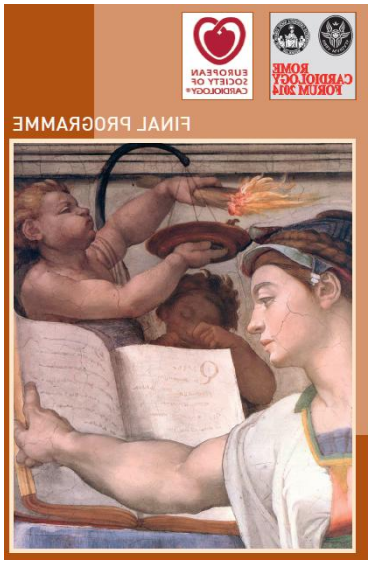

Rome Cardiology Forum 2014
An ESC Update
Programme in Cardiology
Rome, 29-31 2014

Beyond HDL: new therapeutic targets

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SAPIENZA
UNIVERSITÀ DI ROMA

Conflict of interest

Grants, consulting fees and/or honoraria and delivering lectures from:

Aegerion, BMS, Genzyme, Kowa, Merck , Novartis, Pfizer, Recordati, Roche, and Sanofi-Aventis

Meta-analysis of Comparative Efficacy of Increasing Dose of *Atorvastatin* Versus *Rosuvastatin* Versus *Simvastatin* on Lowering Levels of Atherogenic Lipids (from VOYAGER)

Percentage of patients achieving low-density lipoprotein cholesterol (LDL-C) goals with increasing statin doses

Drug	LDL-C Goal <70 mg/dl			LDL-C Goal <100 mg/dl		
	Baseline LDL-C <130 mg/dl	Baseline LDL-C 130–160 mg/dl	Baseline LDL-C ≥160 mg/dl	Baseline LDL-C <130 mg/dl	Baseline LDL-C 130–160 mg/dl	Baseline LDL-C ≥160 mg/dl
Rosuvastatin (mg)						
5	NA	0%*	3.2%	NA	66.7%*	38.0%
10	47.2%	33.0%	11.4%	82.0%	75.9%	56.8%
20	81.1%	57.2%	20.5%	94.6%	90.1%	64.5%
40	83.5%	67.6%	31.7%	97.3%	95.4%	74.1%
Atorvastatin (mg)						
10	28.4%	8.8%	2.0%	71.4%	62.1%	28.7%
20	64.7%	26.4%	4.1%	91.2%	83.8%	45.0%
40	72.8%	45.2%	9.8%	97.4%	91.1%	56.6%
80	76.4%	52.4%	18.1%	94.2%	86.4%	71.4%
Simvastatin (mg)						
10	NA	0%*	0%	NA	50.0%*	8.8%
20	10.3%	7.0%	1.6%	51.0%	57.3%	24.0%
40	31.3%	19.9%	1.5%	87.5%	76.7%	34.2%
80	NA	NA	4.0%	NA	NA	38.5%

*32258 patients included in the meta-analysis

Am J Cardiol 2010;105:69 –76

The PCSK9 (Proprotein Convertase Subtilisin/Kexin type 9) Story

- From discovery to clinical applications
- Strategies for inhibition of PCSK9

PCSK9 : Rapid Progress from Discovery to Clinic

2003

- PCSK9 (NARC-1) discovered

Seidah et al. PNAS 2003; 100: 928-33

- PCSK9 GOF mutations (missense mutations) cause Autosomal Dominant Hypercholesterolemia

Abifadel et al. Nat Genet 2003; 34: 154-6

PCSK9 : Rapid Progress from Discovery to Clinic

2005

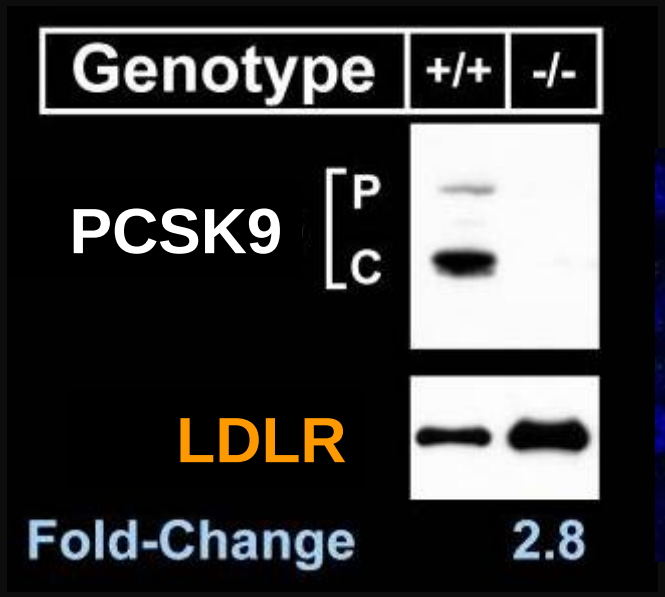
- PCSK9 KO mice ↓ LDL-C

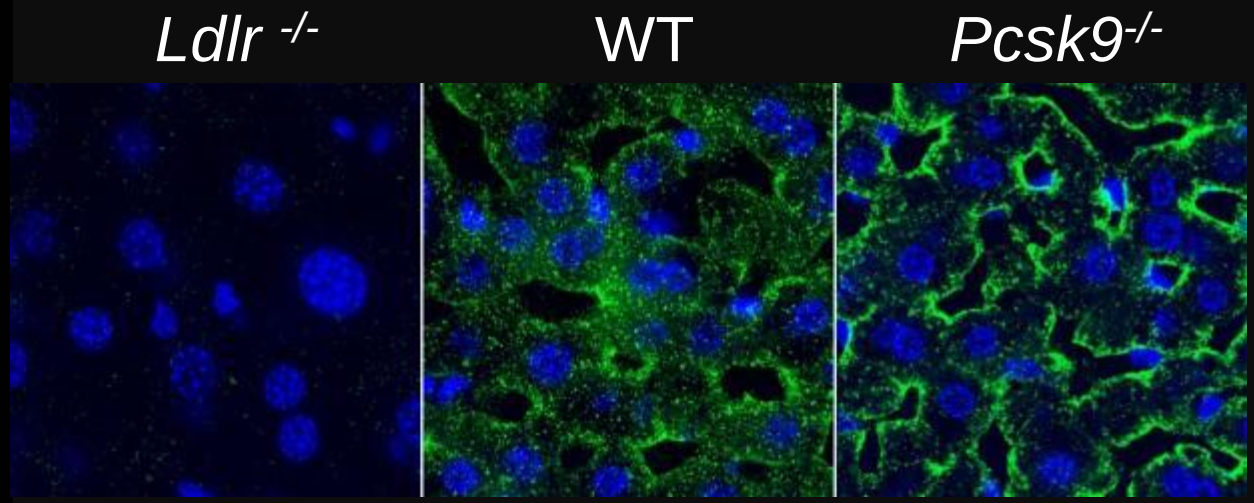
Rashid et al. PNAS 2005; 102: 5374-9

- PCSK9 LOF mutations (nonsense mutations) associated with low LDL-C and large reduction in the incidence of CHD

Cohen et al. Nat Genet 2005; 37:161-5
& N Engl J Med 2006; 354:1264-72

LDLR Protein Levels are Increased in Livers of Mice with No PCSK9

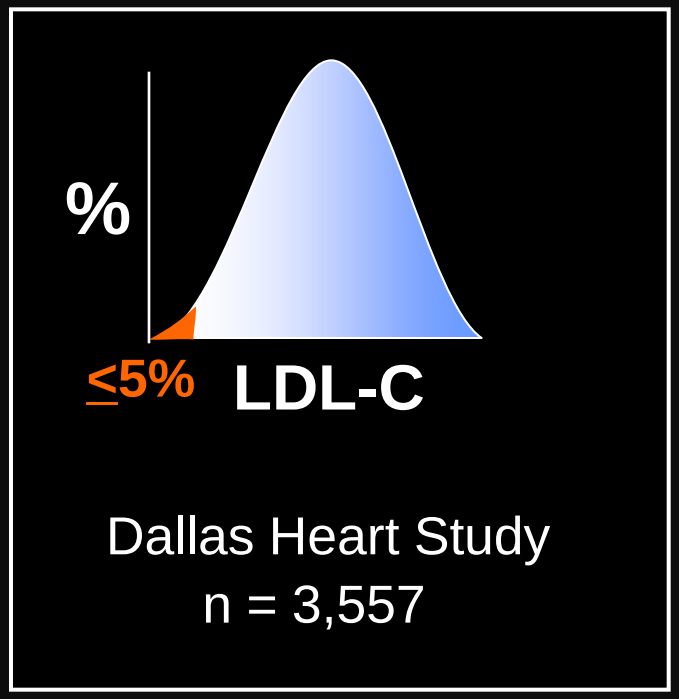




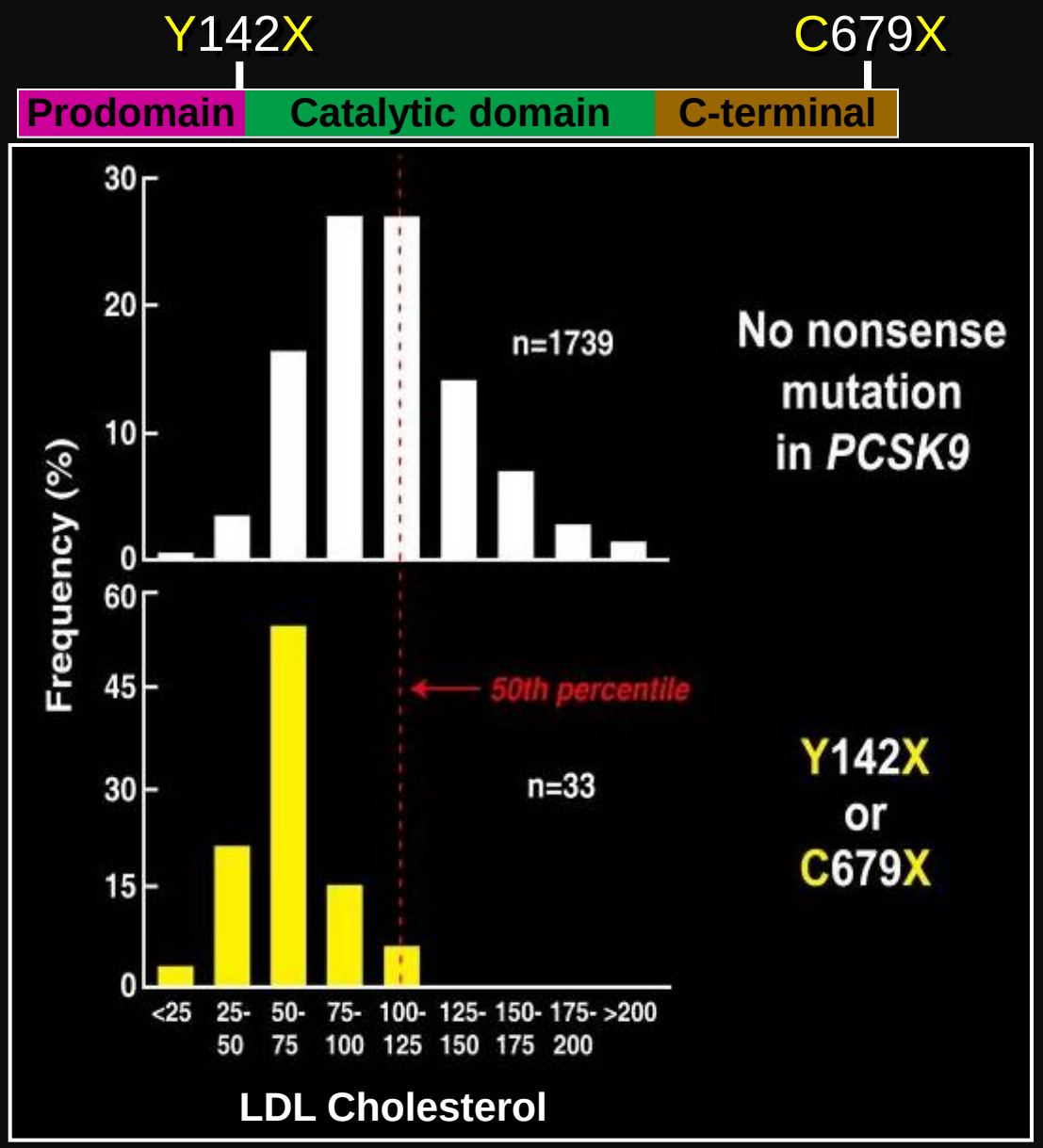
P and C denote the proprotein and cleaved forms of PCSK9

Rashid S et al. PNAS 2005; 102: 5374-9

LOF (Nonsense) Mutations in PCSK9

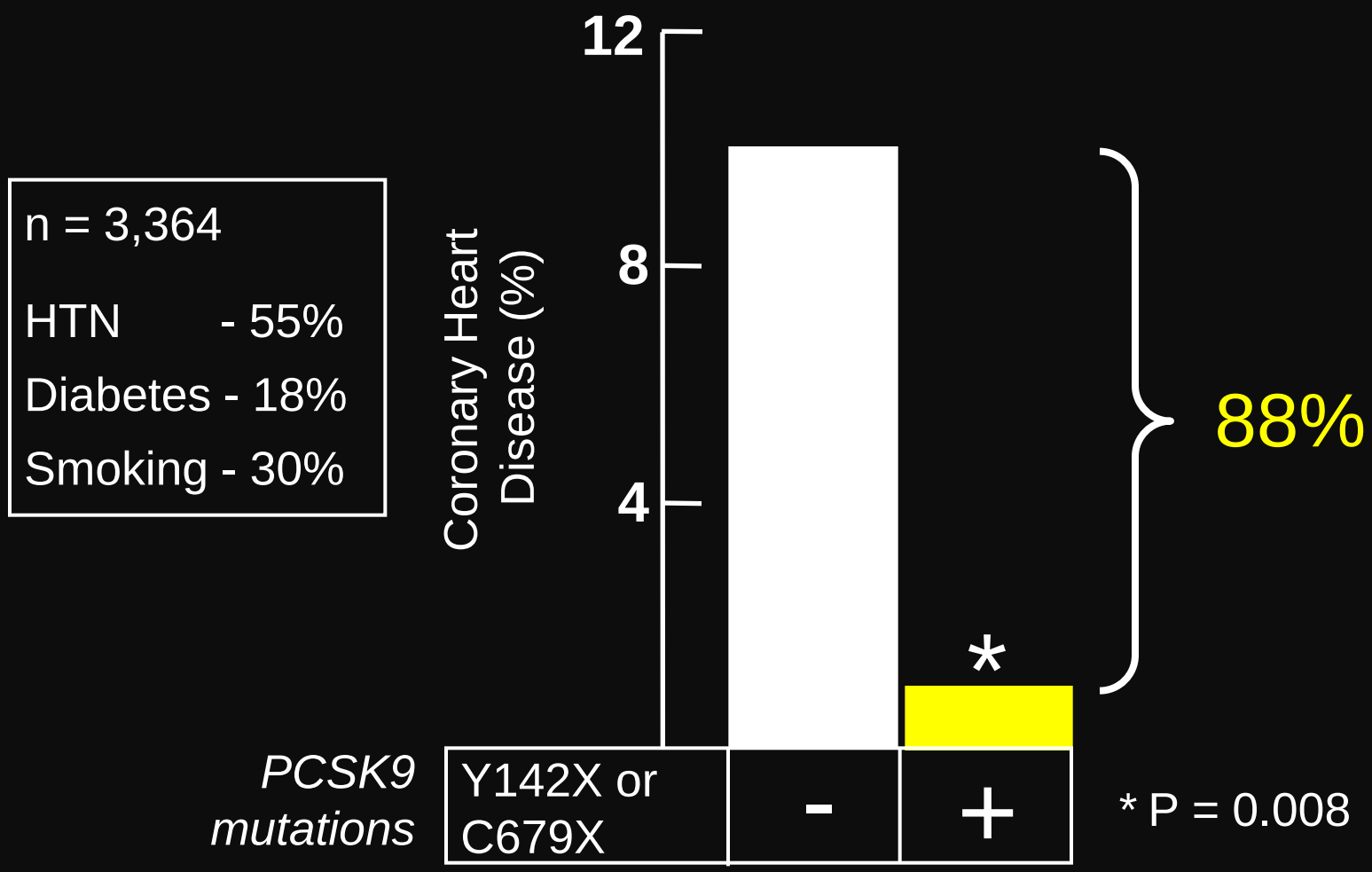


Dallas Heart Study
n = 3,557



Cohen et al. 2005. Nat. Genet. 37:161-165.

ARIC: 28% Reduction in LDL - 88% Reduction in CHD in AA with *PCSK9 (Y142X or C679X)*



Cohen et al. 2006. N. Engl. J. Med. 354:1264-1272.

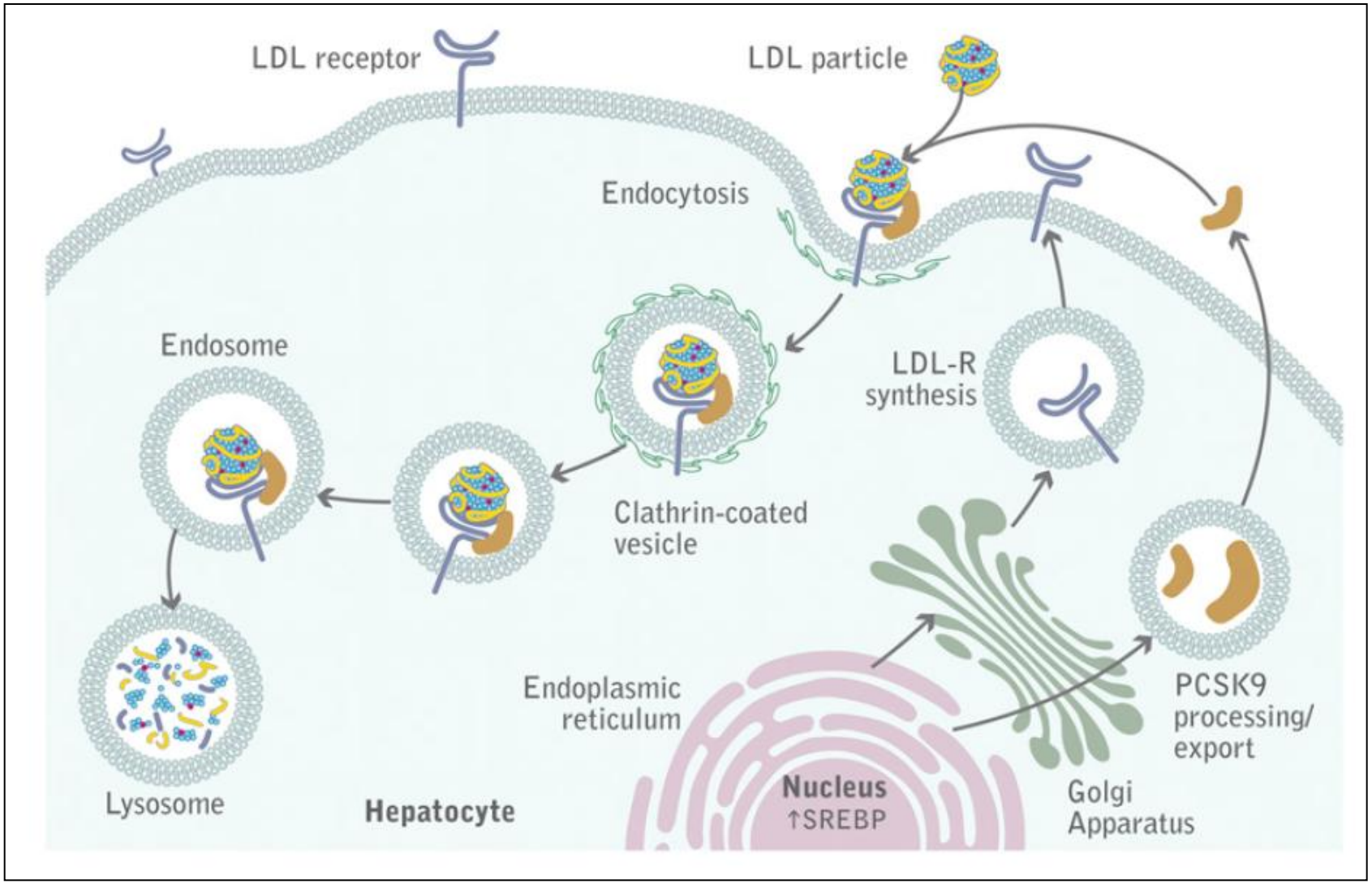
PCSK9 LOF, LDL-C and Risk of CHD

Population	PCSK9 Mutation	LDL-C Reduction	CHD Reduction
ARIC Study [1] (US)	Y142X or C679X	28%	88%
	R46L	15%	47%
3 independent Danish Studies [2]	R46L	14%	34%

1. Cohen et al. 2006. N Engl J Med; 354:1264-1272

2. Benn et al. 2010. J Am Coll Cardiol; 55: 2833-42

PCSK9-mediated degradation of LDLR



J Lipid Res 2012. 53: 2515–2524

Anti-PCSK9 therapeutic agents

- Inhibition of PCSK9 binding to LDLR
  - Monoclonal antibodies (mAb)
  - Small peptide molecules
  - Adnectins
- Inhibition of PCSK9 synthesis (gene silencing)
  - Antisense oligonucleotides (ASO)
  - Small interfering RNA (siRNA)
- Inhibition of PCSK9 autocatalytic processing
  - Small molecule inhibitors

PCSK9 inhibitors in development

Compound	Company	Phase of clinical development
mAbs		
Alirocumab (REGN727/ SAR236553)[1]	Sanofi/Regeneron	Phase 3
AMG 145[2]	Amgen	Phase 3
RN-316 (PF-04950615)[3]	Pfizer/Rinat	Phase 2 (completed)
RG 7652[4]	Roche/Genentech	Phase 2 (on hold – looking for partner)
LY3015014[5]	Eli Lilly	Phase 2
LGT209[6]	Novartis	Phase 2 (discontinued)

http://clinicaltrials.gov/ct2/results?term=REGN727%2F+SAR236553&Search=Search
http://clinicaltrials.gov/ct2/results?term=AMG+145&Search=Search
http://clinicaltrials.gov/ct2/results?term=PF-04950615&Search=Search

http://clinicaltrials.gov/ct2/show/NCT01890967?term=LY3015014&rank=3&submit_fld_opt=
http://clinicaltrials.gov/ct2/results?term=LGT209&Search=Search

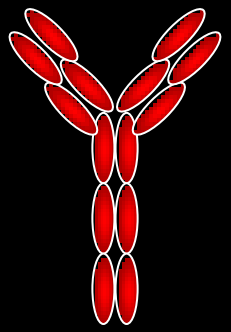
PCSK9 inhibitors in development

Compound	Company	Phase of clinical development
(si)RNA		
ALN-PCS[1]	Alnylam Pharmaceuticals	Phase I (IV formulation) Pre-clinical (SC formulation)
Adnectin		
BMS-962476[2]	BMS	Phase I
Mimetic Peptides		
EGF-A peptide[3]	Department of Cardiovascular and Metabolic Disease Research, Schering-Plough Research Institute	Pre-clinical
Prodomain and C-terminal domain interaction disruption[4]	Department of Cell Biology and Anatomy, School of Medicine, University of South Carolina	Pre-clinical

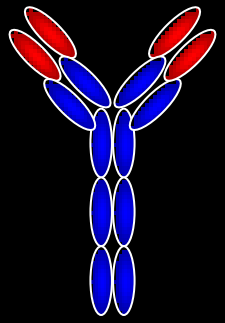
http://clinicaltrials.gov/ct2/results?term=ALN-PCS&Search=Search
http://www.clinicaltrials.gov/ct2/show/NCT01587365

Evolution of therapeutic mAbs

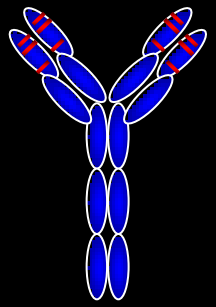
Mouse



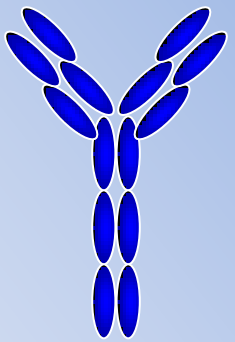
Chimeric



Humanised

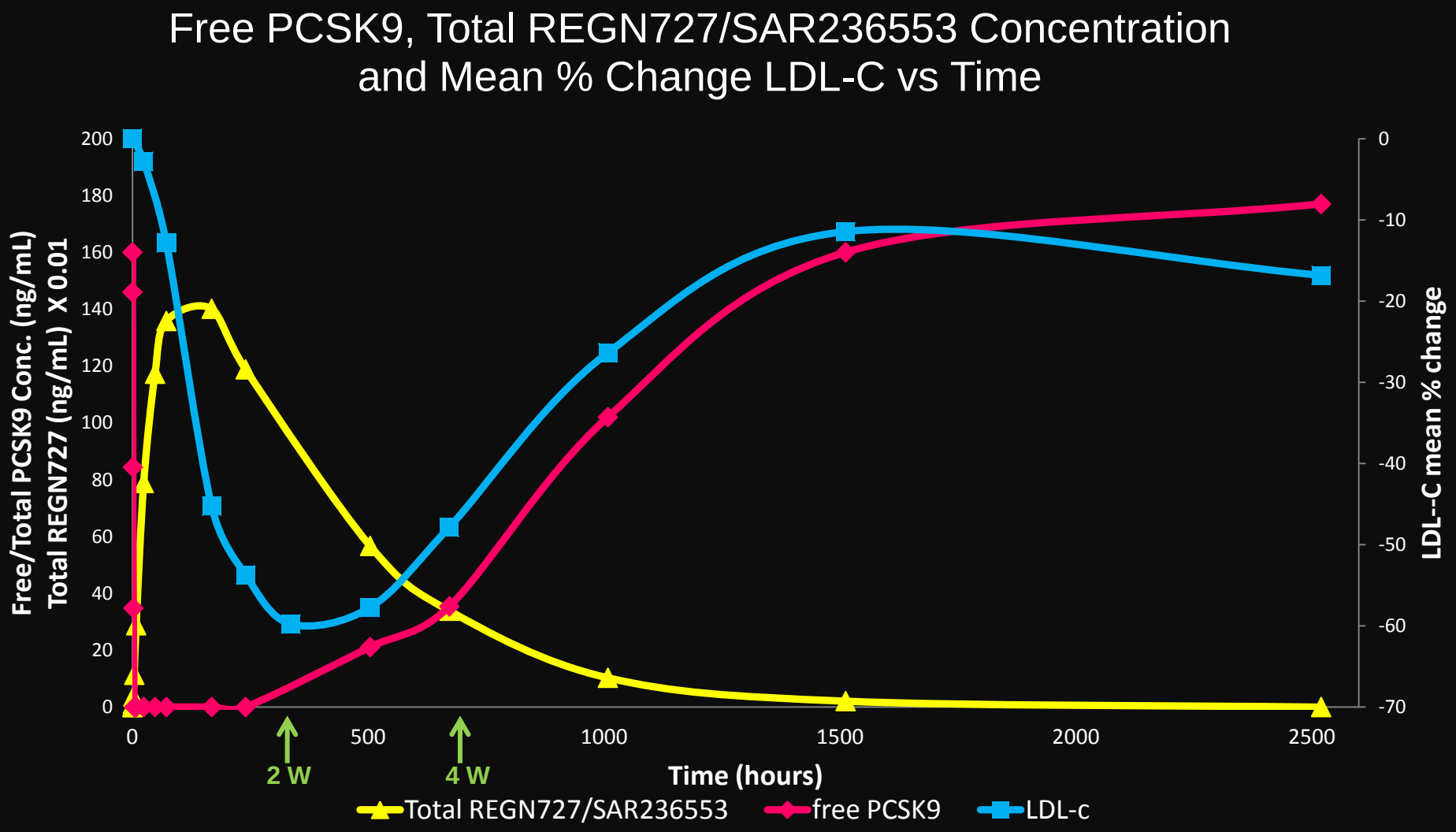


Human



- **Human variable**
- **Human constant**
- **Decreased risk of immunogenicity**

Alirocumab : relationship between mAb levels, PCSK9 and LDL-C

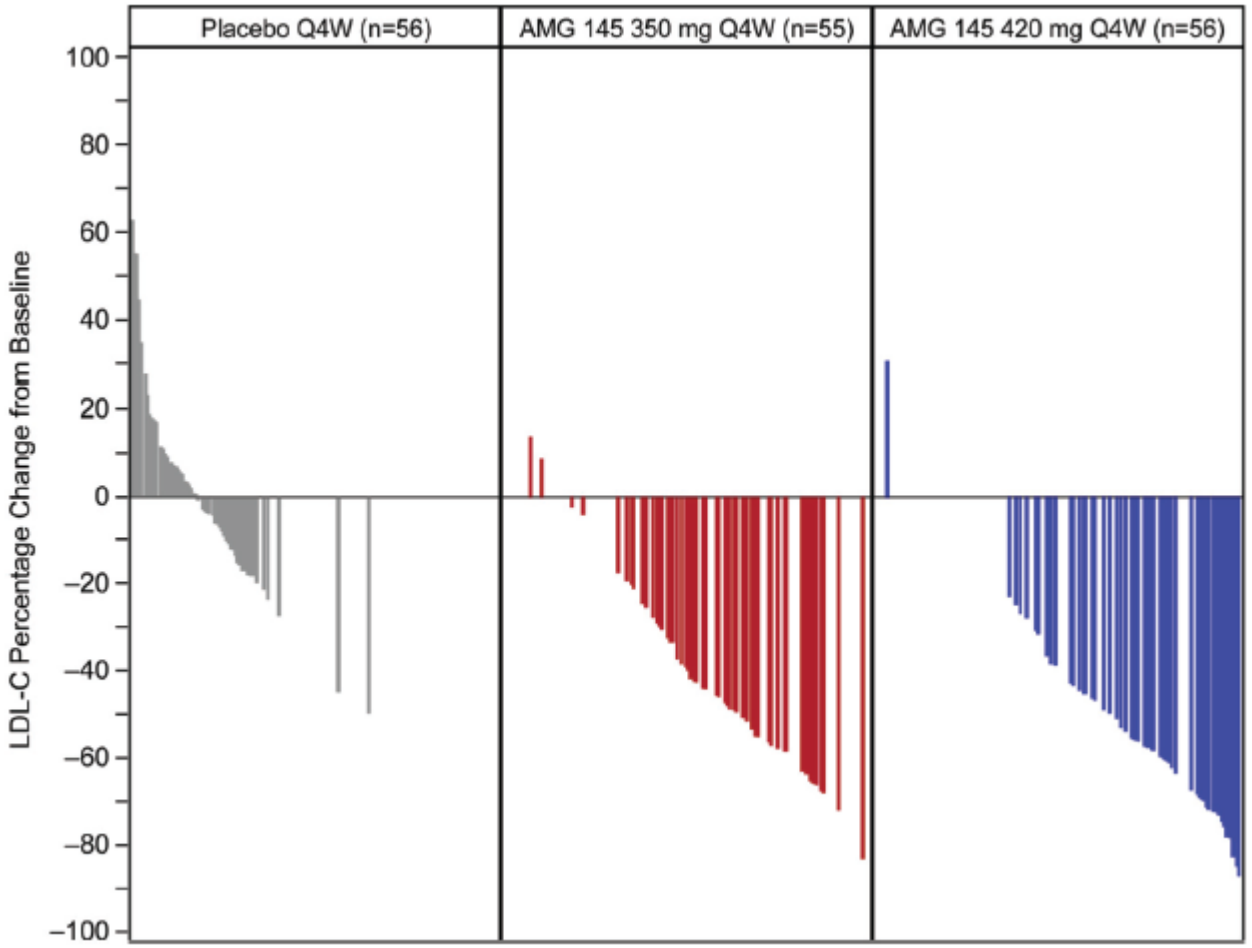


Summary of LDL-C lowering trials with PCSK9 inhibitors in patients with heterozygous familial hypercholesterolemia

	Patient population	Duration	Dosage	Baseline LDL-C (mg/dl)	LDL-C % change	Major side effects
Stein et al	HeFH (n=21)	57 days	placebo	133.2 ± 20.7		
(2012)			50 mg/every 3wk	125.0 ± 12.1	- 41.4	13% increase in CPK (>3 x ULN) but also in those taking atorvastatin
			100 mg/every 3wk	135.8 ± 41.1	- 57.6	
			150 mg/ every 3wk	140.2 ± 26.2	- 55.7	
Stein et al	HeFH (n=77)	12 weeks	placebo	150.0 ± 34.0	-10.6	
(2012)			150 mg/ every 4wk	166.7 ± 50.2	- 28.9	injection-site reactions, infections, gastrointestinal disorders; no change sin liver or muscle enzymes
			200 mg/ every 4 wk	169.8 ± 56.7	- 31.5	
			300 mg/ every 4 wk	139.7 ± 24.7	- 42.5	
			150 mg/every 2 wk	147.1 ± 32.4	- 67.9	
Raal et al (2012)	HeFH (n=167)	12 weeks	placebo	162.1 ± 42.5	1.1	
			350 mg/ every 4 wk	158.3 ± 46.3	-42.7	injection site pain, skin burning, headache, 3% increase CPK (>5 x ULN) and 1.8 % increase in liver enzymes (<3 x ULN)
			420 mg/ every 4 wk	150.5 ± 34.7	-55.2	

Wk, week; increase of liver transaminase >3X ULN

Low-Density Lipoprotein Cholesterol–Lowering Effects of AMG 145, a Monoclonal Antibody to PCSK9 in Patients With Heterozygous Familial Hypercholesterolemia. The RUTHERFORD study



Circulation 2012;126: 2408-2417

RUTHERFORD: safety and tolerability

Adverse events (AEs) Patients incidence, n (%)	Placebo (n=56)	AMG 145 300 mg Q4W (n=55)	AMG 145 420 mg Q4W (n=56)
Treatment –emergent AEs, n (%)	33 (58.9)	32 (58.2)	37 (66.1)
Most common AEs			
Nasopharyngitis, n (%)	6 (10.7)	7 (12.7)	7 (12.5)
Injection site pain, n (%)	1 (1.8)	5 (9.7)	2 (3.6)
Headache, n (%)	5 (8.9)	3 (5.5)	3 (5.4)
Serious AEs	0	0	2 (3.6)
Deaths, n (%)	0	0	0
Treatment-related AEs, n (%)	6 (10.7)	13 (23.6)	8 (14.3)
Injection-site reaction, n (%)	3 (5.4)	6 (10.9)	2 (3.6)
Muscle-related AEs, n (%)	2 (3.6)	2 (3.6)	4 (7.1)
CK elevation (>5xULN), n (%)	0	1 (2)	4 (2)

***Circulation* 2012;126: 2408-2417**

PRELIMINARY COMMUNICATION

ONLINE FIRST

Effect of a Monoclonal Antibody to PCSK9 on Low-Density Lipoprotein Cholesterol Levels in Statin-Intolerant Patients

The GAUSS Randomized Trial

David Sullivan, MD
Anders G. Olsson, MD, PhD
Rob Scott, MD
Jae B. Kim, MD
Allen Xue, PhD
Val Gebski, MStat
Scott M. Wasserman, MD
Evan A. Stein, MD, PhD

Context An estimated 10% to 20% of patients cannot tolerate statins or adequate doses to achieve treatment goals. Plasma proprotein convertase subtilisin/kexin type 9 (PCSK9) binds to low-density lipoprotein (LDL) receptors, promoting their degradation and increasing LDL cholesterol levels. In phase 1 studies, a human monoclonal antibody to PCSK9, AMG145, was well tolerated and reduced LDL cholesterol levels.

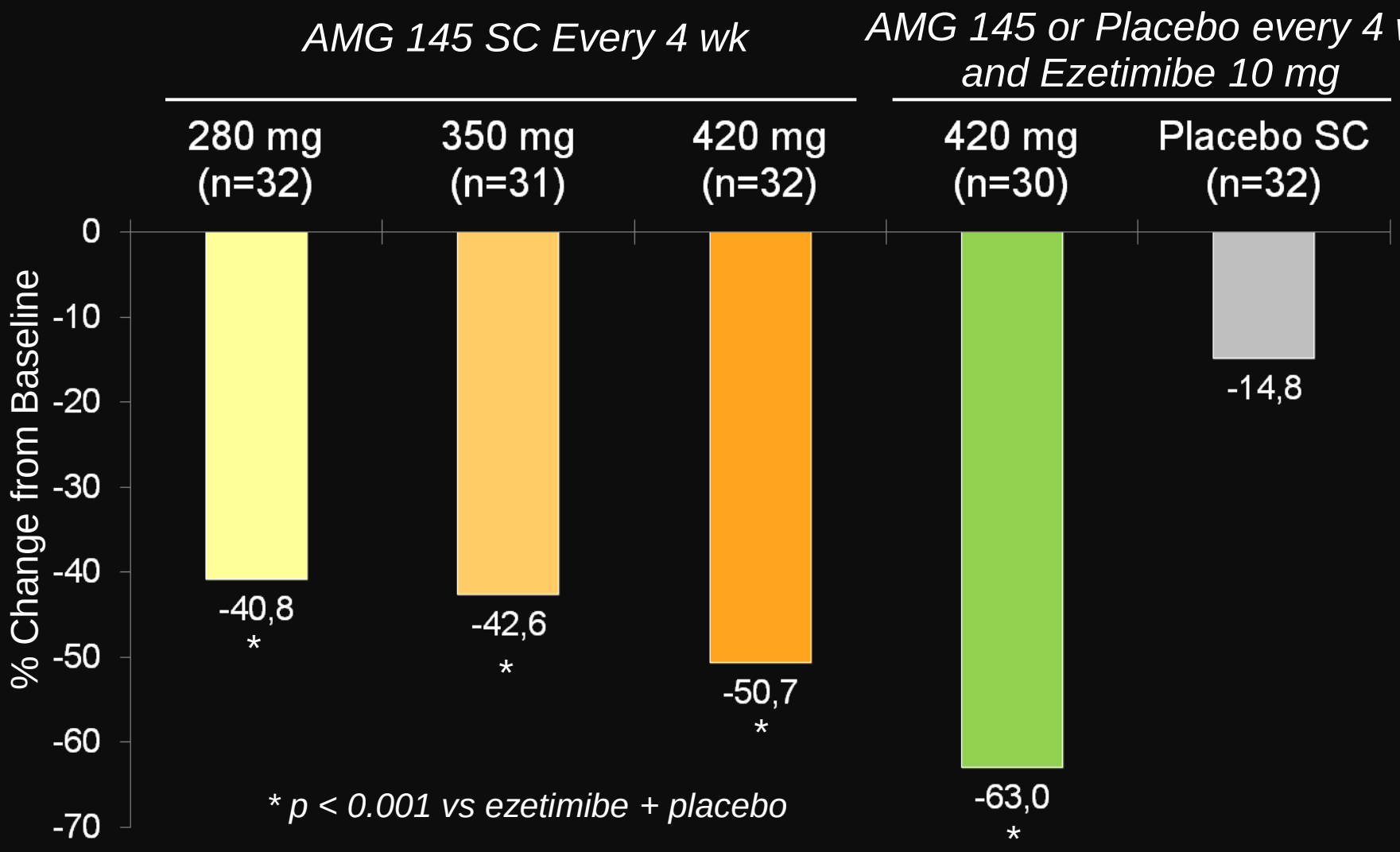
Objective To assess the efficacy and tolerability of AMG145 in patients with statin intolerance due to muscle-related side effects.

Design, Setting, and Patients A 12-week, randomized, double-blind, placebo- and ezetimibe-controlled, dose-ranging study conducted between July 2011 and May 2012 in statin-intolerant adult patients at 33 international sites.

JAMA 2012; November 5, online

GAUSS : Evolocumab in statin-intolerant patients

% change in LDL-C (by UC) from baseline to week 12



Sullivan et al. JAMA 2012; 308: 2497-506

Summary of Phase 3 clinical development programs for Alirocumab and Evolocumab

Patient segment	Alirocumab	AMG 145	Select observations
Monotherapy	ODYSSEY MONO (N=100)	MENDEL-2 (N=600)	• Primary endpoint is Week 24 for alirocumab vs Week 12 for AMG 145
Combination therapy	ODYSSEY COMBO I (N=306) ODYSSEY COMBO II (N=660) ODYSSEY OPTIONS I (N=350) ODYSSEY OPTIONS II (N=300)	LAPLACE-2 (N=1,700)	• Patient populations in ODYSSEY COMBO trial must be on max-tolerated statin and have “high CV risk” • Primary endpoint is Week 24 for alirocumab vs Week12 for AMG 145
HeFH	ODYSSEY FH I (N=471) ODYSSEY FH II (N=250) ODYSSEY HIGH FH (N=105)	RUTHERFORD-2 (N=300)	• Offers insights into both FH and high FH patient segments • Primary endpoint is at Week 24 for alirocumab vs Week 12 for AMG 145
HoFH	None to date	TESLA & TAUSSIG (N=67 & N=75)	
Statin-intolerant patients	ODYSSEY ALTERNATIVE (N=250)	GAUSS-2 (N=300)	• No evidence of a “statin-challenge” in the GAUSS-2 trial • Primary endpoint is at Week 24 for alirocumab vs Week 12 for AMG 145
Outcomes studies	ODYSSEY OUTCOMES (N=18,000)	FOURIER (N=22,500)	• FOURIER – patient population: history of CVD or at high risk of CVD; primary endpoint is time to CV death, MI, hospitalization for unstable angina, stroke, or coronary revascularization • ODYSSEY OUTCOMES – Patient population: recently hospitalized for ACS; primary endpoint is time to first occurrence of one of the following clinical events: CHD death, non-fatal MI, fatal and non-fatal ischemic stroke, unstable angina requiring hospitalization
Safety studies	ODYSSEY LONG TERM (N=2,100)	DESCARTES (N=900) OSLER (N=1,400)	

Alirocumab = REGN727/SAR236553; Alirocumab and AMG 145 are in clinical development and have no marketing authorization yet

PCSK9 Monoclonal Antibody Therapy

- Evidence to date
  - Very effective lowering of LDL-C, non HDL-C, ApoB
  - Positive effects on Lp(a), TG
  - Lipid effects in monotherapy and additive to other LDL lowering drugs
  - No short-term safety issues (months)
- Unanswered issues
  - Longer term lipid efficacy (dosing interval)
  - Longer term safety profile
  - Immune effects over time
  - CVD outcome trial efficacy
- Other issues
  - Relevance of other therapies at very low LDL-C levels
  - Cost

Candidate populations for PCSK9 inhibition

Priorities

- Patients with heterozygous FH (HoFH?)
  (LDLR, apoB mutations)

Other candidates

- high-risk patients not at LDL-C goal on maximum tolerated lipid-lowering treatment

Questions to be addressed:

PCSK9 has been postulated to play a role in multiple other tissues and pathways including hepatic and adipose tissue, sodium channel regulation, pancreatic islet cell function and nervous system development

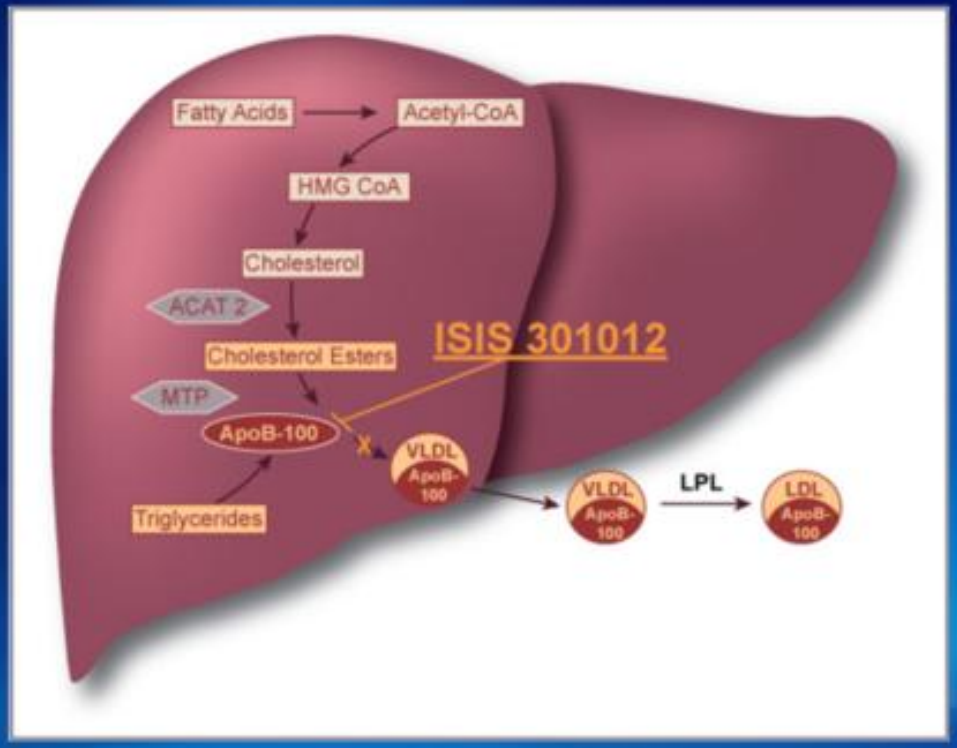
- Mice lacking PCSK9 develop necrotic hepatic lesions and adipose tissue hypertrophy
- Mice lacking PCSK9 also develop glucose intolerance due to lipid accumulation in pancreatic beta cells?.
- PCSK9 may also reduce the expression of the epithelial sodium channel (ENaC) which could lead to increased sodium reabsorption and hypertension
- PCSK9 also down regulates CD81 which is important for the entry of the hep C virus into hepatocytes
- CNS dysfunction (PCSK9 initially called Neural Apoptosis-Regulated Convertase-1 or NARC-1)?

New Approaches to LDL Reduction

What is in development?

- Cholesterol Absorption Inhibitors
- Squalene Synthase (SSI) inhibitors
- **Apo B mRNA antisense drugs**
- **Microsomal Triglyceride Transfer Protein (MTP) inhibitors (lomitapide)**
- Thyroxin Receptor Agonists
- **PCSK9 Inhibitors**

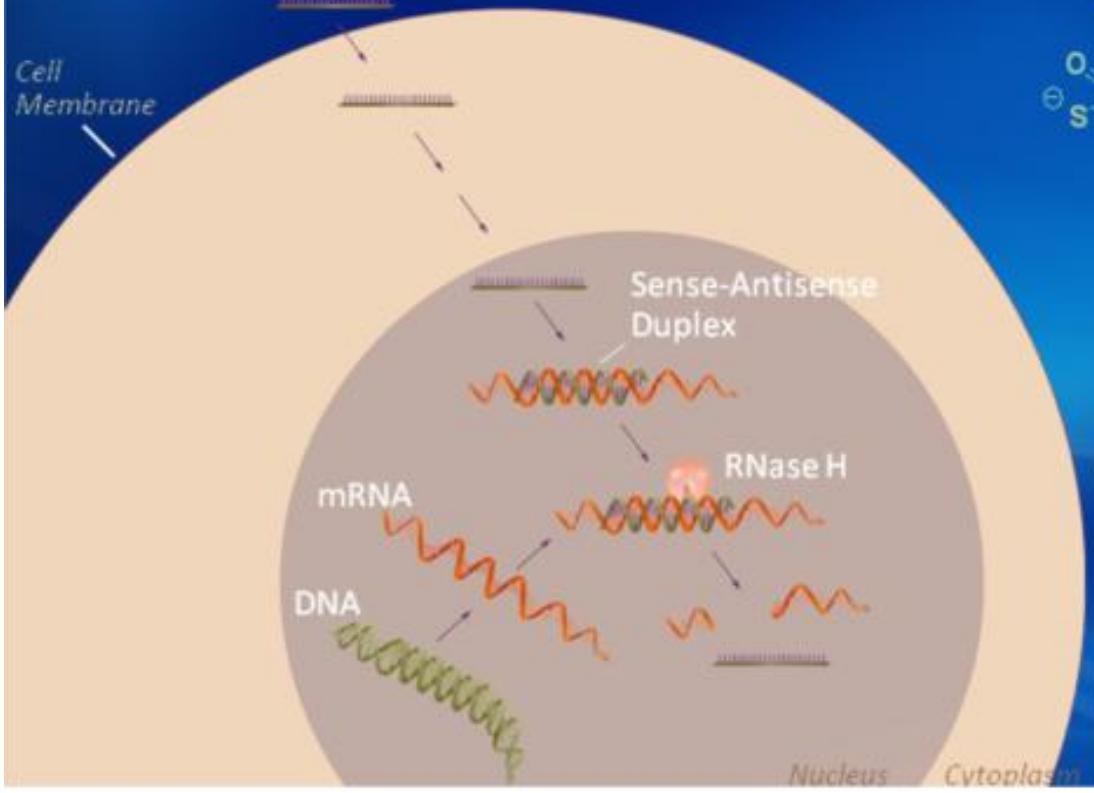
Human ApoB-100 is an Ideal Target for a 2nd Generation Antisense Drug



- **ApoB-100:**
  - Is expressed in the liver
  - Is essential for the synthesis and transport of VLDL and LDL-C
  - Plays a crucial role in lipid management
  - Is a biologically validated, but undruggable target for small molecules
- **An apoB-100 inhibitor should have**
  - A unique lipid lowering profile
  - A complementary mechanism with potential for additive effects when co-administered with statins
- **Status of ISIS 301012:**
  - Phase 2 program in progress
  - Symphony Genisis collaboration

Antisense: A Novel Approach to Drug Discovery by Inhibition of Translation of a Specific Targeted Protein

RNase H Dependent Mechanism of Action



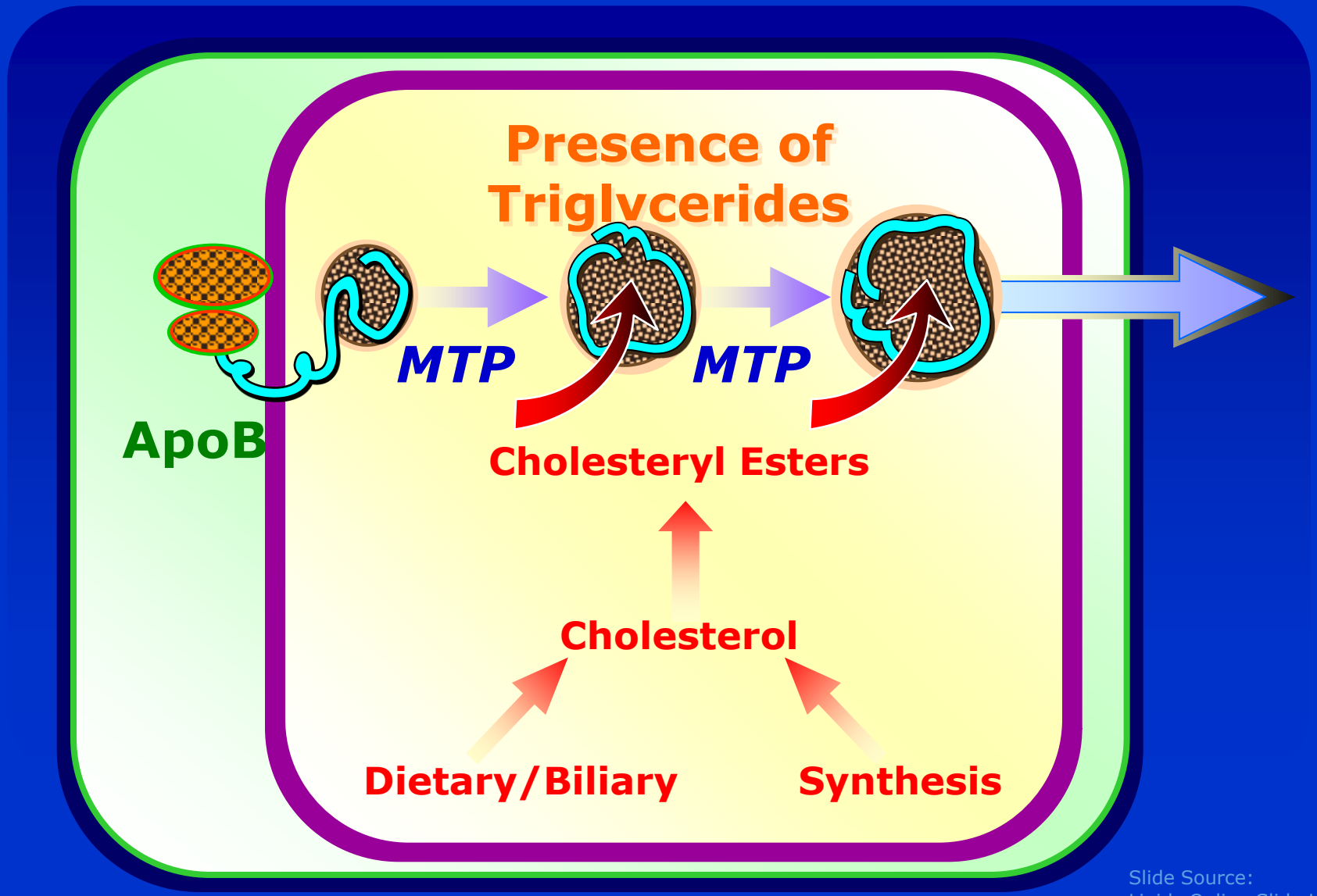
2nd Generation Antisense Drugs

- ~20X more potent
- 1X/week to 1X/quarter dosing
- Better tolerated
- Lower cost of therapy

Summary of LDL-C lowering trials with mipomersen in patients with heterozygous familial hypercholesterolemia

Study	Patient population	Duration	Dosage	Baseline LDL-C (mg/dl)	LDL-C % change	Major side effects
Raal *et al*	HoFH (n=51)	26 weeks	Placebo	401.4 ± 142.8	-3.3	
(2010)			200 mg/wk	440.0 ± 139.0	-25	injection-site reactions, 12% increase of ALT
Akdim *et al*	HeFH (n=44)	6 weeks	placebo	170.6 ± 46.3	0	
(2010)			50 mg/wk	206.5 ± 77.2	-13	injection-site reactions, 11% increase of liver transaminases
			100 mg/wk	173.7 ± 38.6	-11	
			200 mg/wk	163.7 ± 30.9	-21	
			300mg/wk	173.7 ± 34.7	-34	
Visser *et al*	HeFH (n=21)	13 weeks	placebo	155 ± 31	1.0	
(2010)			200 mg/wk	155 ± 37	-22	injection-site reactions, flu-like illness, 10% with mild steatosis
Stein *et al*. (2012)	HeFH (n=124)	26 weeks	Placebo	142.8	5	
			200 mg/wk	152.8	-28	
Tardiff *et al* (2011)	Severe HeFH (n=58)	26 weeks	Placebo	248.6	13	
			200 mg/wk	275.6	-36	

Assembly and Secretion of VLDL



Efficacy and safety of a microsomal triglyceride transfer protein inhibitor in patients with homozygous familial hypercholesterolaemia: a single-arm, open-label, phase 3 study

	Baseline (n=29)	Week 26 (n=23)			Week 56 (n=23)			Week 78 (n=23)		
		Concentrations	Change from baseline (%)	p value†	Concentrations	Change from baseline (%)	p value‡	Concentrations	Change from baseline (%)	p value‡
Total cholesterol, mmol/L	11·1 (3·5)	6·1 (2·9)	−46% (−56 to −35)	<0·0001	7·1 (3·7)	−39% (−51 to −27)	<0·0001	7·3 (3·9)	−35% (−48 to −22)	<0·0001
LDL cholesterol, mmol/L	8·7 (2·9)	4·3 (2·5)	−50% (−62 to −39)	<0·0001	5·1 (3·2)	−44% (−57 to −31)	<0·0001	5·4 (3·4)	−38% (−52 to −24)	0·0001
VLDL cholesterol, mmol/L	0·5 (0·3)	0·3 (0·3)	−45% (−61 to −29)	<0·0001	0·4 (0·4)	−28% (−48 to −10)	0·0185	0·4 (0·4)	−31% (−54 to −7)	0·0389
Non-HDL cholesterol, mmol/L	10·0 (3·4)	5·1 (2·8)	−50% (−61 to −39)	<0·0001	5·9 (3·6)	−44% (−57 to −31)	<0·0001	6·2 (3·8)	−39% (−53 to −25)	<0·0001
Triglycerides, mmol/L	1·0 (0·4 to 2·9)	0·5 (0·1 to 1·7)	−45% (−61 to −29)	<0·0001	0·7 (0·2 to 2·9)	−29% (−47 to −11)	0·0157	0·7 (0·2 to 4·1)	−31% (−54 to −8)	0·0368
ApoB, g/L	2·6 (0·8)	1·3 (0·7)	−49% (−60 to −38)	<0·0001	1·5 (0·8)	−45% (−57 to −33)	<0·0001	1·5 (0·9)	−43% (−56 to −29)	<0·0001
Lipoprotein (a), µmol/L	2·4 (0·6 to 2·1)	1·7 (0·3 to 7·1)	−15% (−30 to 0·9)	0·0003	2·0 (0·5 to 8·6)	−19% (−31 to −8)	0·0111	2·6 (0·6 to 7·0)	−1% (−17 to 6)	0·5827
HDL cholesterol, mmol/L	1·1 (0·3)	1·0 (0·4)	−12% (−20 to −4)	0·0001	1·2 (0·4)	1% (−13 to 15)	0·954	1·1 (0·3)	−5% (−13 to 3)	0·1396
ApoA-I, g/L	1·2 (0·3)	1·0 (0·2)	−14% (−17 to −4)	0·0003	1·1 (0·3)	1% (−11 to 13)	0·568	1·1 (0·3)	−4% (−10 to 3)	0·1155

Data are mean (SD), median (range) for triglycerides and lipoprotein (a) at baseline, weeks 26, 56, and 78, or mean (95% CI) for percent change. †p values from mixed model. ‡p values from one-sample t test.

Table: **Lipid and lipoprotein concentrations at baseline and weeks 26, 56, and 78 (end of study)**

The median dose of lomitapide was 40 mg a day

Cuchel M et al. Lancet November 2, 2012 online

Efficacy and safety of a microsomal triglyceride transfer protein inhibitor in patients with homozygous familial hypercholesterolaemia: a single-arm, open-label, phase 3 study

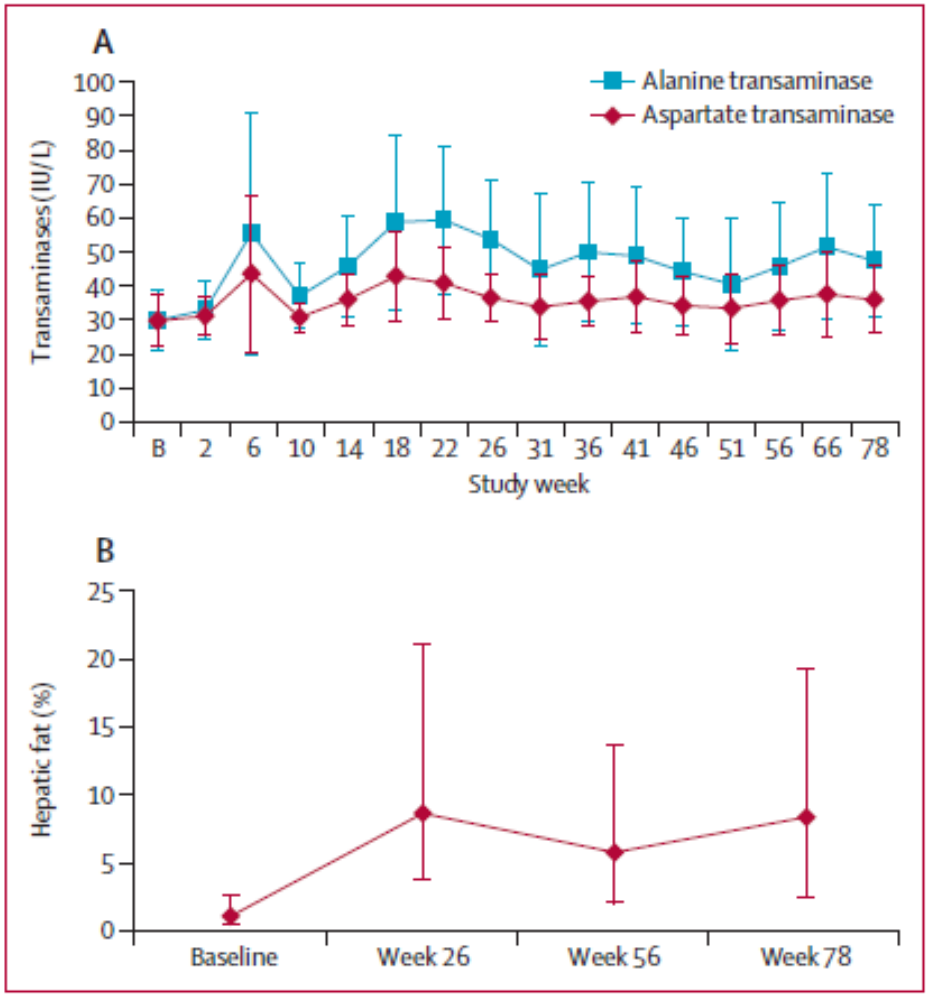


Figure 2: **Alanine transaminase and aspartate transaminase levels and percentage of hepatic fat in the liver**

A. Laboratory reference ranges for ALT levels were 10–40 U/L in men and 10–33 U/L in women; reference ranges for AST were 10–43 U/L in men and 10–36 U/L in women

B. Percentage of fat in the liver, as measured by NMRS at baseline and 26, 56, and 78 weeks of lomitapide treatment (n=20)

Gastrointestinal symptoms were the most common adverse event. Four patients had transaminases levels of more than five times the upper limit of normal, which resolved after dose reduction or temporary interruption of lomitapide

Lancet, November 2, 2012 on line

Conclusions

- Inhibition of PCSK) has been proved to be an usefull target for the treatment elevated LDL-C
- The administration of Ab anti-PCSK9 causes a 60-70% reduction of LDL-C HeFH, resistant to conventional therapies, without relevant AE.
- Potentially relevant therapies might also be those dirceted towards the inhibition of VLDL synthesis (mainly indicated in patients which are receptor negative).