig. S6, A and B). MEDI5884 concentrations over time (fig. S7) and PK analysis revealed greater than dose-proportional increases in observed C_{max} and AUC for MEDI5884 (table S5), with PD effects largely consistent with exposure. These results demonstrated that considerable and lasting HDL-C elevation can be achieved in humans through neutralization of EL with MEDI5884.

To provide a more detailed characterization of the changes to lipoprotein profiles that occur after EL neutralization with MEDI5884, nuclear magnetic resonance (NMR) spectroscopy was also performed. In accordance with a mechanism involving the stabilization of HDL particles, EL neutralization resulted in an increase in HDL particle number (HDL-P) (Fig. 5A). However, the magnitude of this increase was not directly

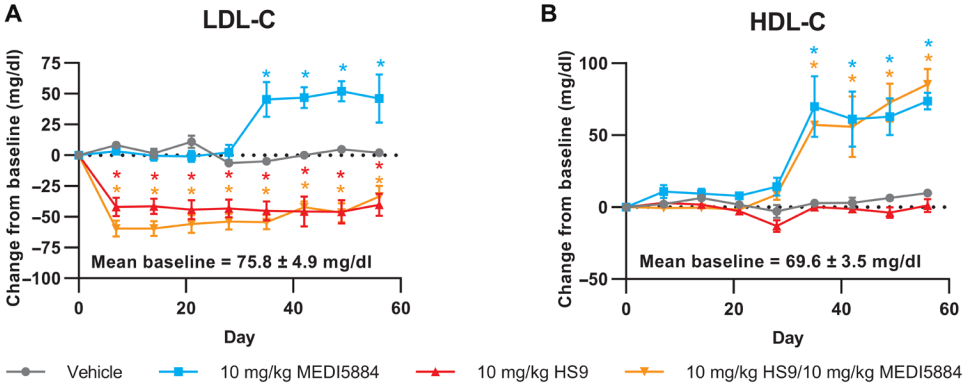

Fig. 3. Effect of combined EL and PCSK9 inhibition on LDL-C and HDL-C in NHPs. Healthy cynomolgus monkeys were administered weekly, subcutaneous injections of PCSK9 inhibitor HS9 (10 mg/kg, $n = 8$) or vehicle ($n = 8$) throughout the study starting on day 0. Four animals from each group were then administered subcutaneous doses of MEDI5884 (10 mg/kg, $n = 4$) or vehicle ($n = 4$) on day 28 and day 42. LDL-C (**A**) and HDL-C (**B**) were measured in plasma samples collected at the indicated time points. Data are presented as average change from baseline (day 0) measurements ± SEM. The mean baseline value ($n = 16$) is indicated in each plot. *$P < 0.05$ relative to change in vehicle, determined by ANOVA.

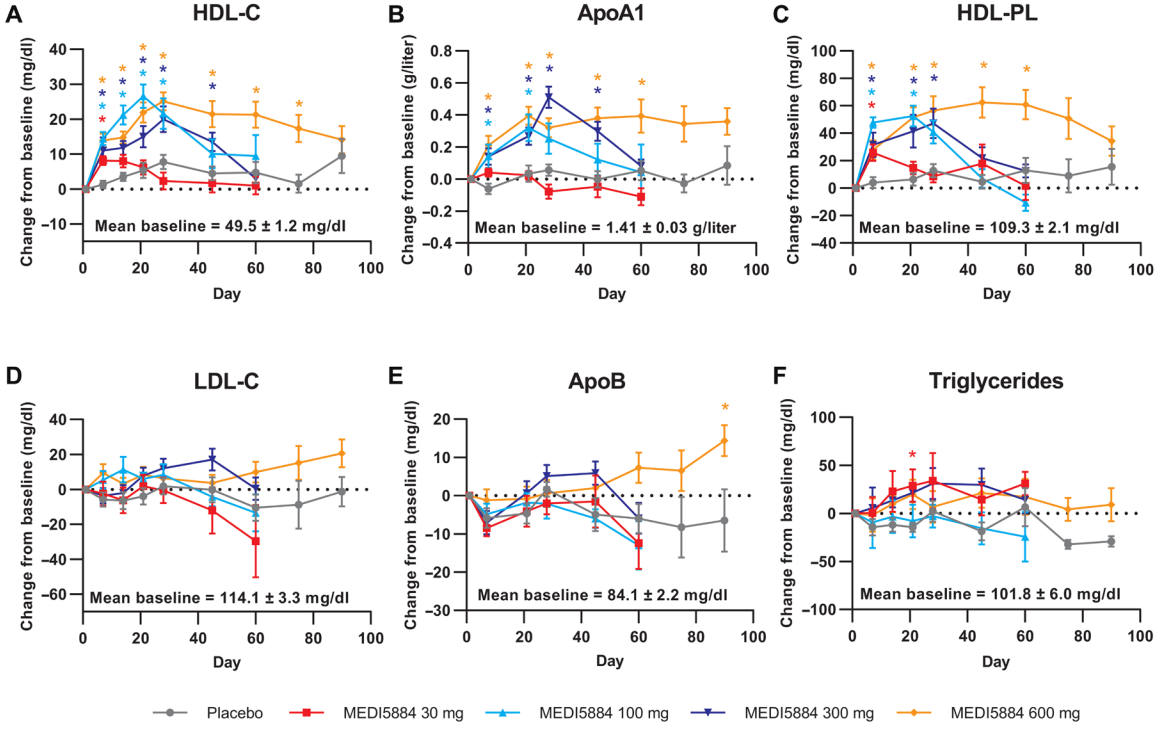

Fig. 4. Effect of MEDI5884 on lipoprotein metabolism in healthy human participants. Healthy human volunteers were administered a single subcutaneous injection of 30 mg ($n = 12$), 100 mg ($n = 12$), 300 mg ($n = 12$), or 600 mg ($n = 12$) of MEDI5884 or placebo ($n = 16$). HDL-C (**A**), ApoA1 (**B**), HDL-PL (**C**), LDL-C (**D**), ApoB (**E**), and triglycerides (**F**) were measured in plasma samples collected at the indicated time points. Data are presented as average change from baseline measurements ± SEM. The mean baseline value ($n = 64$) is indicated in each plot. *$P < 0.05$ relative to change in placebo, determined by ANOVA.

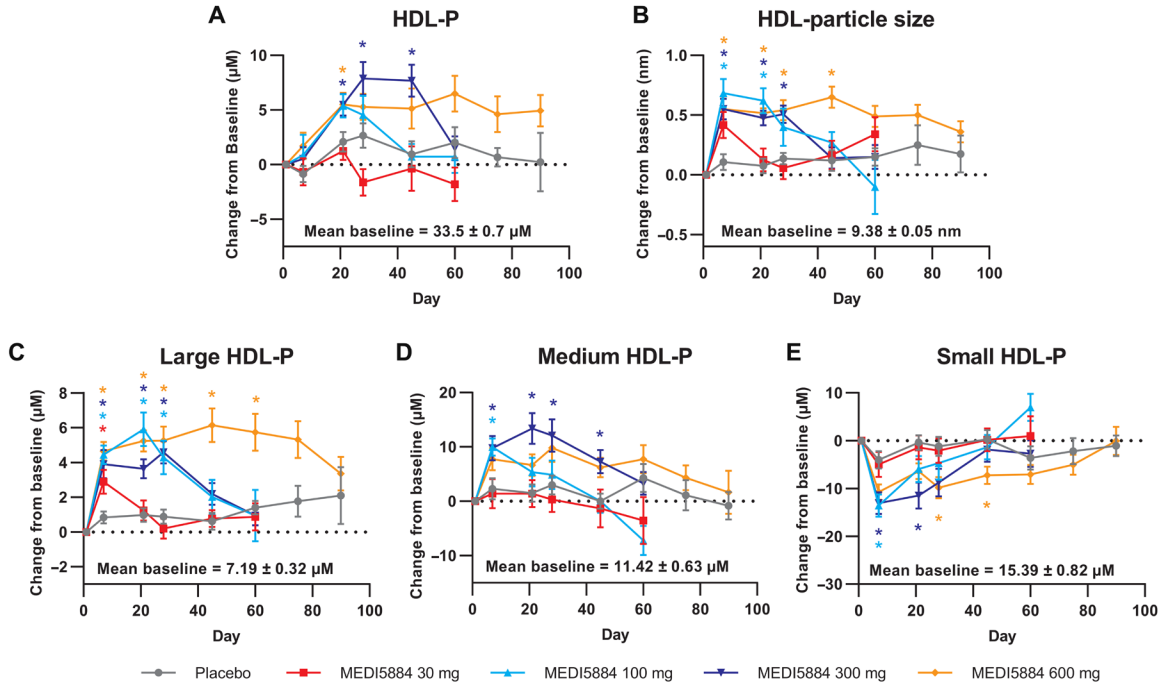

Fig. 5. Effect of MEDI5884 on HDL particle metabolism in healthy human participants. Healthy human volunteers were administered a single subcutaneous injection of 30 mg ($n = 12$), 100 mg ($n = 12$), 300 mg ($n = 12$), or 600 mg ($n = 12$) of MEDI5884 or placebo ($n = 16$). HDL particle number (HDL-P) (**A**), HDL-particle size (**B**), large HDL-P (**C**), medium HDL-P (**D**), and small HDL-P (**E**) were measured in plasma samples collected at the indicated time points. Data are presented as average change from baseline measurements ± SEM. The mean baseline value ($n = 64$) is indicated in each plot. *$P < 0.05$ relative to change in placebo, determined by ANOVA.

proportional to the observed change in HDL-C, indicating that particle number was not the only factor contributing to the collective elevation in HDL-C. Our analysis demonstrated that the average HDL particle size was also increased after MEDI5884 treatment, reaching a plateau within 1 week, with the duration of the change being generally dose-related (Fig. 5B). Further analysis revealed that the change in size was due to increases in both large and medium HDL particles and a pronounced, acute decline in the small HDL subfraction (Fig. 5, C to E). These data clearly establish that the increase in HDL-C resulting from EL neutralization is the consequence of increases in both HDL particle number and average particle size, reinforcing a key role for EL in the processes dictating retention and maturation of HDL.

In addition, NMR analysis was used to assess the potential impact of EL inhibition on LDL, intermediate-density lipoprotein (IDL), and VLDL particle remodeling. In agreement with the measurements of LDL-C and ApoB, we found that LDL particle number was mostly unchanged during the course of the study, with possible increases only evident in the 600 mg dose group at the very latest time point (Fig. 6A). Despite this marginal effect on LDL abundance, a small but measurable increase in average LDL particle size was apparent 1 week after MEDI5884 administration (Fig. 6B), resulting mainly from an acute decrease in the small LDL subfraction (Fig. 6, C and D). IDL and VLDL particle concentrations were not affected in a dose-dependent manner (fig. S8, A and B). The dynamic alterations in LDL particles are consistent with the observed changes to LDL-C and demonstrate that the effect of EL neutralization on lipoprotein metabolism is not restricted to HDL.

Effect of MEDI5884 on HDL cholesterol efflux and anti-inflammatory properties in man

To test the function of HDL particles after EL neutralization in humans, cholesterol efflux assays were performed on ApoB-depleted plasma collected at baseline and 28 days after MEDI5884 treatment (100, 300, and 600 mg and placebo groups). Consistent with the observed changes in HDL-C, global efflux was increased at the 28-day time point in participants treated with MEDI5884 (Fig. 7A), whereas ABCA1-dependent efflux was unchanged (Fig. 7B), likely reflecting the small average increase in HDL particle size observed after EL neutralization. Moreover, the magnitude of increase in cholesterol efflux was proportionally similar to the observed changes in HDL-P, suggesting that the HDL molecules retained their ability to efflux cholesterol from macrophages. To expand on our characterization of HDL functionality, assays were also performed to test the anti-inflammatory properties of HDL after EL neutralization. In these experiments, HDL isolated 28 days after MEDI5884 treatment demonstrated an enhanced ability to suppress palmitate-induced expression of both *serum amyloid A3* (*Saa3*) and *C-C motif chemokine ligand 2* (*Ccl2*) from 3T3-L1 adipocytes relative to HDL collected before dosing (Fig. 7, C and D). Collectively, these data suggest that EL neutralization with MEDI5884 provides a mechanism to promote increases in both cholesterol efflux capacity and anti-inflammatory potential, two key predictors of CV risk benefit.

DISCUSSION

In this work, we demonstrate that MEDI5884-mediated neutralization of EL drives sustained elevations in functional HDL in both

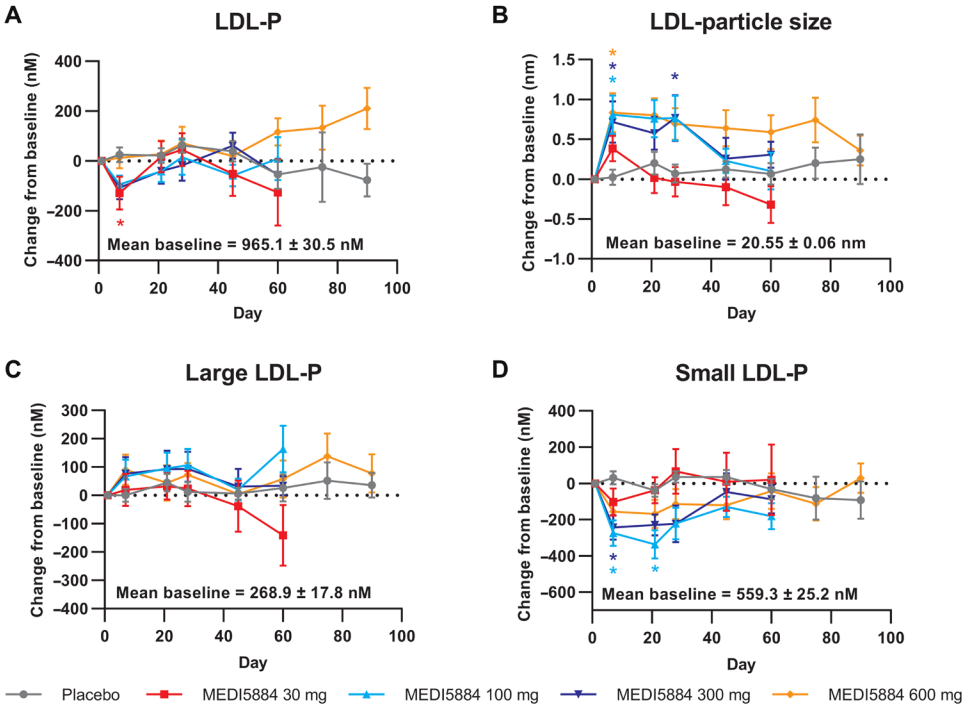

Fig. 6. Effect of MEDI5884 on LDL particle metabolism in healthy human participants. Healthy human volunteers were administered a single subcutaneous injection of 30 mg ($n = 12$), 100 mg ($n = 12$), 300 mg ($n = 12$), or 600 mg ($n = 12$) of MEDI5884 or placebo ($n = 16$). LDL particle number (LDL-P) (**A**), LDL-particle size (**B**), large LDL-P (**C**), and small LDL-P (**D**) were measured in plasma samples collected at the indicated time points. Data are presented as average change from baseline measurements ± SEM. The mean baseline value ($n = 64$) is indicated in each plot. *$P < 0.05$ relative to change in placebo, determined by ANOVA.

NHPs and in healthy human volunteers. MEDI5884 displays PK/PD characteristics supportive of a once-monthly dosing regimen to maintain this increase in HDL-C that could offer additional benefit to patients suffering from pathologies associated with coronary atherosclerosis. Despite the cardioprotective activities associated with HDL and its reported inverse relationship with CVD risk in epidemiological studies, most therapeutic interventions targeting HDL elevation have failed to demonstrate clinical efficacy (*27*–*29*). Perhaps, most notable were the CV outcomes trials performed to investigate inhibition of cholesterol ester transfer protein (CETP). CETP plays a key role in regulating RCT by catalyzing the transfer of esterified cholesterol (CE) from HDL to LDL and VLDL and was considered an ideal target to address lipid control due to the combination of increased HDL-C and reduced LDL-C observed with CETP deficiency in man (*14*, *30*, *31*). Although pharmacological inhibition of CETP had a similar effect on lipoprotein metabolism, with some raising HDL-C by more than 100% and most showing marked increases in cholesterol efflux capacity (*32*–*34*), four separate phase 3 studies have failed to show a meaningful degree of CV risk reduction as the result of CETP inhibition (*35*–*38*). It is important to consider, however, that the inhibition of CETP interferes with what is likely a key step in the RCT process. Therefore, although HDL-C increases, the CETP-dependent mechanism to transfer CE to ApoB-containing lipoproteins is lost, which may impede a substantial portion of the LDL-mediated path that returns cholesterol to the liver from the periphery and diminish the potential importance of increasing HDL-C (*39*). By instead targeting EL to raise HDL-C, the CETP-dependent component important to fully functional RCT remains intact, allowing transfer of CE from HDL to ApoB-containing lipoproteins. The increase in LDL size that results from MEDI5884 treatment may provide circumstantial evidence for enhanced transfer of CE from HDL to LDL, indicating a key, distinguishing advantage over CETP inhibitors in this regard. Further studies should be performed to formally investigate the capacity of HDL particles to mobilize RCT after EL neutralization and to determine whether MEDI5884 effectively circumvents this potential liability inherent to CETP inhibition.

Similarly, findings have been controversial in trials using recombinant HDL (rHDL) administration as a strategy to increase HDL and reduce coronary atheroma volume (*40*–*42*). One limitation common to each of the rHDL approaches is the requirement for serial infusions, making them impractical as an option for chronic treatment. Furthermore, the observed elevations in ApoA1 with rHDL treatments tend to be temporary, evident for less than 24 hours after infusion, and do not represent persistent increases in HDL (*40*, *43*). It may be unreasonable to expect substantial plaque regression to occur in a chronic disease after such a short treatment regimen. For instance, it has been demonstrated that LDL-C lowering with statins, the current standard of care to treat atherosclerosis, is also insufficient to provoke reductions in plaque volume when treatment is limited to short periods of 8 to 12 weeks (*44*). Instead, the acute benefits of statins are derived from improved plaque stability, a clinical readout that has yet to be assessed in rHDL trials. Whereas promoting atherosclerotic cardiac artery regression would seem ideal, there may be additional benefits to acute rHDL treatments that are not obvious in short-term imaging studies. Unlike rHDL approaches that offer only transient increases in HDL-C, MEDI5884 was able to maintain HDL-C elevations, thereby offering an avenue to assess the impact of persistent HDL-C raising in long-term studies of plaque regression, more comparable to those performed with LDL-C–lowering therapies. In future trials, it will be important to demonstrate that repeat dosing of MEDI5884 is capable of supporting HDL-C elevations for the prolonged periods necessary to be therapeutically relevant.

Although HDL-C is the most common surrogate for HDL abundance as it relates to CVD, it is not necessarily reflective of net HDL functionality. Rather, HDL particle number and function have proven in multiple clinical trials to represent better predictors of CV risk than HDL-C even when adjusted for additional risk factors (*45*, *46*). MEDI5884 treatment increased both HDL particle number and average HDL particle size, presumably due to a change in kinetics that favor HDL retention, consistent with the mechanism of EL action. Neutralization of EL does not appear to result in HDL

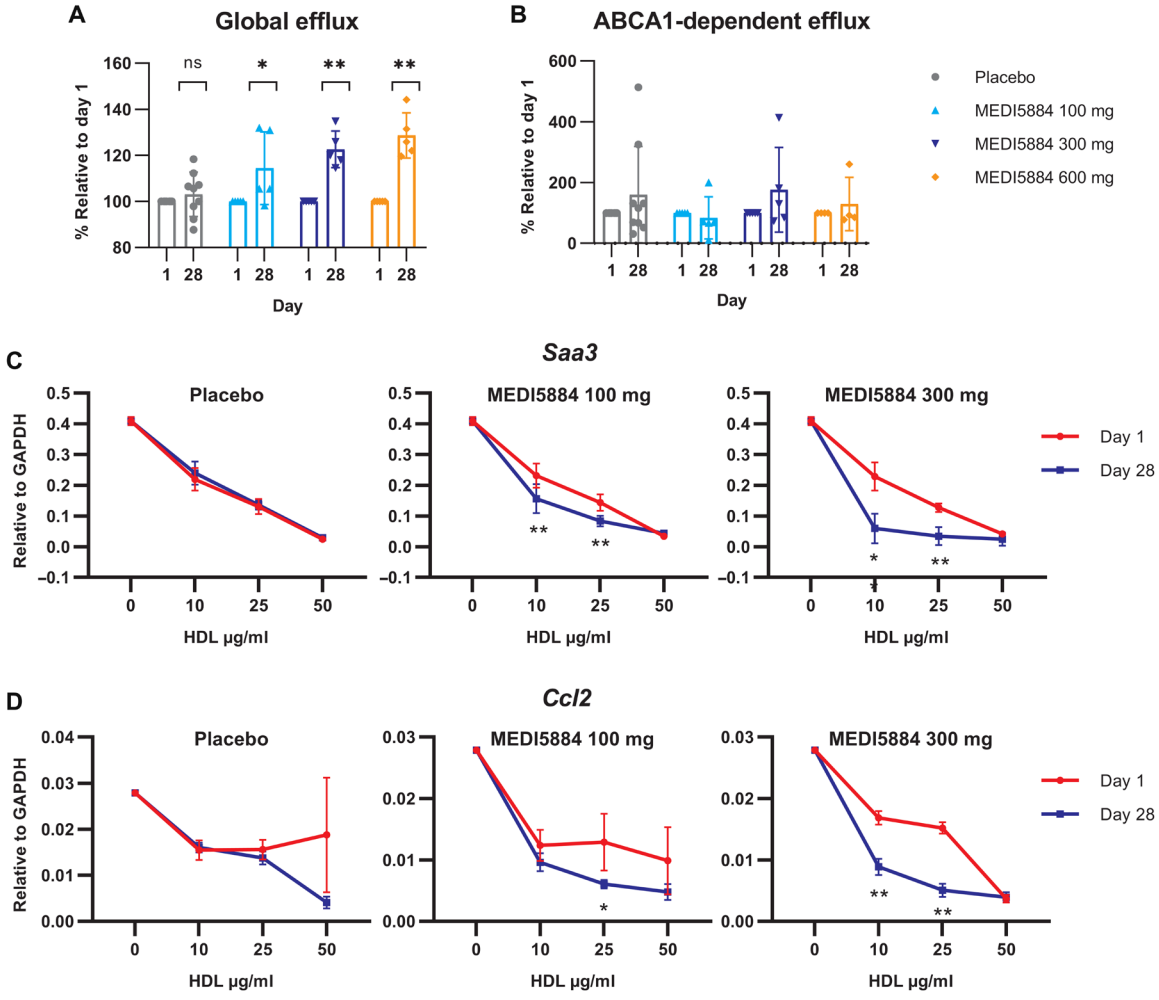

Fig. 7. Improvements in cholesterol efflux capacity and anti-inflammatory properties in healthy human participants after MEDI5884 treatment. Global (**A**) and ABCA1-dependent (**B**) cholesterol efflux capacity was measured in plasma samples collected at baseline (day 1) and on day 28 from consenting healthy human volunteers in the 100 mg ($n = 5$), 300 mg ($n = 5$), 600 mg ($n = 5$), or placebo ($n = 9$) groups. Data are presented as average % efflux relative to day 1 measurements ± SD. *Saa3* (**C**) and *Ccl2* (**D**) gene expressions were measured by quantitative RT-qPCR in palmitate-stimulated 3T3-L1 adipocytes pretreated with or without protein HDL (10, 25, or 50 µg/ml) isolated from plasma samples collected at baseline (day 1) and on day 28 from consenting healthy human volunteers in the 100 mg ($n = 4$) or 300 mg ($n = 4$) of MEDI5884 or placebo ($n = 5$) groups. Data are presented as mean expression relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) ± SEM. *$P < 0.05$ and **$P < 0.005$, determined by ANOVA. ns, not significant.

dysfunction as demonstrated by an improvement in anti-inflammatory properties and total efflux capacity, a key component of an intact RCT process associated with reduced CV risk (*4*, *5*). Additional studies should be performed to assess other aspects of RCT and better characterize HDL as cholesterol acceptors in the context of EL neutralization, including assessments of cholesterol influx, cholesterol ester transfer to ApoB-containing particles, as well as cholesterol delivery to the liver. Although technically challenging, in vivo assessments of RCT would prove most definitive toward validating EL neutralization as an approach capable of driving substantial CV improvements. Furthermore, although our study was limited to an assessment of cholesterol efflux and anti-inflammatory capacities, the impact of EL neutralization on other elements of HDL functionality and composition should also be the subject of further investigation to more thoroughly define the possible impact of MEDI5884 on HDL beyond its role in RCT and inflammation.

It is also notable that we observed statistically significant LDL-C elevations after MEDI5884 treatment in monkeys and, to a lesser extent, in healthy humans. Although the mechanism of EL action predicts a more specific effect on HDL particles due to substrate preference for HDL-PL, it may not be unexpected to see a parallel increase in LDL-C. It is possible that by increasing HDL-C and cholesterol efflux capacity in the presence of functional CETP, MEDI5884 coordinately leads to additional transfer of CE to LDL particles, which manifests in an increase in LDL-C. Therefore, an increase in LDL-C could indicate an enhancement in this component of RCT, which would be favorable and predictive of CV benefit. The observed increase in LDL-C was accompanied by a small but consistent increase in LDL particle size, which might also be expected if additional CE is being transferred from HDL. Further, we demonstrated that the increase in LDL-C can be mitigated by coadministration of a PCSK9 inhibitor. This is an important confirmation of the NMR

result indicating that MEDI5884 does not increase proatherogenic, small-dense LDL particles that show reduced affinity for the LDL receptor, increased half-life, and an ability to enter the subendothelial space (*47*). In light of this, it is intriguing to consider dual inhibition of EL and PCSK9 and the potential for synergy that may arise from combined action of the two complementary mechanisms, both targeting different aspects of RCT. Alternatively, the increase in LDL-C and LDL particle size could reflect a direct role for EL in regulating LDL particle metabolism. LDL and VLDL can act as substrates for EL in vitro (*48*), whereas overexpression in animal models of elevated ApoB-containing lipoproteins showed that EL accelerates LDL turnover (*25*, *26*). In addition, EL has been described to facilitate lipoprotein clearance through a nonenzymatic bridging activity (*49*), thus implicating that increases in LDL-C might be a direct consequence of EL neutralization. Further, NHP studies combining MEDI5884 and CETP inhibitors could shed light on the possible role of enhanced RCT on LDL-C concentration after EL neutralization.

We acknowledge several limitations in the studies presented here. First, our analysis was restricted by relatively small sample sizes in both the NHP and human studies with regard to exploratory endpoints of HDL functionality. The initial NHP study included only three animals in each group, and we only gained consent to assess secondary measures of HDL activity in five partcipants from each MEDI5884 treatment group in the human study, severely hindering statistical power in assays that can be inconsistent and variable. Thus, our analysis only included functional readouts of cholesterol efflux capacity and anti-inflammatory properties. Ideally, a more thorough characterization of HDL function would have been performed, including antioxidant and vasoprotective assessments, to better establish the presumed benefits that come with EL neutralization. Further, our contention that EL neutralization represents a more clinically favorable means to HDL-C elevation is predicated on an intact CETP axis. As we have not measured CETP mass or activity in our samples, we acknowledge that these assessments in the context of EL neutralization should be performed in future studies. In addition, the human study presented here was conducted in healthy participants only, not the true patient population likely to be taking lipid-lowering medication, which carries additional CV risk factors and possibly harbors dysfunctional HDL. It will be important to expand upon this analysis in forthcoming human studies with MEDI5884 that will be larger in size and will be more representative of the intended target population.

Based largely on the results of CETP inhibitor trials, HDL-targeted therapies have gone out of favor due to a lack of translation in the clinical setting. However, previously attempted approaches to HDL-C elevation have limitations that preclude definitive rejection of HDL raising as a viable therapeutic target. MEDI5884 overcomes many of these liabilities by offering the ability to durably elevate functional HDL, without interfering with the potentially critical CETP component of RCT, and could represent a therapeutic approach capable of reducing residual CV risk while categorically addressing the HDL flux hypothesis.

MATERIALS AND METHODS

Study design

The studies presented here were designed to characterize the ability of MEDI5884 to neutralize EL and assess the pharmacological consequence (efficacy and safety) of EL neutralization in mice, NHPs, and in healthy human volunteers. Results of in vitro experiments were validated across independent, repeat studies. Rodent pharmacology studies were conducted in C57BL/6J mice in groups of five or more per treatment and randomized by body weight. The results from these studies were validated across independent, repeat studies. NHP pharmacology studies were performed in cynomolgus monkeys in groups of three or four per treatment. The two NHP studies included here were performed independently, with aspects of the combination pharmacology study confirming the results of the initial PK/PD study. The clinical trial in healthy human volunteers was conducted in males and females of nonchildbearing potential between the ages of 18 and 55 years, with a body mass index between 18 and 30 kg/m^2 at the time of screening. The study was randomized into groups of at least 12, placebo-controlled, and blinded to both the partcipants and investigators. All data points collected in each of the above studies were included with no outliers excluded in our calculations.

Generation and optimization of MEDI5884

A panel of anti-EL monoclonal antibodies, originally derived using hybridoma technology, was humanized and affinity-optimized by Shionogi Inc. One of these IgG antibodies, mAb h55A1, was humanized using the variable region light chain (VL) framework from a human immunoglobulin germline kappa chain of a pseudo gene, which encodes an N-linked glycosylation motif. Several h55A1 variants composed of the rehumanized VL with the glycosylation site removed, paired with affinity-optimized variable heavy chain (VH) variants and constant region isotypes (IgG_1, IgG4P, and IgG_1 triple mutation), were generated by MedImmune and selected for further evaluation. MEDI5884 is an IgG4P, engineered for reduced effector function, composed of the affinity-optimized VH from mAb h55A1-S6 paired with the rehumanized VL from mAb h55A1-F1 with the N-glycosylation motif removed.

Preparation of conditioned medium

The cDNAs of hEL and cyno EL were cloned into the expression vector pEBNA3 (MedImmune/AstraZeneca) with His-tag or the expression vector pCDNA3.1 (Life Technologies) with 3′ double epitope tag (C2 tag). The cDNAs of mouse EL, rat EL, and rabbit EL were cloned into the expression vector pCDNA3.1 or pCDN3.3 (Life Technologies) with 3′ double epitope tag (C2 tag). The cDNAs of human hepatic lipase and human lipoprotein lipase were cloned into the expression vector pcDNA3.1 (Life Technologies). The expression vectors were transfected in to G22 Chinese hamster cells (MedImmune/AstraZeneca) (*50*) using polyethylenimine (Sigma-Aldrich) and cultured for 3 days. The cells were harvested by centrifugation and washed with phosphate-buffered saline (PBS). The cell pellet was suspended in proprietary culture medium containing heparin (20 U/ml; Sigma-Aldrich). The cell suspension was incubated at 37°C for 45 min, and then the supernatant was concentrated 20- to 25-fold using Vivaspin20 filters [30 kDa molecular-weight cutoff (MWCO); GE Healthcare (GE)].

Binding activity assay

EL binding activity of MEDI5884 was measured by biolayer interferometry on an Octet384 instrument (ForteBio). MEDI5884 (300 nM) prepared in the same proprietary culture medium used to generate the conditioned medium (CM) was captured on protein A biosensors (ForteBio), then washed with fresh culture medium to remove

any unbound protein (Baseline step), subsequently dipped into CM containing active hEL, cyno EL, mouse EL, rat EL, or rabbit EL, and incubated for the indicated time (association step). The loaded biosensors were subsequently dipped into fresh culture medium (dissociation step). The binding profile is illustrated by the instrument sensorgrams.

Binding affinity assay

The apparent binding affinity of MEDI5884 to hEL and cyno EL were determined at 25°C by surface plasmon resonance using the ProteOn XPR36 instrument (Bio-Rad). Anti–C2-tag antibody or anti–His-tag antibody was immobilized on a GLC biosensor chip (Bio-Rad) using a standard amine coupling kit (Bio-Rad). C2-tagged hEL protein and His-tagged cyno EL CM were captured on the sensor chip by an anti–C2-tag antibody and an anti–His-tag antibody, respectively. MEDI5884 was injected at concentrations of 100 to 6.25 nM (1:2 dilutions). The association constant (k_a) and dissociating constant (k_d) were calculated by Langmuir fit of ProteOn Manager 3.1 software (Bio-Rad). The equilibrium dissociation constant (K_D) was calculated using the following equation: $K_D = k_d/k_a$.

In vitro neutralizing assays

The neutralizing activity of MEDI5884 against EL was evaluated using human HDL (1 mg/ml) as the substrate. HDL was isolated by sequential density gradient ultracentrifugation from plasma pooled from healthy human donors (n = 5) enrolled in the AstraZeneca Research Specimen Collection Program. For hepatic lipase and lipoprotein lipase assays, human VLDL (75 μg/ml; Cell Biolabs) was used as the substrate. CM containing active EL, lipoprotein lipase, or hepatic lipase was incubated in half-area 96-well microplates with assay buffer [20 mM tris-HCl, 150 mM NaCl, 4 mM $CaCl_2$, and 0.5% bovine serum albumin (Sigma-Aldrich)] and human HDL or VLDL in the presence or absence of MEDI5884 at concentrations ranging from 1000 nM to 31.6 pM for 2 hours at 37°C. After this incubation, free fatty acid release was measured in each well using the NEFA-HR(2) assay kit (Wako Diagnostics). Absorbance at both 550 and 660 nm was measured using a Molecular Devices SpectraMax M5 plate reader. The value obtained from subtracting the 660-nm reading from the 550-nm reading was used for further analysis. The percent activity "A_x" at an antibody concentration of "x" was calculated using the following equation

$$A_x = [(E_x - V_0)/(E_0 - V_0)] \times 10$$

where "E_x" is the mean values of absorbance unit with an inhibitor in the presence of an enzyme, and "E_0" and "V_0" are the mean values of absorbance unit without an inhibitor and in the absence of an enzyme, respectively. The IC_{50} values were calculated and plotted using GraphPad Prism.

Recombinant adenovirus

For cytomegalovirus-driven expression of hEL in vivo, recombinant, replication-deficient adenoviral vectors were generated using the AdEasy system (Agilent).

Mouse studies

Rodent studies were conducted at MedImmune LLC (USA) according to protocols reviewed and approved by the Institutional Animal Care and Use Committee of the testing facility and in compliance with national laws and regulations concerning humane care and use of laboratory animals and the AstraZeneca Animal Welfare and Bioethics policies.

Eight-week-old male C57BL/6J mice (Jackson Laboratories), caged five per group, were given ad libitum access to water and regular chow diet containing 12% fat, 62% carbohydrates, and 26% protein with a total energy content of 3.0 kcal/g (Research Diets). Animals were maintained under standardized conditions of temperature (21° to 22°C) and humidity (40 to 60%) with light between 7 a.m. and 7 p.m. After the 1-week acclimation period, 4-hour fasted mice were randomized by body weight, and baseline blood samples were collected by retroorbital bleed before receiving a single dose (2.5×10^8 plaque-forming units) of Ad-hEL adenovirus in 200 μl of PBS by tail vein injections. This dose showed stable expression averaging 2 to 3 μg/ml of hEL 4 days after injection in previous experiments. Four days after virus administration, mice were fasted for 4 hours before blood collection by retroorbital bleed. Mice then received a single dose (30 mg/kg) of MEDI5884 or R347 IgG isotype control antibody in 200-μl acetate buffer [25 mM acetate and 75 mM NaCl (pH 5)] by subcutaneous injection. Twenty-four hours after antibody administration, mice were fasted for 4 hours and then injected with heparin (100 U/kg; Sagent Pharmaceuticals) intravenously. Five minutes later, mice were euthanized using CO_2, and blood was collected by cardiac puncture. After each blood collection, plasma was obtained by centrifugation at 10,000 rpm for 10 min at 4°C. Plasma HDL-C were measured using a Cobas cc13 analyzer (Roche Inc.).

EL measurements

hEL concentrations in mouse and human plasma were measured using the Meso Scale Diagnostics (MSD) immunoassay platform. Briefly, wells on a 96-well plate were coated with MEDI5884 and incubated overnight at 4°C. The next day, wells were washed with PBS containing 0.05% Tween-20 wash buffer and blocked with I-Block Buffer (Applied Biosystems) for 1 hour at room temperature. Plates were washed, and then recombinant EL protein standard (Origene Technologies) and mouse plasma samples were added to corresponding wells and incubated for 1 hour at room temperature. After washing, a biotinylated EL detection antibody (Origene Technologies) was added to corresponding wells and incubated for 1 hour at room temperature. Plates were washed, and then a streptavidin, sulfo-TAG antibody (MSD) was added to corresponding wells and incubated for 1 hour at room temperature. After washing, wells were incubated with Read buffer (MSD). Plates were read using the MESO Sector S 600 plate reader, and data were analyzed using the MSD Discovery Workbench software analysis program.

Cynomolgus monkey studies

Naïve, male cynomolgus monkeys (*Macaca fascicularis*) were obtained by Covance Research Products, socially housed, and acclimated for 3 weeks before the study. Animals were allowed access to water ad libitum and fed Certified Primate Diet #5048 (Purina Mills), supplemented with fruit, vegetables, or other dietary enrichments. Animals ranged from 2 to 3 years of age and weighed between 2.4 and 3.0 kg at the initiation of dosing. For the single-dose PK/PD study, animals (n = 3 per group) received a single subcutaneous dose of MEDI5884 at 0.5, 6, or 30 mg/kg on day 0. Blood samples were taken at the following times, before dosing when appropriate: predose (day 0), then 0.5, 1, 2, 3, 7, 14, 21, 28, 35, 42, 49, and 56 days. For the combination study (n = 4 per group), animals receiving

PCSK9 antibody HS9 (10 mg/kg) were injected subcutaneously starting on day 0 and then once weekly throughout the remainder of the study. Vehicle for HS9 was 20 mM histidine and 240 mM sucrose (pH 6.0). Animals receiving MEDI5884 (10 mg/kg) were first dosed subcutaneously on day 28 and then again on day 42. Vehicle for MEDI5884 was composed of a buffer, stabilizer, and surfactant. Blood samples were taken at the following times, before dosing when appropriate: predose (day 0), then 0.5, 1, 2, 3, 7, 14, 21, 28, 35, 42, 49, and 56 days. Blood samples were transferred to Covance-Madison Clinical Pathology Department and processed for lipid profile analysis (LDL-C, HDL-C, total-C, TG, or phospholipid) according to Covance standards of practice. ApoA1 and ApoB were measured using the Beckman Coulter AU680 clinical analyzer.

Cynomolgus monkey PK evaluation

The method used an indirect enzyme-linked immunosorbent assay format to measure the concentrations of MEDI5884 and control mAb in cynomolgus monkey serum. Standards, controls, and test samples were incubated with sheep anti-human IgG (H+L), which had been immobilized on a microtiter plate. After incubation, unbound material was washed away. MEDI5884 and control mAb were detected using goat anti-human IgG (H+L)–horseradish peroxidase conjugate and visualized with the addition of a tetramethylbenzidine substrate solution. The color development was stopped, and the intensity of the color was measured at 450/650 nm. The data were analyzed using a weighted four-parameter curve. Concentrations of MEDI5884 and control mAb in unknown samples were determined by computer interpolation from the plot of the standard data.

Cynomolgus monkey PK interpretation

Individual PK parameters were evaluated from serum concentration–time data by noncompartmental analysis using Phoenix WinNonlin 6.3, a component of Phoenix v1.3. Descriptive statistics was generated for PK parameters for MEDI5884 for each dose evaluated. PK parameters that were derived were maximum concentration (C_{max}), area under the PK curve up to the last sampling time and infinity ($AUC_{0\text{-last}}$ and $AUC_{0\text{-infinity}}$, respectively), time to achieve C_{max} (T_{max}), apparent clearance (CL/F), and half-life ($t_{1/2}$).

Clinical trial participants

Volunteers in the clinical trial were healthy adult men or women of nonchildbearing potential (50% Japanese-American), aged 18 through 55 years, with body mass index of 18.0 to 30.0 kg/m^2 (inclusive) at the time of screening. Females of childbearing potential and lactating females were excluded. Japanese-Americans were of Japanese descent. All participants provided written informed consent, and the studies were approved by an Independent Ethics Committee and performed in accordance with guidelines established by the Declaration of Helsinki.

Clinical trial study design and treatments

This was a first-in-human, phase 1, randomized, blinded (subject/investigator blinded, MedImmune unblinded), placebo-controlled, dose escalation study to evaluate the safety, tolerability, PK, PD, and immunogenicity of a single SC dose of MEDI5884 in healthy participants (NCT03001297). This study was conducted at one site in the United States. This study planned to enroll a total of up to 64 participants. Participants in cohorts 1 to 4 were randomized in a 3:1 ratio to receive, sequentially, 30, 100, 300, or 600 mg MEDI5884 (n = 6 per cohort) or placebo (n = 2 per cohort). The placebo formulation consisted of an aqueous buffer, sugar, and surfactant that differed in composition to the proprietary formulation used for the drug product. There was an optional Japanese-American cohort (cohort 5); participants in this cohort were also randomized in a 3:1 ratio to receive, sequentially, 30, 100, 300, or 600 mg MEDI5884 (n = 6 per dose level) or placebo (n = 2 per dose level).

Human PK evaluation

A validated immunoassay for the quantitative measurement of MEDI5884 in human serum was performed. Briefly, anti-idiotypic antibodies to MEDI5884 were used in a sandwich immunoassay format conducted on the Gyrolab Workstation. The Gyrolab method for the determination of MEDI5884 in serum was based on a sequential flow-through sandwich method. A Gyrolab Bioaffy 200 CD microlaboratory consists of 14 available segments, each segment having eight individual microstructures giving 100 and 12 available structures for protein quantification. Each microstructure contained a column prepacked with streptavidin-coated beads, which were functionalized with a biotinylated capture reagent in the first step of the assay. Standards and control samples containing the MEDI5884 were passed through the streptavidin bead column and captured by the biotinylated capture reagent. The fluorophore-labeled detection reagent was then added to bind to the captured MEDI5884. The concentration of detection reagent bound to the MEDI5884 (hence the concentration of the MEDI5884) was calculated from the fluorescence measured by the fluorescence detector in the Gyrolab Workstation. The amount of fluorescence detected was directly proportional to the amount of analyte in the sample. The lower limit of quantification of the assay was 0.0125 μg/ml.

Human PD evaluation

Lipid panels measuring HDL-C, total-C, non–HDL-C, LDL-C (direct), and TGs, immunoassays for ApoA1 and ApoB, and NMR were all performed on plasma samples at LipoScience Inc. (LabCorp) using validated assays. NMR analysis was performed as previously described (*51*). Briefly, the proton NMR spectrum of the plasma samples was measured with a 400-MHz NMR clinical analyzer. The resulting digitized spectrum for each sample was quantified using analysis software that extracts the amplitudes of individual subclass signals and converts the values to a concentration (nanomolar). Individual subclass signal amplitudes were derived computationally from the recorded plasma methyl signal envelope through a linear least-squares deconvolution process using singular value decomposition, as described previously (*52*). HDL-PL was measured at Pacific Biomarkers using a validated assay. Briefly, HDL was isolated from human plasma using polyethylene glycol (PEG) precipitation as described in the "Efflux assays" section below. Phospholipids were then detected in the HDL fraction using the Wako Phospholipids C assay.

Safety and tolerability

Participants were confined to the clinical research facility under continuous medical observation for 48 hours after administration of study drug. Participants were followed for up to 120 days depending on dose. Safety assessments included monitoring of adverse events that were evaluated for seriousness, severity, and possible relationship to study drug. Participants were also assessed with clinical chemistry and

hematology, urinalysis, physical exams, vital signs, and antidrug antibodies.

Efflux assays

Cholesterol efflux capacity assays were performed as previously described (*53*, *54*). Briefly, ApoB-depleted plasma was prepared by precipitation of ApoB-containing lipoproteins using PEG. Briefly, for each serum sample, 100 parts serum was mixed with 40 parts PEG [20% (v/v) in glycine buffer (pH 7.4)]. The mixture was incubated at room temperature for 20 min and then centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant containing serum HDL was collected and used for analysis of cholesterol efflux capacity.

Global and ABCA1-mediated cholesterol efflux capacity was measured using J774 mouse macrophage cells (American Type Tissue Culture Collection) in the presence and absence of adenosine 3′,5′-monophosphate (cAMP). Cells were preincubated with [^{3}H]-cholesterol and Acyl-CoA cholesterol acyltransferase (ACAT) inhibitor (Sandoz 58-035) but not preloaded with mass cholesterol overnight. Cells were then incubated overnight in Dulbecco's modified Eagle's medium (Life Technologies) containing 0.2% (w/v) bovine serum albumin with or without cpt 8-(4-Chlorophenylthio)-cAMP (Sigma-Aldrich). After washing, the cells were incubated for 4 hours with ApoB-depleted serum added at 2.8% (v/v). [^{3}H]-cholesterol released to serum after incubation with cells for 4 hours was measured by liquid scintillation counting. Cholesterol efflux results are expressed as the radiolabel released as a percentage of [^{3}H]-cholesterol within cells before addition of serum. All efflux values were corrected by subtracting the small amount of radioactive cholesterol released from cells incubated with serum-free medium. ABCA1-dependent efflux from J774 cells was determined as the difference in efflux from cAMP-treated (global efflux) and untreated cells.

HDL isolation and anti-inflammatory assays

Anti-inflammatory assays were performed as previously described (*55*, *56*). HDL was isolated by sequential ultracentrifugation, whereas palmitate (16:0) was purchased from Sigma-Aldrich (#P-0500) and prepared by conjugation with albumin (Sigma-Aldrich, #A8806) in a 3:1 ratio. 3T3-L1 murine preadipocytes, obtained from American Type Tissue Culture Collection (#CRL-3242), were propagated and differentiated according to standard procedures. Fully differentiated 3T3-L1 adipocytes were pretreated with HDL (10, 25, or 50 μg/ml protein) for 6 hours, washed three times by PBS, and then incubated with 250 μM palmitate for 24 hours. Real-time quantitative reverse-transcription polymerase chain reaction (RT-qPCR) was performed using the TaqMan Master kit (Applied Biosystems) in the ABI prism 7900HT system. TaqMan assays for *Saa3*, *Ccl2*, and *Gapdh* were obtained from Applied Biosystems (Assay-on-Demand; Mm00441203-m1, Mm00441242-m1, and Mm99999915-g1).

Statistical analysis

Data from the rodent pharmacology studies were analyzed by one-way ANOVA followed by a Tukey's test for multiple comparisons. For each parameter and for each time point in the NHP studies, an ANOVA model was fitted to the data, and mean comparisons were made for each point relative to baseline values. An exact *P* value correction was made according to the Edwards/Berry method. Data derived from the clinical trial were analyzed by ANOVA, and *P* values were corrected with a Dunnett procedure for multiple testing. Efflux and RT-qPCR data were analyzed by two-way ANOVA followed by a Sidak's test for multiple comparisons. In all cases, $P < 0.05$ was considered significant. Raw data are provided in data file S1.

SUPPLEMENTARY MATERIALS

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Fig. S1. In vitro binding specificity of MEDI5884.
Fig. S2. Effect of MEDI5884 on total-C and non–HDL-C in NHPs.
Fig. S3. PK profile of MEDI5884 in NHPs.
Fig. S4. Effect of combined EL and PCSK9 inhibition on ApoB and ApoA1 in NHPs.
Fig. S5. Study flow diagram describing clinical trial performed in healthy human participants.
Fig. S6. Effect of MEDI5884 on total-C and non–HDL-C in healthy human participants.
Fig. S7. PK profile of MEDI5884 in healthy human participants.
Fig. S8. Effect of MEDI5884 on VLDL/chylomicrons and IDL particles in healthy human participants.
Table S1. MEDI5884 does not inhibit lipoprotein lipase or hepatic lipase in vitro.
Table S2. Summary of PK characteristics of MEDI5884 in NHPs.
Table S3. Baseline characteristics of subjects enrolled in phase 1 clinical trial.
Table S4. Summary of adverse events reported during phase 1 clinical trial.
Table S5. Summary of PK characteristics of MEDI5884 in healthy human participants.
Data file S1. Raw data.

View/request a protocol for this paper from *Bio-protocol*.

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Blocking endothelial lipase with monoclonal antibody MEDI5884 durably increases high density lipoprotein in nonhuman primates and in a phase 1 trial

John E. Le LayQun DuMinal B. MehtaNicholas BhagrooB. Timothy HummerJudith FalloonGlenn CarlsonAnton I. RosenbaumChaoYu JinHolly KimkoLan-Feng TsaiSteven NovickBill CookDavid HanChang Yeop HanTomas VaisarAlan ChaitSotirios K. KarathanasisChristopher J. RhodesBoaz HirshbergMelissa M. DamschroderJudith HsiaJoseph S. Grimsby

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Limiting lipase to control cholesterol

Strategies to reduce low-density lipoprotein cholesterol (LDL-C) while promoting high-density lipoprotein cholesterol (HDL-C) are of interest for combating atherosclerosis. Le Lay *et al.* developed a monoclonal antibody that neutralizes endothelial lipase, an enzyme that hydrolyzes HDL. Antibody treatment in nonhuman primates increased plasma HDL-C, and pretreatment with a PCSK9-neutralizing antibody reduced LDL-C. Results from a phase 1 study showed that the endothelial lipase-neutralizing antibody was safe and elevated HDL-C. This approach may help reduce cardiovascular risk.

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