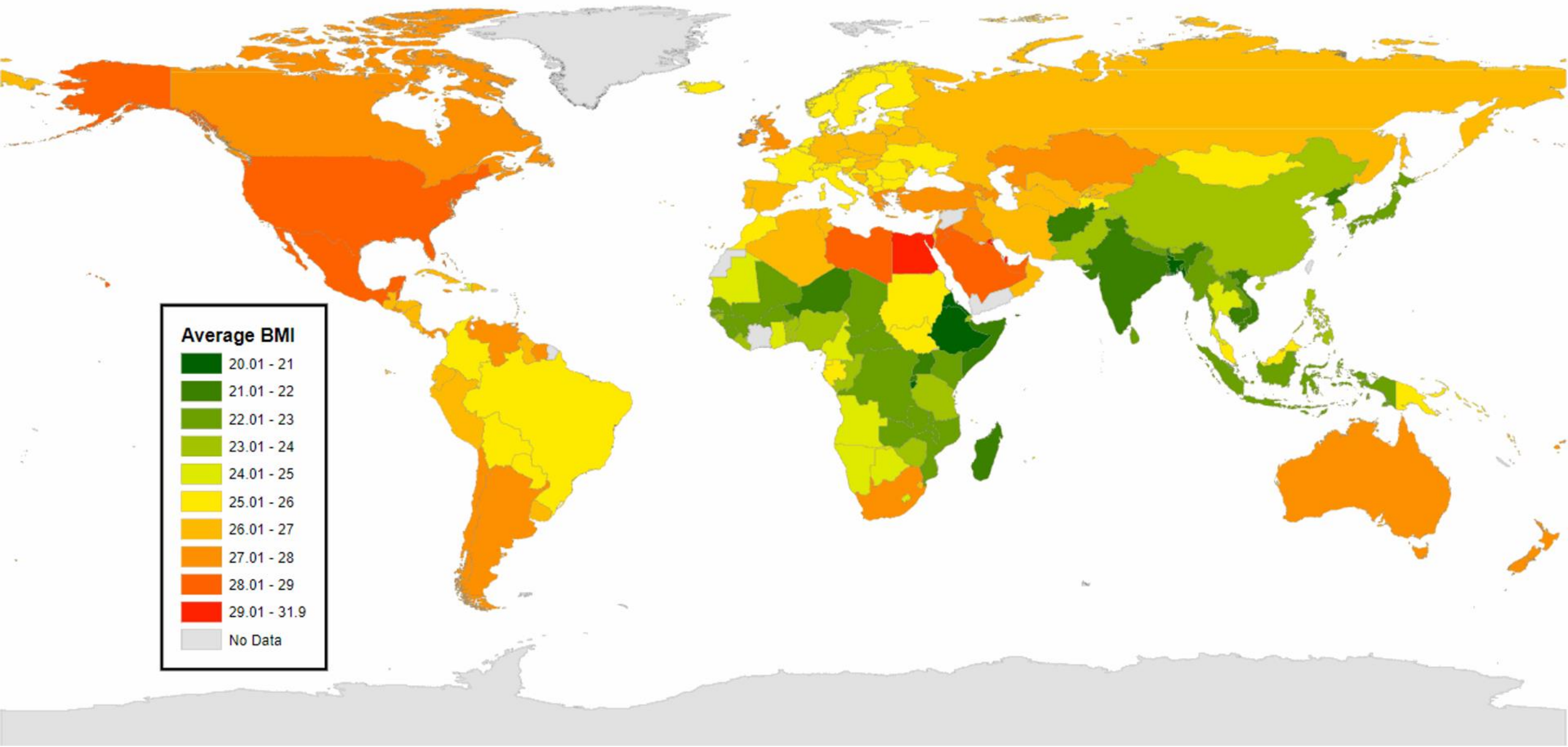
Optimal Lipid control
& Role of Oligonuleotides

Dr.S.Venkatesan
Cardiologist
Madras medical college
Chennai

9-02-2020

Average Body Mass Index (BMI) per country, 2014



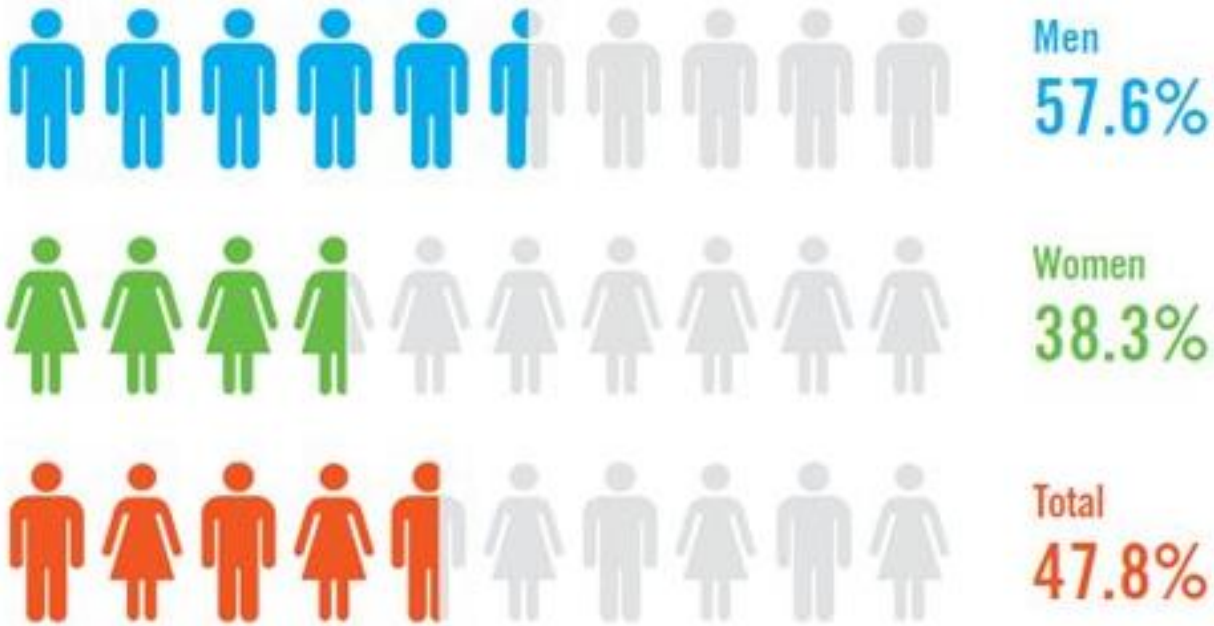
Prevalence of Dyslipidemia

- Approximately half of adults aged 30 years or older had dyslipidemia.
- About 6 out of every 10 men and 4 out of every 10 women were dyslipidemic.

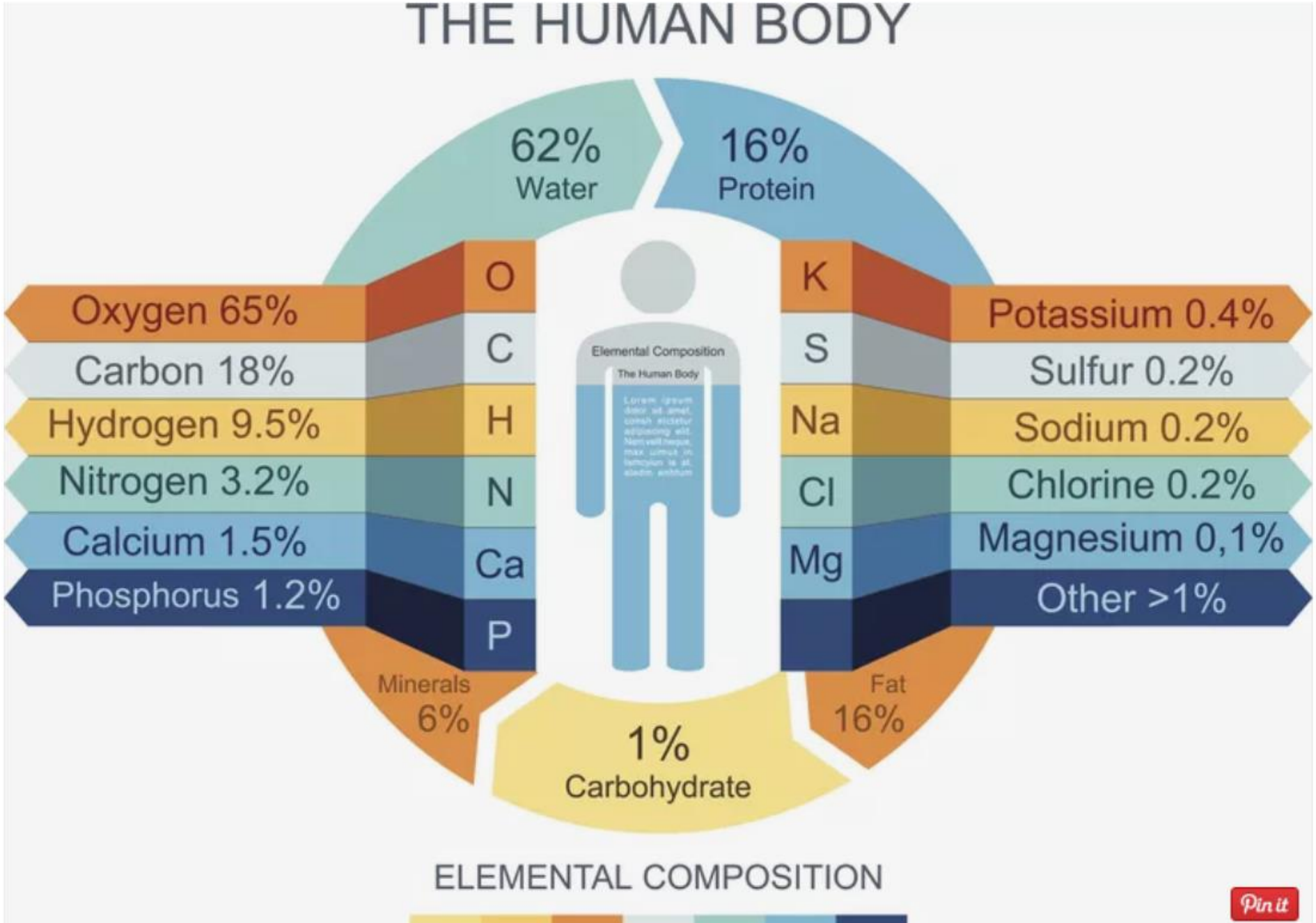
1/2

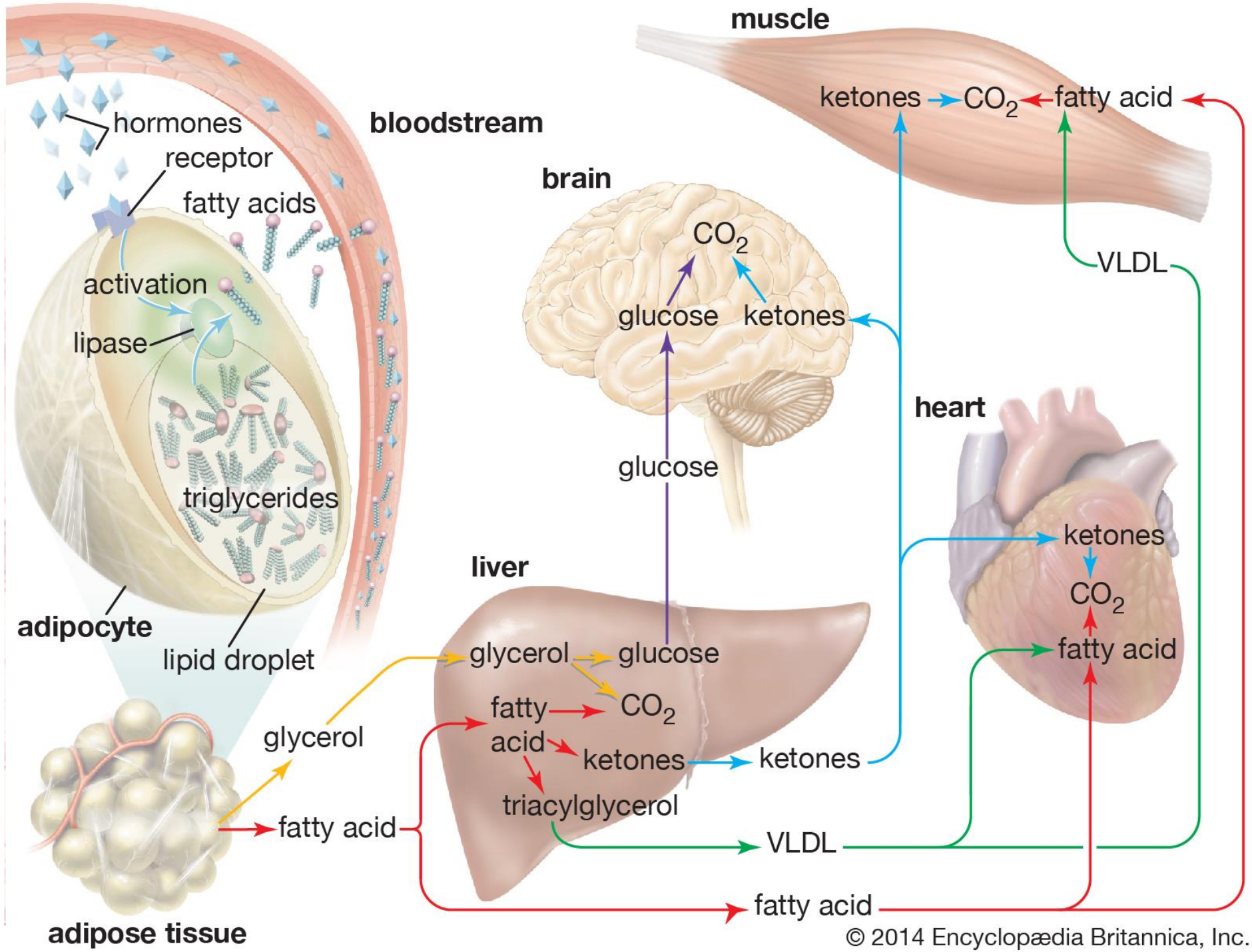
of people aged 30 years or older

Prevalence (People aged 30 years or older)



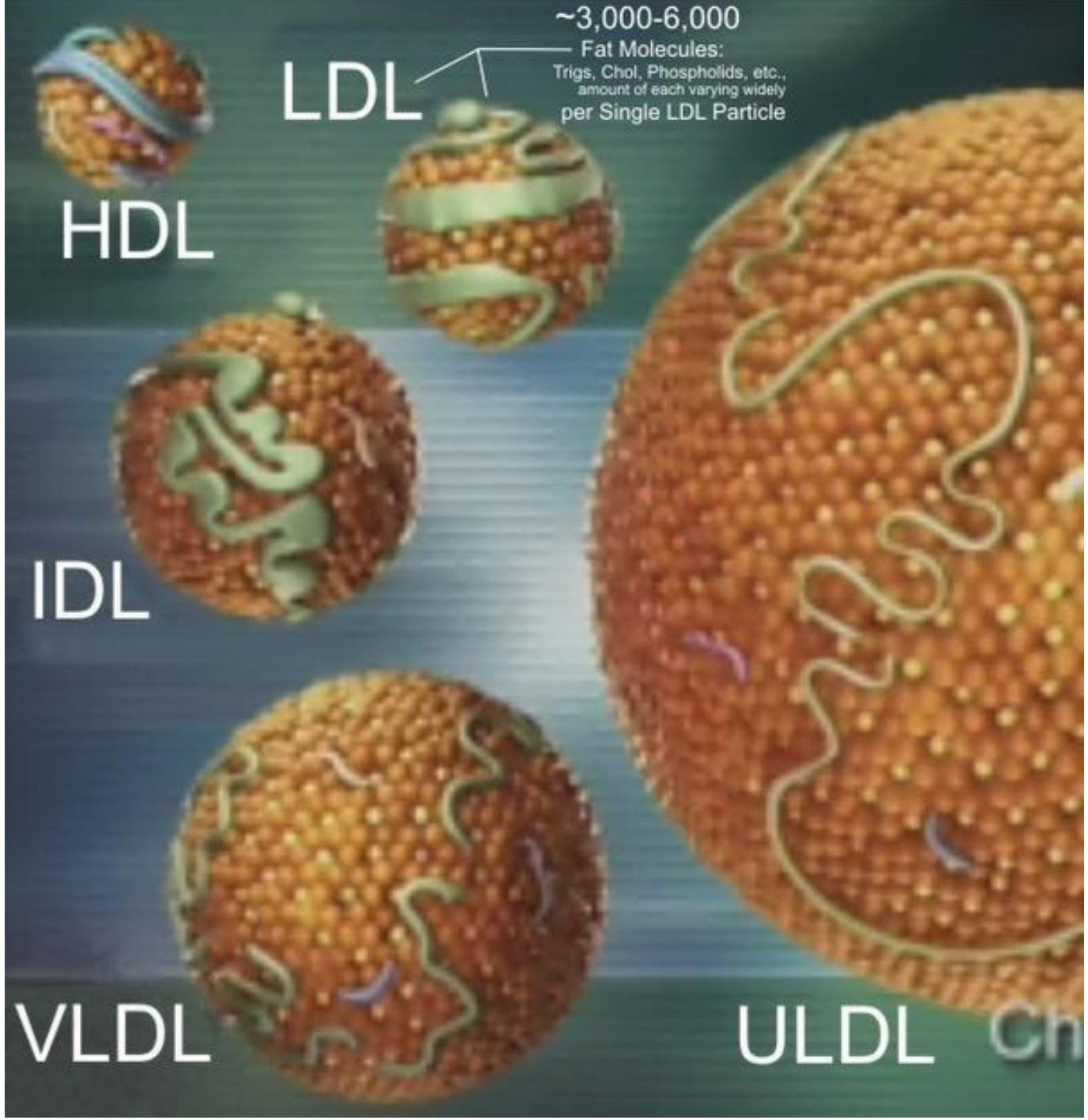
16,081,940 people


THE HUMAN BODY
62%
Water
16%
Protein
Oxygen 65%
Carbon 18%
Hydrogen 9.5%
Nitrogen 3.2%
Calcium 1.5%
Phosphorus 1.2%
O
C
H
N
Ca
P
K
S
Na
Cl
Mg
Potassium 0.4%
Sulfur 0.2%
Sodium 0.2%
Chlorine 0.2%
Magnesium 0,1%
Other >1%
Elemental Composition
The Human Body
Minerals
6%
Fat
16%
1%
Carbohydrate
ELEMENTAL COMPOSITION
Pin it

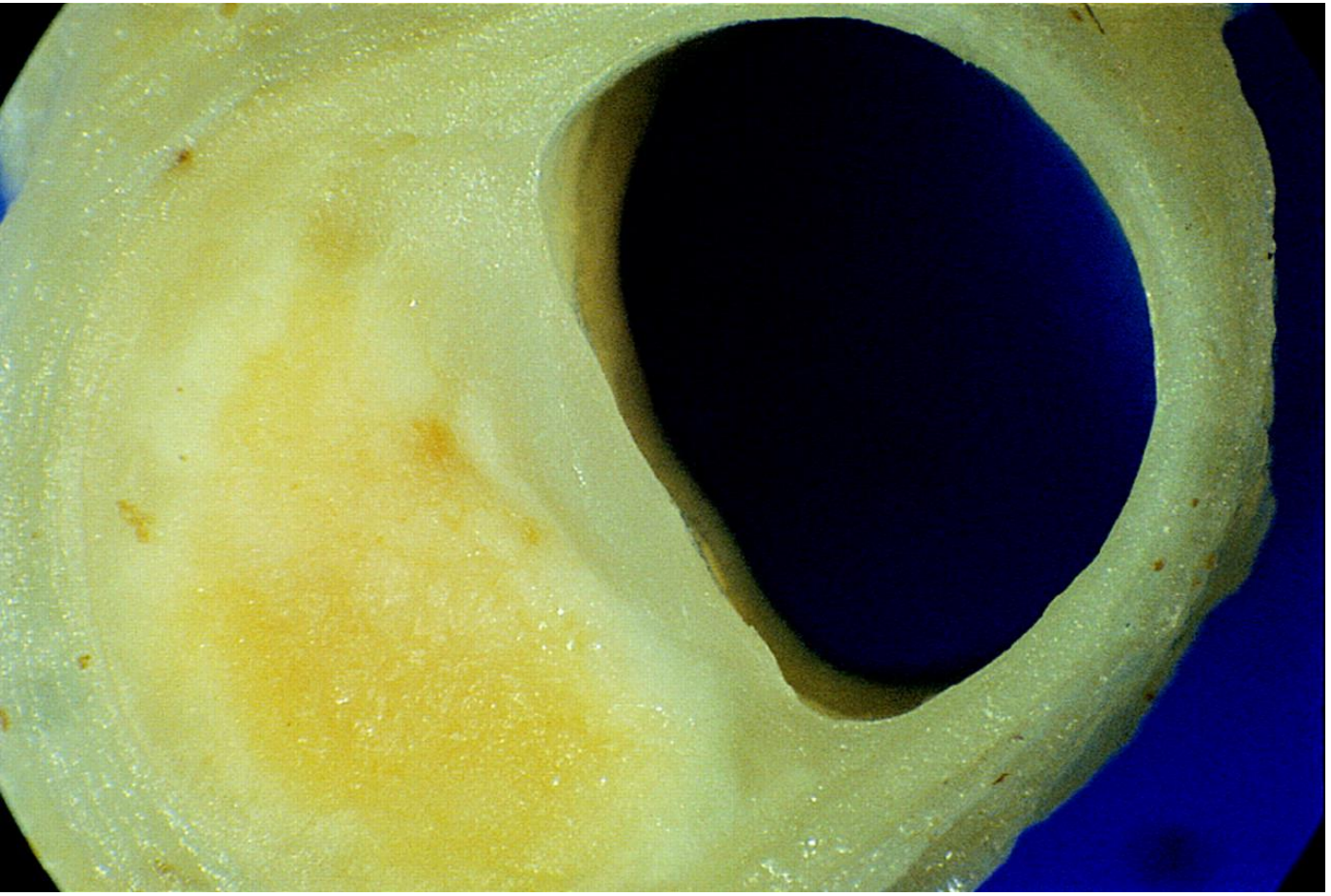


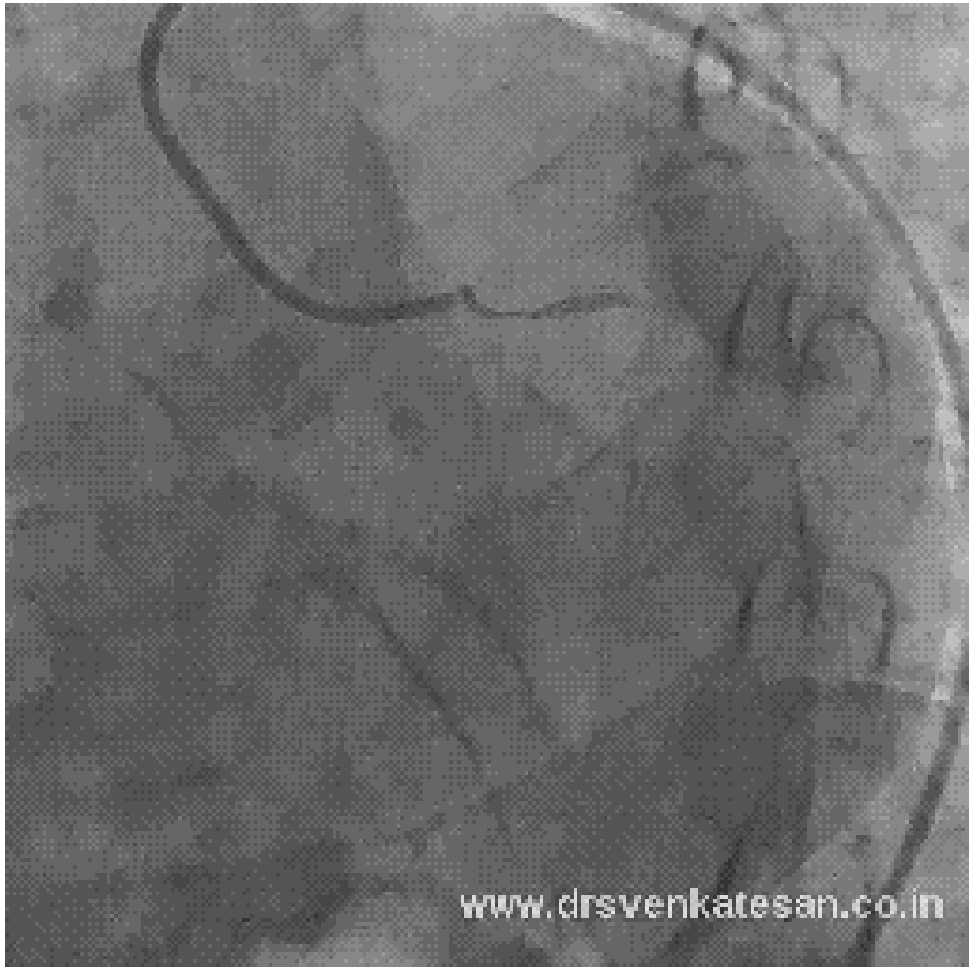
Less than 200mg /dl

Circulate in the blood




RISK
FACTOR




www.drsvenkatesan.co.in

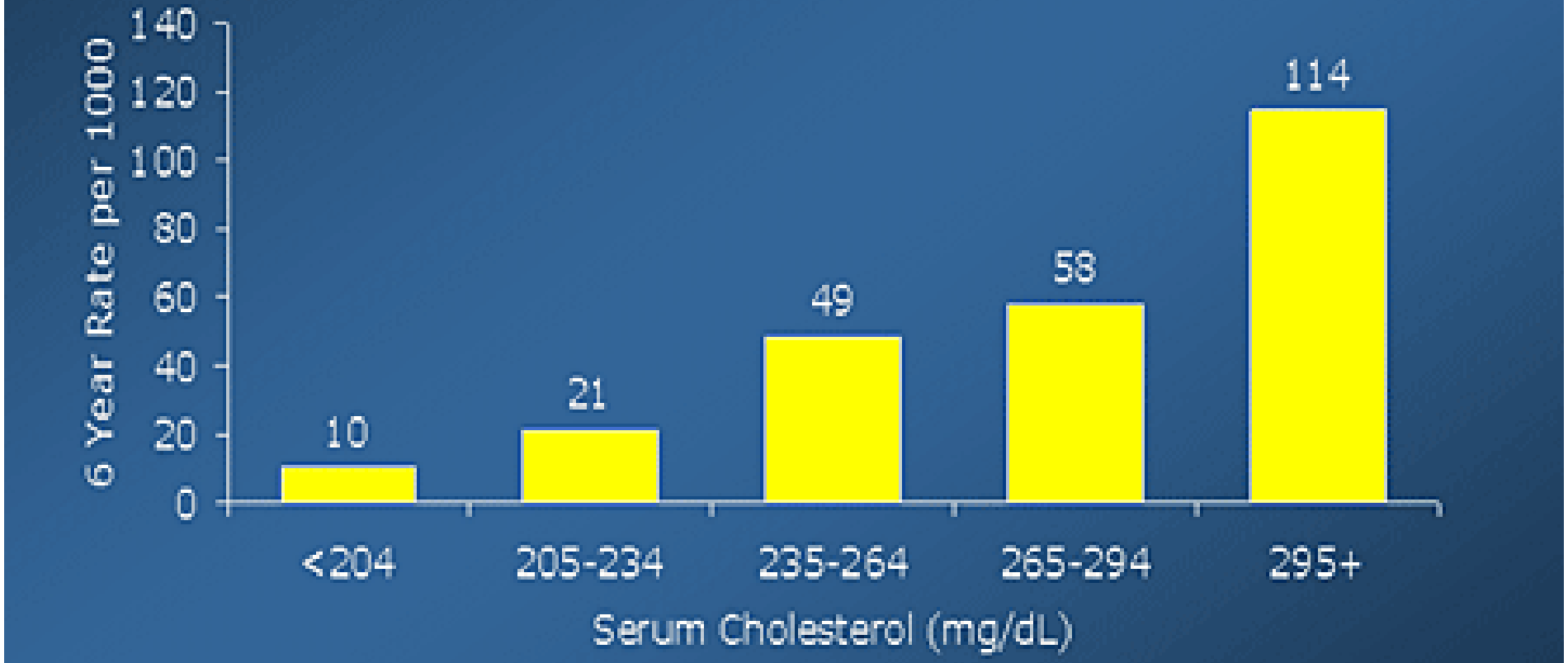
FRAMINGHAM

MRFIT

MONICA

INTERHEART

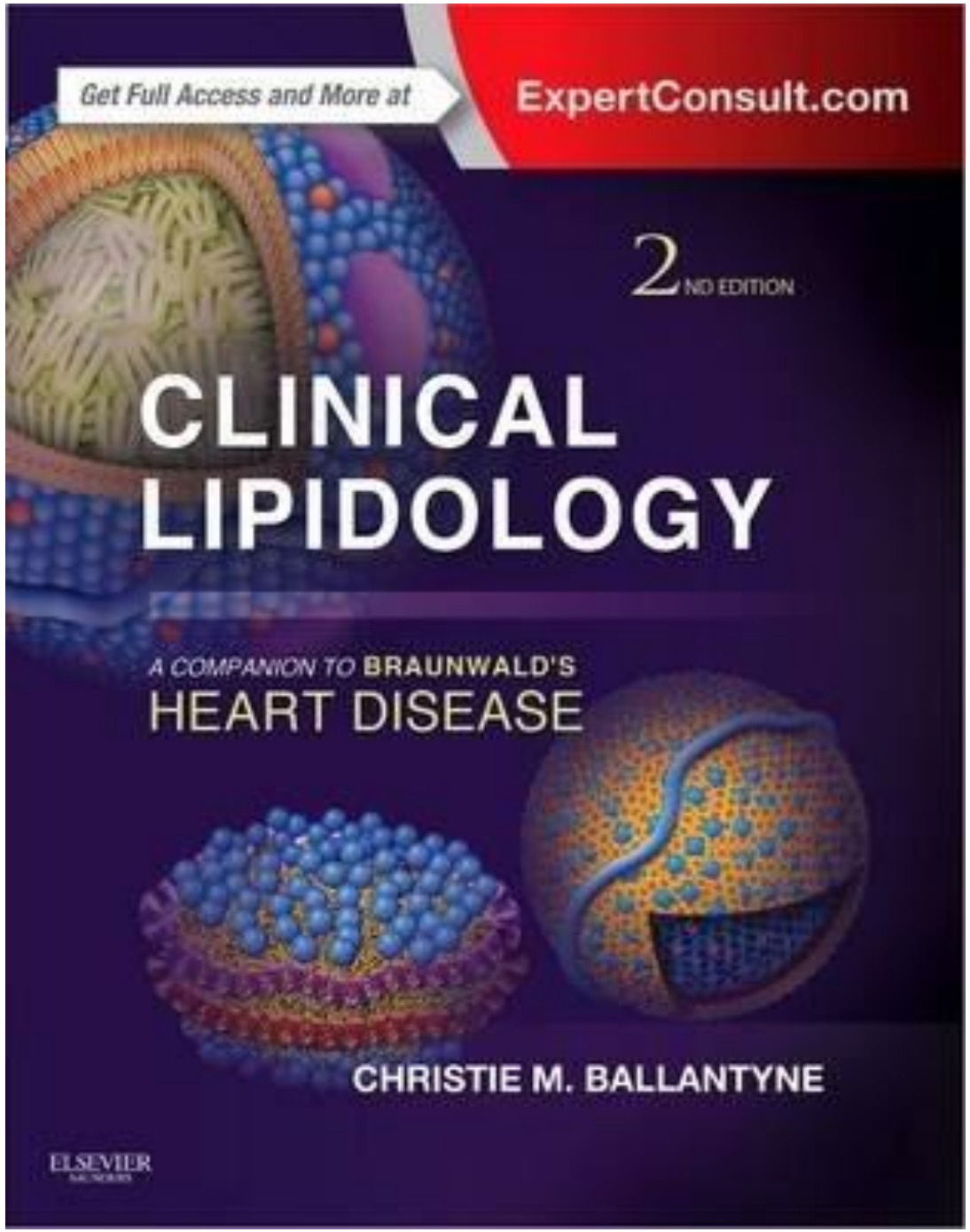
Risk of Premature CHD: The Framingham Heart Study



Men aged 30-49 years at Framingham Exam 2; 1951-1953

INTERHEART: Apolipoprotein B/A-1 and MI

Deciles:	1	2	3	4	5	6	7	8	9	10
Cont	1210	1206	1208	1207	1210	1209	1207	1208	1208	1209
Cases	435	496	610	720	790	893	1063	1196	1366	1757
Median	0.43	0.53	0.60	0.66	0.72	0.78	0.85	0.93	1.04	1.28



MASTERS in Lipidology Course

NATIONAL LIPID ASSOCIATION

AMERICAN BOARD OF CLINICAL LIPIDOLOGY

ABCL

EST. 2004

Pharmacological strategies

Resins

Niacin

Fibrates

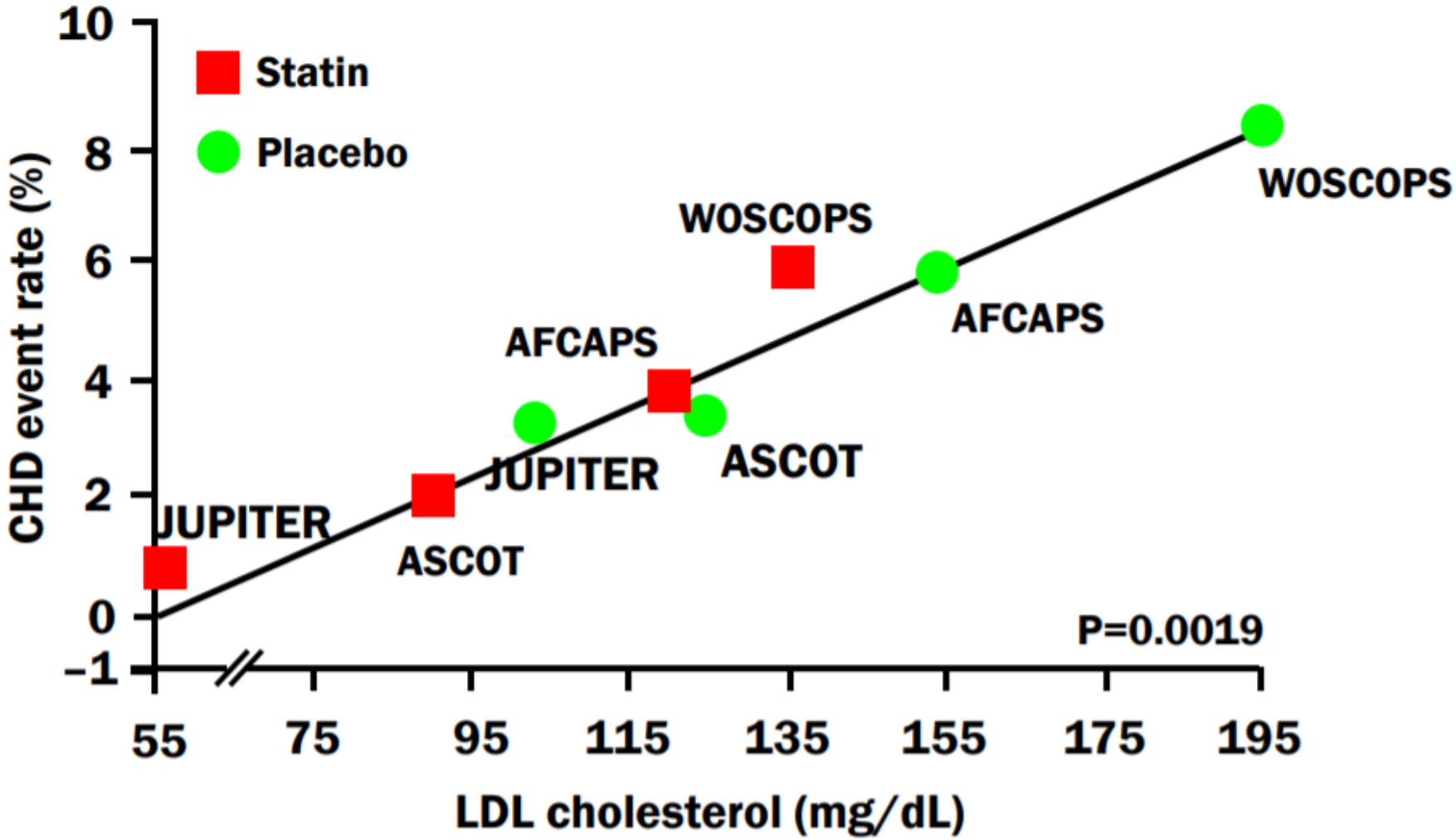
Eztemibe

Statins

PCSK inhibitors


Date:
Doctor's Name:
Rx
PRESCRIPTION
Statins

HMG-CoA Reductase Inhibitor Evidence: Primary Prevention and LDL-C on Therapy



NATIONAL LIPID ASSOCIATION

INTERNATIONAL ATHEROSCLEROSIS SOCIETY

NCEP/ATP-3

Are we reaching the target ?

Total cholesterol	Less than 200 mg/dL
Low-density lipoprotein	Less than 100 mg/dL
High-density lipoprotein	60 mg/dL or higher
Triglycerides	Less than 150 mg/dL

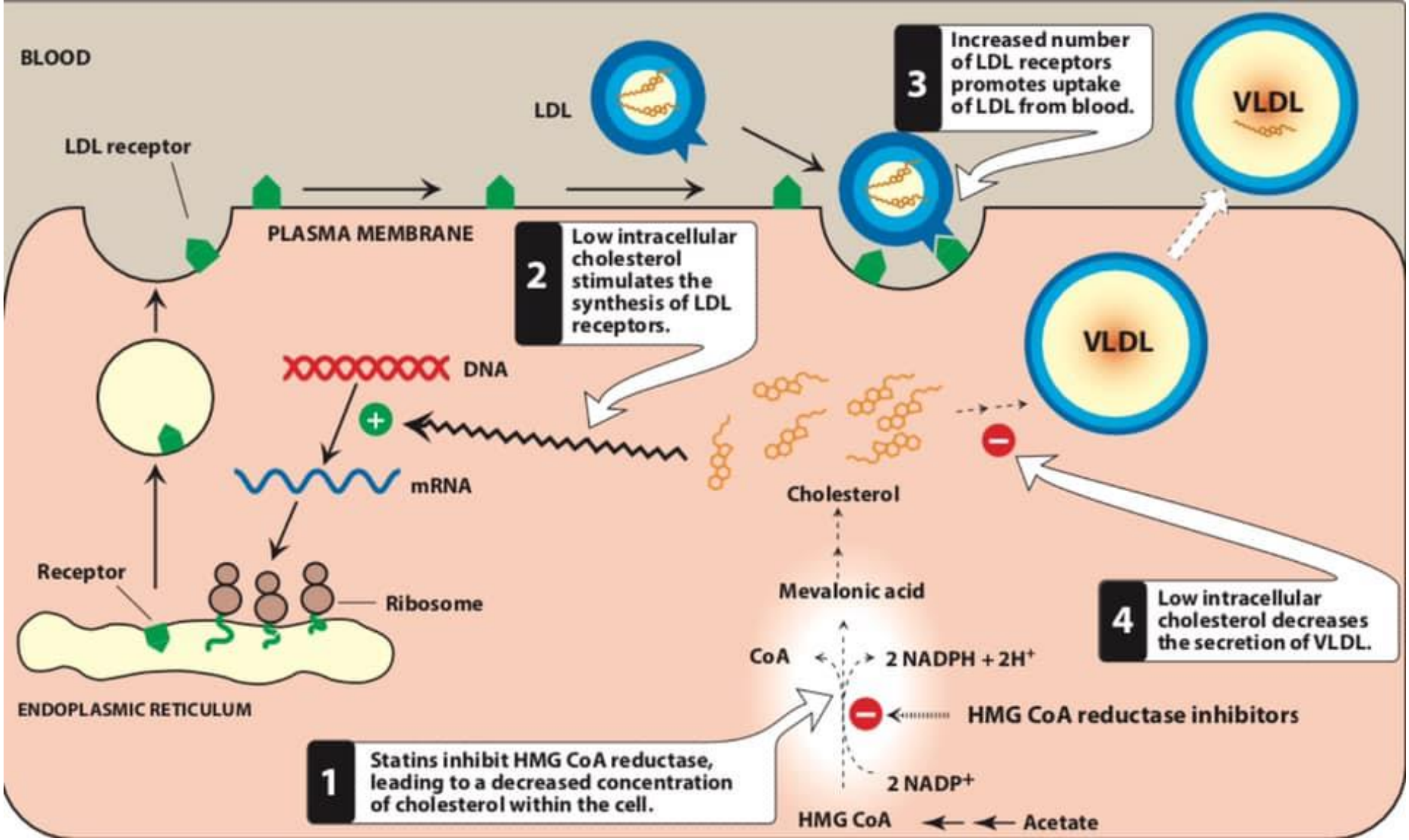
LDL : How low is low ?

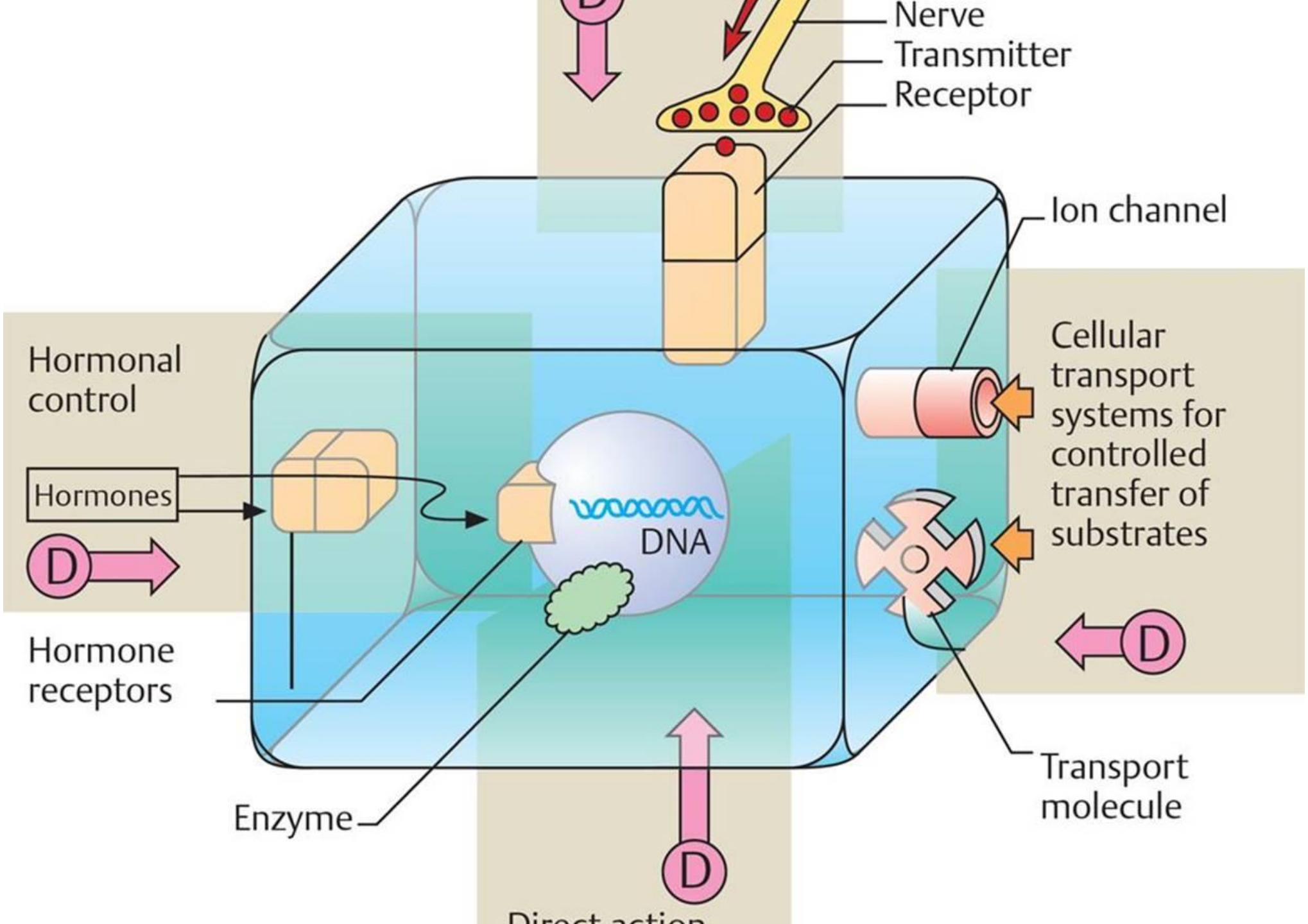
Free falling target LDL

100mg 70mg 55mg 40mg . . .

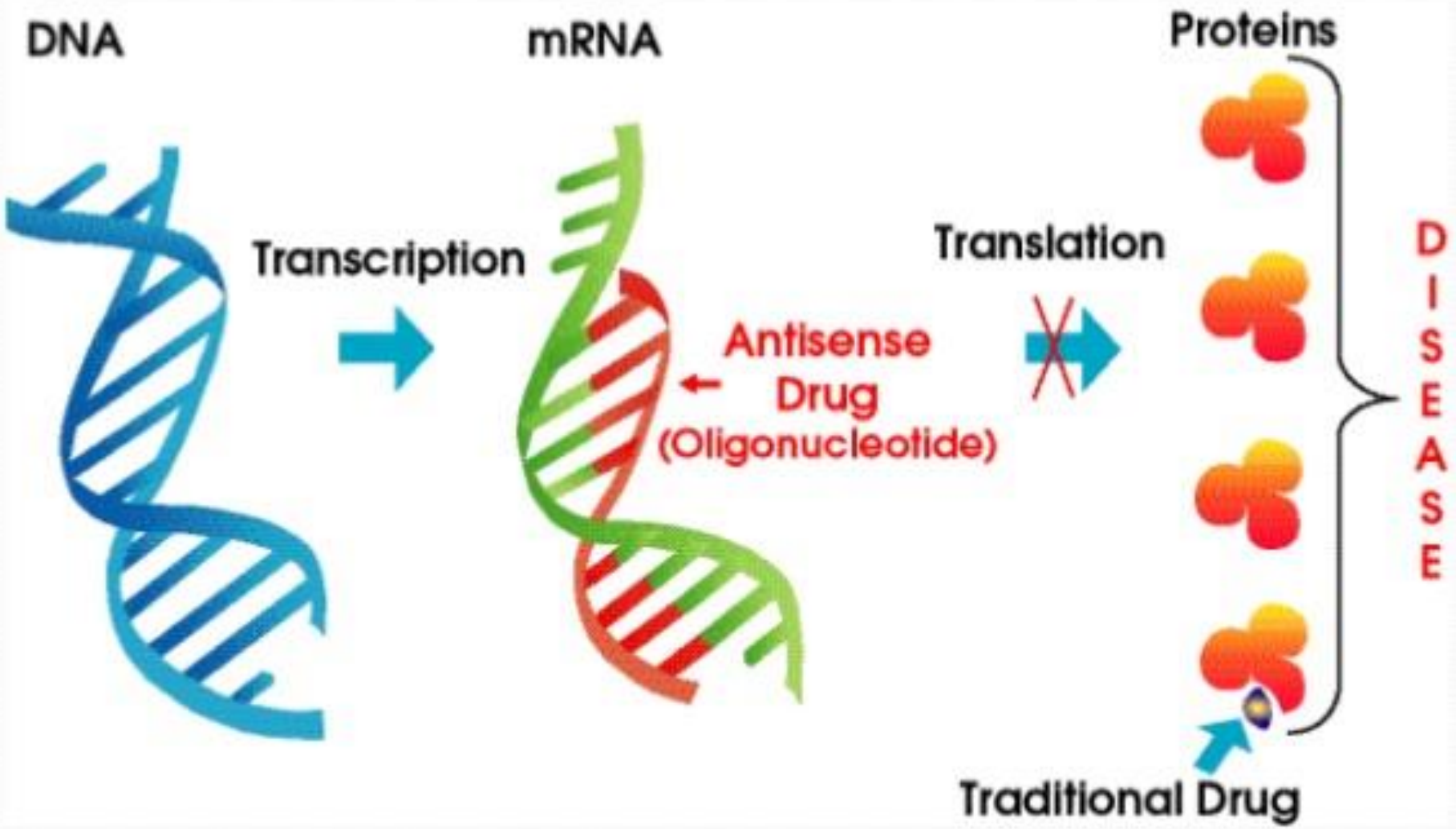
What is the need to look beyond statins ?

Going beyond the conventional pharmacotherapy


BLOOD
LDL
LDL receptor
3 Increased number of LDL receptors promotes uptake of LDL from blood.
VLDL
PLASMA MEMBRANE
2 Low intracellular cholesterol stimulates the synthesis of LDL receptors.
VLDL
DNA
mRNA
Cholesterol
Receptor
Ribosome
Mevalonic acid
4 Low intracellular cholesterol decreases the secretion of VLDL.
CoA
2 NADPH + 2H+
ENDOPLASMIC RETICULUM
HMG CoA reductase inhibitors
1 Statins inhibit HMG CoA reductase, leading to a decreased concentration of cholesterol within the cell.
2 NADP+
HMG CoA
Acetate


Nerve
Transmitter
Receptor
Ion channel
Hormonal control
Hormones
D
Hormone receptors
DNA
Cellular transport systems for controlled transfer of substrates
D
Transport molecule
Enzyme
D

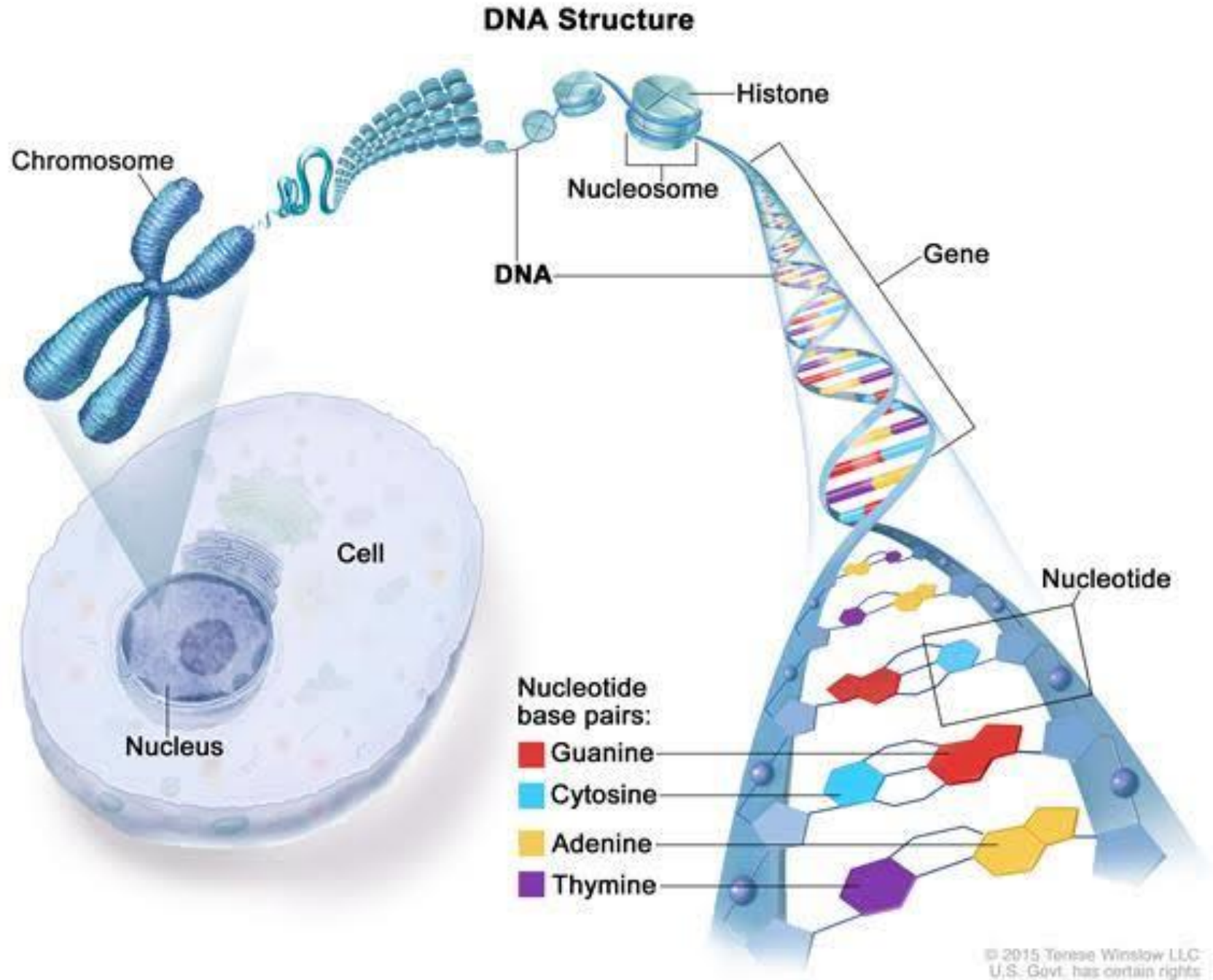
Instead of attacking the receptors enzymes, proteins , what about directly Inhibit , Interfere ,silence and put off the receptor or enzyme synthesis itself ?



Translational Arrest

How to do it ?

Oligonucleotides


DNA Structure
Chromosome
Histone
Nucleosome
DNA
Gene
Cell
Nucleotide
Nucleus
Nucleotide base pairs:
Guanine
Cytosine
Adenine
Thymine
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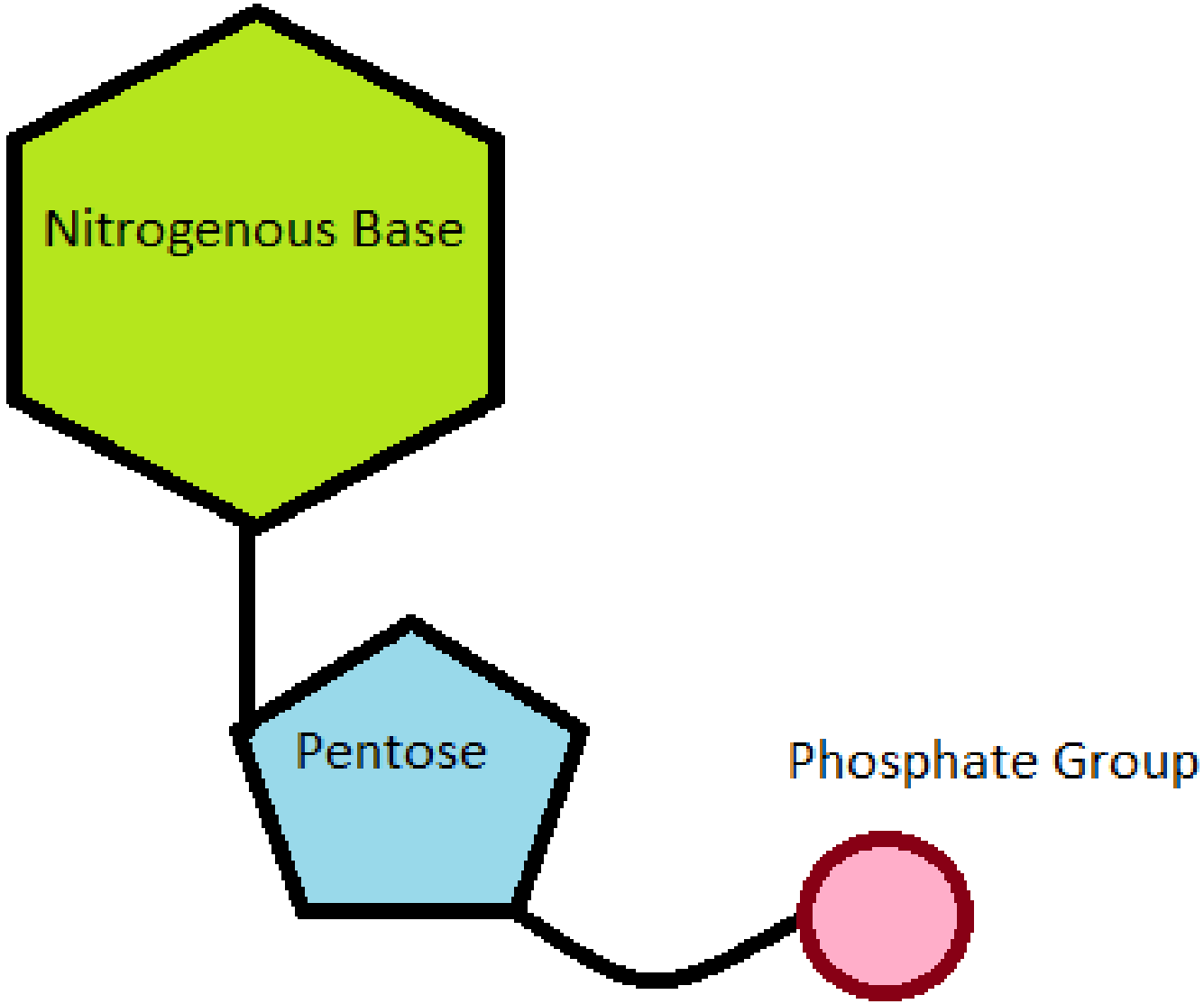
U.S. Govt. has certain rights

Nucleotide



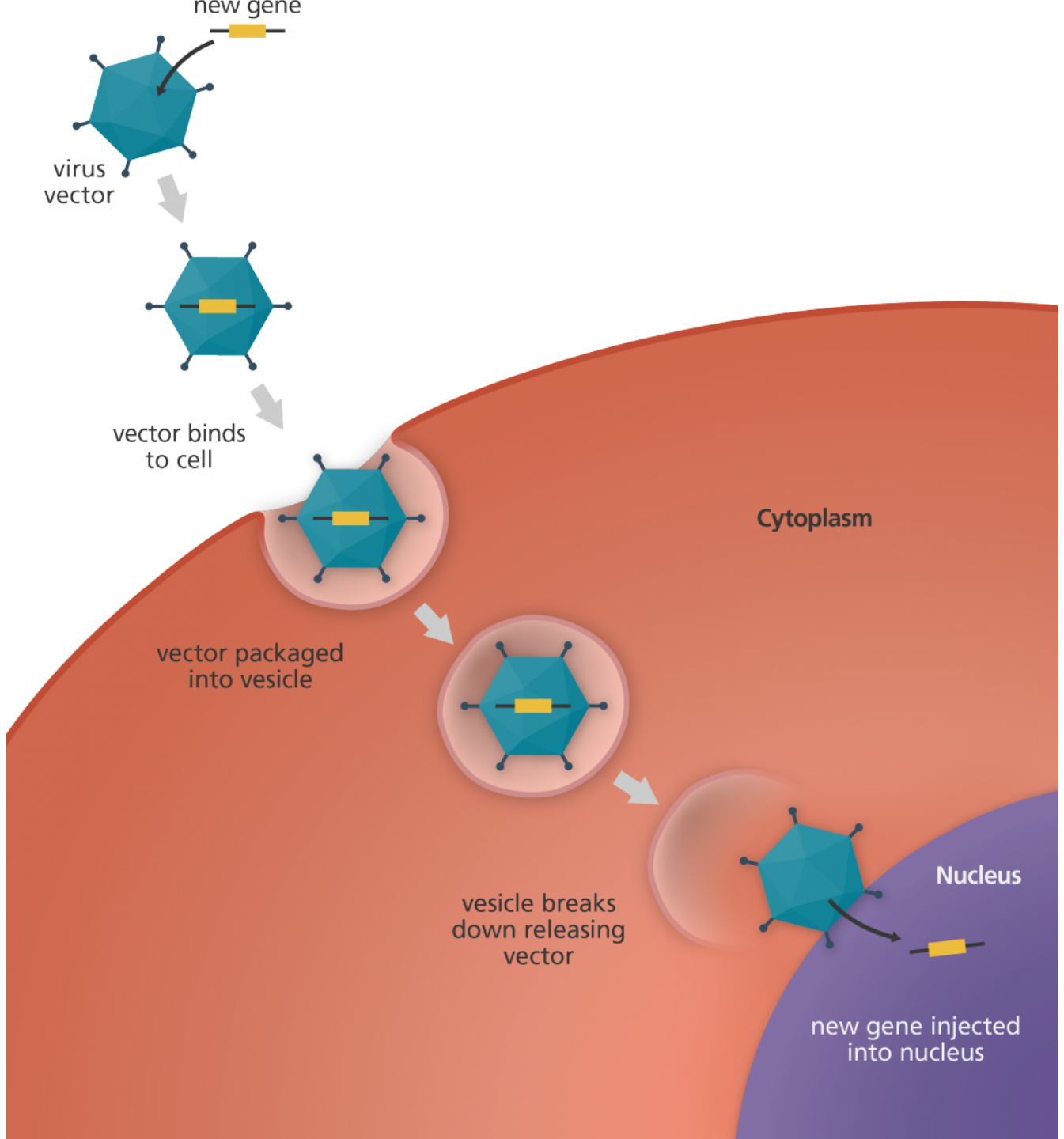
Gene therapy vs Gene manipulating drugs

ONs Is it gene therapy ?

Gene therapy

Genetic defects

Mutation

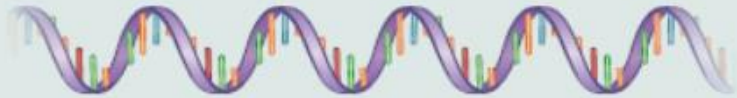


Targeting strategies for oligonucleotides

DNA

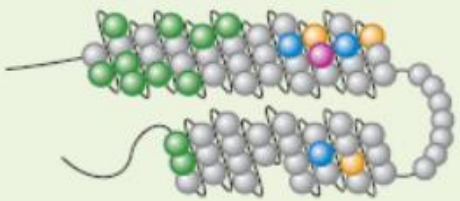
TFO
DNA strand-invading ON
Synthetic guide RNA for CRISPR/Cas

RNA



Antisense (RNaseH) ON
Splice-switching ON
Antagomir (anti-miRNA)
miRNA mimic
siRNA
DNAzyme

Protein
Structural or enzyme



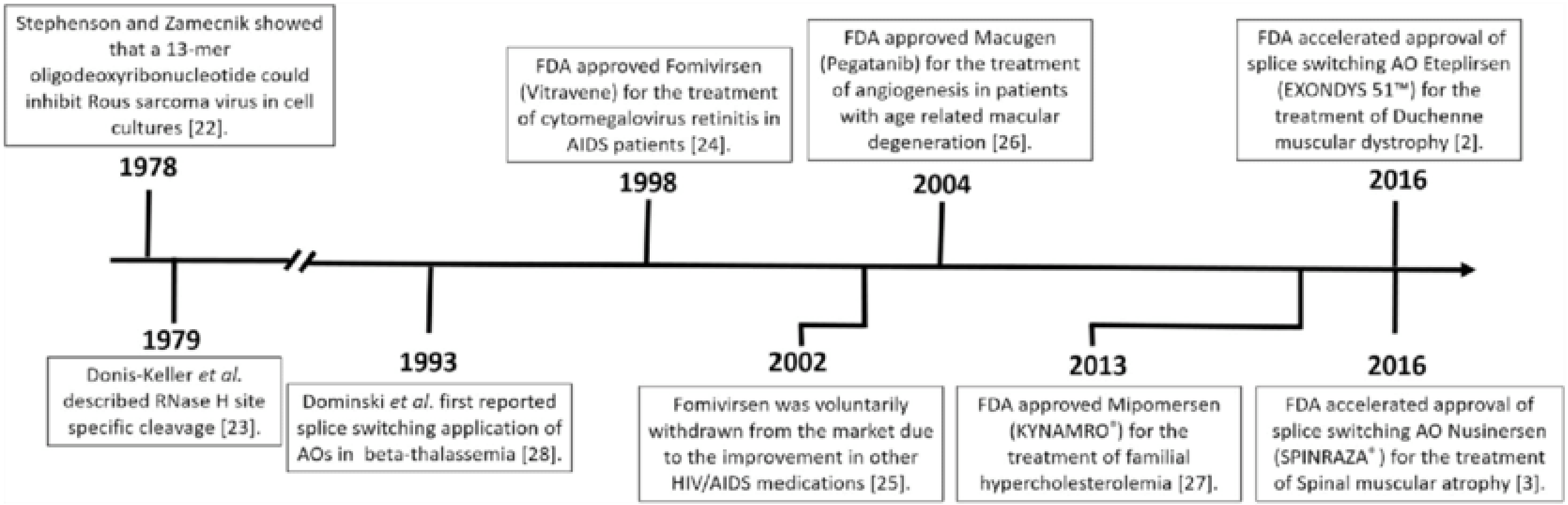
Aptamer
Decoy
Innate immunity (nucleic acid sensor)

Posttranslational modifications
Carbohydrates
Lipids
Metabolites

Aptamer

Is this a new concept ?

How has it evolved ?


Stephenson and Zamecnik showed that a 13-mer oligodeoxyribonucleotide could inhibit Rous sarcoma virus in cell cultures [22].
1978
1979
Donis-Keller et al. described RNase H site specific cleavage [23].
1993
Dominski et al. first reported splice switching application of AOs in beta-thalassemia [28].
FDA approved Fomivirsen (Vitravene) for the treatment of cytomegalovirus retinitis in AIDS patients [24].
1998
2002
Fomivirsen was voluntarily withdrawn from the market due to the improvement in other HIV/AIDS medications [25].
FDA approved Macugen (Pegatanib) for the treatment of angiogenesis in patients with age related macular degeneration [26].
2004
2013
FDA approved Mipomersen (KYNAMRO®) for the treatment of familial hypercholesterolemia [27].
FDA accelerated approval of splice switching AO Eteplirsen (EXONDYS 51™) for the treatment of Duchenne muscular dystrophy [2].
2016
2016
FDA accelerated approval of splice switching AO Nusinersen (SPINRAZA®) for the treatment of Spinal muscular atrophy [3].

January 17, 2012 | vol. 109 | no. 3 | pp. 647–996

PNAS

Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide

(tridecamer deoxyribonucleotide/hybridization competitor/hybridon)

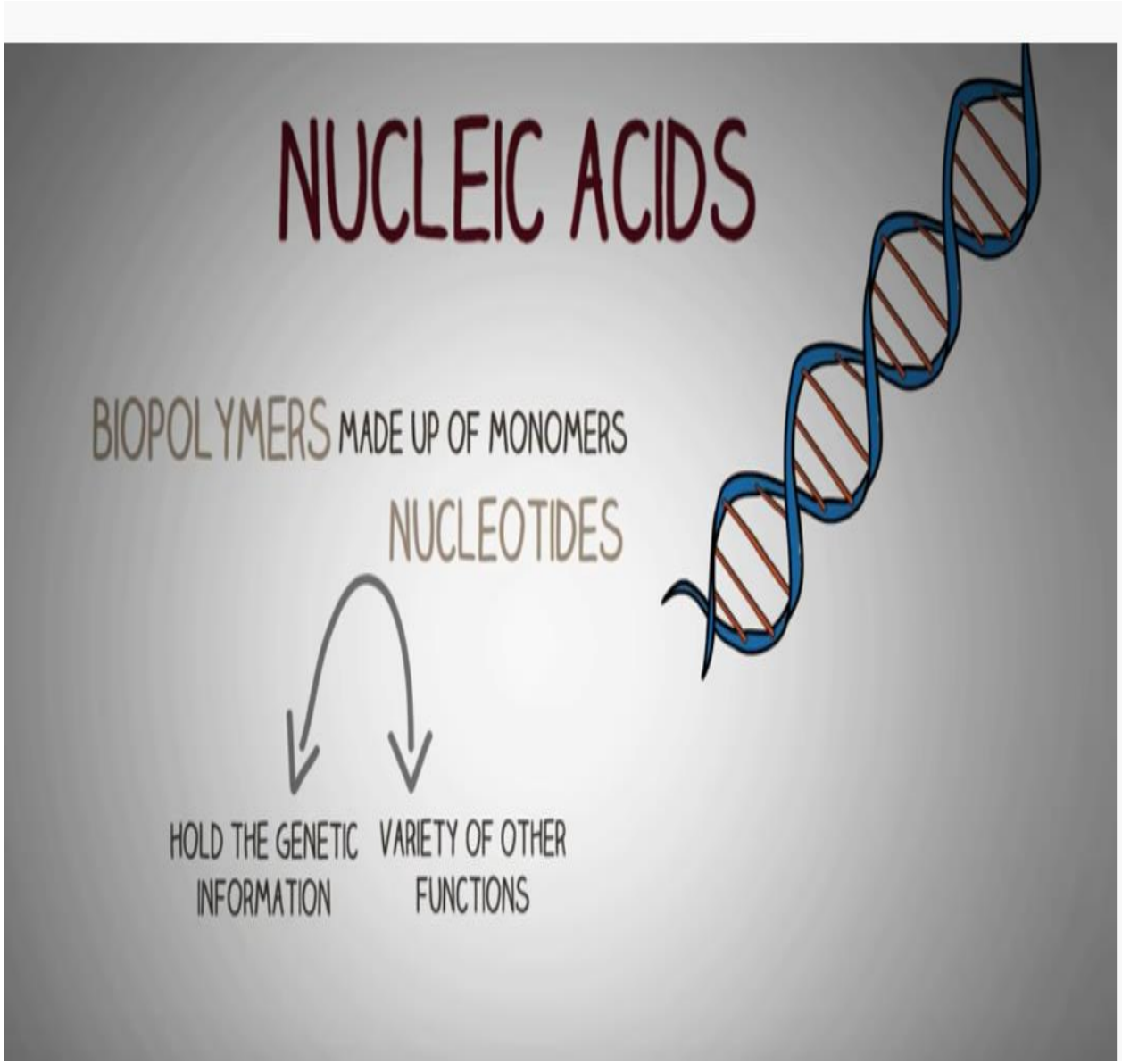
PAUL C. ZAMECNIK AND MARY L. STEPHENSON

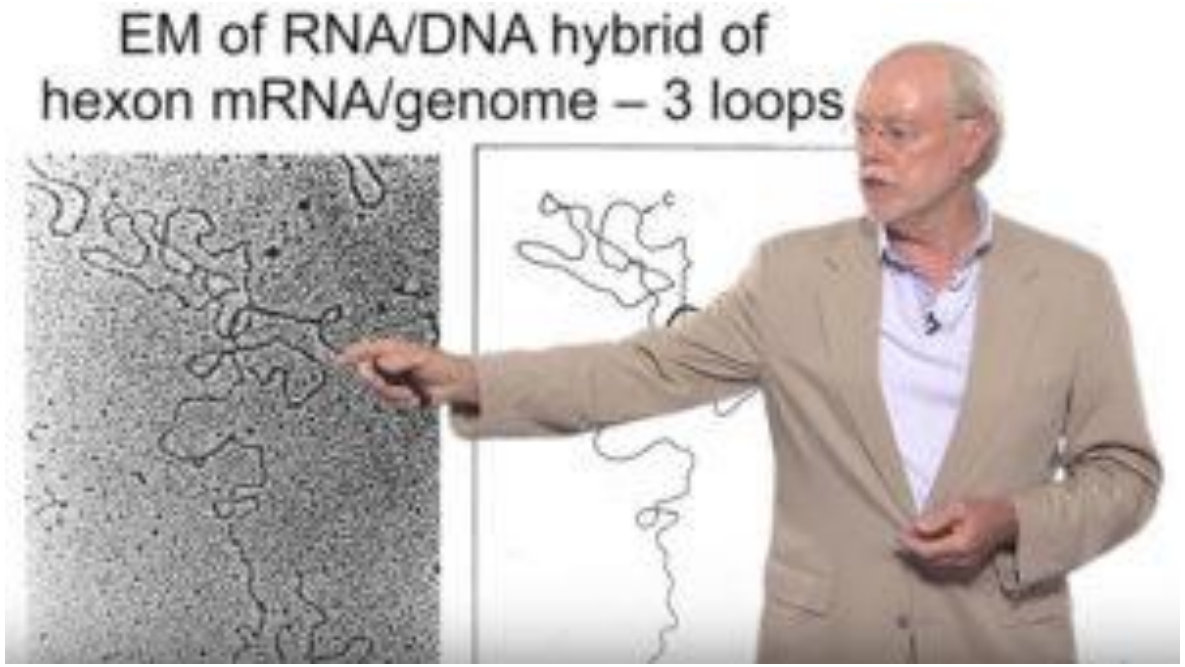
The John Collins Warren Laboratories of the Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital, Boston, Massachusetts 02114

Contributed by Paul C. Zamecnik, November 10, 1977

ABSTRACT **The tridecamer d(A-A-T-G-G-T-A-A-A-A-T-G-G), which is complementary to 13 nucleotides of the 3′- and 5′-reiterated terminal sequences of Rous sarcoma virus 35S RNA, was added to chick embryo fibroblast tissue cultures infected with Rous sarcoma virus. Inhibition of virus production resulted. The inference emerges that the tridecamer and its counterpart with**

Massachusetts General Hospital. Chick embryo fibroblasts were obtained as primary explants from 10-day-old Spafas chicks, and were used at early passages. Tissue cultures were grown as monolayers, using 10% tryptose, 5% irradiated fetal calf serum, and Dulbecco's modified Eagle's medium, without antibiotics


NUCLEIC ACIDS
BIOPOLYMERS MADE UP OF MONOMERS
NUCLEOTIDES
HOLD THE GENETIC INFORMATION
VARIETY OF OTHER FUNCTIONS



Phillip A. Sharp

The Nobel Prize in Chemistry 1993

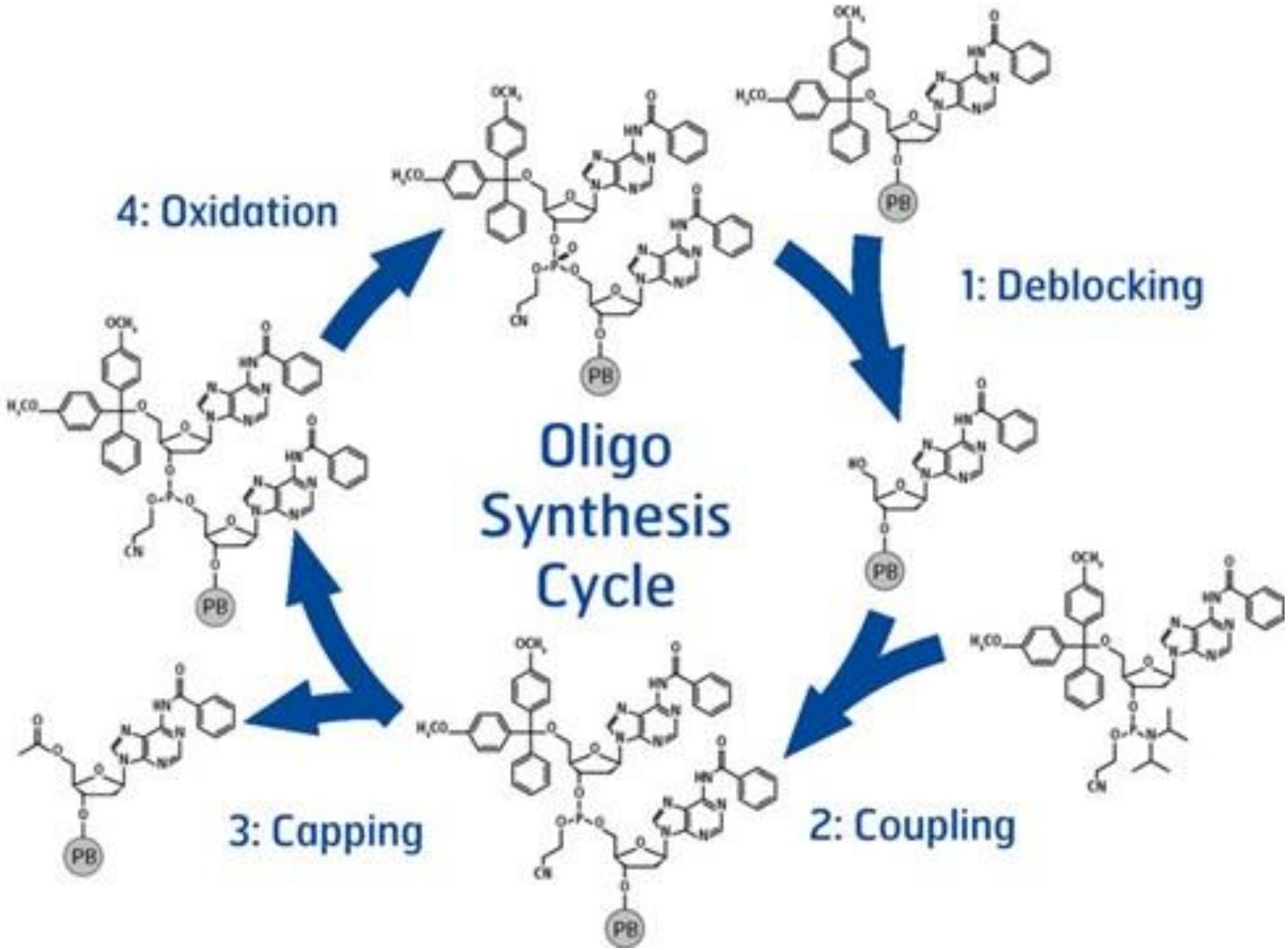
Kary B. Mullis

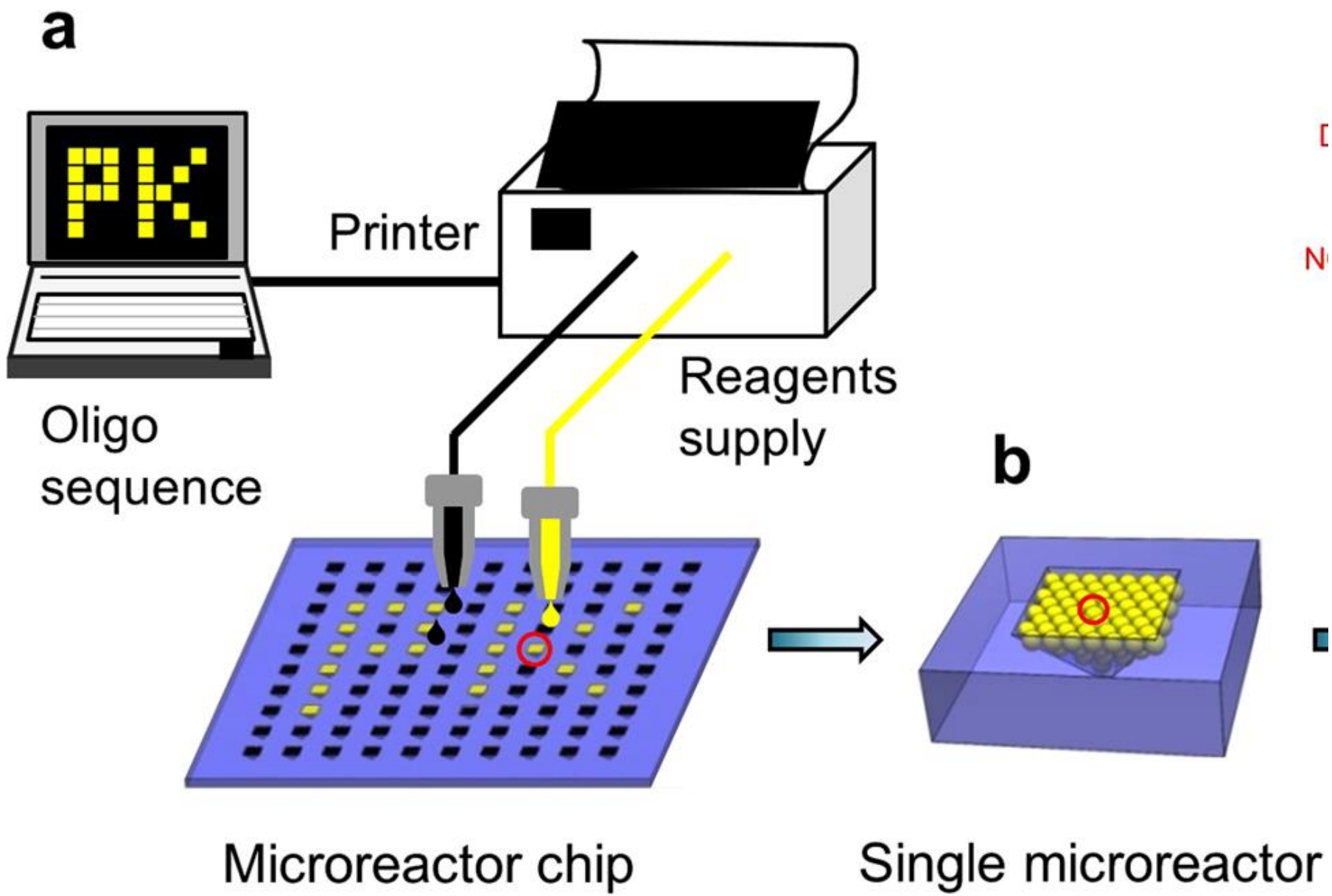
Prize share: 1/2

Michael Smith

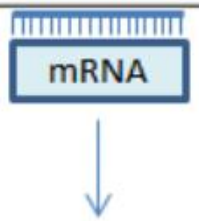
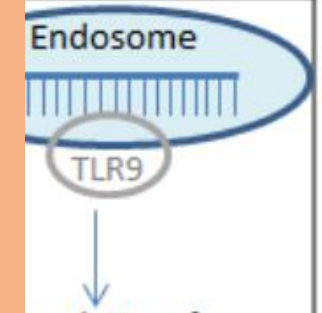
Prize share: 1/2

DNA splicing
Liquid chromatography
PCR


4: Oxidation
1: Deblocking
Oligo
Synthesis
Cycle
3: Capping
2: Coupling
PB


a
Printer
Oligo
sequence
Reagents
supply
b
Microreactor chip
Single microreactor

Classifications of major oligonucleotide therapies

	Antisense oligonucleotides	siRNA	miRNA (mimic)	Aptamer	CpG oligonucleotides
Typical structure	Single-stranded DNA/RNA 	Double-stranded RNA 	Double-stranded RNA 	Single-stranded DNA/RNA 	Single-stranded DNA 
Mechanism of action	 mRNA degradation, Suppression of transcription, etc.				 imulation of mune system
Characteristics	A variety of mechan e.g. mRNA degradat splicing regulation, i miRNA.				innate immune oligonucleotides which is considered effect.
DDS/ miscellaneous	Chemically modified oligonucleotides are often used and DDS is not usually required.	oligonucleotides have recently been developed but DDS is generally required.		used in order to extend blood circulation time.	ith antigen as an adjuvant.

Antisense ON
Small Interfering RNA
miRNA mimic
Aptamer

Oligonucleotide

Nucleic acids
Structure designed
10 to 30 nucleotide

Can be synthesised /Purified /Stablised

How to reach the cell nucleus ?

Non viral delivery of drugs

- **Naked DNA delivery systems,**
- **Polymeric delivery systems,**
- **Liposomal delivery systems**

Target site & cells

It is the weakest link (Recall stem cell fiasco !)

Intrathecal /Intravitreal /

Being over come : Subcutaneous is practical

Target molecule

DNA

RNA

mRNA

APO-B
Apo C 2
PCSK

Classifications of major oligonucleotide therapies

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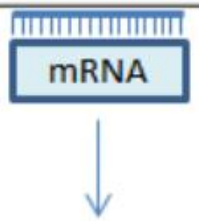
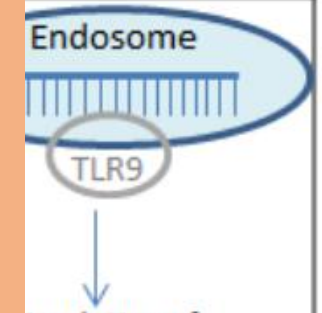
Mechanism of action	 mRNA degradation, Suppression of transcription, etc.				 imulation of mune system
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Antisense ON
Small Interfering RNA
miRNA mimic
Aptamer

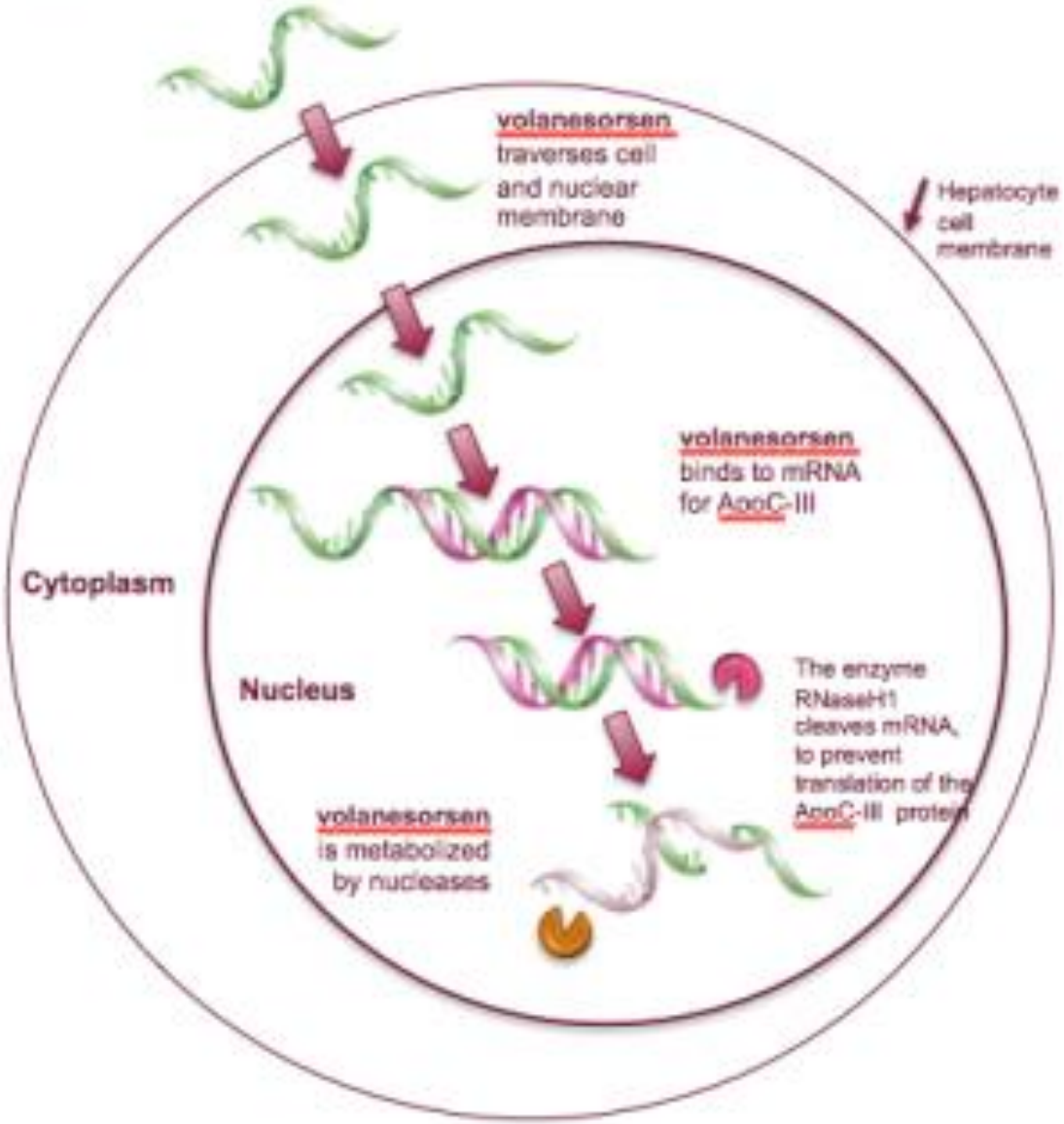
ON in Dyslipidemia

KYNAMRO® (Mipomersen)	APOB	2'-MOE modified Gapmer	Ionis	Hypercholesteremia
IONIS-APOCIIIRX (volanesorsen)	APOC3	2'-MOE modified Gapmer	Ionis/Akcea	Chylomicronemia
IONIS-APO(a)-LRX	APO(a)	2'-MOE modified Gapmer	Ionis/Akcea	Very high Lp(a)
IONIS-ANGPTL3-LRX	ANGPTL3	2'-MOE modified Gapmer	Ionis/Akcea	Mixed dyslipidemias
anti-miR-33	mir-33a/b	2'F/MOE-modified mixmer	Regulus	Atherosclerosis etc.
anti-miR-208	miR-208a	LNA-modified mixmer	miRagen/ Servier	Hypertrophic cardiomyopathy

KYNAMRO® (Mipomersen)	APOB	2'-MOE modified Gapmer	Ionis	Hypercholesteremia
IONIS-APOCIIIRX (volanesorsen)	APOC3	2'-MOE modified Gapmer	Ionis/Akcea	Chylomicronemia

Figure 1: Volanesorsen Mechanism of Action

Preventing Formation of ApoC-III by a Second Generation Antisense Oligonucleotide (ASO)



Attributes of Antisense Drugs

- Highly specific, with reduced potential for off-target binding
- No known drug/drug interactions, not metabolized by CYP450 pathways
- Unable to cross placenta and blood/brain barrier

APPROACH Trial NEJM

August 8, 2019

N Engl J Med 2019; 381:531-542

Antisense-mediated inhibition of hepatic *APOC3* mRNA with volanesorsen led to decreased plasma apolipoprotein C-III and triglyceride levels.


waylivra®
(volanesorsen) injection
285 mg/1.5 mL

Apo-lipoprotein B100

TGL

PCSK –mRNA

Mipomersen

Antisense ON therapy

mRNA coding for Apolipoprotein B-100

Blocking translation of the gene product.

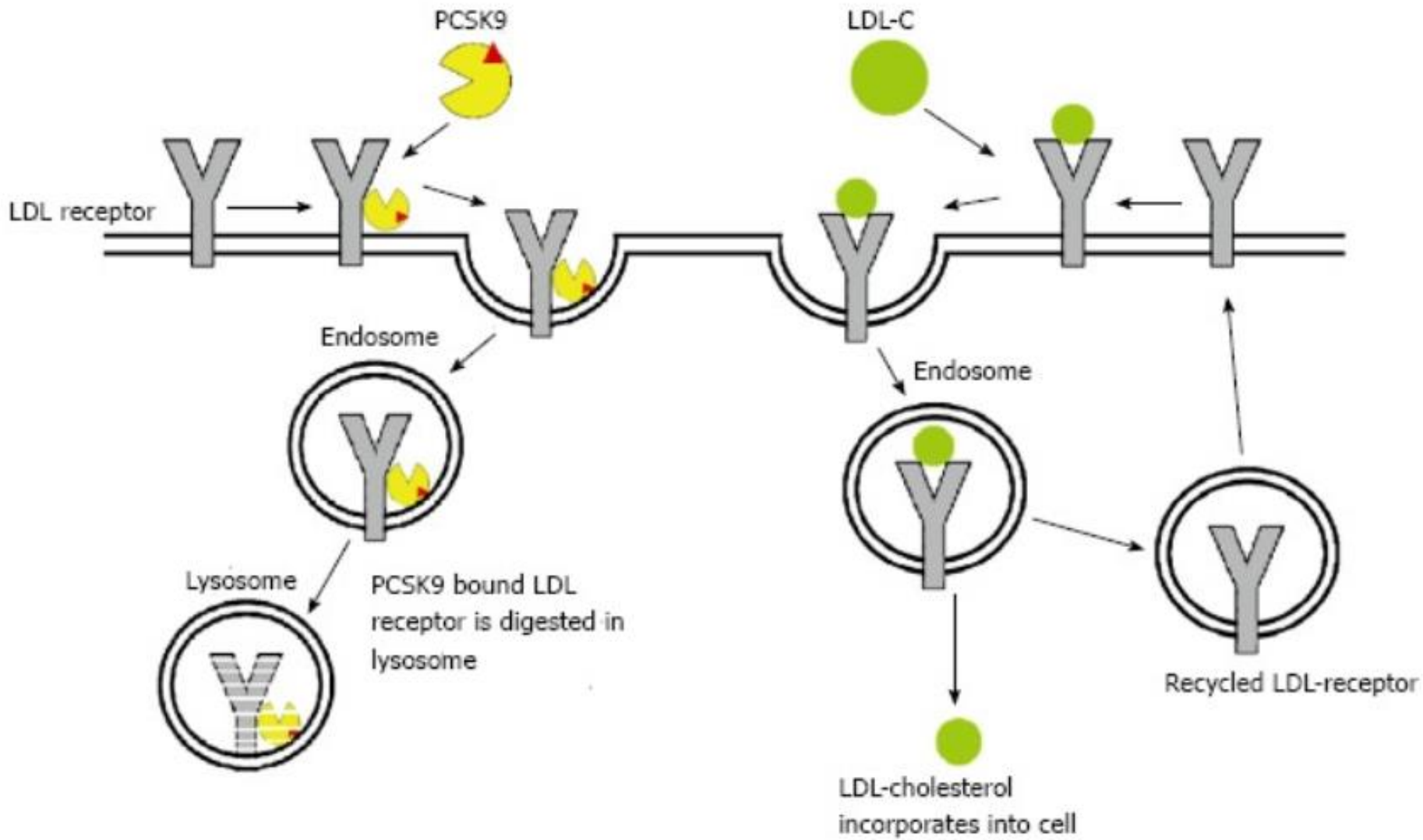
The reduction in production of the atherogenic lipoproteins VLDL, IDL, LDL, and lipoprotein(a).

What is the price of Kynamro

According to the manufacturer, Kynamro costs around $176,000 each year, which is about $3,667 a week. According to Red Book, 1 vial of Kynamro has an Average Whole Sale Price (AWP) of $5,759.65.

PCSK9 is still favourite & (Powerful)target

Monoclonal antibodies
Mimetic peptides or Adnectins
Inhibition of PCSK autocatalytic sites.
Gene silencing through antisense ONs
Small interfering RNA


PCSK9
LDL-C
LDL receptor
Endosome
Endosome
Lysosome
PCSK9 bound LDL receptor is digested in lysosome
Recycled LDL-receptor
LDL-cholesterol incorporates into cell

PCSK blocking (mab)

VS

PCSK synthesis Inhibition

Another potential and significant complication with drugs that are monoclonal antibodies is the development of anti-drug antibodies that may interfere with clinical efficacy and increase adverse events

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Inclisiran in Patients at High Cardiovascular Risk with Elevated LDL Cholesterol

Kausik K. Ray, M.D., Ulf Landmesser, M.D., Lawrence A. Leiter, M.D., David Kallend, M.D., Robert Dufour, M.D., Mahir Karakas, M.D., Tim Hall, M.D., Roland P.T. Troquay, M.D., Traci Turner, M.D., Frank L.J. Visseren, M.D., Peter Wijngaard, Ph.D., R. Scott Wright, M.D., and John J.P. Kastelein, M.D., Ph.D.

ABSTRACT

BACKGROUND

In a previous study, a single injection of inclisiran, a chemically synthesized small interfering RNA designed to target *PCSK9* messenger RNA, was found to produce sustained reductions in low-density lipoprotein (LDL) cholesterol levels over the course of 84 days in healthy volunteers.

METHODS

We conducted a phase 2, multicenter, double-blind, placebo-controlled, multiple-ascending-dose trial of inclisiran administered as a subcutaneous injection in patients

The autho
Appendix.
Dr. Ray at
vascular D
of Primary
rial Colleg
Dunstan's
Kingdom,

Drs. Ray

2017

Imperial College London

Phase 2 Trial

INCLISIRAN

Twice a year Injection

FDA approval awaited very soon

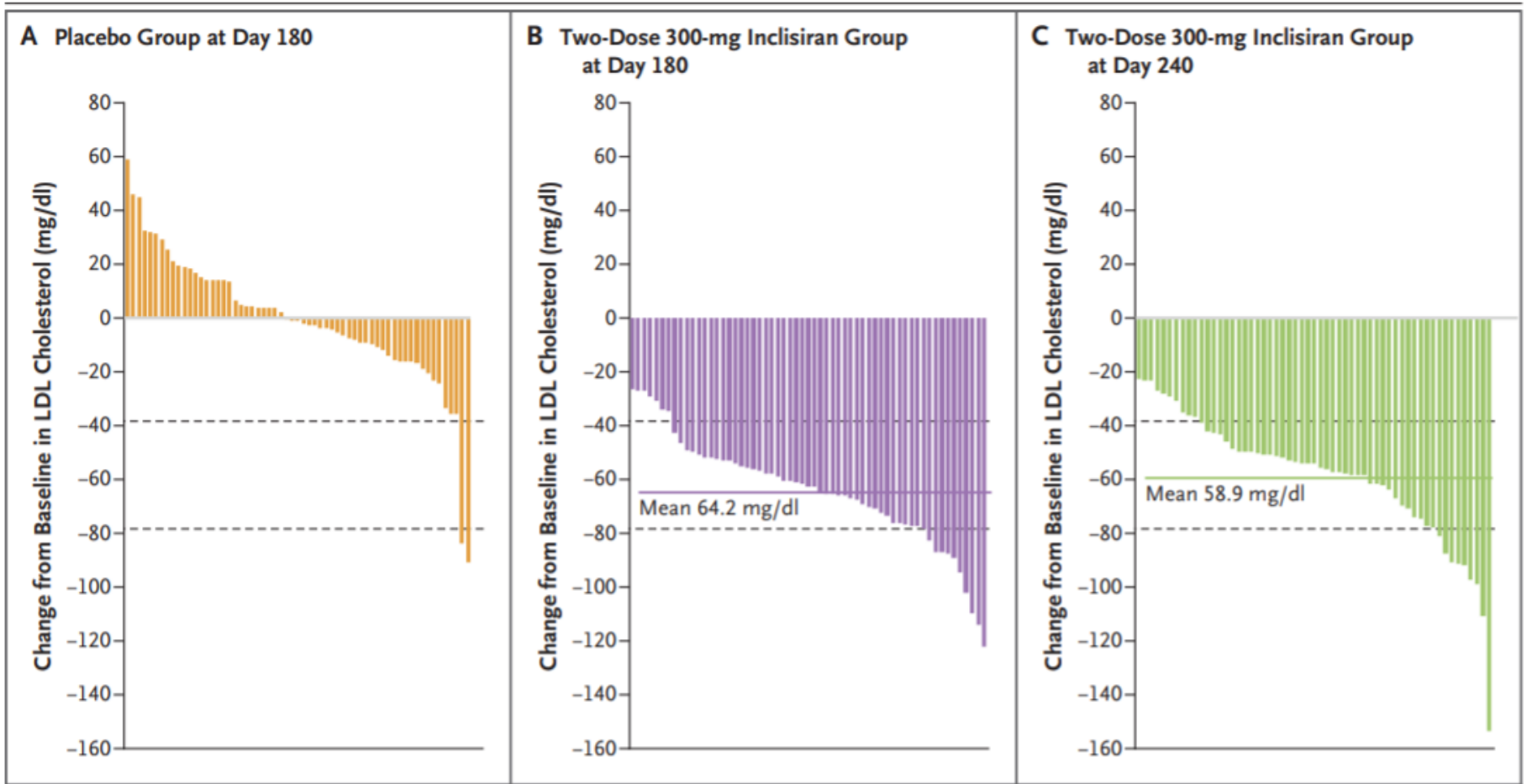


Figure 2. Changes in LDL Cholesterol Levels.

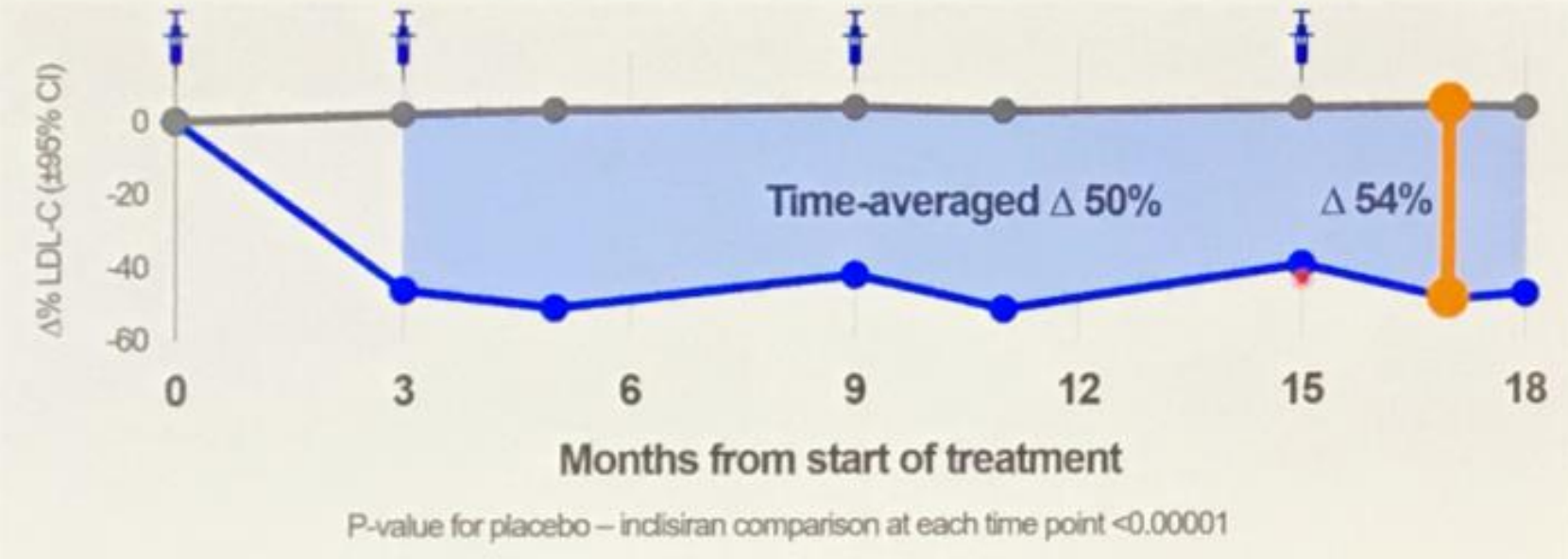
The change in LDL cholesterol level from baseline to day 180 is shown for each patient randomly assigned to the two-dose placebo group (61 patients) (Panel A) and the two-dose 300-mg inclisiran group (59 patients) (Panel B); the changes from baseline to day 240 are also shown for the two-dose 300-mg inclisiran group (59 patients) (Panel C). Dashed lines represent LDL cholesterol reductions of 39 mg per deciliter and 78 mg per deciliter. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586.

Impact of inclisiran on LDL-C over 18 months in patients with ASCVD or risk-equivale

ORION-11: Efficacy

Durable, potent and consistent effect over 18 months

Percent change in LDL-C over time – observed values ITT patients



P-value for placebo – inclisiran comparison at each time point <0.00001

1. All 95% confidence intervals are less than ±2% and therefore are not visible outside data points

16

ORION-11: Exploratory endpoint

Adverse cardiovascular events

Cardiovascular TEAEs Safety population[1,2]	Placebo N = 804	Inclisiran N = 811
Pre-specified exploratory CV endpoint[3]	83 (10.3%)	63 (7.8%)
Cardiovascular death	10 (1.2%)	9 (1.1%)
Fatal or non-fatal MI and stroke[4]	30 (3.7%)	12 (1.5%)
Fatal or non-fatal MI	22 (2.7%)	10 (1.2%)
Fatal or non-fatal stroke	8 (1.0%)	2 (0.2%)

1. Safety population includes all patients who received at least 1 dose of study medication 2. Patients may be counted in more than one category 3. MedDRA-defined cardiovascular basket of non-adjudicated terms including those classified within cardiac death, and any signs or symptoms of cardiac arrest, non-fatal MI and/or stroke 4. Post hoc analysis of hard endpoints

Metabolism & Pharmacokinetics

Toxicity of Oligonucleotide Therapeutics

Nucleic acid has unique molecule
Immunogenicity
Cross reactive with native DNA
Even cell death possible
Hepatocyte paralysis ? & Other Liver toxicity

Still very early days . . .

The future of gene modifying drugs


More than 200 drugs are underway

NOVARTIS

SANOFI GENZYME

DYNAVAX

DYNAVAX TECHNOLOGIES

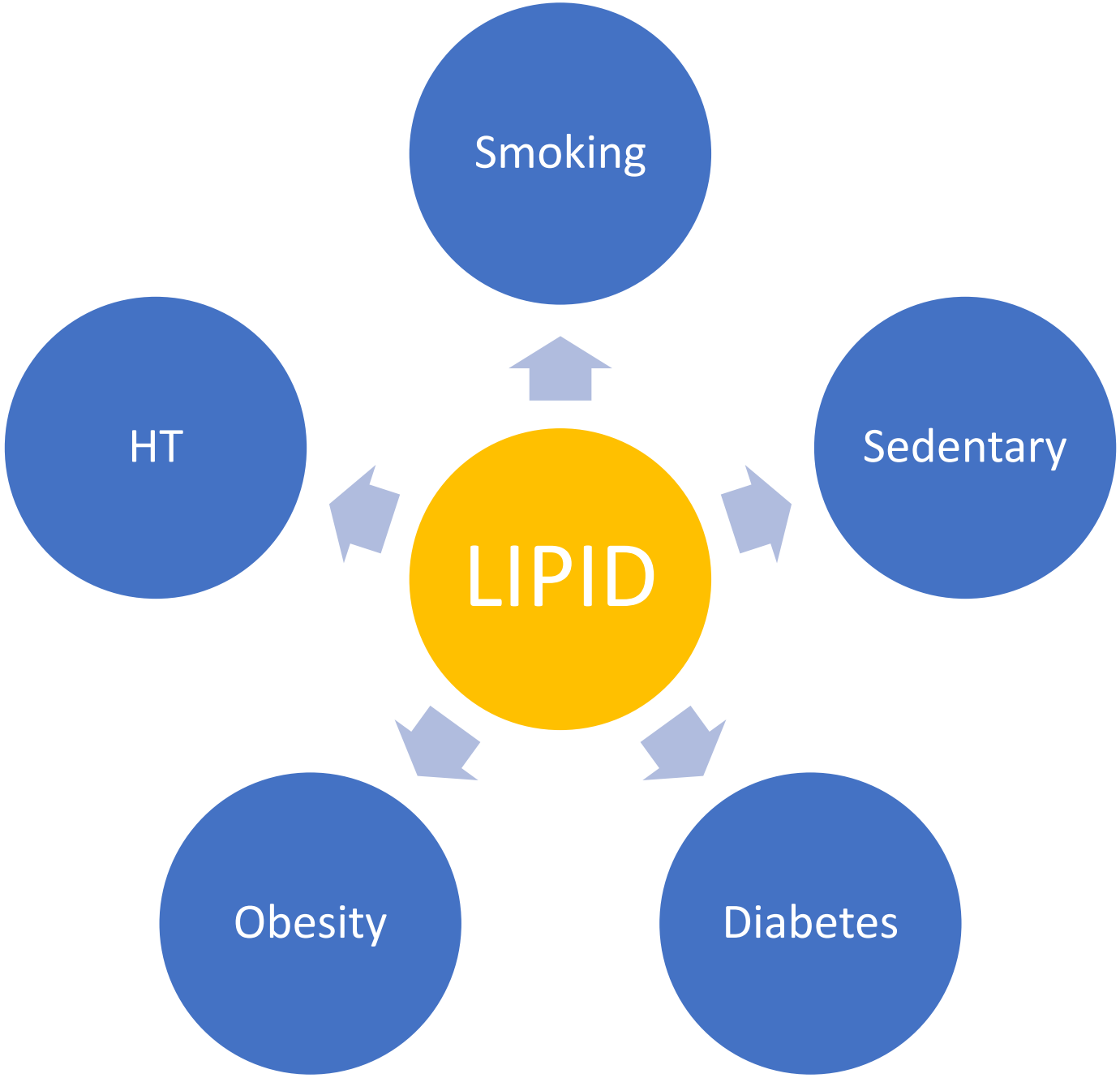
IONIS™

PHARMACEUTICALS

Oligonucleotide based Pharmacotherapy

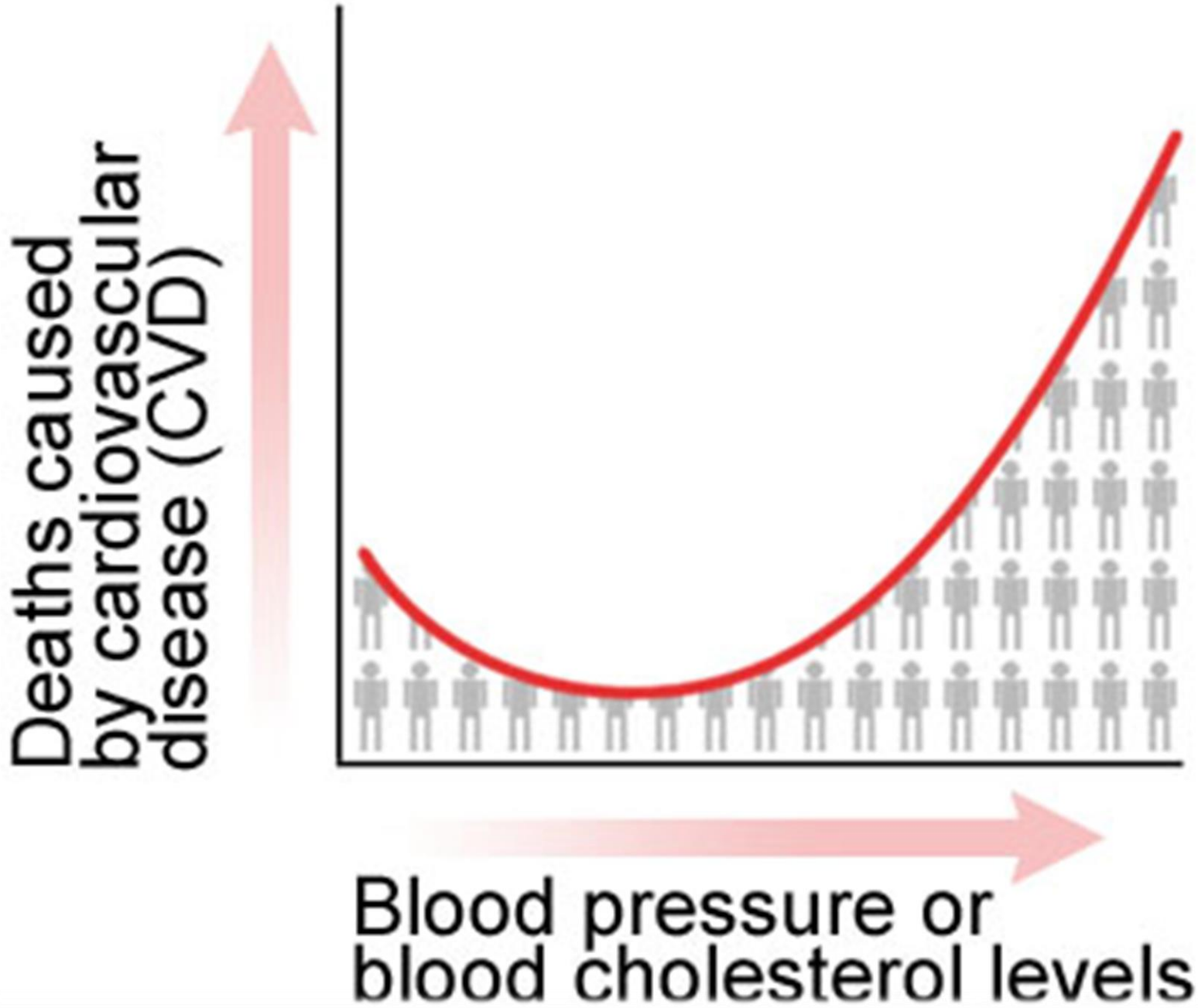
The broad diversity of the mechanism
Various designs
Chemical modifications
Endless dimensions.

Concluding comments


Smoking
HT
Sedentary
LIPID
Obesity
Diabetes

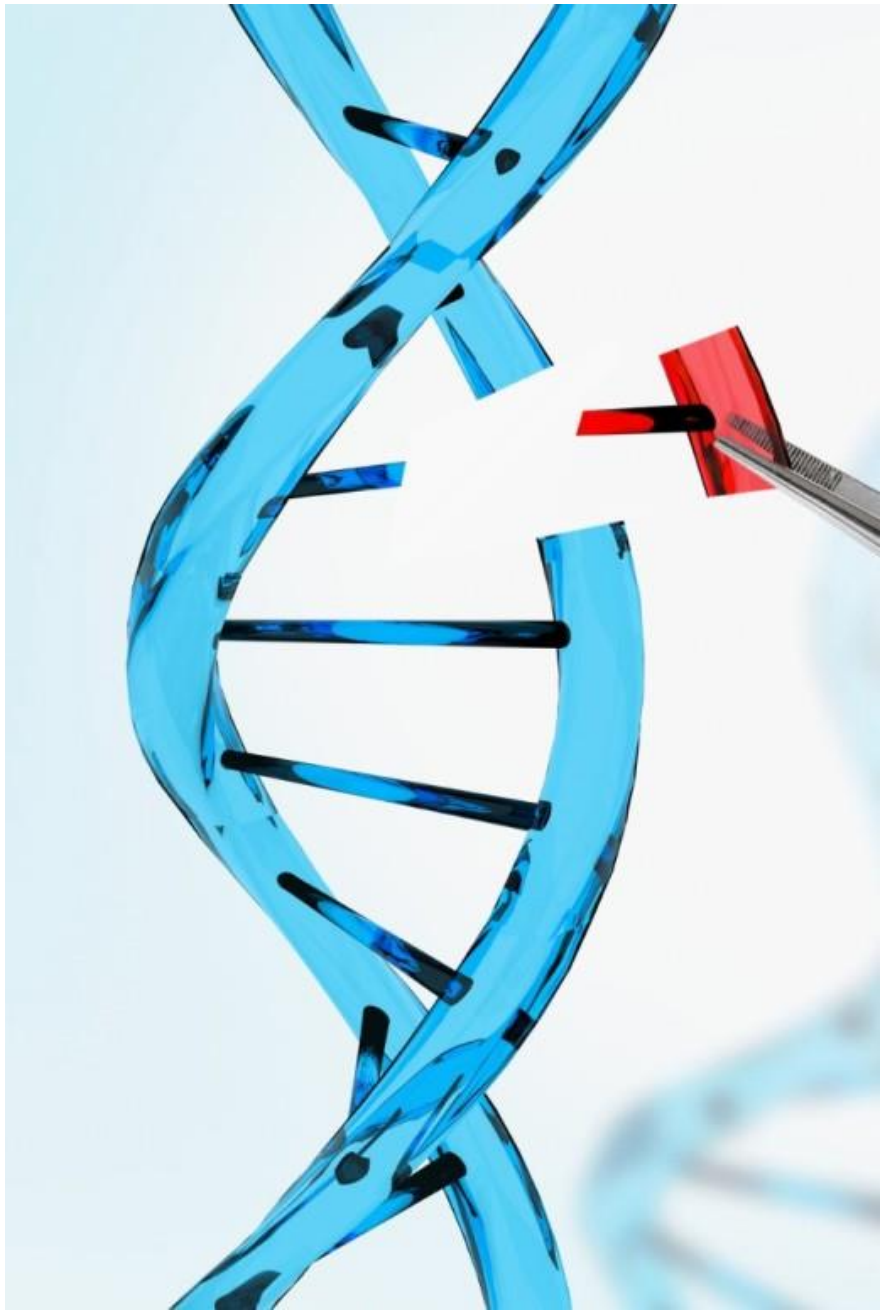
Why fat may not be our enemy ?

As we are waging a war against lipids
We must learn to live In harmony with lipids


Deaths caused by cardiovascular disease (CVD)
Blood pressure or blood cholesterol levels


STATINS


CAUTION
CAUTION
CAUTION
CAUTION
CAUTION
CAUTION



How to conquer Atherosclerosis ?

Are the strategies cost effective ?

Praluent®
(alirocumab) Injection 75mg/mL 150mg/mL

Repatha™
(evolocumab) injection 140 mg/mL

15000 $/year

Best site to block cholesterol metabolism

Is not HMG COA , PCSK, or SiRNA receptors

www.drsvekatesan.com

Thank you