CVS
PHARMACOLOGY

Modified NO: 8

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Novel Lipid-Lowering Agents for Reducing Cardiovascular Risk: Beyond Statins

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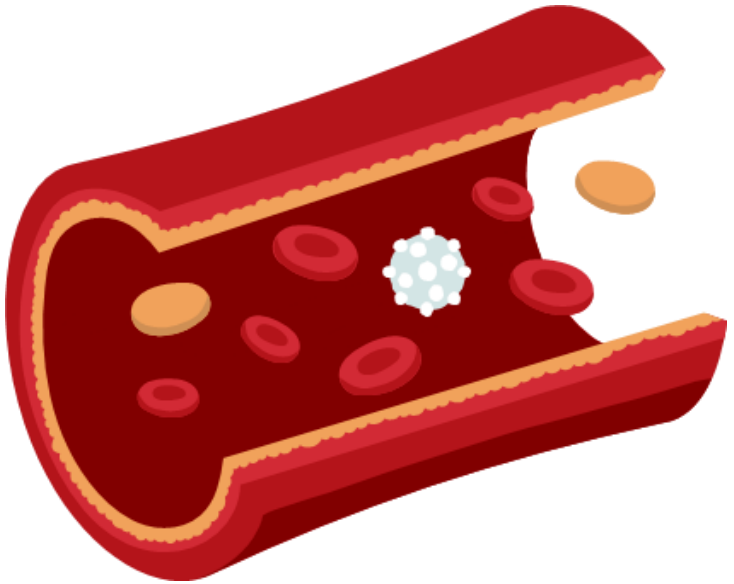


- All the five categories of a drugs which were discussed in the previous lecture (**statins , Fibrates, Niacin , Ezitemibe, bile acid sequestrants**) , have been in the market since long time. However, these drugs **did not offer a complete coverage for all patients with hyperlipidemia** , Neither through managing their situation nor through reducing the LDL towards the target levels that we need.
- This opened the door to **newer drugs** , that have emerged to the market in the **last 5 years** .

- **In this modified we will review some new Lipid-Lowering Agents :**

- 1. **Bempedoic acid**
- 2. **Evolocumab and alirocumab** (PCSK9 inhibition by monoclonal antibodies)
- 3. **Inclisiran** (PCSK9 inhibition by RNA silencing)
- 4. **Volanesoren** (ApoC-III inhibitor)
- 5. **Evinacumab and Vupanorsen** (ANGPTL3 inhibitors)

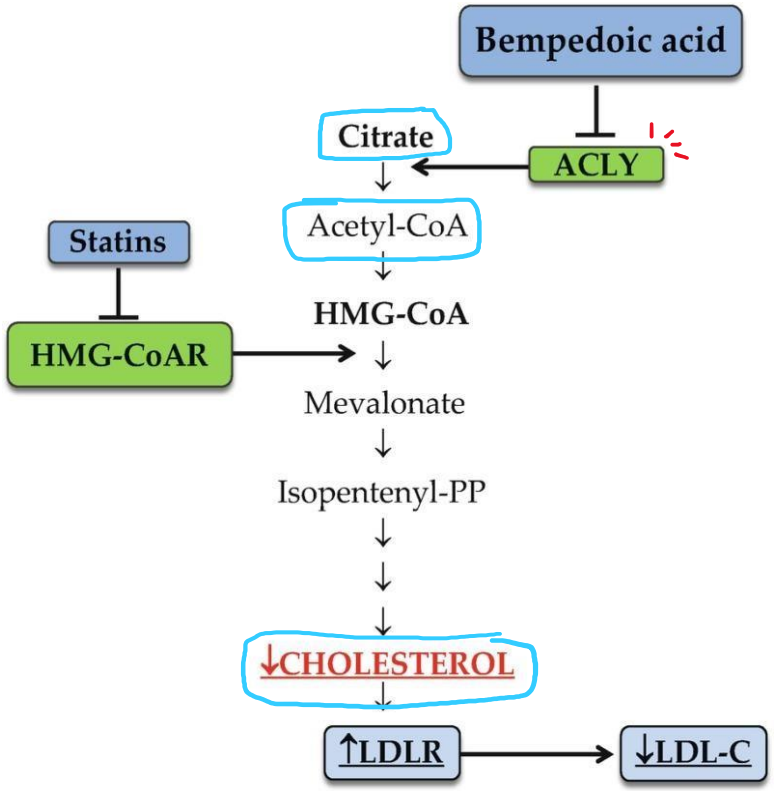
LDL-C LOWERING AGENTS

- Let's start with Bempedoic acid 🎯

Bempedoic acid

- Bempedoic acid is a small molecule that acts as a **selective antagonist of ACLY.**

- **What is ACYL ?**
- ATP-citrate lyase (ACLY) is an enzyme that catalyzes the ATP-dependent conversion of citrate to acetyl-CoA.
- Acetyl-CoA, is the precursor of **(HMG- CoA**), which is crucial for the biosynthesis of **cholesterol**.
- Thus, **inhibition of ACLY leads to a reduction of acetyl-CoA and cholesterol** synthesis, resulting in an increased number of LDLRs, causing a subsequent reduction of plasma cholesterol.
- It also may cause a slight increase in HDL , carrying cholesterol from foam cells to the liver .



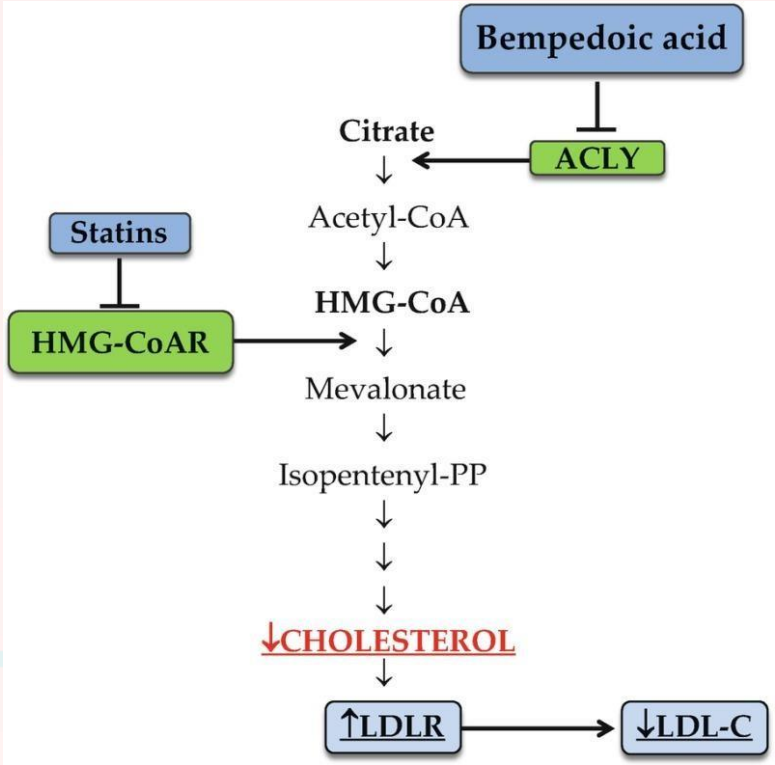
Bempedoic acid

- Luckily, the bempedoic acid is a **pro drug** , requires activation by an enzyme that is **mainly expressed in the liver : " very-long-chain acyl-CoA synthetase-1"**
- This property minimizes the exposure of the active drug to tissues other than the liver , such as the skeletal muscle.
- So this property will reduce the side effects
- As a compensatory mechanism, the **GI will increase Cholesterol absorption**, however, we can **inhibit this by giving Ezetimibe** (remember that it binds to cholesterol and inhibits its absorption) .
- Keep in mind that we use **Ezetimibe with Statins and Bempedoic acid**

Bempedoic acid

- Everything is explained in the previous slides

- ATP-citrate lyase (ACLY) catalyzes the ATP-dependent conversion of citrate and coenzyme A (CoA) to oxaloacetate and acetyl-CoA.
- Acetyl-CoA, the precursor of 3-hydroxy-3-methylglutaryl-CoA **(HMG-CoA**), is crucial for the biosynthesis of cholesterol.
- Thus, inhibition of ACLY leads to a reduction of acetyl-CoA and cholesterol synthesis, resulting in an increased number of LDLRs, causing a subsequent reduction of plasma cholesterol.
- Bempedoic acid is a small molecule that acts as a **selective antagonist of ACLY.**
- It is administered as a prodrug and requires activation by very-long-chain acyl-CoA synthetase-1, which is an enzyme mainly expressed in the liver.
- This property minimizes the exposure of the active drug to the non-hepatic tissue, such as the skeletal muscle



Bempedoic acid side effects

- **The most obvious side effects seen in clinical trials :**

- **Hyperuricemia** > due to the increase in uric acid levels
- **Gout** in 3% of the population
- **Anemia** > bc the drug reduces the hemoglobin
- May cause **Muscle pain** (unknown mechanism)
- Bempedoic acid is associated with increase of **blood urea nitrogen, creatinine, and uric acid** , because this drug inhibits the transporters (or secretory channels) that secrete those solutes within the kidneys, as a result, they will accumulate in the blood
- **Hyperglycemia is not observed**

- Everything is explained in the previous slides

Bempedoic acid

- Bempedoic acid was associated with **increase** of blood urea nitrogen, creatinine, and uric acid. It also resulted in a **decrease** in hemoglobin.
- Gout incidence was **higher** in the bempedoic acid group (3.4 – 3.5 %) compared with the placebo group (1.5 – 2 %).
- New-onset diabetes/hyperglycemia incidence was **lower** in the bempedoic acid group compared with that in the placebo group

Now we will talk about PCSK9 inhibitors.

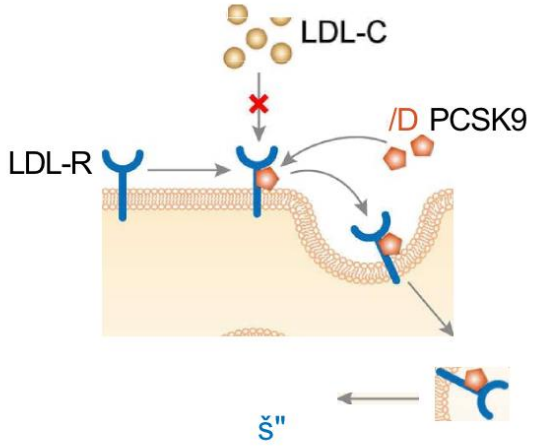
- These drugs will **increase the LDL Receptors**, thus **decreasing LDL-C**, without causing side effects , such as myopathy (which is seen with statins) , nor hyperuricemia (which is seen with Bempedoic acid) .

- **What is PCSK9?**
-
- Proprotein convertase subtilisin/kexin type 9 (PCSK9), an **enzyme** predominantly produced in the liver, **binds to the LDL receptor (LDLR) present on the surface of the hepatocytes, leading to its degradation** and a subsequent increase in plasma LDL-C levels
- So inhibition of this enzyme causes an increase in LDLR number and a subsequent decrease in plasma LDL-C levels.

- We have 2 ways to inhibit this enzyme :
- 1. monoclonal antibodies developed against PCSK9
- 2. inhibition by RNA silencing
- **Both have the same target (pcsk9) , with different mechanism of action**

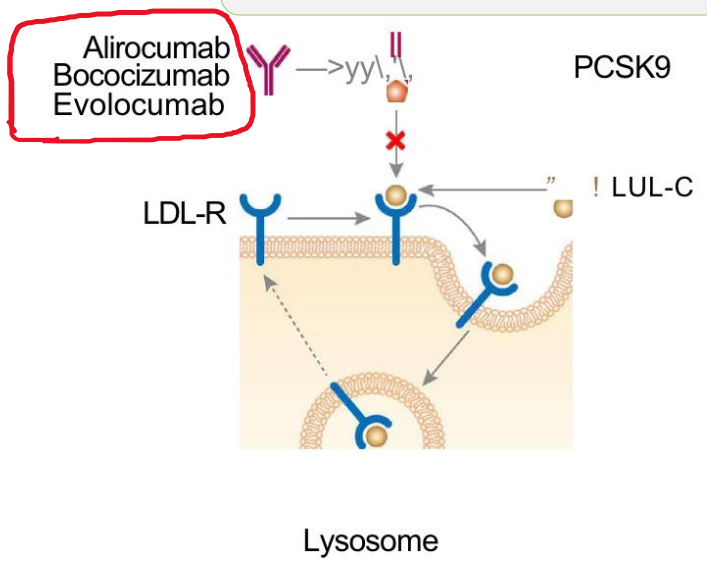
- **1. monoclonal antibodies developed against PCSK9 :**
- **evolocumab and alirocumab are** monoclonal antibodies developed against PCSK9 .
- Normally the PCSK9 will internalize the LDLRs , resulting in their lysosomal degradation.
- So these monoclonal AB will inhibit this lysosomal degradation of LDLR .
- Keep in mind that **STATINS , BEMPEDOIC ACIDS , evolocumab and alirocumab >> they all increase the LDLR, However evolocumab and alirocumab without or with minimal Side effects , unlike the others .**

- Without the drug



Physiology of PCSK9

- With the drug



Mechanism of action of anti-PCSK9 mAb

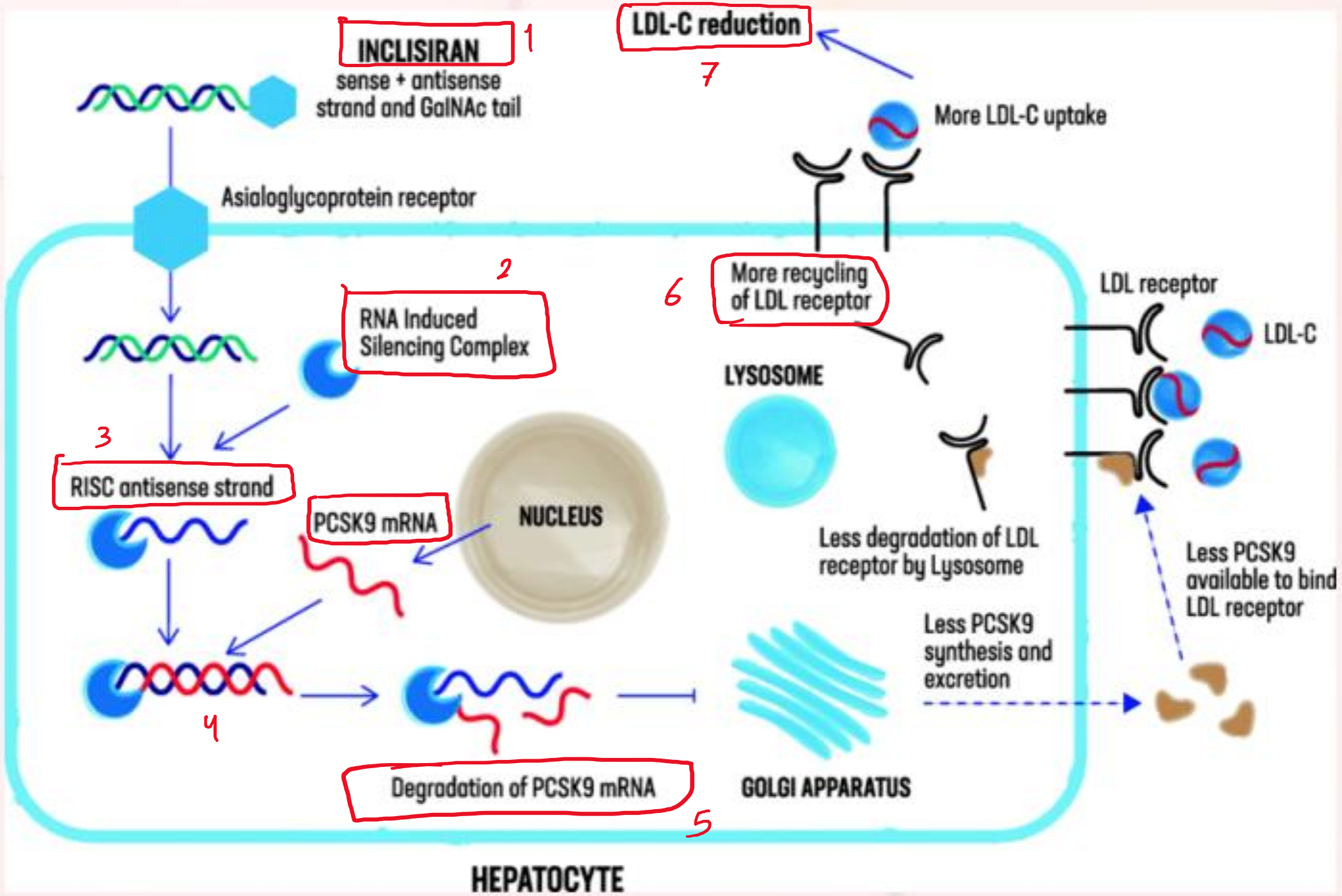
- Everything is explained in the previous slides

PCSK9 inhibition by monoclonal antibodies

- Proprotein convertase subtilisin/kexin type 9 (PCSK9), an enzyme predominantly produced in the liver, binds to the LDL receptor (LDLR) present on the surface of the hepatocytes, leading to its degradation and a subsequent increase in plasma LDL-C levels
- Thus, inhibition of PCSK9 causes an increase in LDLR number and a subsequent decrease in plasma LDL-C levels
- Among the several monoclonal antibodies developed against PCSK9, **evolocumab and alirocumab** have been approved for clinical use

- 2. **PCSK9 inhibition by RNA silencing by Inclisiran :**

- **Inclisiran** is a **synthetic small interfering RNA (siRNA),** which works by targeting the PCSK9 mRNA
- is conjugated to (**GalNAc**), which targets the **siRNA to the liver**. (GaLNAc will carry on the siRNA to its target)
- siRNA is a **double stranded RNA** with a sequence of 22-26 nucleotide
- Once it enters the cell , the " RNA induced silencing complex " , will open up the 2 strands ending with one strand called " RISC antisense strand " , which will bind to the mRNA of the target protein (**our target here is the PCSK9 mRNA**) and degrade it >> preventing the translation of this mRNA >> no protein is produced


INCLISIRAN
1
sense + antisense strand and GalNAc tail
Asialoglycoprotein receptor
2
RNA Induced Silencing Complex
3
RISC antisense strand
PCSK9 mRNA
NUCLEUS
4
Degradation of PCSK9 mRNA
5
GOLGI APPARATUS
HEPATOCYTE
LDL-C reduction
7
More LDL-C uptake
6
More recycling of LDL receptor
LYSOSOME
Less degradation of LDL receptor by Lysosome
LDL receptor
LDL-C
Less PCSK9 synthesis and excretion
Less PCSK9 available to bind LDL receptor

PCSK9 inhibition by RNA silencing

- **Inclisiran** is a synthetic small interfering RNA (siRNA), which works by targeting the PCSK9 and is conjugated to triantennary N-acetylgalactosamine carbohydrates (GalNAc), which targets the siRNA to the liver
- **Inclisiran shows comparable effects to that of PCSK9 monoclonal antibodies**
- **Side effect**: The inclisiran group reported a higher rate of **injection-site reaction** compared with the placebo group (17.0% vs. 1.7%), which was graded as mild

- **Inclisiran is very expensive, around 3000 JD**
- It is an **injectable drug that is given 3 times a year**
- So the best thing about Inclisiran and evolocumab and alirocumab, is that we don't have to worry about the compliance issue, bc they **are not frequently taken** , compared to the Inclisiran, The monoclonal ABs are taken a little **more** frequent.
- Both are given in a **loading dose**
- **Side effects of inclisiran** : injection-site reaction only
- **Side effects of evolocumab and alirocumab:** flue like syndrome (remember they are monoclonal Abs)

- Note : Inclisiran can be given with Statins to increase its activity

- **Now we are moving to ApoC-III inhibitor (Volanesoren)⭐**

- **What is Apolipoprotein C-Ⅲ (apoC3)?**

- It is an enzyme that **inhibits the lipo protein lipase LPL**, (the enzyme responsible for the lipolysis of TG in the very-low-density lipoprotein (VLDL) and chylomicron particles) .
- Logically, we want to " **inhibit the inhibitor** " , in order to **increase the expression of LPL, thus increasing TG (triglycerides) lipolysis.**
- The drug that inhibits APOC3 is called: **Volanesoren**
- This drug works **more on Triglycerides** (reduces VLDL, chylomicrones) , unlike the previous drugs that work on lowering **LDL cholestrol**

- **From where did they come up with the idea of this drug?**

- They discovered that some people who have a polymorphism in APOC3 (loss function mutation) , were protected against CVs diseases (especially if the disease is related to VLDL) , and had **lesser amounts of TGs.**

- Everything is explained in the previous slides

ApoC-III inhibitor

- Apolipoprotein C-III (apoC3) is a key regulator of TG metabolism.
- It is a potent inhibitor of lipoprotein lipase (LPL), the enzyme responsible for the lipolysis of TG in the very-low-density lipoprotein (VLDL) and chylomicron particles.

- Check this out

- loss-of-function mutations in the APOC3 gene are associated with
- 40% lower plasma TG levels and a 40% lower risk of CVD

- **What is the mechanism of action of (Volanesoren) ?**

- an antisense oligonucleotide (ASO) (**siRNA**) , but our target this time is **APOC3 mRNA .**

  Remember : **Inclisiran** has nearly the same mechanism of action but it's target is : **PCSK9 mRNA .**

  It is very beneficial in treating in patients with **elevated plasma TG levels and in patients with familial chylomicronemia syndrome (FCS) .**

  It is an **injectable** drug

ApoC-III inhibitor: Volanesoren

- Volanesorsen is an **antisense oligonucleotide (ASO)** targeting **apoC3 mrna**

- Volanesorsen has been tested in patients with elevated plasma TG levels and in patients with familial chylomicronemia syndrome (FCS), an autosomal recessive disease of chylomicron metabolism

- In 2019, volanesorsen was approved by the European Union (EU) for the treatment of adult patients with FCS

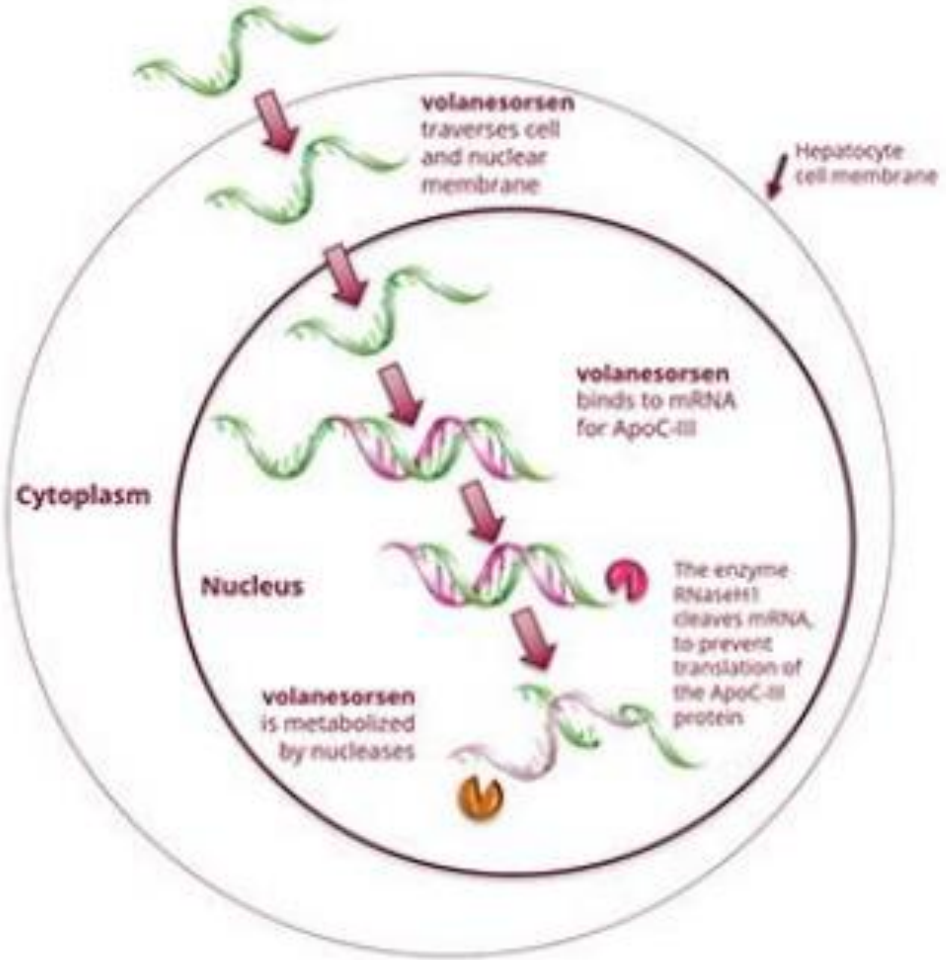
- **Common adverse events are thrombocytopenia and injection-site reactions, but not very common**

- Check this out

- The doctor said that it is **double stranded and has the same mechanism of as Indisiran, to simplify things**
- **But after digging, and as shown in this pic, it is a single stranded RNA that will bind to the target mRNA, then degraded by the complex > no APOC3 > more LPL > reduced TG.**
- **You can watch this video for clarification:**
- https://youtu.be/QdJB9HoZmj4?si=yxPz29BtWOKNq1OE

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

- **Finally, Let's review the last group of drugs in this lecture :**
  - **ANGPTL3 inhibitors**
  - **(Evinacumab and Vupanorsen)** 

- **What is ANGPTL3?**

- It is an enzyme that regulates plasma TG and HDL-C levels by **inhibiting lipoprotein lipase (LPL) and endothelial lipase, respectively.**
- **Inhibition of ANGPTL3** preserves the function of LPL and EL > resulting in a **decline in TG, LDL-C and HDL-C plasma levels independently of LDLR function** (it works more on TG than LDL)

- **We can inhibit ANGPTL3 by two mechanisms:**

1. **Evinacumab** a monoclonal antibody neutralizing levels in the serum Influenza like effect was observed in 11%
2. **Vupanorsen** an antisense oligonucleotide inhibiting production in hepatocytes

- Don't get confused !
- **Evinacmab** is a monoclonal AB against ANGPLT3
- **Evolocumab is a monoclonal AB against PCSK9**
- **Volanesoren is an antisense oligonucleotide (ASO)** targeting APOC3 mRNA
- **Vupanorsen** an antisense oligonucleotide targeting ANGPTL3

ANGPTL3 inhibitor

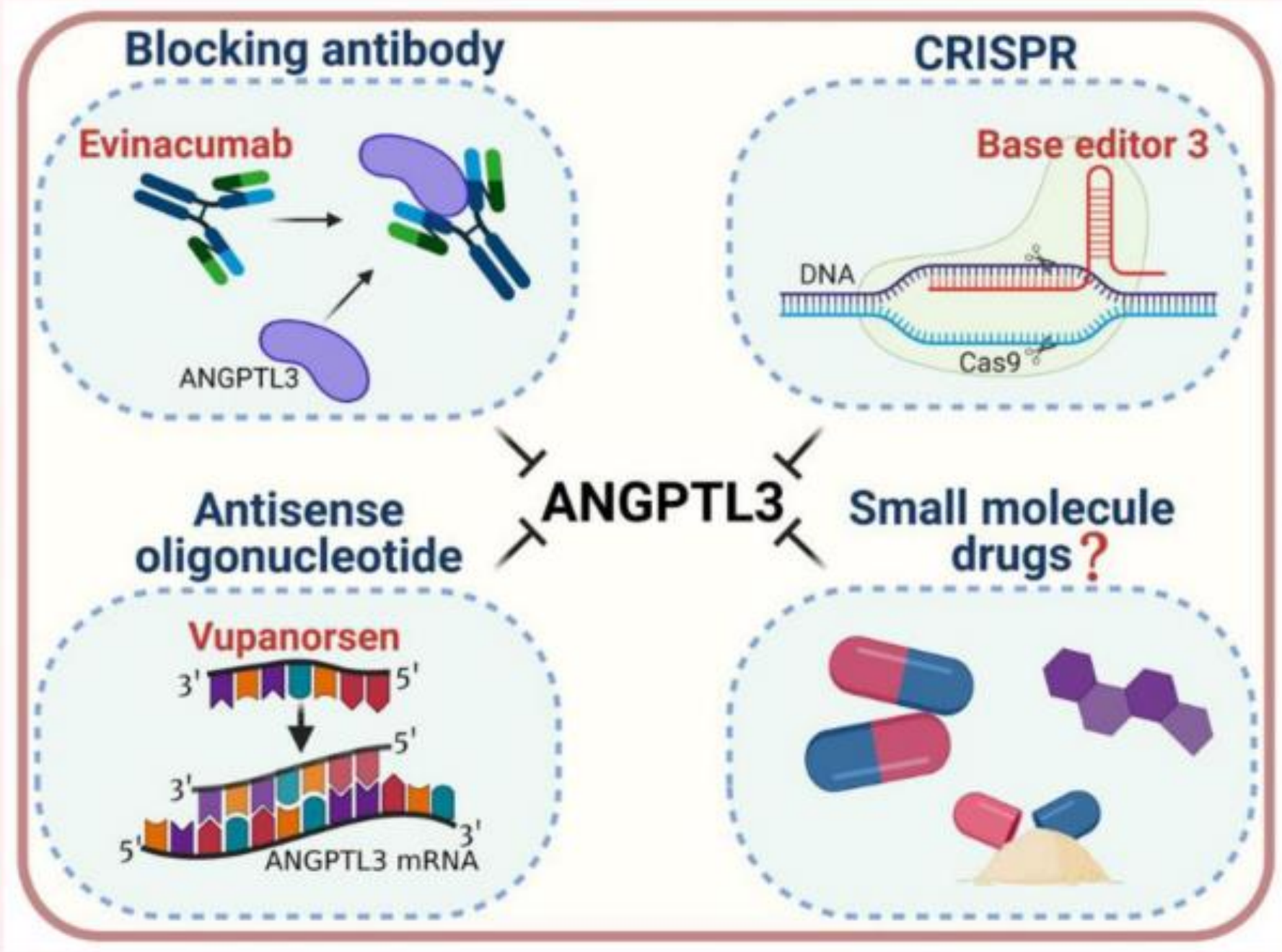
- Everything is explained in the previous slides

- ANGPTL3 regulates plasma TG and HDL-C levels by inhibiting lipoprotein lipase (LPL) and endothelial lipase, respectively.
- Inhibition of ANGPTL3 preserves the function of LPL and EL with a subsequent decline in TG, LDL-C and HDL-C plasma levels independently of LDLR function
- Therefore, it was proposed that blocking ANGPLT3 might produce a beneficial effect on cardiovascular risk and future outcomes.
- therapies targeting ANGPTL3 were developed by two mechanisms:

1. **Evinacumab** a monoclonal antibody neutralizing levels in the serum

Influenza like effect was observed in 11%

1. **Vupanorsen** an antisense oligonucleotide inhibiting production in hepatocytes


Blocking antibody
Evinacumab
ANGPTL3
CRISPR
Base editor 3
DNA
Cas9
ANGPTL3
Antisense oligonucleotide
Vupanorsen
3'
5'
5'
3'
3'
5'
ANGPTL3 mRNA
Small molecule drugs?

- **All drugs in this lecture (new LDL-C LOWERING AGENTS) are not considered first line therapy**
- **They all have activities, but we use them as Add On Drugs to Statins (and others) ,rather than being new drugs .**

Agent	Mechanism of Action	Main Lipid Lowering Effect	Administration Scheme	Side-Effects	Comment
Statin	HMG-CoA inhibition	LDL - C	1x/day p.o.	Myopathy, increased liver enzymes	Side-effects are rare, novel statins like rosuvastatin and atorvastatin can be taken in the morning because of long t 1/2
Ezetimibe	NPC1L1 protein inhibition	LDL-C	1x/day p.o.	Diarrhoea	Side-effects are rare
PCSK9i (alirocumab/ evolocumab)	PCSK9 inhibition	LDL-C	2x/month (1x/ month) s.c.	Injection site reactions	Side-effects are rare, not more than placebo
Inclisiran	siRNA targeting mRNA PCSK9	LDL-C	2x/year s.c.	Injection site reactions	Side-effects are rare, not more than placebo (still under investigation)
Bempedoic acid	Inhibiting ACL and AMPK	LDL-C	1/day p.o.	Not greater than placebo	Alernative to SAMS?
Icosapent ethyl	LPL?	TGs	1/day p.o.	?	Benefit of long-term use of this agent still needs to be proven; many pleiotropic effects
Volanesorsen	Antisense oligonucleotide to apo C-III	TGs	2x/year s.c.	Thrombocytopenia and injection-site reactions	Treatment of ultra rare LPL deficiency
ANGPTL3	Monoclonal anti-ANGPLT3 antibody and ASO	TGs, LDL-C	2x/year s.c.?	Not yet fully determined	Studies are ongoing
Pemafibrate	Peroxisome proliferator-activated receptor alpha modulator	TGs	1/day p.o.	Liver enzymes?	Clinical data as well as long-term efficacy and safety need to be investigated
Pelacarsen	ASO to apolipoprotein(a)	Lp(a)	2x/year s.c.?	?	The agent is in phase III trial

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; LDL-C, Low density lipoprotein cholesterol; NPC1L1, Niemann-pick-C1 like-1 protein; PCSK9i, inhibitor of proprotein kexin serin convertase type 9; p.o., peroral therapy; s.c., subcutaneous therapy; ACL, Adenosine triphosphate-citrate lyase; AMPK, adenosine monophosphate-activated protein kinase; SAMS, statin associated muscular symptoms; TGs, Triglycerides; LPL, lipoprotein-lipase; Apo-CIII, Apolipoprotein CIII; ANGPTL3, Angiopoietin-Like 3; ASO, anti sense oligonucleotide; Lp(a), lipoprotein (a).
Notes: ? indicates unknown side effects.

ضعْ عُنُقي على السّكين ألهِب أضلُعي
لن تستَطيعَ حصارَ فكرِيَ ساعةً
أو نزَعَ إيماني و نورَ يقيني
سأعيشُ مُعتصِمًا بحبلِ عقيدَتي
سأموتُ مبتَسِمًا ليحيا ديني

قالَها فارِسُ جباليا المُصاب ..

Additional sources :

1. Volanesorsen : https://youtu.be/QdJB9HoZmj4?si=PPGFa2lcfb34JmGz

VERSIONS	SLIDE #	BEFORE CORRECTION	AFTER CORRECTION
V1→ V2	12	Reduce LDLR	INCREASE LDLR
V2→V3			

امسح الرمز و شاركنا بأفكارك لتحسين أدائنا!!