Clinical pharmacological properties of mipomersen (Kynamro), a second generation antisense inhibitor of apolipoprotein B

Stanley T. Crooke & Richard S. Geary

Isis Pharmaceuticals, Carlsbad, CA, USA

Correspondence

Dr Stanley T. Crooke MD PhD, Isis Pharmaceuticals, 2855 Gazelle Court, Carlsbad, CA 92010, USA.
Tel.: +1 760 603 2301
Fax: +1 760 603 4651
Email: scrooke@isisph.com

Keywords

antisense inhibitor, familial hypercholesterolaemia, LDL-cholesterol, mipomersen

Received

30 July 2012

Accepted

20 September 2012

Accepted Article

Published Online

26 September 2012

Mipomersen is a second generation antisense oligonucleotide that targets apolipoprotein B. It has been studied thoroughly in clinical trials (more than 800 subjects), including four randomized double-blind placebo controlled phase 3 studies involving 391 patients, and is in registration for the treatment of severe hypercholesterolaemia. The pharmacokinetic and pharmacodynamic properties of mipomersen are well characterized. Mipomersen is rapidly and extensively absorbed after subcutaneous administration and has an elimination half-life of approximately 30 days across species. It is cleared by nuclease metabolism and renal excretion of the metabolites. Mipomersen reduces all apolipoprotein B containing atherogenic particles and displays dose dependent reductions between 50–400 mg week⁻¹, both as a single agent and in the presence of maximal lipid lowering therapy. No drug–drug interactions have been identified. Mipomersen is a representative of second generation antisense drugs, all of which have similar properties, and is thus representative of the behaviour of the class of drugs.

Introduction

Antisense technology supports the design of chemically modified oligonucleotides (typically 16–20 nucleotides) that bind to RNA molecules via Watson–Crick hybridization [1, 2]. In principle and in practice, essentially any RNA can be targeted including pre- and mRNA, pre-ribosomal RNA, non-coding RNAs, and small nuclear and nucleolar RNAs [3]. Once bound, antisense oligonucleotides (ASOs) can work by quite a number of mechanisms, but the most frequently used mechanism is the recruitment of RNase H1, an enzyme that degrades RNA in a DNA–RNA like duplex [2, 4].

Second generation antisense drugs are oligonucleotides that are the products of an extensive oligonucleotide medicinal chemistry effort. They are comprised of two 'wings' at the 5' and 3' ends of the oligonucleotides with 2' methoxyethyl substituted nucleotides, a gap of deoxy nucleotides designed to support RNase H1 activity and phosphorothioates at each internucleotide linkage [5, 6]. The 2' methoxyethyl substitution differentiates the second and first generations, and increases the affinity

(and thereby potency) of ASOs significantly. The addition of 10 methoxyethyl nucleotides results in a multiple log increase in affinity. The 2' methoxyethyl substitution also results in significant additional nuclease resistance resulting in drugs that typically have elimination half-lives of 2–4 weeks in all species. The substitution also reduces pro-inflammatory effects. The phosphorothioate modification enhances nuclease resistance as well and provides low affinity protein binding that facilitates distribution to peripheral tissues [7–9].

Mipomersen is a second generation ASO that targets apolipoprotein B (apoB100) (Figure 1). It is 20 nucleotides long and has a molecular weight of approximately 7000 Daltons. It is typically administered subcutaneously in buffered saline once weekly. It has been studied extensively in animals and man, including lifetime carcinogenicity tests in two species, four phase 3 studies and many other clinical trials. It is currently undergoing regulatory review in the US and Europe for the treatment of severe forms of familial hypercholesterolaemia (FH) in the US and homozygous FH (HoFH) and severe heterozygous FH (HeFH) in the European Union. As Table 1 demonstrates, this is a clearly

Figure 1
Structure of mipomersen

Table 1
The effects of mipomersen on atherogenic lipids and HDL-C in phase 3 studies (mean % change from baseline). TG and Lp(a) are presented as the median % change from baseline [29]]

(n)	Mipomersen treated group only Patient population	Baseline LDL-C (mg dl ⁻¹)	% change from baseline			TG†	HDL-C‡
			LDL-C*	Apo B*	Lp(a)*		
51	HoFH	439	–25%	–27%	–32%	n.a.	+15%
58	Severe HC	276	–36%	n.a.	–39%	n.a.	+6%
124	HeFH with CAD	153	–28%	–26%	–21%	–14%	+3%
158	HC at high risk for CAD	123	–37%	–38%	–24%	–26%	+2%

P values (vs. placebo) *P ≤ 0.001 all populations, †P < 0.05 all populations, ‡P < 0.05 in HoFH population. Apo B apolipoprotein B; CAD coronary artery disease; HC hypercholesterolaemia; HDL-C high density lipoprotein cholesterol; HeFH heterozygous familial hypercholesterolaemia; HoFH homozygous familial hypercholesterolaemia; LDL-C low density lipoprotein cholesterol; Lp(a) lipoprotein (a); TG triglycerides.

unmet medical need as all the patients were on maximum lipid lowering therapy and had an average of more than two prior cardiovascular events. It is chemically synthesized and is not a natural product.

ApoB100 is the structural core of atherogenic lipoproteins, including low density lipoprotein cholesterol (LDL-C) and lipoprotein (a) (Lp(a)), and is recognized as a risk factor for atherosclerosis. ApoB100 is synthesized and packaged into lipoprotein particles principally in the liver of all species [10–12]. Circulating LDL-C, which typically constitutes 60–70% of serum cholesterol in man, is widely recognized as a major risk factor for coronary heart disease

(CHD) and has been implicated in the inflammation associated with the pathogenesis of atherosclerosis. Similarly, Lp(a) has been shown to be an independent, causal, genetic risk factor for CHD.

Results of phase 3 and open label extension studies

Based on extensive evaluations of dose–response relationships in phase 1 and phase 2 studies (see below), a subcutaneous dose of 200 mg week⁻¹ of mipomersen was

Table 2

Effects of mipomersen on atherogenic lipoproteins and HDL-C in the open label phase 3 extension study [20]

Lipoprotein Mean (95%CI)	Baseline (n = 141)	% change from baseline Week 26 (n = 130)	Week 52 (n = 110)	Week 76 (n = 58)	Week 104 (n = 26)
LDL-C	233 (147)	-28± (-32,-25)	-27± (-31,-23)	-28± (-34, -2)	-26± (-35,-17)
Apo B	175 (81)	-29± (-32,-26)	-28± (-32,-24)	-30± (-35, -6)	-31± (-39,-23)
Lp(a)	61 (55)	-21± (-25,-17)	-16± (-23,-10)	-20± (-26,-14)	-14± (-27, 0)

Baseline values are the mean (SD) in mg dl⁻¹. *P* values were determined by the Wilcoxon Rank Sign test. **P* value < 0.05, †*P* value < 0.01, ‡*P* value < 0.001. Apo B apolipoprotein B; LDL-C low density lipoprotein cholesterol; Lp(a) lipoprotein (a).

chosen as the phase 3 dose. Four identically designed phase 3 studies were performed in which patients taking maximally tolerated doses of lipid lowering drugs were treated with either placebo or mipomersen for 26 weeks. The primary efficacy endpoint was percent change in LDL-C from baseline to week 28 (2 weeks after the last dose). Secondary endpoints included reductions in apoB and other apoB containing atherogenic particles, including Lp(a). All studies were randomized double-blind placebo-controlled with a 2:1 randomization. No dose adjustments were allowed. The only difference in the studies was the nature of the patients treated. One study evaluated the effects of mipomersen in patients with HoFH, another in patients with HeFH with the presence of coronary artery disease (CAD), a third in patients with severe hypercholesterolaemia (severe HeFH), and the fourth in more routine hypercholesterolaemic patients (non-FH) with high risk of CHD.

Intent to treat analyses showed that mipomersen produced robust and statistically significant and clinically meaningful reductions in all measured apoB containing atherogenic lipoproteins (Table 1). Mean absolute reductions of LDL-C >100 mg dl⁻¹ were seen in severe FH patients (both HoFH and severe HeFH patients). Across studies mipomersen has shown consistent efficacy and demonstrated a unique ability to lower all atherogenic particles including LDL, apoB100 and Lp(a), with no deleterious effect on HDL (slight increase in all studies) [13–19].

Mipomersen was well tolerated in patients with elevated cardiovascular risk and on maximal lipid lowering therapy. In phase 3 studies, 37–85% of the patients at enrolment had experienced at least one major cardiovascular event and most were taking maximum doses of statins, ezetimibe and other concomitant medications. The most common side effect was mild to moderate injection site reactions. Flu like symptoms were more frequent in the mipomersen group than in the placebo group and 8.4% of patients experienced two consecutive increases at least 7 days apart in alanine aminotransferase (ALT) concentrations (ALT ≥ three times ULN) during treatment. There was no evidence of liver toxicity, such as increases in bilirubin and many of the ALT elevations decreased while continuing treatment [13–19].

A total of 141 patients with a diagnosis of FH rolled over into an open label long term extension study that demonstrated persistent efficacy (Table 2) [20] and the increases in liver fat that occurred in some patients either stabilized or declined on continuing treatment as did ALT increases. While some of these patients had dose adjustments or doses held during treatment, many maintained continuous dosing. These data are consistent with observations in the mouse that showed that with continuing treatment compensatory mechanisms reduced fat in the liver [21].

Dose–response relationship

In all species studied, mipomersen demonstrated a dose dependent reduction of apoB100 mRNA in the liver which correlated with a dose dependent reduction of apoB100 and apoB100 containing lipid particles in blood [21–23]. In man, in phase 1 normal volunteer studies and in phase 2 studies in patients with hypercholesterolaemia (HC) doses of 50–400 mg week⁻¹ were evaluated. Similarly, in combination with statins, doses of 30–400 mg week⁻¹ were evaluated in patients with HC. In all studies, the results were highly consistent (Figure 2). Importantly, the effects of mipomersen were essentially dose proportional and at 400 mg week⁻¹ many patients had plasma apoB concentrations below the limit of detectability (approximately 30 mg dl⁻¹). The dose that results in 50% reduction in apoB and LDL-C (ED₅₀) was approximately 240 mg week⁻¹ after 13 weeks of treatment [18, 24–26].

Pharmacokinetics

The pharmacokinetics of mipomersen are consistent across all species studied, including mouse, rat, monkey and humans, with rapid transfer of the injected drug from plasma to tissue. In addition, the half-life of the drug, once in tissues of distribution, is consistent across species. Table 3 shows the elimination half-lives of mipomersen in various tissues in the rat, monkey and man [27, 28].

Absorption

After subcutaneous administration in buffered saline, mipomersen is rapidly and extensively absorbed,

Figure 2 Sustained reduction in atherogenic lipids. Dose-dependent and prolonged effects of mipomersen on LDL cholesterol and apoB. LDL cholesterol and apolipoprotein B are presented as mean % change from baseline. Error bars represent $\pm$ SEM. Time period ranges from day 1 to day 175 [26]. $\blacktriangle$, Placebo; $\blacktriangle$, 50 mg week⁻¹; $\blacksquare$, 100 mg week⁻¹; $\blacklozenge$, 200 mg week⁻¹; $\blacklozenge$, 300 mg week⁻¹; $\blacklozenge$, 400 mg week⁻¹

Table 3 Estimated elimination half-life (in days) of apoB ASOs in mice, monkeys and man

Species	Compound	Dose	Elimination half-life (days) Plasma	Liver	Liver : plasma trough concentration ratio
Mouse	ISIS 147764	25 mg kg ⁻¹	NA	20	NM
Mouse	ISIS 301012	2.5–25 mg kg ⁻¹	NM	24*	5861 $\pm$ 2342
Monkey	ISIS 301012	4 mg kg ⁻¹	31.3 $\pm$ 0.3†	34†	5825 $\pm$ 2882
Human	ISIS 301012	200 mg (~2.7 mg kg ⁻¹)	31 $\pm$ 11‡	NA	NM

*Measured in CD-1 mice (internal data). †Animals received 30 mg kg⁻¹ week⁻¹ doses of mipomersen for 1 year. For determination of plasma elimination half-life, plasma samples were collected at various time points up to 182 days after last dose ($n = 3$). ‡Data presented are mean $\pm$ SD ($n = 8$). NA, not applicable; NM, not measured.

Table 4 Pharmacokinetic summary of mipomersen s.c. injection in human volunteers

Cohort	Dose	Dose	n	AUC(0,48 h) ($\mu\text{g ml}^{-1} \text{h}$)	C_{max} ($\mu\text{g/ml}$)	t_{max} (h)	Apparent distribution $t_{1/2}$ (h)	%BAV
A	50 mg	First	3	7.01 $\pm$ 0.49	1.40 $\pm$ 0.29	2.67 $\pm$ 0.58	1.84 $\pm$ 0.49	69.2 $\pm$ 9.63
		Last	6	7.68 $\pm$ 1.8	1.0 $\pm$ 0.31	3.75 $\pm$ 2.23	3.44 $\pm$ 1.38	
B	100 mg	First	3	14.5 $\pm$ 2.91	1.45 $\pm$ 0.83	3.00 $\pm$ 1.00	4.22 $\pm$ 2.71	77.0 $\pm$ 12.1
		Last	3	19.6 $\pm$ 2.65	2.58 $\pm$ 1.41	3.33 $\pm$ 1.15	2.96 $\pm$ 1.30	
C	200 mg	First	3	38.3 $\pm$ 29.8	2.73 $\pm$ 1.66	4.17 $\pm$ 3.40	3.59 $\pm$ 1.22	53.0 $\pm$ 17.2
		Last	6	35.4 $\pm$ 8.0	2.06 $\pm$ 0.95	4.64 $\pm$ 3.97	7.60 $\pm$ 2.13	
D	400 mg	First	4	106 $\pm$ 17	7.60 $\pm$ 0.81	4.00 $\pm$ 1.63	4.33 $\pm$ 0.49	74.5 $\pm$ 4.7
		Last	2	113	7.15	7.50	5.48	

Data are presented as mean $\pm$ SD, except for cohort D at MD22. C_{max} = Maximal plasma concentration. t_{max} = Time C_{max} observed. AUC(0,48 h) = Area under the plasma concentration-time curve from time 0 to 48 h. %BAV = plasma bioavailability (%) following s.c. administration.

independent of dose. Absolute bioavailability (compared with a 2 h i.v. infusion) ranges from 54–78%, but this likely underestimates the total bioavailability due to insufficient sampling and undetectable concentrations. C_{max} has been consistently observed 3–4 h after dosing (Table 4) [27].

Distribution

As is the case with all second generation ASOs, mipomersen distributes broadly into most tissues with the excep-

tion of the brain in all species studied. Liver, kidney, bone marrow, adipose tissue and lymph nodes accumulate the highest concentrations of the drug [7, 9, 27, 28]. The distribution half-life ranges from 2–5 h (Table 4), representing the transfer from blood into tissue [28].

Mipomersen is extensively protein bound in plasma [7, 9, 27, 28]. More than 85% of mipomersen is protein bound in plasma with albumin being the protein that binds the greatest amount of the drug, but mipomersen also inter-

Figure 3
Schematic of mipomersen metabolism

acts with other serum proteins. The K_d for binding to serum albumin is approximately $150\ \mu\text{M}$ [7]. Plasma protein binding appears to saturate at doses greater than $20\text{--}30\ \text{mg kg}^{-1}$ in mice [27].

Elimination

The elimination of mipomersen is consistent across species and is a function of slow nuclease metabolism in tissues and ultimately excretion primarily in urine [27]. The elimination half-life is approximately 30 days in man and similar in other species (Table 3) [27]. The elimination half-life is unaffected by dose.

Metabolism

Mipomersen is metabolized initially by endonucleases then exonucleases [7, 9] (Figure 3). The metabolites have substantially reduced protein binding (fewer phosphorothioates results in lower affinity for proteins) and as a consequence, are cleared in urine. All of the metabolites have been identified by mass spectral analyses [27] and mass balance studies accounted for approximately 83% of the administered dose in rats at all evaluated time points [27, 28]. Mipomersen is not metabolized by traditional drug metabolizing enzymes, such as CYP450.

Pharmacokinetics/ pharmacodynamics

Studies in multiple species have demonstrated that steady-state tissue concentrations were achieved with mipomersen after approximately 6 months of dosing. Because mipomersen has a prolonged elimination half-life, the plasma parameter that best predicts tissue concentrations, and therefore pharmacological activity, is the plasma

trough concentration. Plasma trough concentrations were shown to predict liver concentrations quite effectively. In human apoB transgenic mice, the liver:plasma trough concentration ratio was approximately 6000:1. The plasma trough EC_{50} was $18 \pm 4\ \text{ng ml}^{-1}$ and the liver EC_{50} was $115 \pm 15\ \mu\text{g gm}^{-1}$ [28]. Similar observations were made in the monkey, though the EC_{50} were somewhat higher in the monkey. In fact, the plasma EC_{50} in the transgenic mouse was found to predict best the human plasma EC_{50} ($14 \pm 21\ \text{ng ml}^{-1}$) observed in human volunteers [28]. Figure 4 shows the plasma concentration–response for mipomersen in healthy volunteers, hypercholesterolaemic patients and hypercholesterolaemic patients taking statins, showing a consistent EC_{50} of $10\text{--}17\ \text{ng ml}^{-1}$.

Drug interactions

To date, drug interactions based on competition for protein binding, distribution or metabolism have not been observed. As mipomersen does not interact with traditional drug metabolizing enzymes, it has been shown to have no effect on cytochrome P450 and to have no pharmacokinetic or pharmacodynamic interactions with simvastatin, ezetimibe or warfarin [29].

Conclusions

Mipomersen is representative of second generation ASOs. As all ASOs of the same design and chemistry share similar properties, differing only in sequence, the data for mipomersen are consistent with observations made with other second generation ASOs. Mipomersen has behaved consistently and predictably in all species and in human volunteers and in patients of various types as a single agent and in combination.

Mipomersen dose dependently reduces apoB100 and all measured apoB100 containing atherogenic lipids and may slightly increase, or has no deleterious effect on, high density lipoprotein cholesterol (HDL-C). At the phase 3 subcutaneous dose of $200\ \text{mg week}^{-1}$ mipomersen has shown sustained efficacy and displays no evidence of drug tolerance in patients treated for as long as 4 years. Absolute reductions in severe FH patients $>100\ \text{mg dl}^{-1}$ predict substantial improvement in cardiovascular risk with a consistent safety profile. Phase 1 and phase 2 experiences show that doses other than $200\ \text{mg week}^{-1}$ may be active and that by increasing the dose, greater reduction in atherogenic lipids can be achieved. Of course, at higher doses some additional side effects might be observed. The unique profile, predictability in response and lack of drug–drug interactions, suggests that mipomersen may be of benefit in patients with severe FH who cannot achieve appropriate lipid concentrations with current agents. Moreover, a study in statin intolerant patients has shown that mipomersen may have a role in these patients as well [30].

Figure 4

Plasma concentration–response relationships for mipomersen. The individual points represent measurements of apoB100 or mipomersen in serum from either the phase 1 study in normal volunteers or phase 2 studies in hypercholesterolaemic patients either as a single agent or in combination with statins [25, 27] (PD: 14 days after last dose). —○—, Observed; —, Predicted

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf and declare STC and RSG had support from Isis Pharmaceuticals, Inc for the submitted work as employees of Isis Pharmaceuticals, Inc, STC is chairman of the Board of Directors and CEO of Isis Pharmaceuticals, Inc, RSG is Senior Vice President of Isis Pharmaceuticals, Inc and both authors have interest in the company through stock options.

We thank Brenda Baker for her help, Tracy Reigle for her help with figures and Donna Parrett for excellent assistance.

REFERENCES

- 1 Crooke ST (ed.). Antisense Drug Technology Principle, Strategies, and Applications, Second edn. Boca Raton: CRC Press, 2007.

- 2 Crooke ST, Vickers T, Lima W, Wu H. Mechanisms of antisense drug action. In: *Antisense Drug Technology Principle, Strategies, and Applications*, Second edn. ed. Crooke ST. Baco Raton: CRC Press, 2007; 3–46.
- 3 Liang X, Vickers T, Crooke ST. Antisense-mediated reduction of eukaryotic non-coding RNAs. In: *Nucleic Acids Sequences to Molecular Medicine*, eds Erdmann WA, Barciszewski J. New York: Springer, 2012; 191–214.
- 4 Lima W, Wu H, Crooke ST. The RNase H mechanism. Mechanisms of antisense drug action. In: *Antisense Drug Technology Principle, Strategies, and Applications*, Second edn. ed. Crooke ST. Baco Raton: CRC Press, 2007; 47–74.
- 5 Swayze EE, Bhat B. The medicinal chemistry of oligonucleotides. Mechanisms of antisense drug action. In: *Antisense Drug Technology Principle, Strategies, and Applications*, Second edn. ed. Crooke ST. Baco Raton: CRC Press, 2007; 143–82.
- 6 Bennett CF. Pharmacological properties of 2'-O-methoxyethyl-modified oligonucleotides. Mechanisms of antisense drug action. In: *Antisense Drug Technology Principle, Strategies, and Applications*, Second edn. ed. Crooke ST. Baco Raton: CRC Press, 2007; 273–303.
- 7 Levin AA, Yu RZ, Geary RS. Basic principles of the pharmacokinetics of antisense oligonucleotide drugs. Mechanisms of antisense drug action. In: *Antisense Drug Technology Principle, Strategies, and Applications*, Second edn. ed. Crooke ST. Baco Raton: CRC Press, 2007; 183–215.
- 8 Hardee GE, Tillman LG, Geary RS. Routes and formulations for delivery of antisense oligonucleotides. Mechanisms of antisense drug action. In: *Antisense Drug Technology Principle, Strategies, and Applications*, Second edn. ed. Crooke ST. Baco Raton: CRC Press, 2007; 217–36.
- 9 Geary RS, Yu RZ, Siwkowski A, Levin AA. Pharmacokinetic/pharmacodynamic properties of phosphorothioate 2'-O-(2-methoxyethyl)-modified oligonucleotides in animals and man. Mechanisms of antisense drug action. In: *Antisense Drug Technology Principle, Strategies, and Applications*, Second edn. ed. Crooke ST. Baco Raton: CRC Press, 2007; 305–26.
- 10 Davidson NO, Shelness GS. Apolipoprotein B: mRNA editing, lipoprotein assembly, and presecretory degradation. *Annu Rev Nutr* 2000; 20: 169–93.
- 11 Marsh JB, Welty FK, Lichtenstein AH, Lamon-Fava S, Schaefer EJ. Apolipoprotein B metabolism in humans: studies with stable isotope-labeled amino acid precursors. *Atherosclerosis* 2002; 162: 227–44.
- 12 Das HK, Leff T, Breslow JL. Cell type-specific expression of the human apoB gene is controlled by two cis-acting regulatory regions. *J Biol Chem* 1988; 263: 11452–8.
- 13 Visser ME, Witztum JL, Stroes ES, Kastelein JJ. Antisense oligonucleotides for the treatment of dyslipidaemia. *Eur Heart J* 2012; 33: 1451–8.
- 14 Ricotta DN, Frishman W. Mipomersen: a safe and effective anti-sense therapy adjunct to statins in patients with hypercholesterolemia. *Cardiol Rev* 2012; 20: 90–5.
- 15 Haddley K. Mipomersen sodium: a new option for the treatment of familial hypercholesterolemia. *Drugs Today (Barc)* 2011; 47: 891–901.
- 16 Crooke RM, Graham MJ. Therapeutic potential of antisense oligonucleotides for the management of dyslipidemia. *Clin Lipidol* 2011; 6: 675–92.
- 17 Bell DA. Mipomersen, an antisense apolipoprotein B synthesis inhibitor. *Expert Opin Investig Drugs* 2011; 20: 265–72.
- 18 Visser ME, Akdim F, Tribble DL, Nederveen AJ, Kwok TJ, Kastelein JJ, Trip MD, Stroes ES. Effect of apolipoprotein-B synthesis inhibition on liver triglyceride content in patients with familial hypercholesterolemia. *J Lipid Res* 2010; 51: 1057–62.
- 19 Raal FJ, Santos RD, Blom DJ, Marais AD, Charng MJ, Cromwell WC, Lachmann RH, Gaudet D, Tan JL, Chasan-Taber S, Tribble DL, Flaim JD, Crooke ST. Mipomersen, an apolipoprotein B synthesis inhibitor, for lowering of LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia: a randomized, double-blind, placebo controlled trial. *Lancet* 2010; 375: 998–1006.
- 20 Duell PB, Santos RD, East C, Guyton JR, Moriarty P, Donovan JM, Mittleman RS. Long-term safety and efficacy of mipomersen in patients with familial hypercholesterolemia uncontrolled by maximally tolerated lipid lowering therapy. *J Clin Lipidol* 2012; 6: 291.
- 21 Crooke RM, Graham MJ, Lemonidis KM, Whipple CP, Koo S, Perera RJ. An apolipoprotein B antisense oligonucleotide lowers LDL cholesterol in hyperlipidemic mice without causing hepatic steatosis. *J Lipid Res* 2005; 46: 872–4.
- 22 Crooke RM, Baker BF, Wedel MK. Cardiovascular therapeutic applications. In: *Antisense Drug Technology Principle, Strategies, and Applications*, Second edn. ed. Crooke ST. Baco Raton: CRC Press, 2007; 601–39.
- 23 Kastelein JJ, Wedel MK, Baker BF, Su J, Bradley JD, Yu RZ, Chuang E, Graham MJ, Crooke RM. Potent reduction of apolipoprotein B and low-density lipoprotein cholesterol by short-term administration of an antisense inhibitor of apolipoprotein B. *Circulation* 2006; 114: 1729–35.
- 24 Akdim F, Visser ME, Tribble DL, Baker BF, Stroes ES, Yu R, Flaim JD, Su J, Stein EA, Kastelein JJ. Effect of mipomersen, an apolipoprotein B synthesis inhibitor, on low-density lipoprotein cholesterol in patients with familial hypercholesterolemia. *Am J Cardiol* 2010; 105: 113–31.
- 25 Akdim F, Stroes ES, Sijbrands EJ, Tribble DL, Trip MD, Jukema JW, Flaim JD, Su J, Yu R, Baker BF, Wedel MK, Kastelein JJ. Efficacy and safety of mipomersen, an antisense inhibitor of apolipoprotein B, in hypercholesterolemic subjects receiving stable statin therapy. *J Am Coll Cardiol* 2011; 55: 1611–18.
- 26 Akdim F, Tribble DL, Flaim JD, Yu R, Su J, Geary RS, Baker BF, Fuhr R, Wedel MK, Kastelein JJ. Efficacy of apolipoprotein B synthesis inhibition in subjects with mild-moderate hyperlipidaemia. *Eur Heart J* 2011; 32: 2650–59.
- 27 Yu RZ, Kim TW, Hong A, Watanabe TA, Gaus HJ, Geary RS. Cross-species pharmacokinetic comparison from mouse to

man of a second-generation antisense Oligonucleotide, ISIS 301012, targeting human apolipoprotein B-100. *Drug Metab Dispos* 2007; 35: 460–63.

28 Yu RZ, Lemonidis KM, Graham MJ, Matson JE, Crooke RM, Tribble DL, Wedel MK, Levin AA, Geary RS. Cross-species comparison of *in vivo* PK/PD relationships for second-generation antisense oligonucleotides targeting apolipoprotein B-100. *Biochem Pharmacol* 2009; 77: 910–19.

29 Yu RZ, Geary RS, Flaim JD, Riley GC, Tribble DL, van Vliet AA, Wedel MK. Lack of pharmacokinetic interaction of mipomersen sodium (ISIS 301012), a 2'-O-methoxyethyl

modified antisense oligonucleotide targeting apolipoprotein B-100 messenger RNA, with simvastatin and ezetimibe. *Clin Pharmacokinet* 2009; 48: 39–50.

30 Visser Wagener G, Baker BF, Geary RS, Donovan JM, Beuers UH, Nederveen AJ, Verheij J, Trip MD, Basart DC, Kastelein JJ, Strokes ES. Mipomersen, an apolipoprotein B synthesis inhibitor, lowers low-density lipoprotein cholesterol in high-risk statin-intolerant patients: a randomized, double-blind, placebo-controlled trial. *Eur Heart J* 2012; 33: 1142–49.