

# New translational perspectives in cardiovascular medicine

Attila Tóth

Division of Clinical Physiology

# What do we learn today?



Beriberi is a relatively common disease in Asia, sailors and prisoners.

1873: a Dutch naval doctor observed that European crew members had significantly fewer cases of beriberi than sailors recruited from the East Indies. When the amount of white rice in the diet of the East Indies sailors was decreased, the rate of beriberi came down.

Beriberi was believed to have been caused by some toxin or infectious agent in the white rice. Kanehiro Takaki, a Japanese naval doctor, was the first to report beriberi as a nutritional deficiency. His reports were based on the fact that the incidence of beriberi reduced in Japanese sailors when they were given additional meat, dry milk, and vegetables.

# What do we learn today?



In 1875, after taking his preliminary examinations, Eijkman became a student at the Military Medical School of the University of Amsterdam, where he was trained as a medical officer for the Netherlands Indies Army, passing through all his examinations with honours.

Christiaan Eijkman was appointed as Director of the “Dokter Djawa School” (Javanese Medical School) in 1888. Eijkman was also Director of the “Geneeskundig Laboratorium” (Medical Laboratory) from January 15, 1888 to March 4, 1896, and during that time he made a number of his most important researches. These dealt first of all with the physiology of people living in tropical regions. He was able to demonstrate that a number of theories had no factual basis.

Eijkman realized that the real cause of beriberi was the deficiency of some vital substance in the staple food of the natives, which is located in the so-called “silver skin” (pericarpium) of the rice. This discovery has led to the concept of vitamins.

Eijkman noticed that when fowl were fed a diet solely consisting of polished white rice, they developed symptoms similar to beriberi. By adding rice polishings, the material removed from whole rice to produce white rice, to the feed, Eijkman was able to cure the fowl of beriberi.

In 1926, pure thiamine, the true anti-beriberi vitamin, was isolated by two Dutch scientists, Barend Jansen and W. F. Donath, working in Java.

# Translational medicine

The term *translational medicine* was introduced in the 1990s but only gained wide usage in the early 2000s. Its definition varies according to the stakeholder. Patients, physicians, and other practitioners tend to use the term to refer to the need to accelerate the incorporation of benefits of research into clinical medicine and to close the gap between “what we know” and “what we practice.” Academics tend to interpret *translational medicine* as the testing of novel concepts from basic research in clinical situations, which in turn provide opportunity for the identification of new concepts. In industry it is used in reference to a process that is aimed at expediting the development and commercialization of known therapies. Although different, these interpretations are not mutually [exclusive](#). Rather, they reflect different priorities for achieving a common goal.

www.britannica.com

Phase 1 (T1): move basic discovery to clinical application

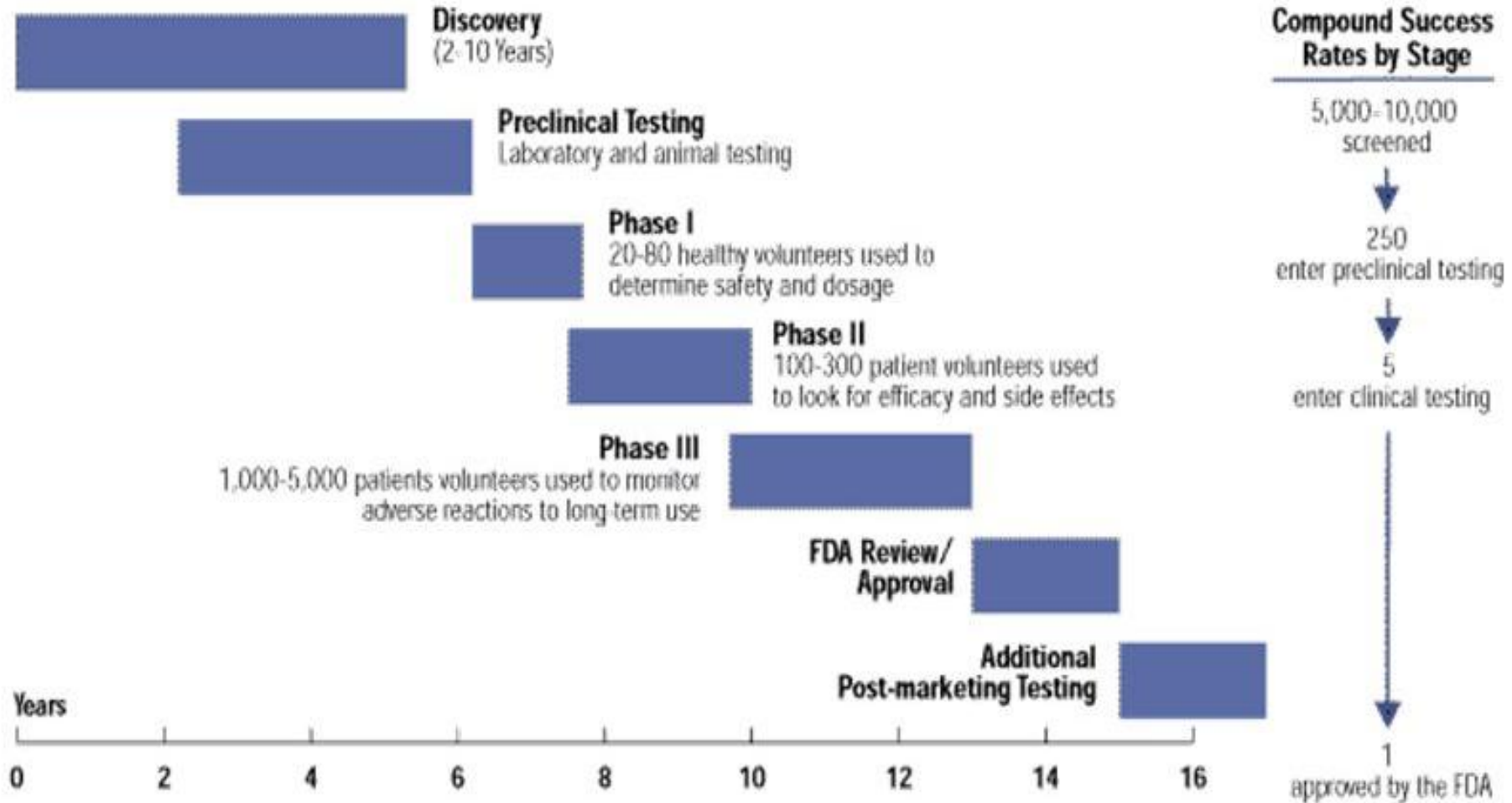
Phase 2 (T2): assess the value of a clinical application to develop therapeutic guidelines

Phase 3 (T3): move evidence-based guidelines into health practice

Phase 4 (T4): evaluate the real world health outcomes.

# Drug development

## COMPOUND SUCCESS RATES BY STAGES

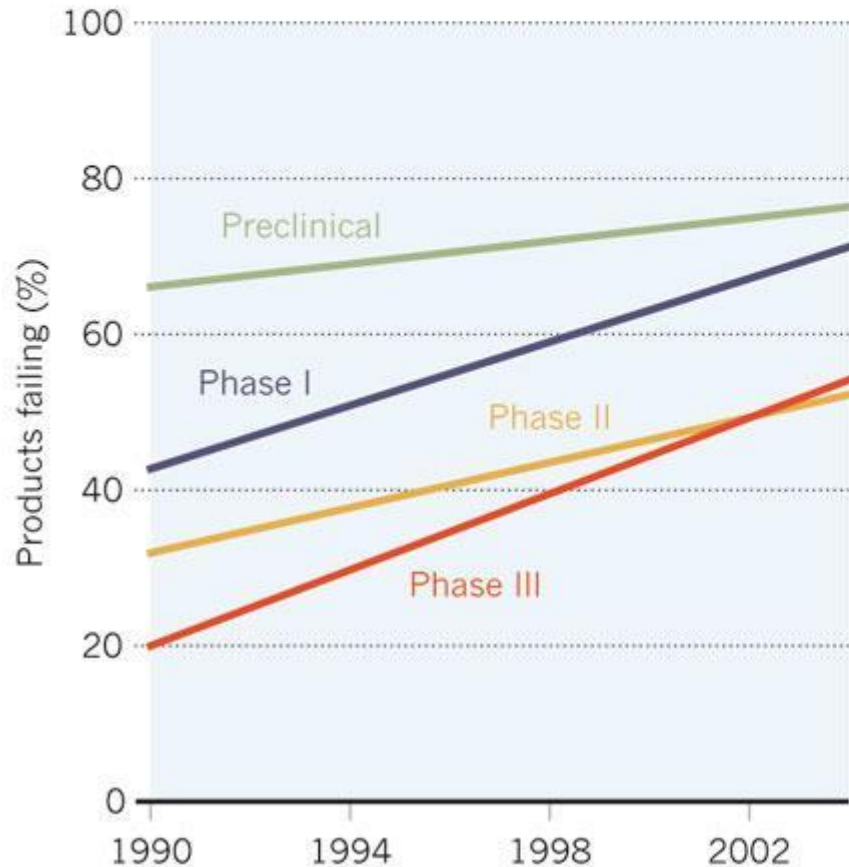


Source: PhRMA, based on data from Center for the Study of Drug Development, Tufts University, 1995.

# THE CLINICAL-TRIAL CLIFF

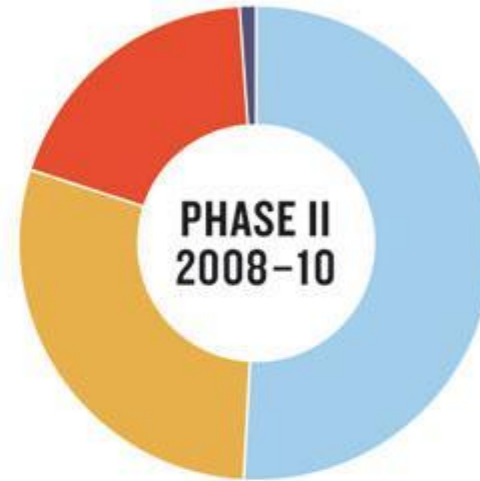
Drug companies are removing more compounds from the pipeline at all levels of testing than ever before.

For projects started between 1990 and 2004, the United States, Europe and Japan have seen sharp rises in the attrition of drugs tested in trials.



Most of the product failures in phase II and III trials are because researchers are unable to demonstrate efficacy or sufficient safety.

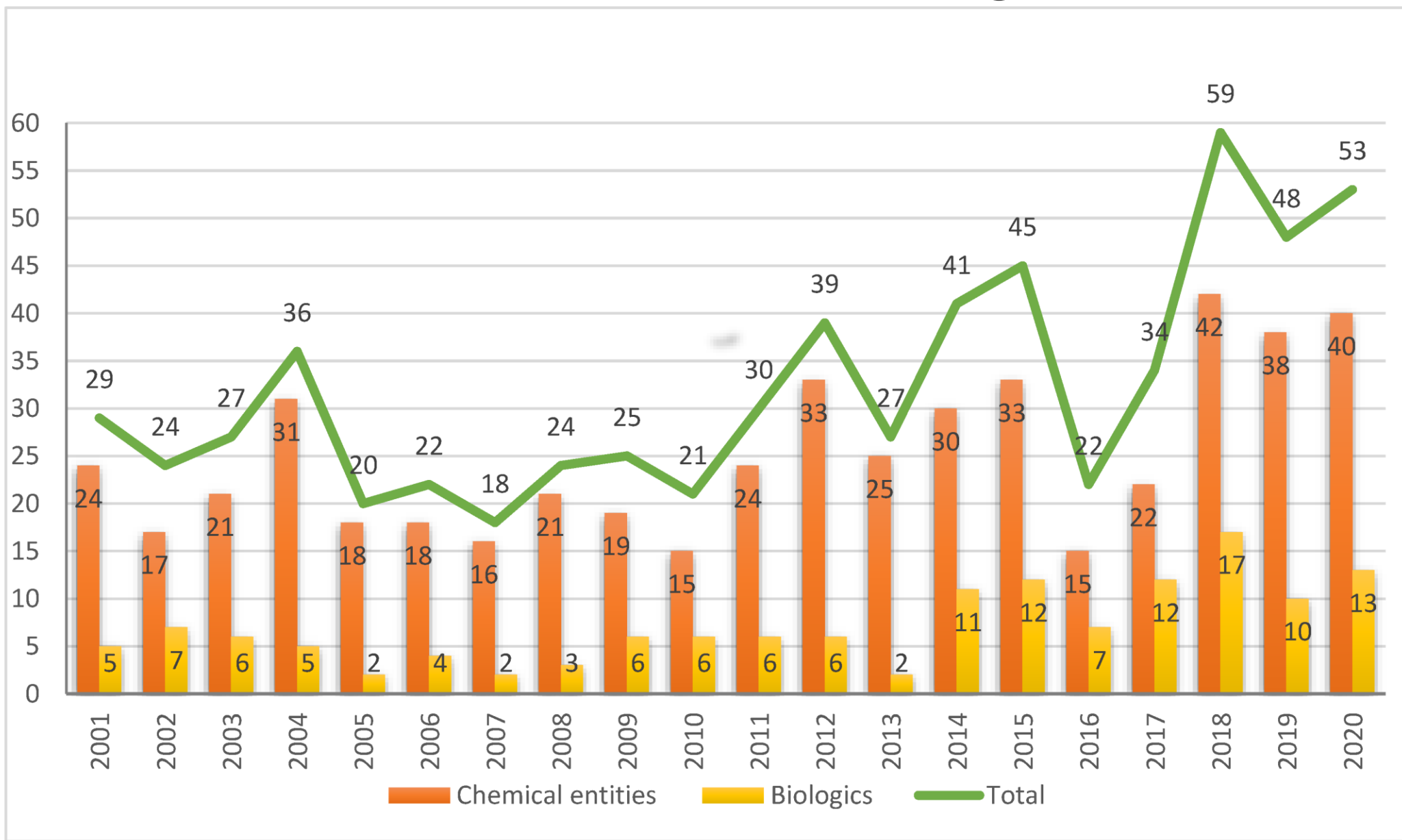
- Efficacy
- Safety
- Strategic
- Pharmacokinetics/ bioavailability
- Commercial/ financial
- Not disclosed



# Modern pharmacology

<b>Properties</b>	<b>Small molecules</b>	<b>Protein-based drugs</b>	<b>siRNA/miRNA-based drugs</b>
Nature of action	Activation or inhibition of targets	Activation or inhibition of targets	Inhibition of targets
Site of target proteins	Extracellular and Intracellular	Mainly extracellular	Virtually any sites
Selectivity and potency	Variable (depending on binding-site and ligand specificity, their affinity and efficacy etc.)	Highly specific and potent	Highly specific and potent
Lead optimization	Slow	Slow	Rapid
Manufacture	Easy	Difficult	Easy
Stability	Stable	Unstable	Unstable
Delivery	Easy	Difficult	Difficult

# Small molecules - biologicals



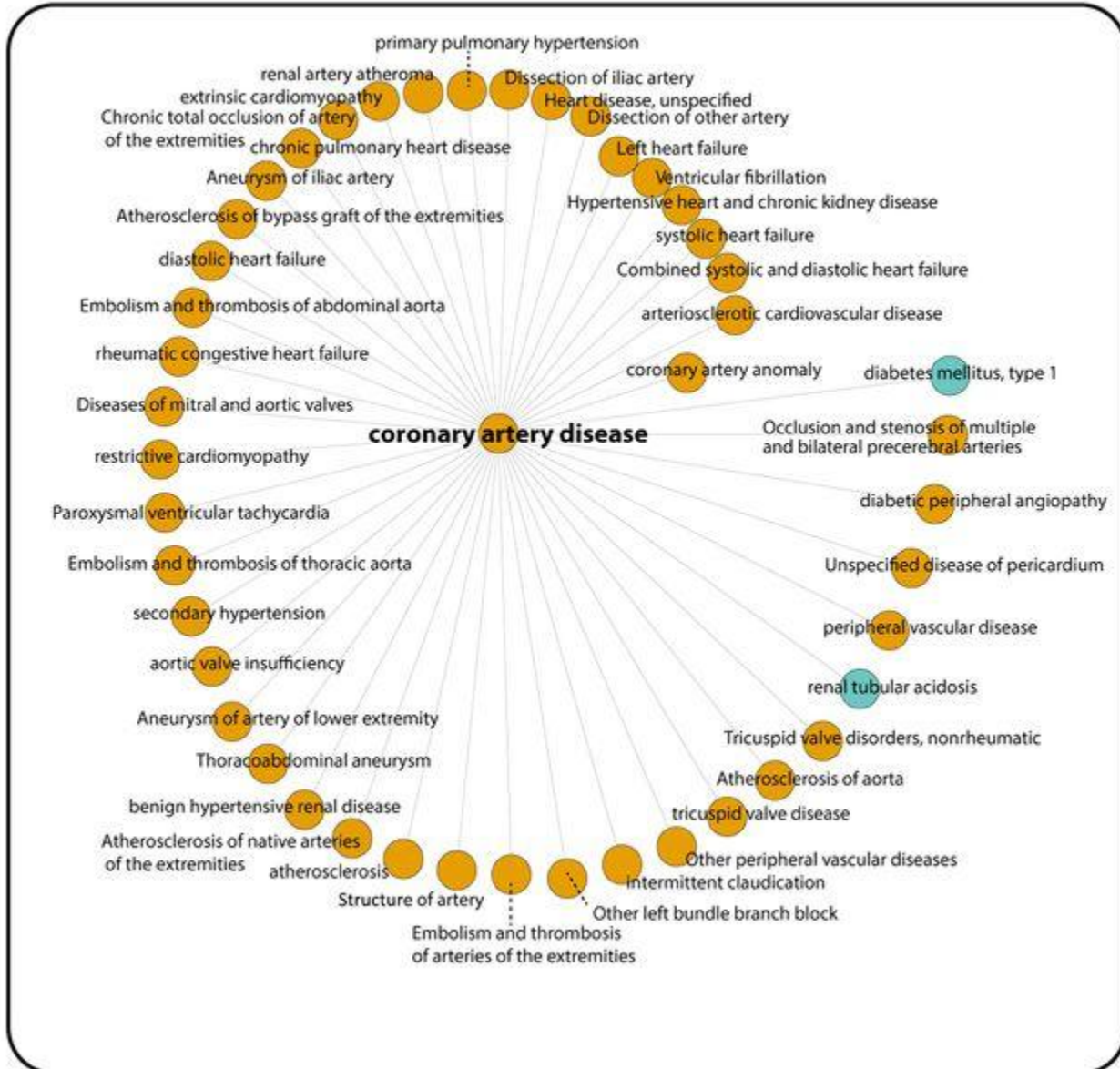
# Top 200 Pharmaceuticals by Retail Sales in 2019

Compiled and Produced by the Njardarson Group (The University of Arizona)

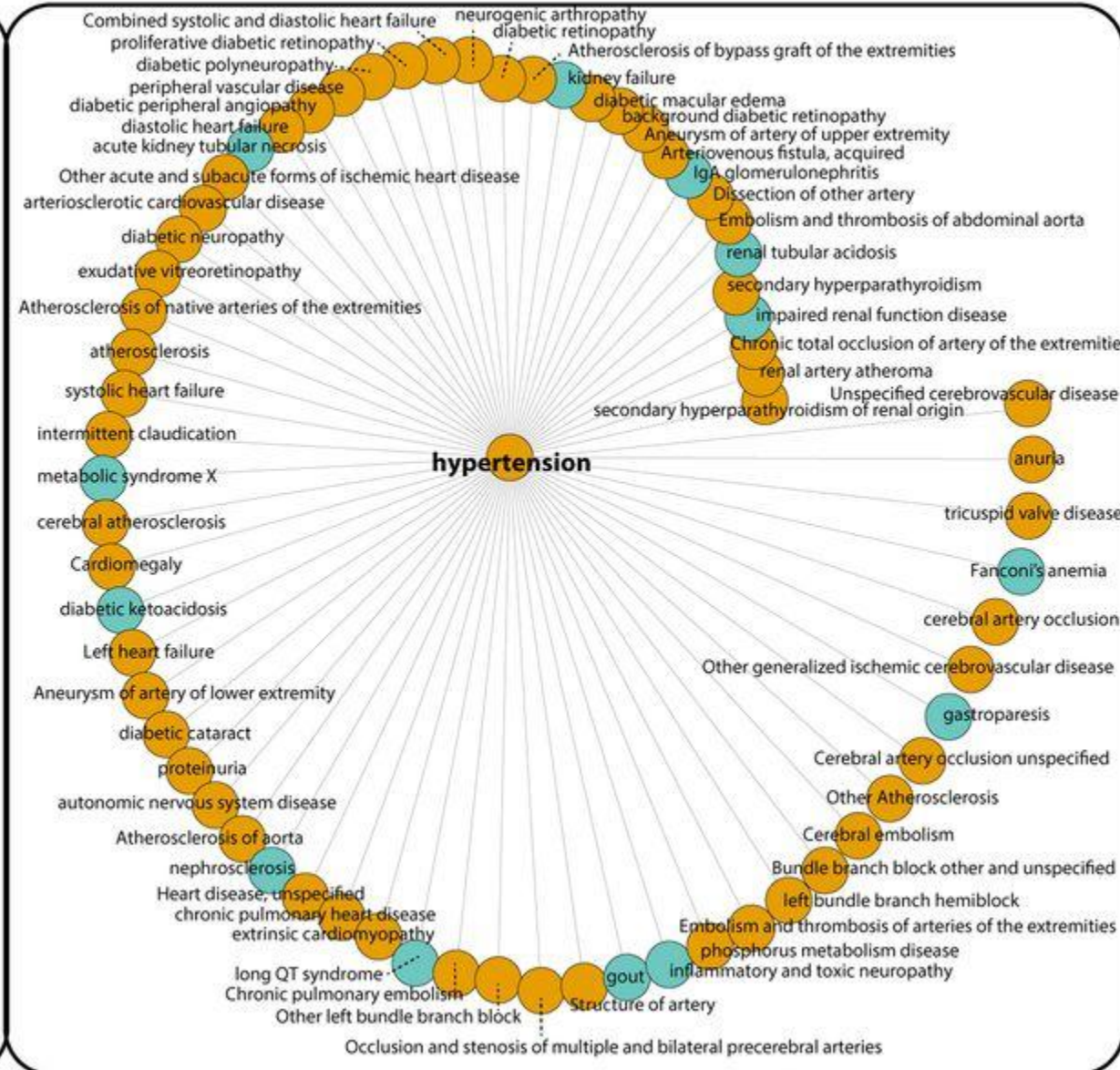
1 Humira (adalimumab)	2 Eliquis (apixiban)	3 Keytruda (pembrolizumab)	4 Revlimid (lenalidomide)	5 Inveceva (nivolumab)	6 Opdivo (nivolumab)	7 Eylea (aflibercept)	8 Avastin (bevacizumab)	9 Enbrel (etanercept)	10 Xarelto (rivaroxaban)	11 Eliquis (apixiban)	12 Stegno (simeprevir)	13 Receptin (trastuzumab)	14 Prevnis 13 (nasal corticosteroid)	15 Remicade (infliximab)	16 Ibuprofen (ibuprofen)	17 Bikanyl (amilofril)	18 Tecidena (telmisartan)	19 Tivicity (dolutegravir)	20 Xolair (omalizumab)
21 Lantus (insulin glargine)	22 Sildenafil (sildenafil)	23 Cymbalta (duloxetine)	24 Ozempic (semaglutin)	25 Sildenafil (sildenafil)	26 Crestor (rosuvastatin)	27 Protonix (pantoprazole)	28 Synthroid (levothyroxine)	29 Celecoxib (celecoxib)	30 Januvia (sitagliptin)	31 Vicodin (hydrocodone bitartrate/paracetamol)	32 Victoza (liraglutide)	33 Gilead (sofosbuvir)	34 Lantus (insulin glargine)	35 Celecoxib (celecoxib)	36 Nevelin (pegaptanib sodium)	37 Acton (doxepin)	38 Toprol XL (metoprolol succinate)	39 Tivicity (dolutegravir)	40 Protonix (pantoprazole)
41 Darzalex (daratumumab)	42 Ozempic (semaglutin)	43 Mavyret (glecaprevir/pasoprevir)	44 Humalog (insulin lispro)	45 Inveceva (nivolumab)	46 Xylopro (propofol)	47 Symbicort (budesonide/formoterol fumarate dihydrate)	48 Novorapid (insulin aspart)	49 Protonix (pantoprazole)	50 Sprycel (dasatinib)	51 Vyvanse (lisdexamfetamine dimesylate)	52 Actemra (tocilizumab)	53 Protonix (pantoprazole)	54 Dupixent (dupilumab)	55 Shingrix (zoster vaccine)	56 Spivro (sildenafil)	57 Xeljanz (tofacitinib)	58 Nexium (esomeprazole)	59 Simponi (tocilizumab)	60 Fomavotil (fomivotril)
61 Advair (salmeterol/fluticasone propionate)	62 Protonix (pantoprazole)	63 Abilify (aripiprazole)	64 Prevacid (lansoprazole)	65 Sildenafil (sildenafil)	66 Opdivo (nivolumab)	67 Imbruvica (venclextinib)	68 Synthroid (levothyroxine)	69 Abilify (aripiprazole)	70 Januvia (sitagliptin)	71 Xarelto (rivaroxaban)	72 Celecoxib (celecoxib)	73 Xarelto (rivaroxaban)	74 Tivicity (dolutegravir)	75 Tybrel (trastuzumab)	76 Toprol XL (metoprolol succinate)	77 Valproic Acid (valproic acid)	78 Protonix (pantoprazole)	79 Lantus (insulin glargine)	80 Actemra (tocilizumab)
81 Estradio (estradiol)	82 Jardiance (empagliflozin)	83 Ozempic (semaglutin)	84 Protonix (pantoprazole)	85 Avastin (bevacizumab)	86 Olanzapine (olanzapine)	87 Xylopro (propofol)	88 Lympoza (sildenafil)	89 Olanzapine (olanzapine)	90 Jardiance (empagliflozin)	91 Jardiance (empagliflozin)	92 Celecoxib (celecoxib)	93 Furosemide (furosemide)	94 Amiloride (amilofril)	95 Vingoil (vitamin E)	96 Copaxone (glatiramer acetate)	97 Desocory (desferrioxamine mesylate)	98 Tivicity (dolutegravir)	99 Ibrance (trastuzumab)	100 Myrbetriq (mirabegron)
101 Infliximab (infliximab)	102 Protonix (pantoprazole)	103 Plavix (clopidogrel)	104 Xarelto (rivaroxaban)	105 Advair (salmeterol/fluticasone propionate)	106 Novorapid (insulin aspart)	107 Keppra (levetiracetam)	108 Synthroid (levothyroxine)	109 Symbicort (budesonide/formoterol fumarate dihydrate)	110 Protonix (pantoprazole)	111 Protonix (pantoprazole)	112 Jardiance (empagliflozin)	113 Trexall (methotrexate)	114 Tivicity (dolutegravir)	115 Tivicity (dolutegravir)	116 Edoxaban (edoxaban)	117 Abilify (aripiprazole)	118 Xarelto (rivaroxaban)	119 Jardiance (empagliflozin)	120 Spring (sildenafil)
121 Mirna (mirna)	122 Jardiance (empagliflozin)	123 Opanol (oxycodone)	124 Gilead (sofosbuvir)	125 Humira (adalimumab)	126 Olanzapine (olanzapine)	127 Jardiance (empagliflozin)	128 Abilify (aripiprazole)	129 Simponi (tocilizumab)	130 Novorapid (insulin aspart)	131 Broc Hista (hydrochlorothiazide)	132 Jardiance (empagliflozin)	133 Revlimid (lenalidomide)	134 Orkambi (cysteamine hydrochloride)	135 Venkoll (venlafaxine)	136 Eylea (aflibercept)	137 Jardiance (empagliflozin)	138 Semultra (sildenafil)	139 Jardiance (empagliflozin)	140 Revlimid (lenalidomide)
141 Celecoxib (celecoxib)	142 Jardiance (empagliflozin)	143 Repatha (evolocumab)	144 Divosa Group (divoson)	145 Celecoxib (celecoxib)	146 Crestor (rosuvastatin)	147 Jardiance (empagliflozin)	148 Tivicity (dolutegravir)	149 Jardiance (empagliflozin)	150 Jardiance (empagliflozin)	151 Jardiance (empagliflozin)	152 Jardiance (empagliflozin)	153 Jardiance (empagliflozin)	154 Jardiance (empagliflozin)	155 Jardiance (empagliflozin)	156 Jardiance (empagliflozin)	157 Jardiance (empagliflozin)	158 Jardiance (empagliflozin)	159 Jardiance (empagliflozin)	160 Jardiance (empagliflozin)
161 Jardiance (empagliflozin)	162 Jardiance (empagliflozin)	163 Jardiance (empagliflozin)	164 Jardiance (empagliflozin)	165 Jardiance (empagliflozin)	166 Jardiance (empagliflozin)	167 Jardiance (empagliflozin)	168 Jardiance (empagliflozin)	169 Jardiance (empagliflozin)	170 Jardiance (empagliflozin)	171 Jardiance (empagliflozin)	172 Jardiance (empagliflozin)	173 Jardiance (empagliflozin)	174 Jardiance (empagliflozin)	175 Jardiance (empagliflozin)	176 Jardiance (empagliflozin)	177 Jardiance (empagliflozin)	178 Jardiance (empagliflozin)	179 Jardiance (empagliflozin)	180 Jardiance (empagliflozin)
181 Jardiance (empagliflozin)	182 Jardiance (empagliflozin)	183 Jardiance (empagliflozin)	184 Jardiance (empagliflozin)	185 Jardiance (empagliflozin)	186 Jardiance (empagliflozin)	187 Jardiance (empagliflozin)	188 Jardiance (empagliflozin)	189 Jardiance (empagliflozin)	190 Jardiance (empagliflozin)	191 Jardiance (empagliflozin)	192 Jardiance (empagliflozin)	193 Jardiance (empagliflozin)	194 Jardiance (empagliflozin)	195 Jardiance (empagliflozin)	196 Jardiance (empagliflozin)	197 Jardiance (empagliflozin)	198 Jardiance (empagliflozin)	199 Jardiance (empagliflozin)	200 Jardiance (empagliflozin)

# The complex picture of cardiovascular diseases

## Coronary Artery Disease



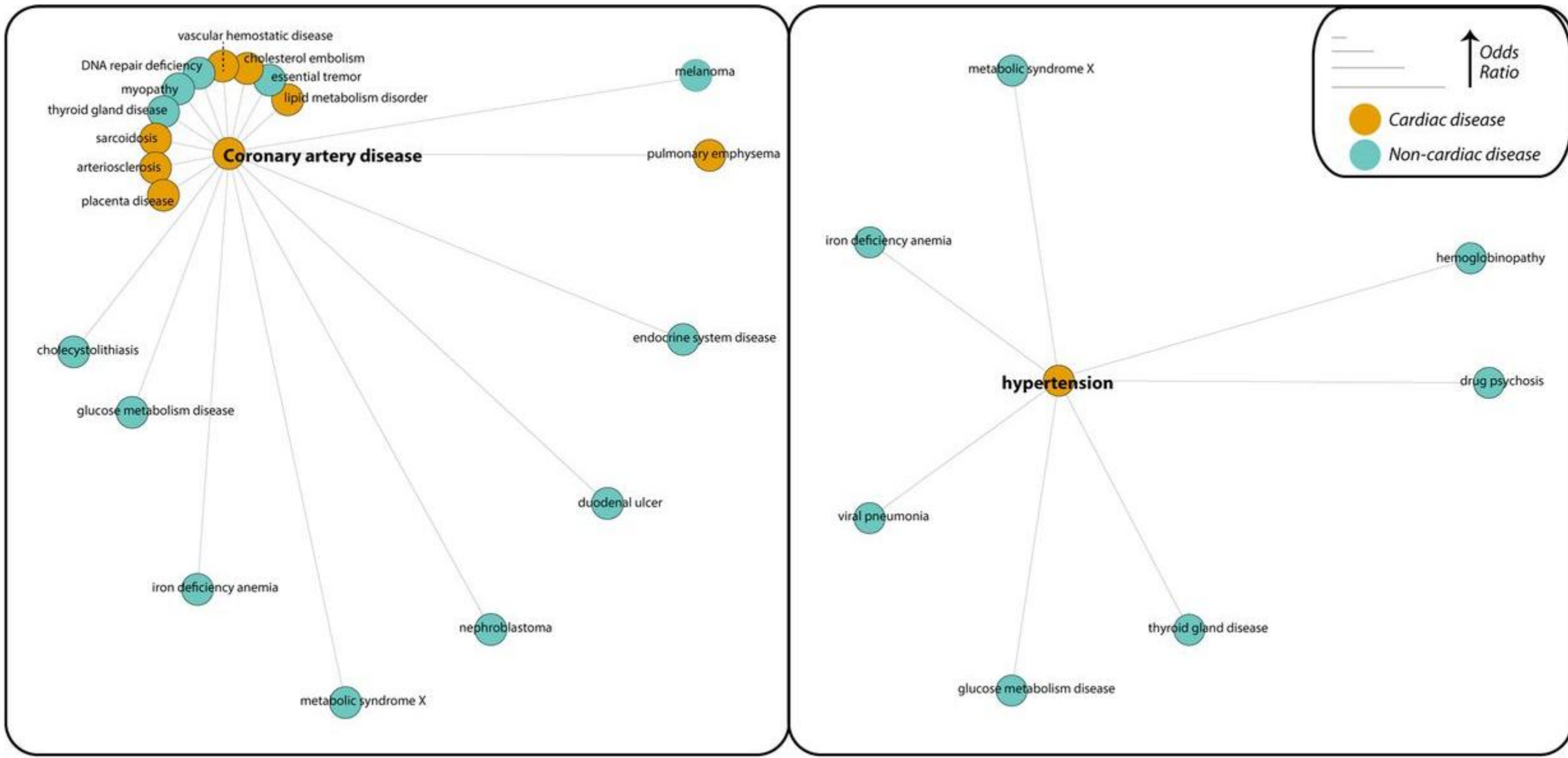
## Hypertension



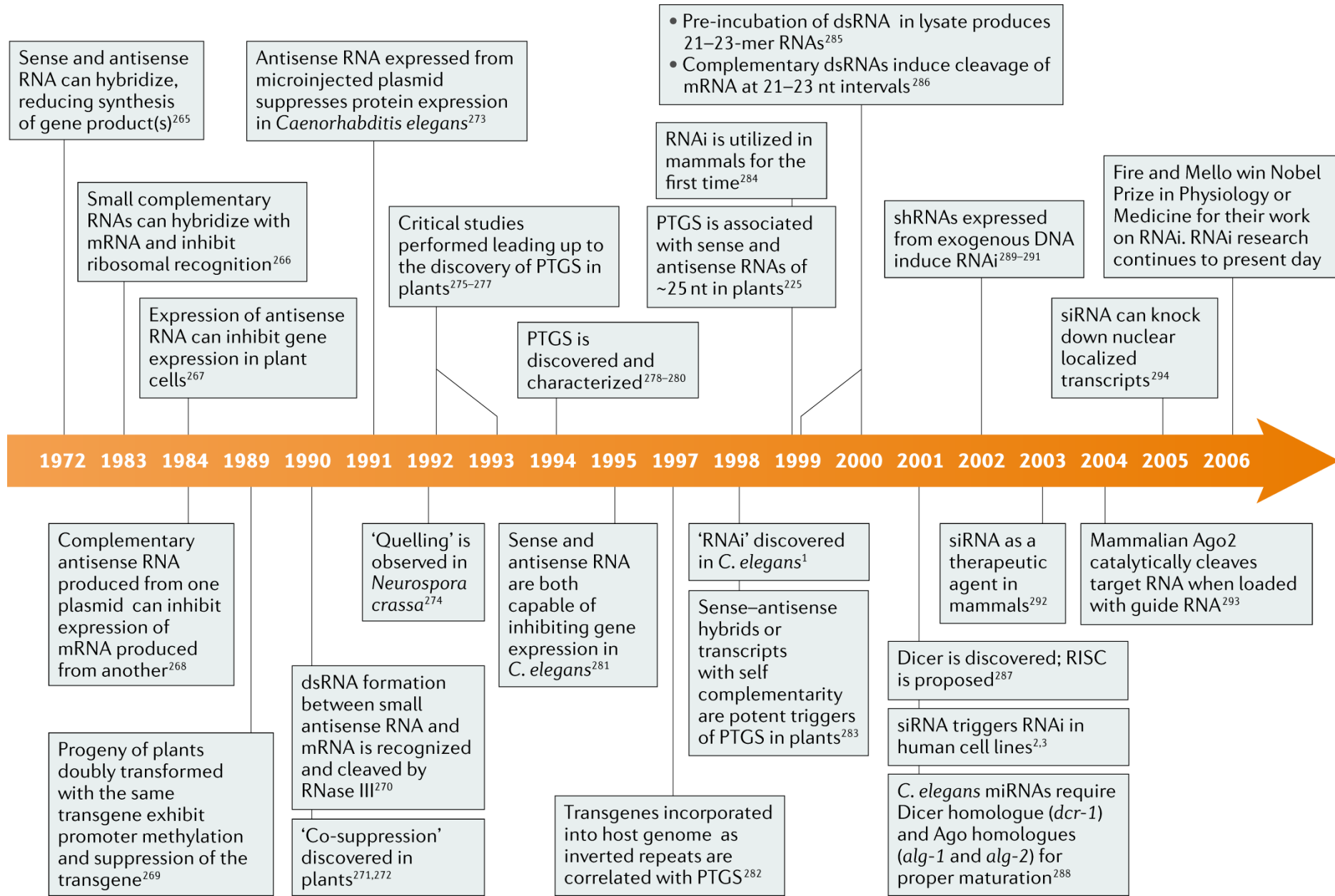
Comorbidities

# The complex picture of cardiovascular diseases

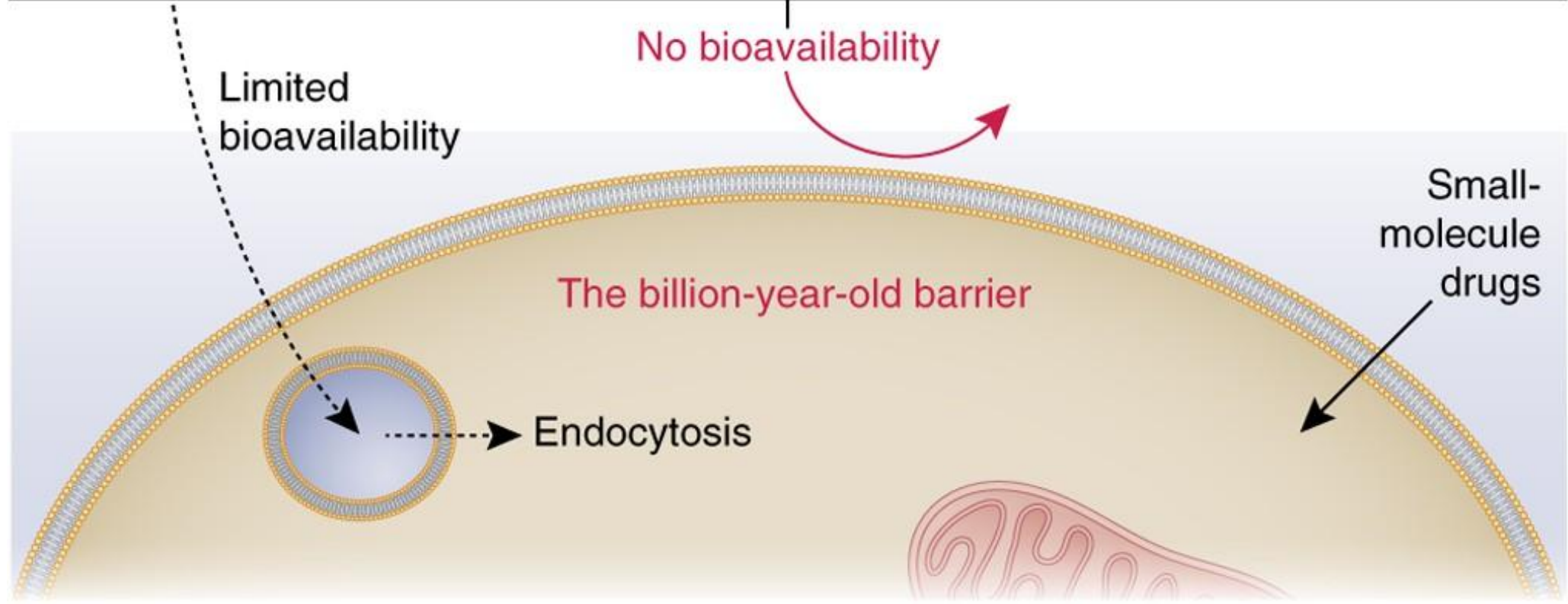
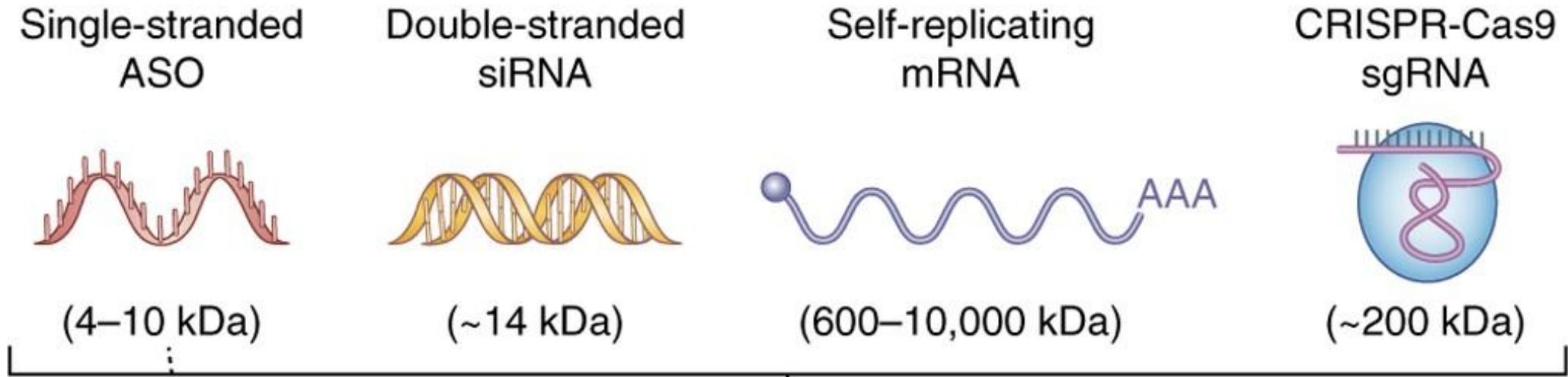
Shared Genetic Architecture



# RNA-based medications – translational view



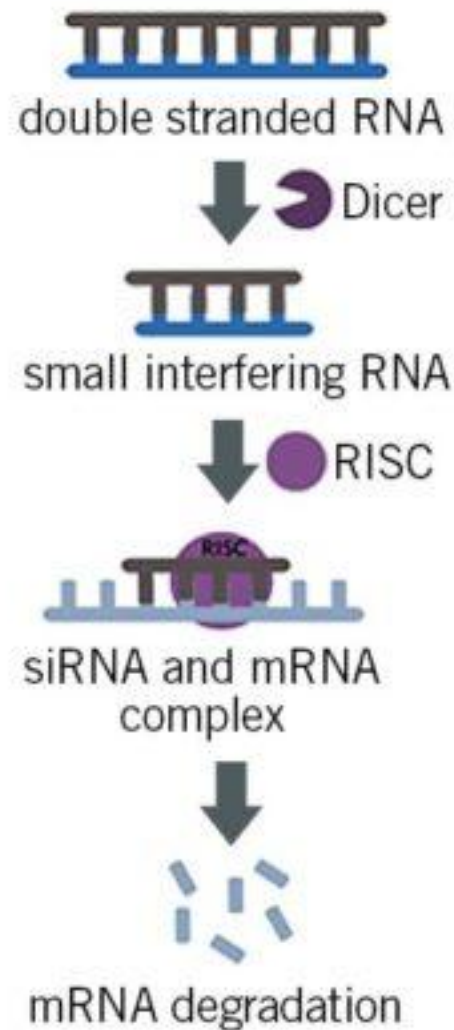
# RNA based therapeutical approaches



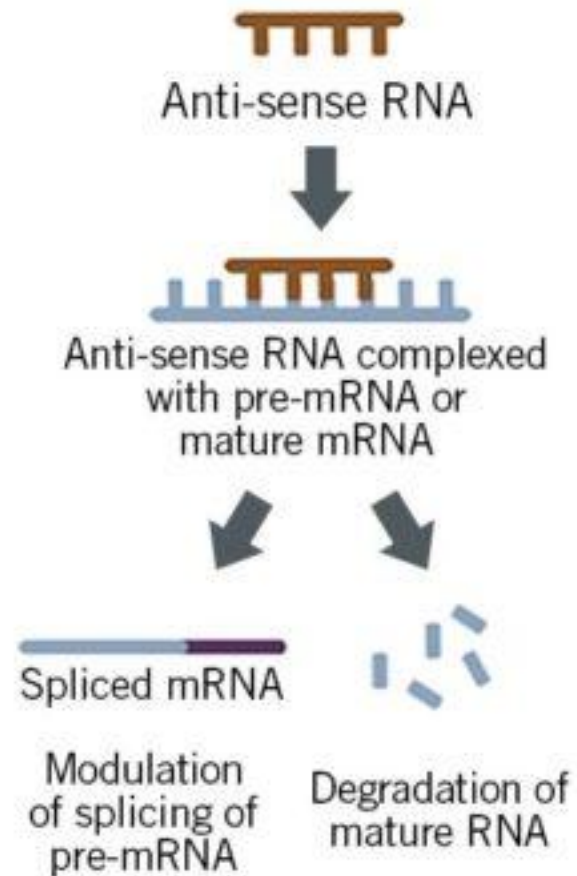


# RNA therapies

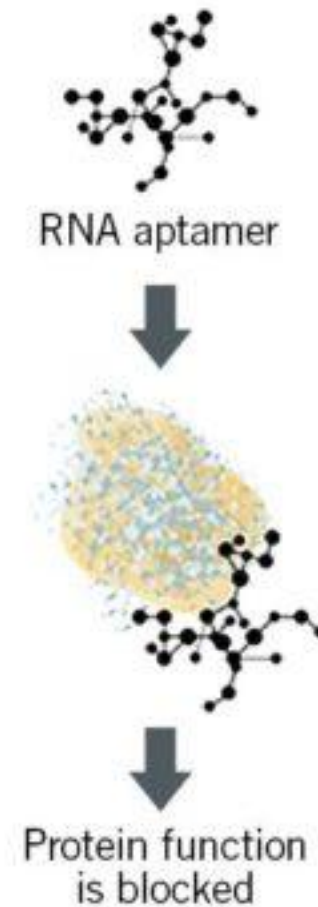
RNA interference



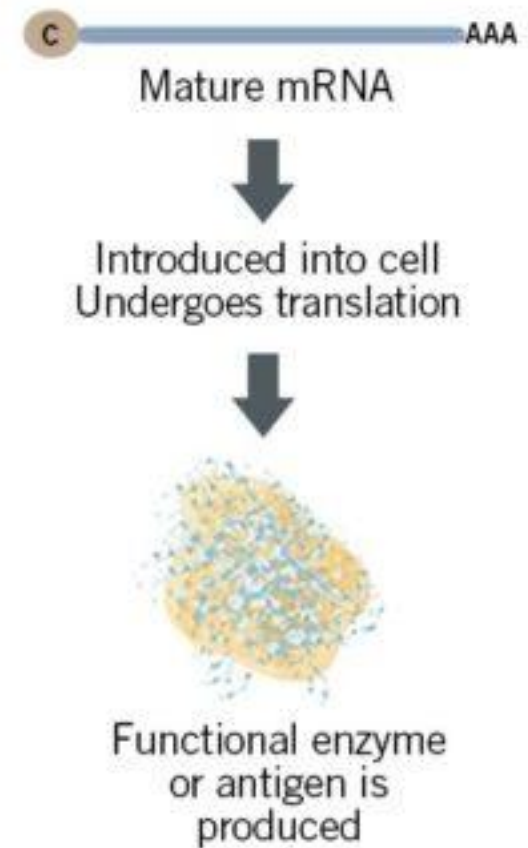
Anti-sense RNA



RNA aptamers



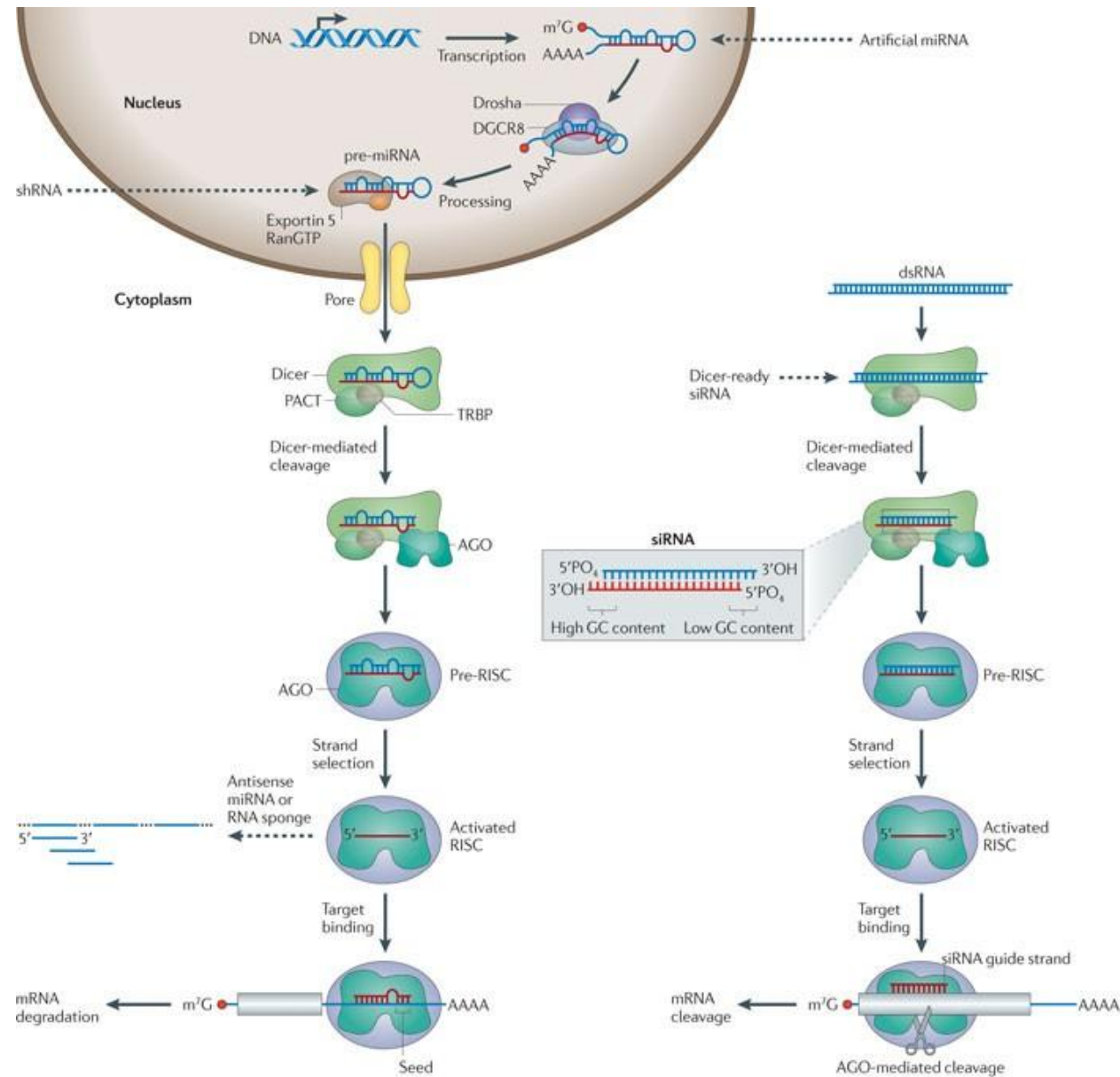
mRNA therapy



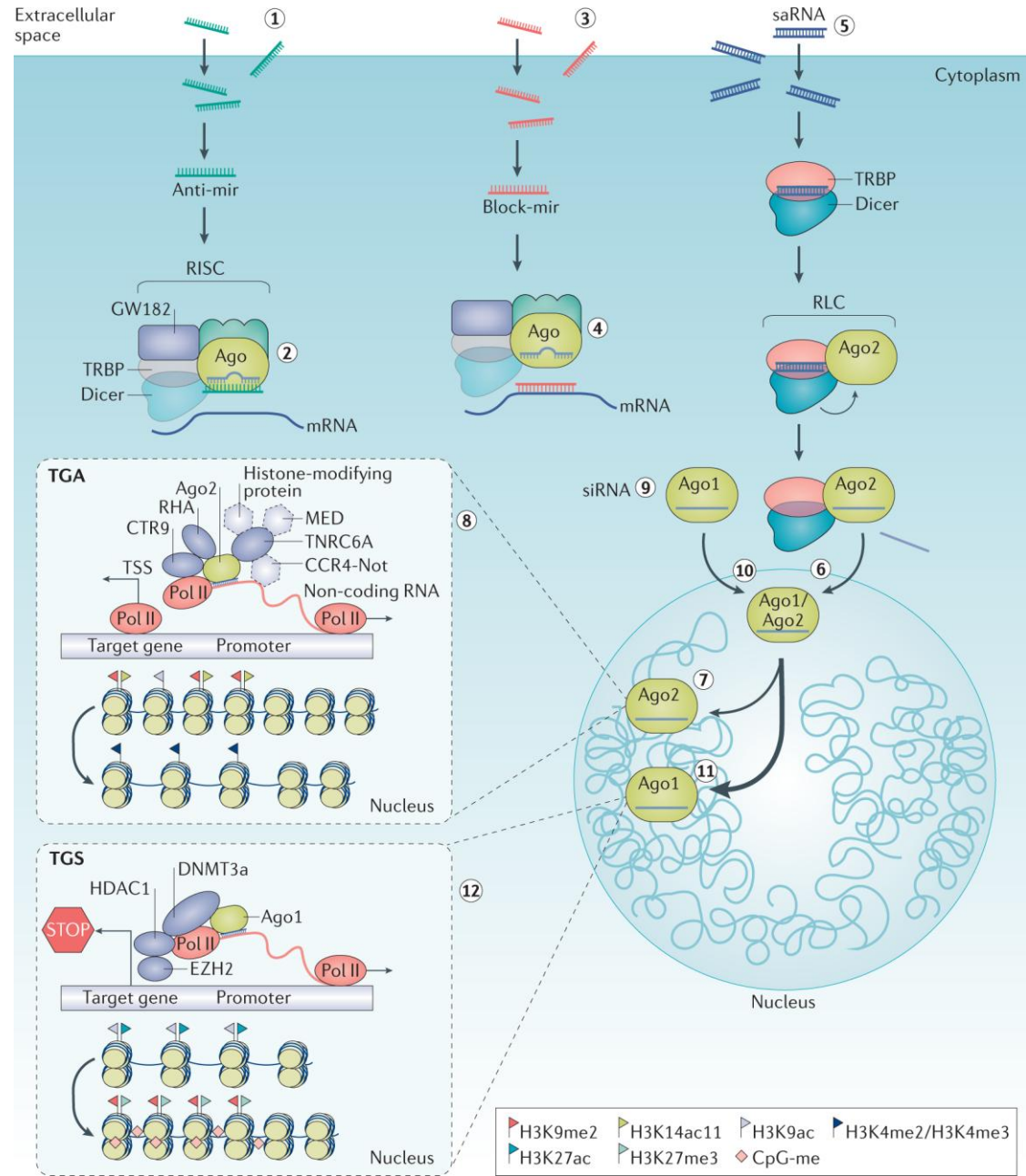
# Short therapeutic RNA types

	<b>siRNA</b>	<b>miRNA</b>
Prior to Dicer processing	Double-stranded RNA that contains 30 to over 100 nucleotides	Precursor miRNA (pre-miRNA) that contains 70-100 nucleotides with interspersed mismatches and hairpin structure
Structure	21-23 nucleotide RNA duplex with 2 nucleotides 3'overhang	19-25 nucleotide RNA duplex with 2 nucleotides 3'overhang
Complementary	Fully complementary to mRNA	Partially complementary to mRNA, typically targeting the 3' untranslated region of mRNA
mRNA target	One	Multiple (could be over 100 at the same time)
Mechanism of gene regulation	Endonucleolytic cleavage of mRNA	Translational repression Degradation of mRNA Endonucleolytic cleavage of mRNA (rare, only when there is a high level of complementary between miRNA and mRNA)
Clinical applications	Therapeutic agent	Drug target Therapeutic agent Diagnostic and biomarker tool

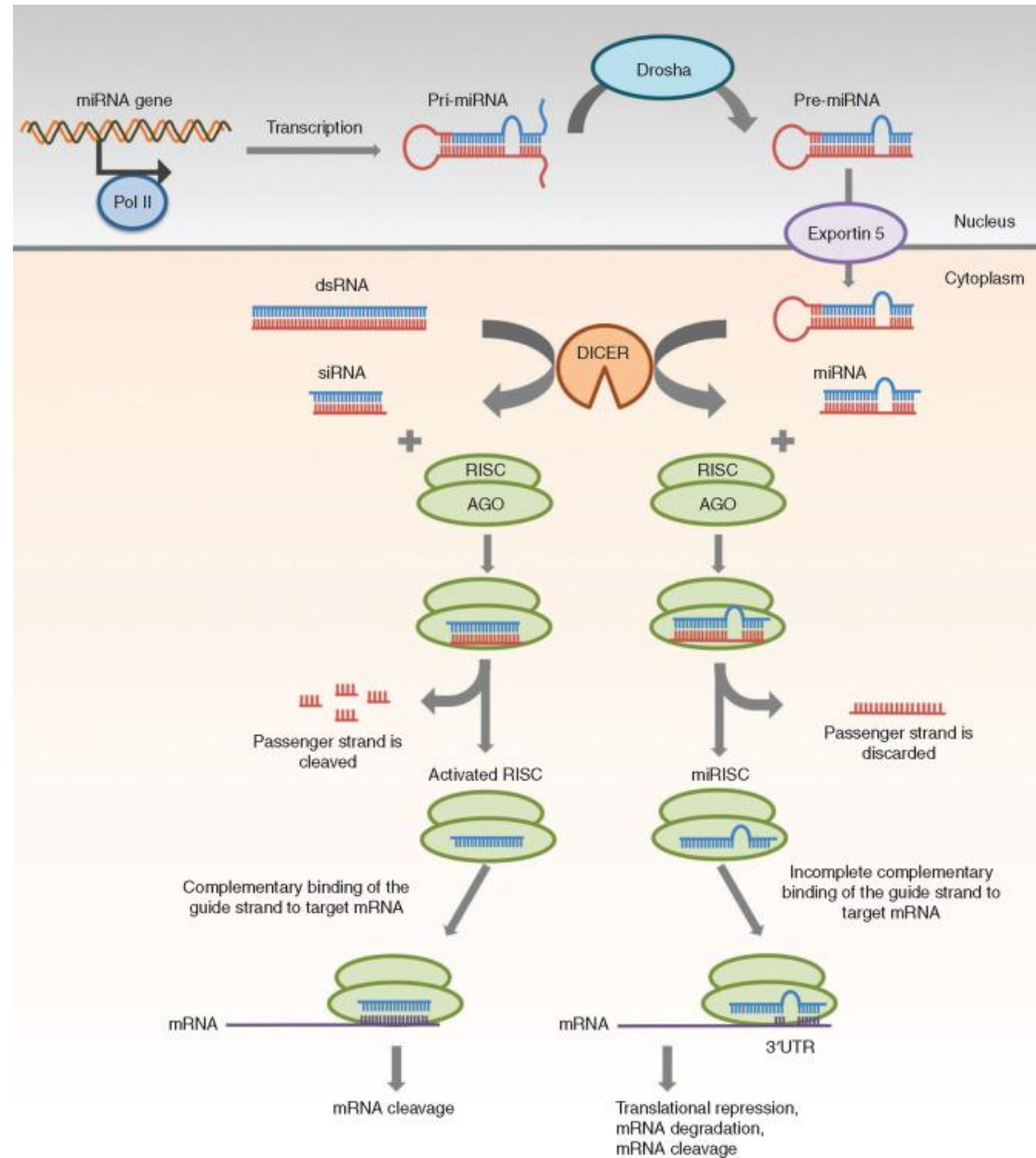
# Small interfering RNA (siRNA; 21-23, 21 mer)



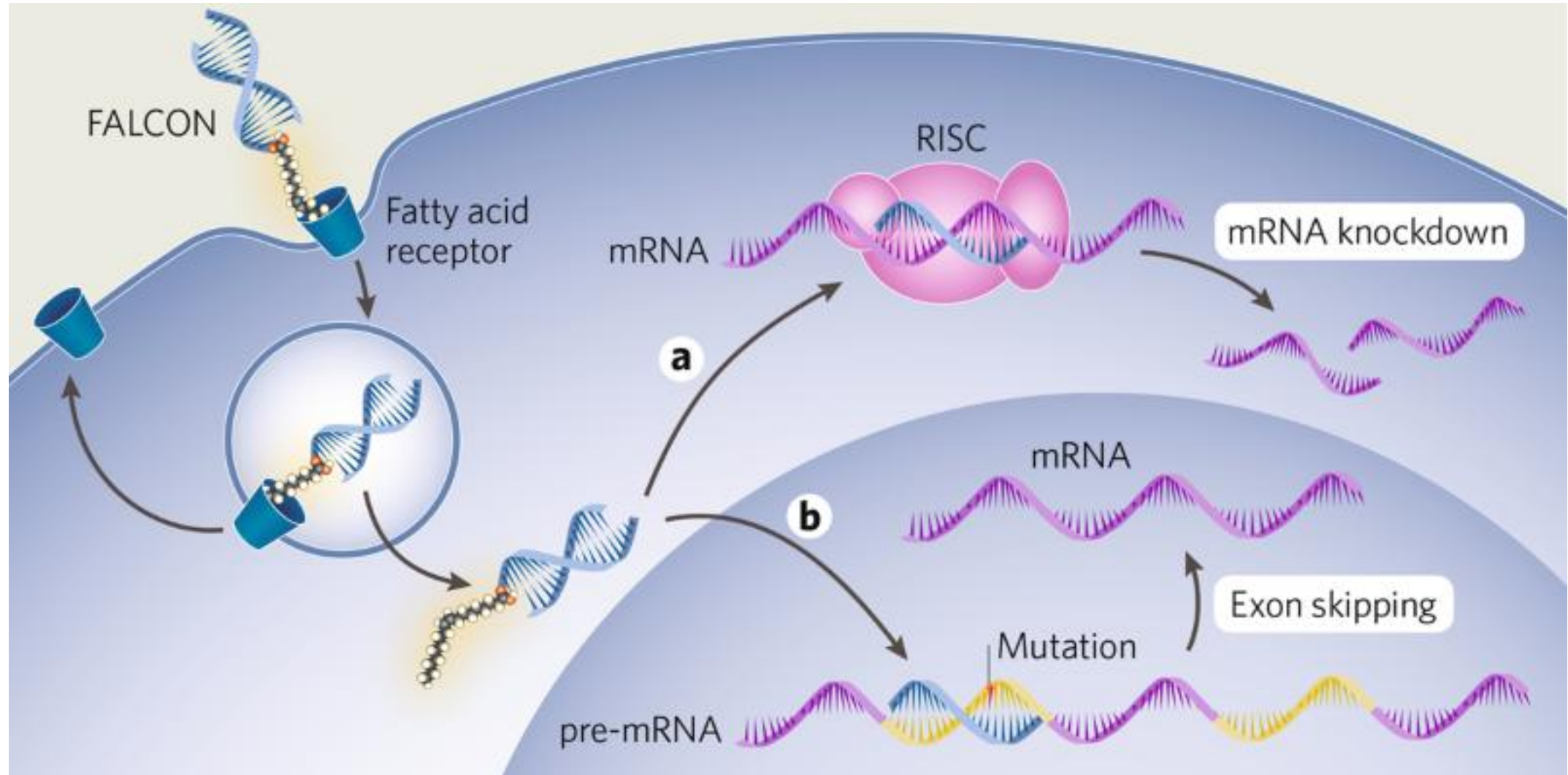
# microRNA (miRNA; 19-25 mer)



# Therapeutic short RNA – simplified mechanism



# Therapeutic RNA application – exon skipping

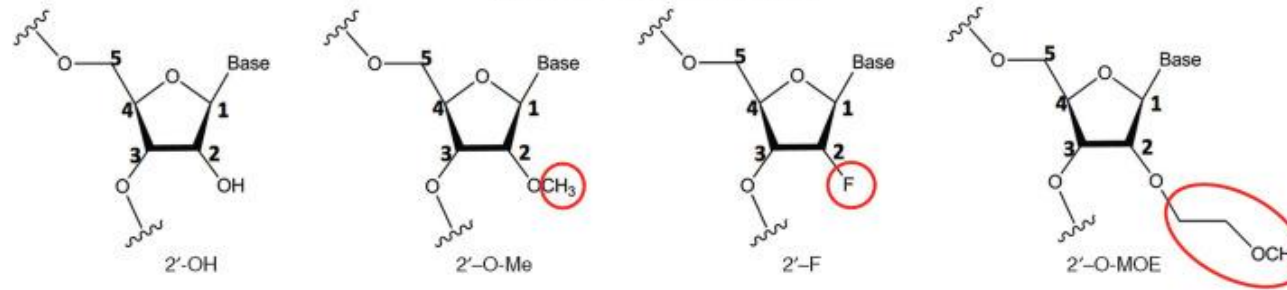


# RNA therapeutics – formulation and pharmacokinetics

Species/formulation	Packaging capacity	Applications and considerations
<b><i>Viral vector</i></b>		
Adenovirus	Up to ~35 kb, usually <10 kb	dsDNA vector with large packaging capacity, transient expression, highly immunogenic
Adeno-associated virus (AAV)	~4.5 kb	ssDNA vector, small packaging capacity, mildly immunogenic, lasting expression in nondividing cells, capsid pseudotyping/engineering facilitates specific cell-targeting
Lentivirus	Up to 13.5 kb (larger inserts will decrease titre)	RNA vector, integration competent and incompetent forms available, less immunogenic than adenovirus or AAV, envelope pseudotyping facilitates cell targeting, clinical production more difficult than for adenovirus or AAV
Herpes simplex virus	150kb	DNA vector, episomal, lasting expression, immunogenic
<b><i>Bacterial vector species</i><sup>*</sup></b>		
<i>Escherichia coli</i> , <i>S. Typhimurium</i> <sup>§</sup>		Delivery of short hairpin RNA or small interfering RNA to gut tissue
<b><i>Non-viral formulations</i><sup>  </sup></b>		
Nanoparticle		Self-assembling, may target specific receptors, requires technical expertise to prepare
Stable nucleic acid lipid particle (SNALP)		Stable for systemic delivery, broad cell-type delivery
Aptamer		Targeting of specific receptors, requires sophisticated screening to develop
Cholesterol		Stable for systemic delivery, broad cell-type delivery
*Representative references.		
*Bacterial minicells can carry plasmids, short interfering RNAs or drugs.		
§ <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium.		
The nucleic acids in non-viral carriers can be any size from small oligonucleotides to large artificial chromosomes.		

# RNA therapeutics – formulation and pharmacokinetics

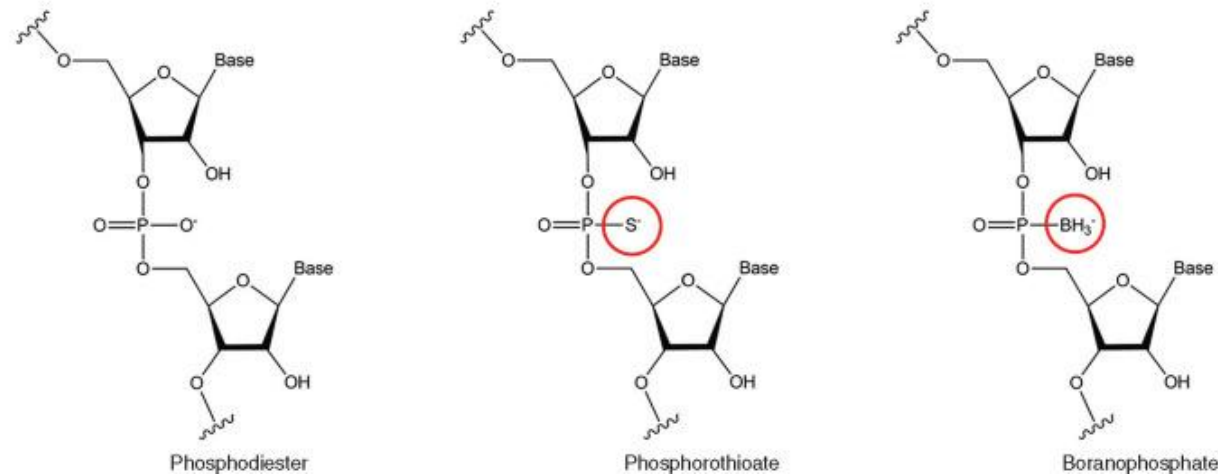
(i) Ribose 2'-OH group modification



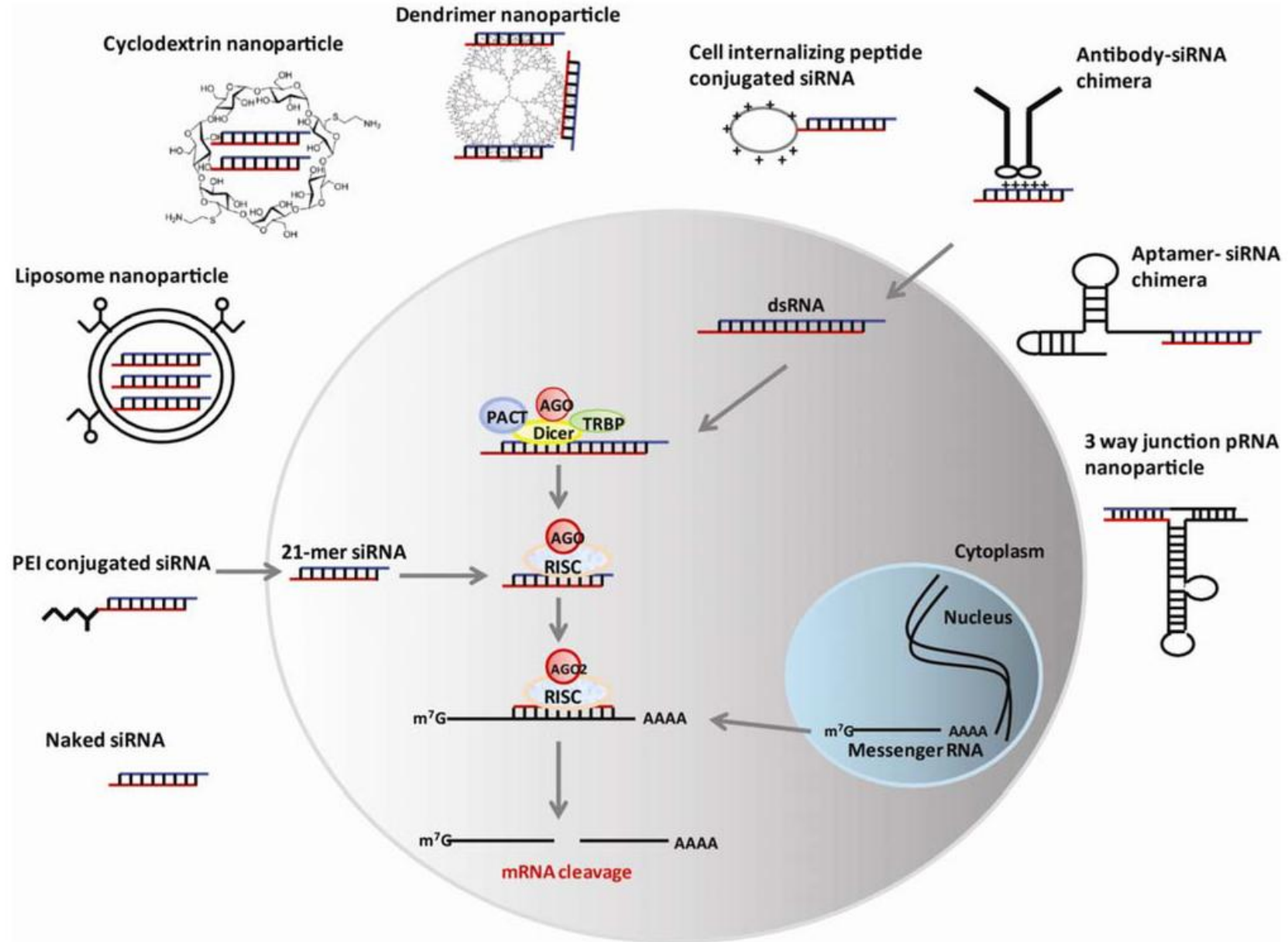
(ii) Locked nucleic acid and unlocked nucleic acid modification



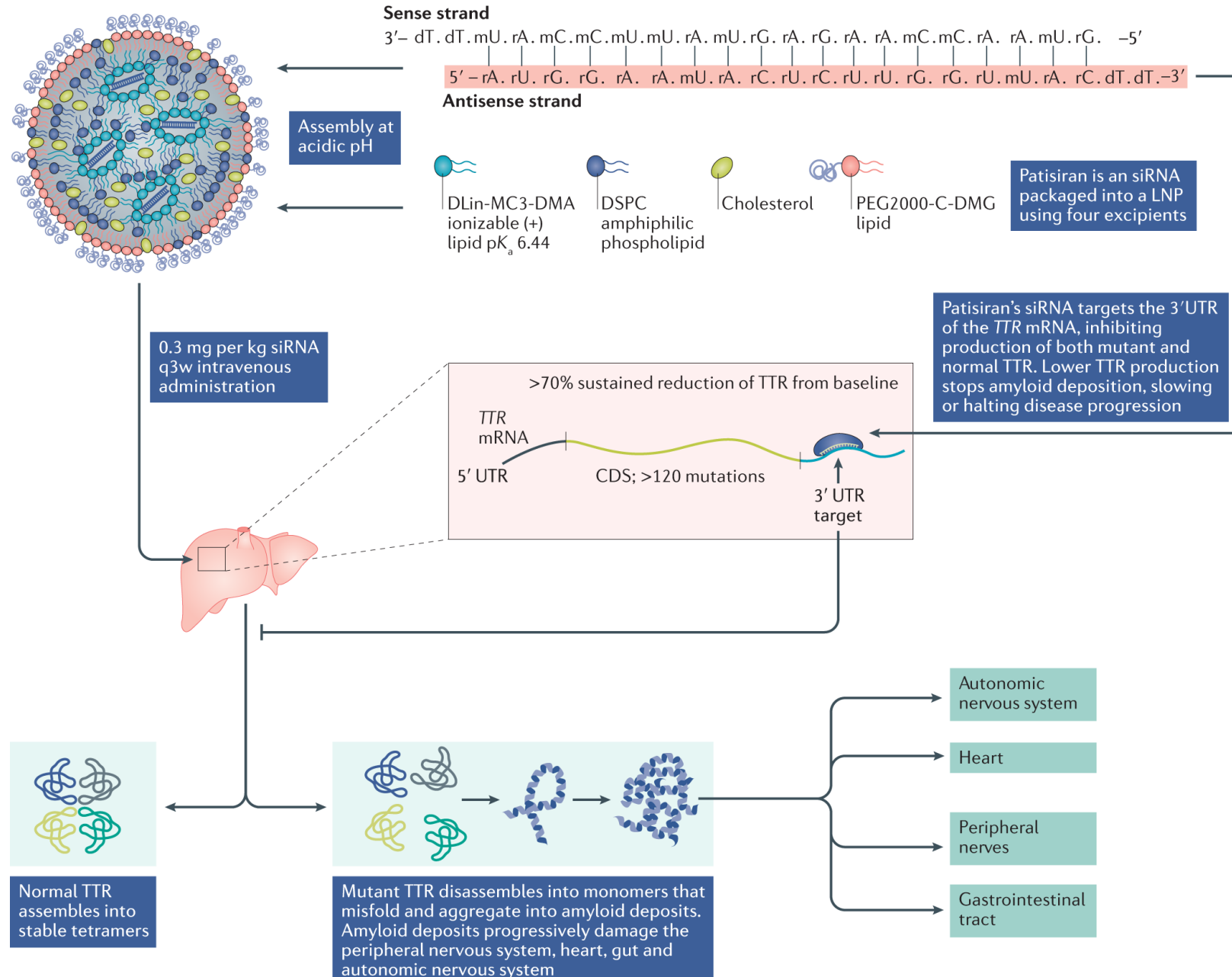
(iii) Backbone modification



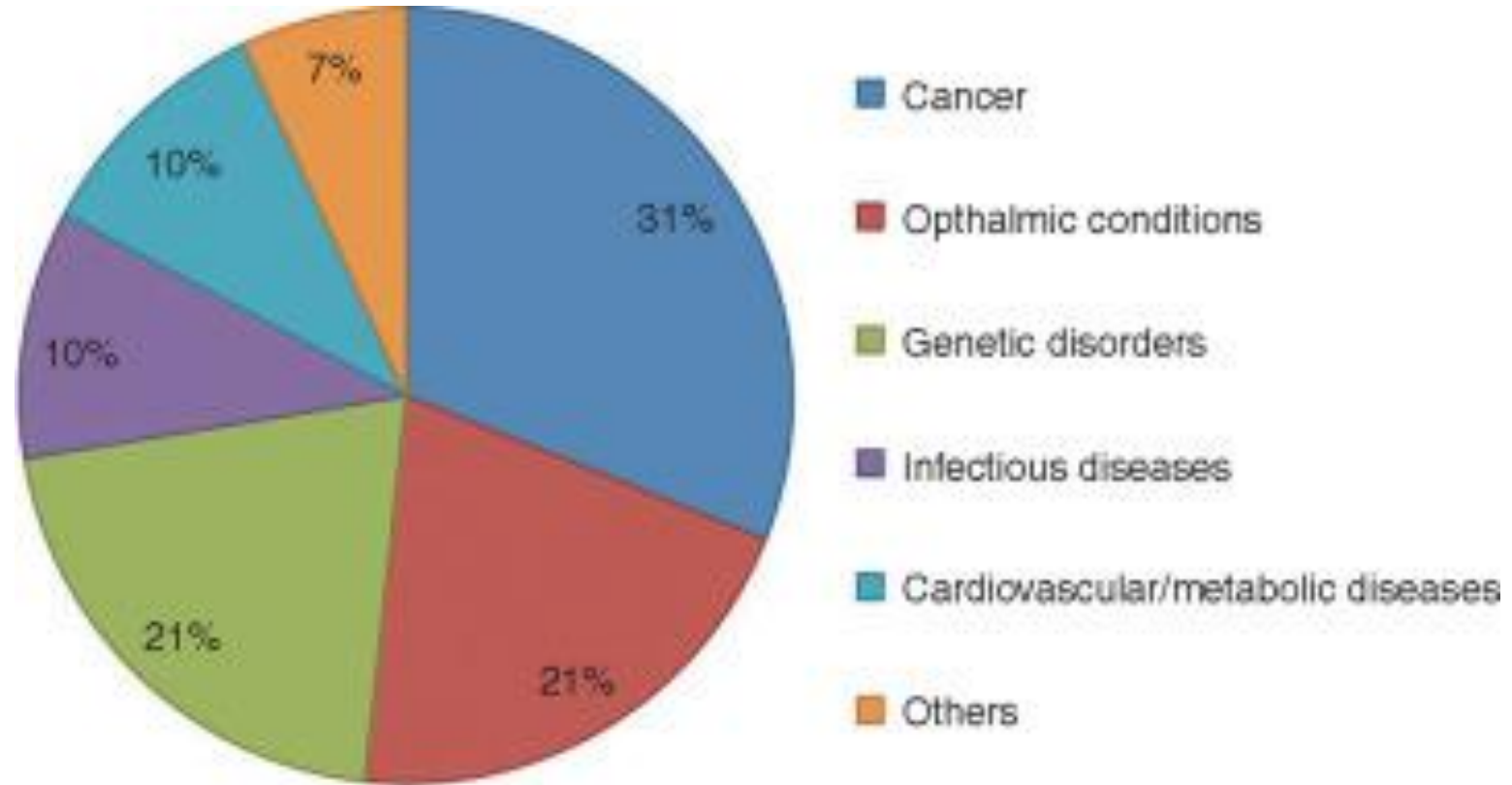
# RNA therapeutics – formulation and pharmacokinetics



# RNA therapeutics – formulation and pharmacokinetics



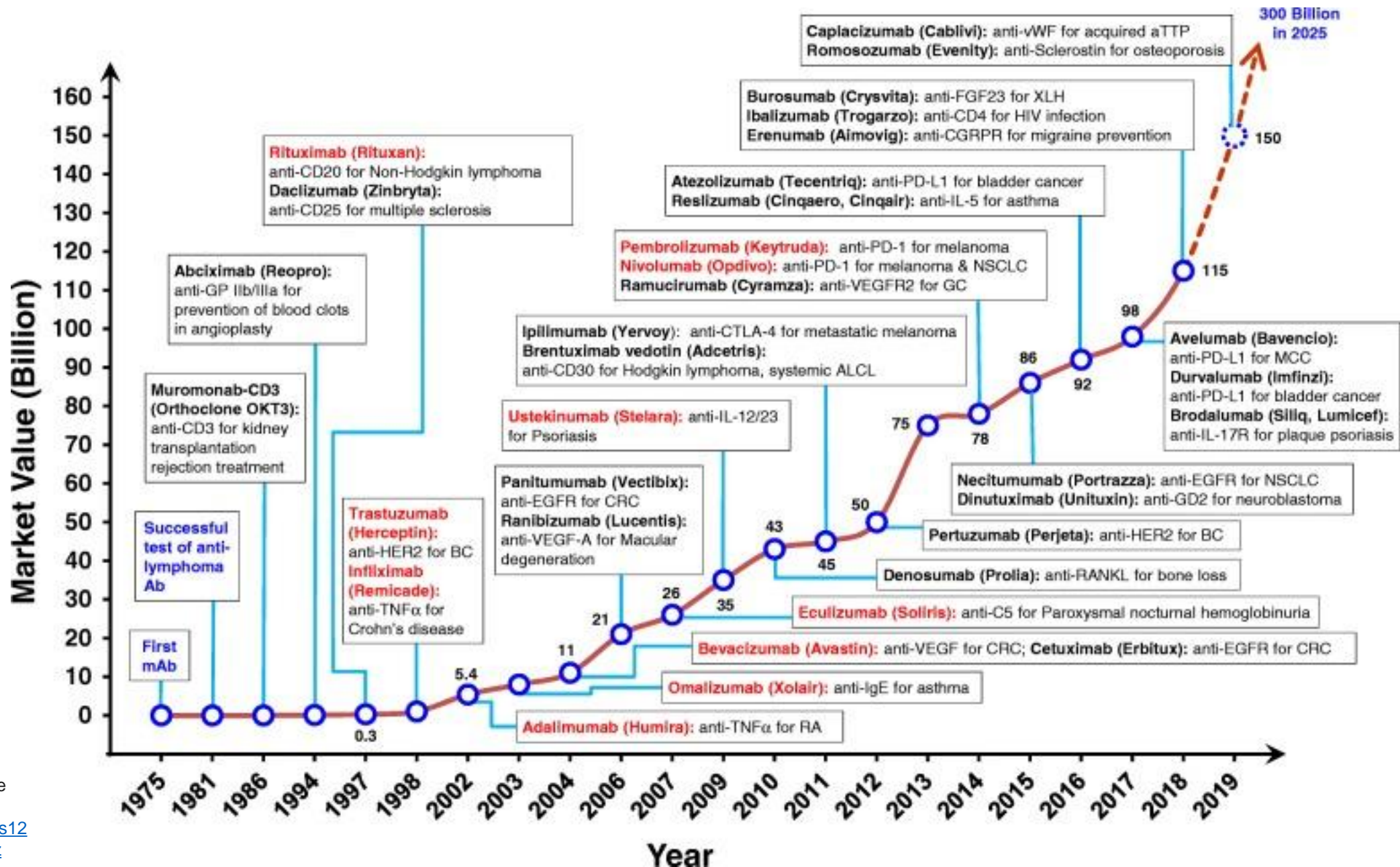
# RNA therapeutics –applications



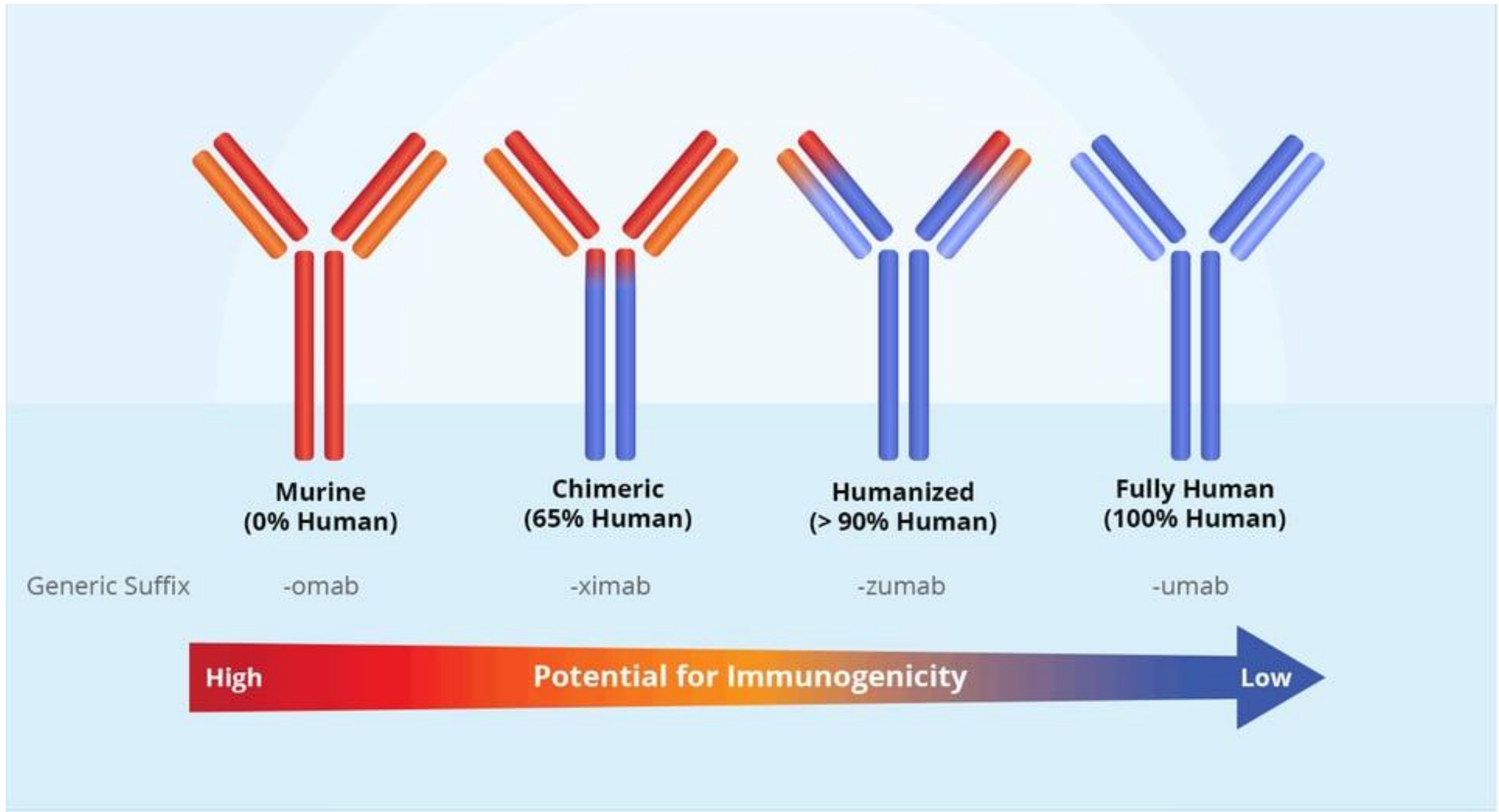
# RNA therapeutics in CVD as summarized by ChatGPT

- Antisense oligonucleotides (ASOs): These are single-stranded RNA molecules that bind to complementary sequences of target RNAs and inhibit their function by various mechanisms, such as degradation, splicing modulation, or translation inhibition. ASOs have been used to treat CVD by targeting genes involved in lipid metabolism, inflammation, fibrosis, and cardiac hypertrophy. Examples of approved ASOs for CVD are mipomersen (for familial hypercholesterolemia) and inclisiran (for hyperlipidemia).
- Small interfering RNAs (siRNAs): These are double-stranded RNA molecules that induce sequence-specific cleavage of target RNAs by the RNA interference (RNAi) pathway. siRNAs have been used to treat CVD by targeting genes involved in lipid metabolism, inflammation, angiogenesis, and cardiac remodeling. Examples of approved siRNAs for CVD are patisiran and givosiran (for hereditary transthyretin-mediated amyloidosis).
- Aptamers: These are single-stranded RNA molecules that fold into complex three-dimensional structures and bind to specific targets with high affinity and specificity. Aptamers have been used to treat CVD by targeting proteins involved in coagulation, platelet aggregation, inflammation, and angiogenesis. Examples of approved aptamers for CVD are pegnivacogin and anivamersen (for acute coronary syndrome).
- Messenger RNAs (mRNAs): These are single-stranded RNA molecules that encode proteins and are translated by ribosomes. mRNAs have been used to treat CVD by delivering therapeutic proteins or antigens to cells or tissues. mRNAs have been used to induce angiogenesis, cardiomyocyte regeneration, immunomodulation, and vaccination. Examples of approved mRNAs for CVD are none yet, but several candidates are in clinical trials.

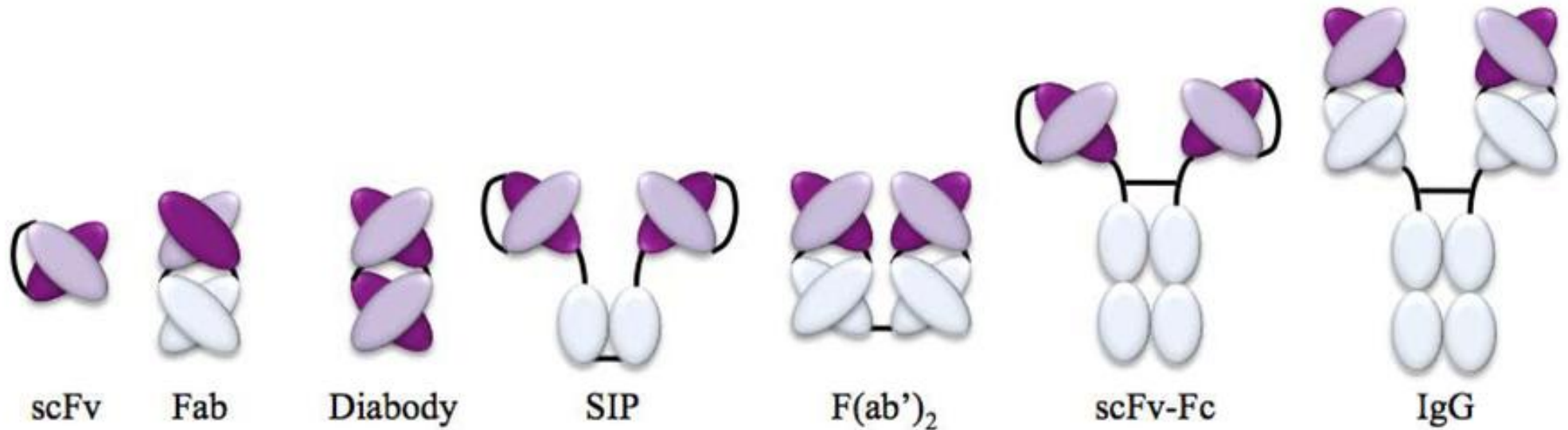
# Monoclonal antibody-based drugs



# Monoclonal antibody-based drugs

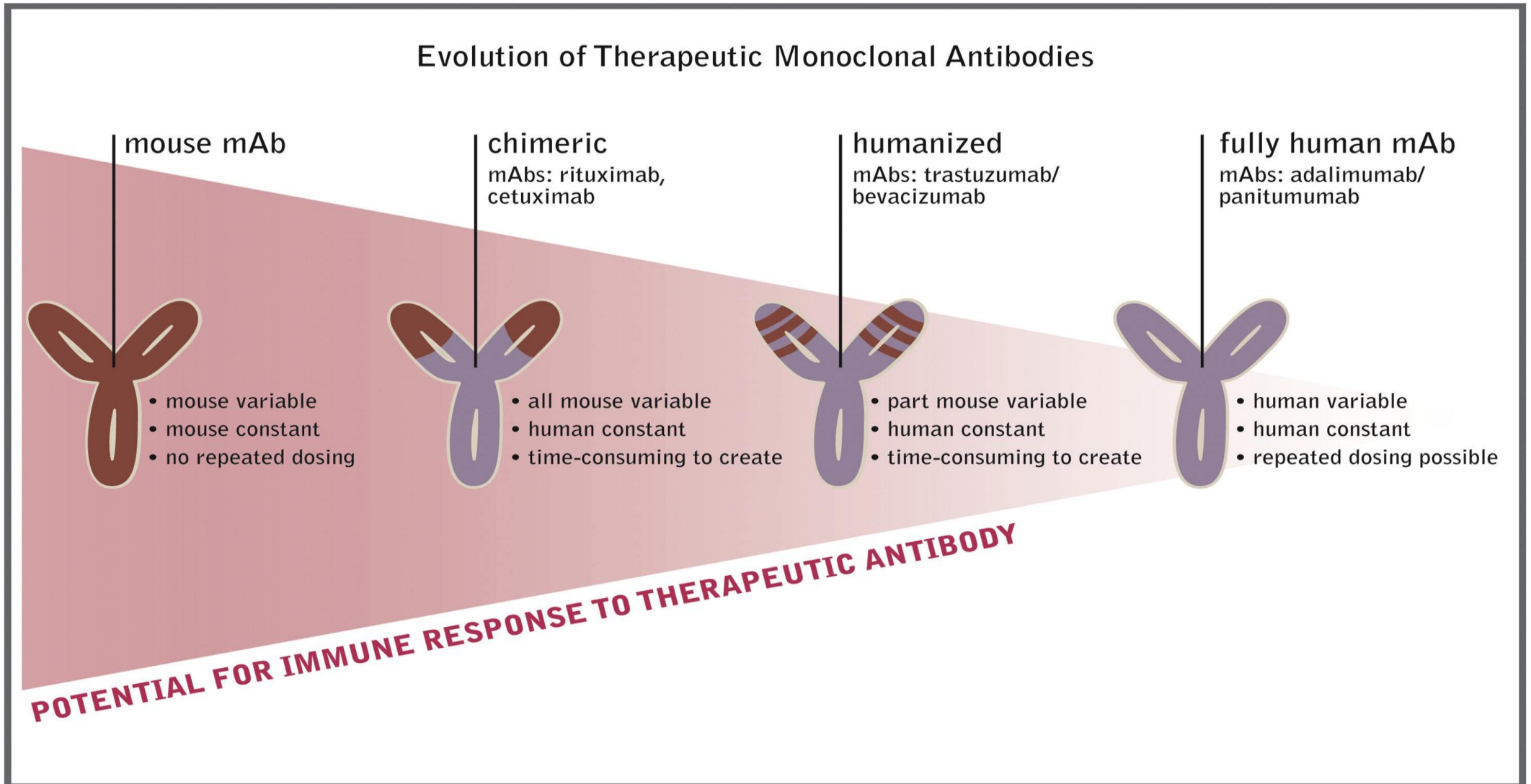


# Monoclonal antibody-based drugs

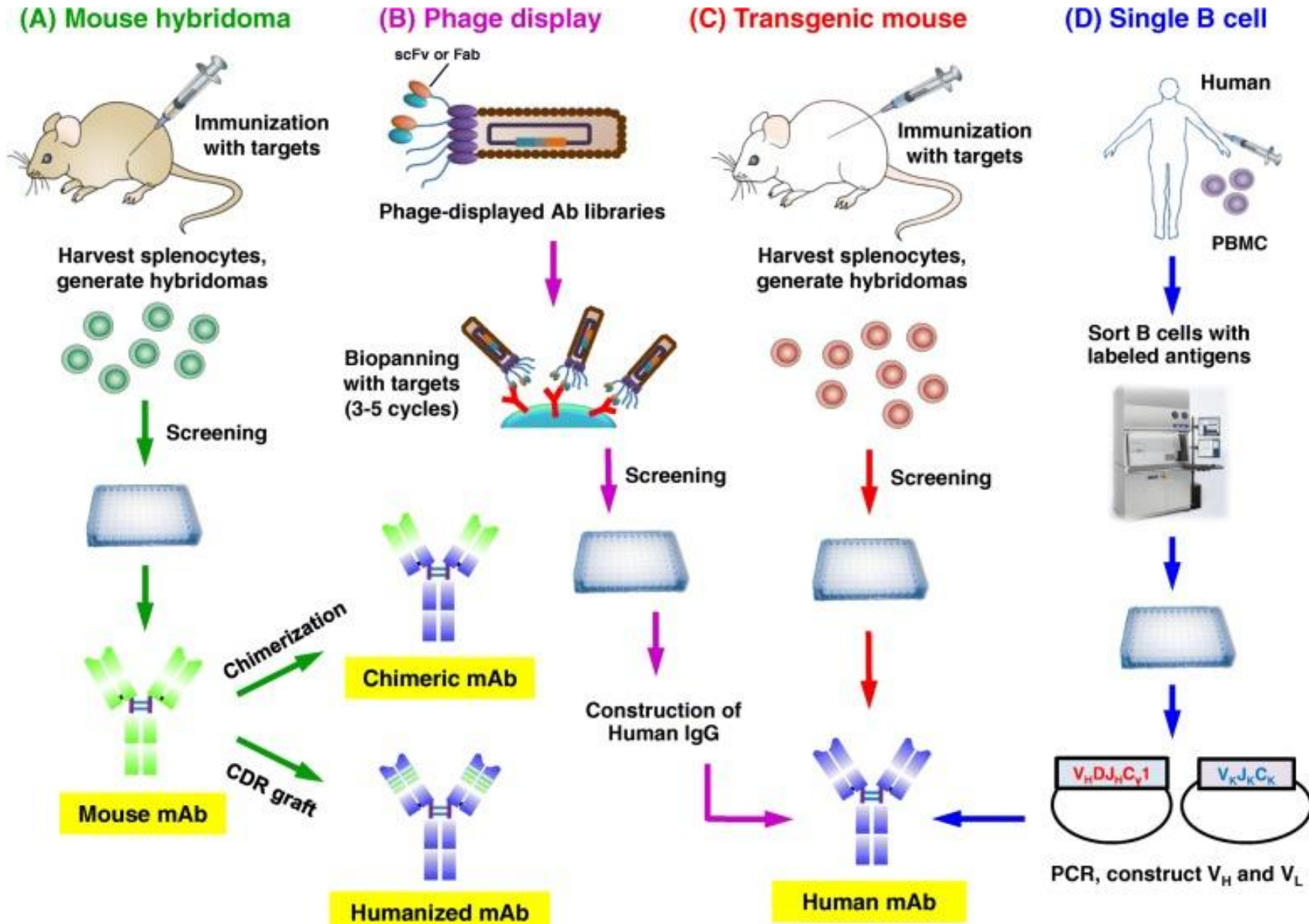


Mw (kDa)	28	50	55	80	110	110	150
Avidity	monovalent			bivalent			
Fc receptors	no FcRn binding					FcRn binding	
Clearance rate	Decreases from left to right						
Tumor uptake	Increases from left to right						

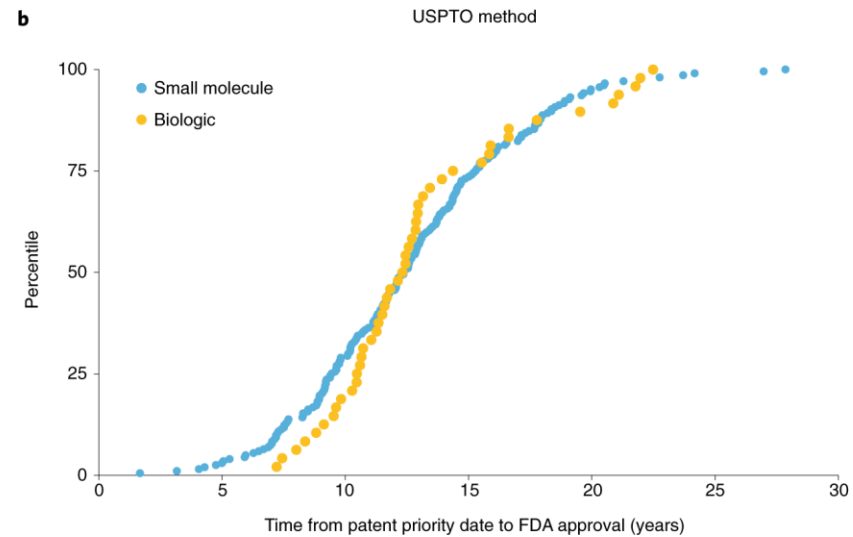
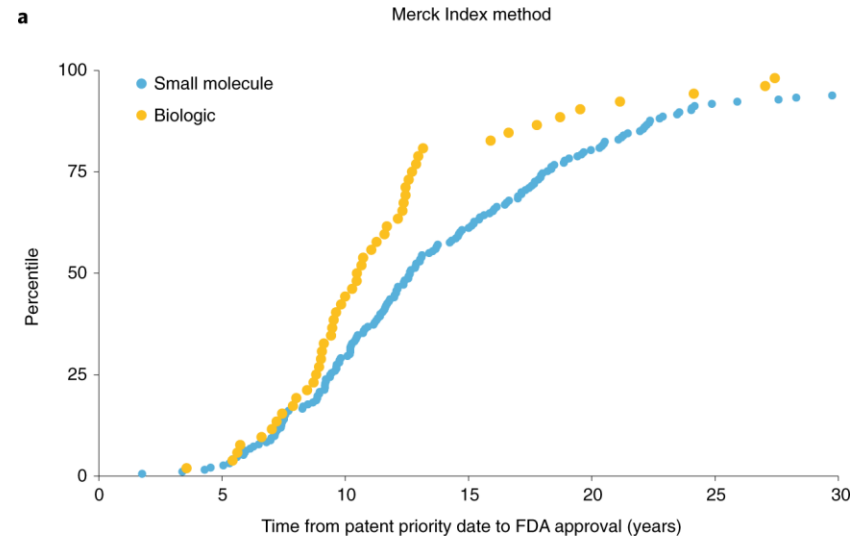
# Monoclonal antibody-based drugs



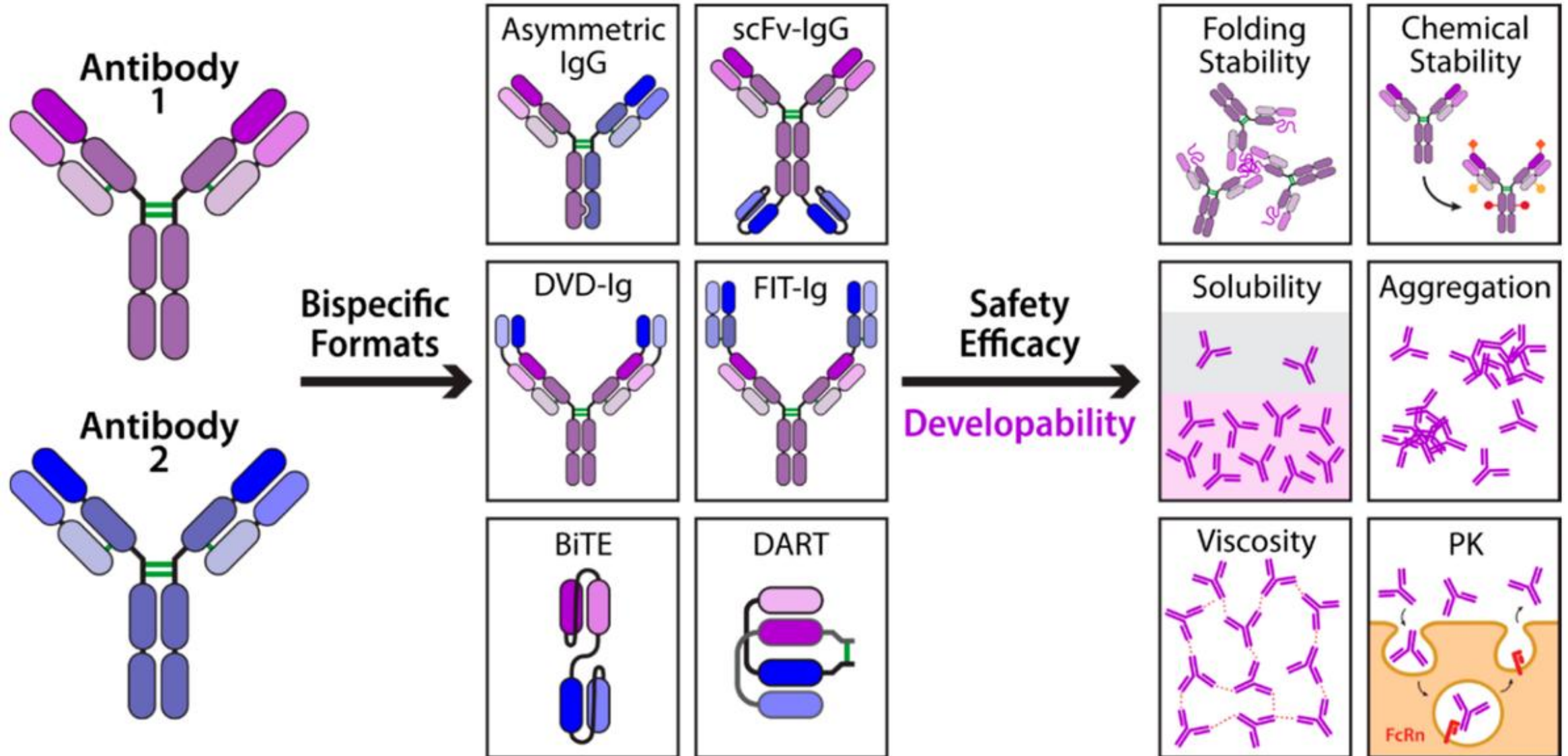
# Monoclonal antibody-based drugs – development strategies



# Monoclonal antibody-based drugs – development time






# Monoclonal antibody-based drugs – pharmacokinetics



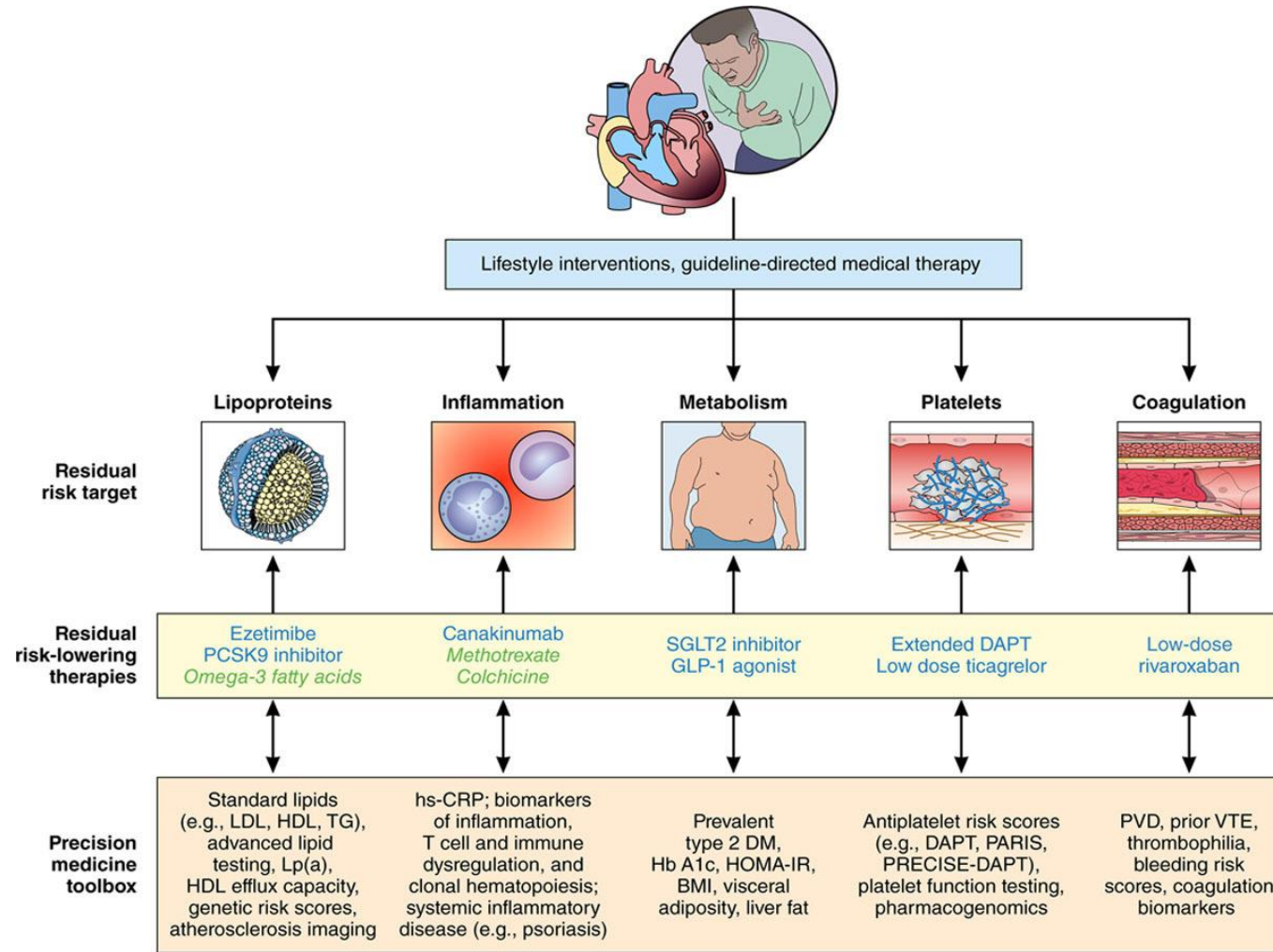
# Monoclonal antibody-based drugs – risks

B. Flühmann et al.

European Journal of Pharmaceutical Sciences 128 (2019) 73–80

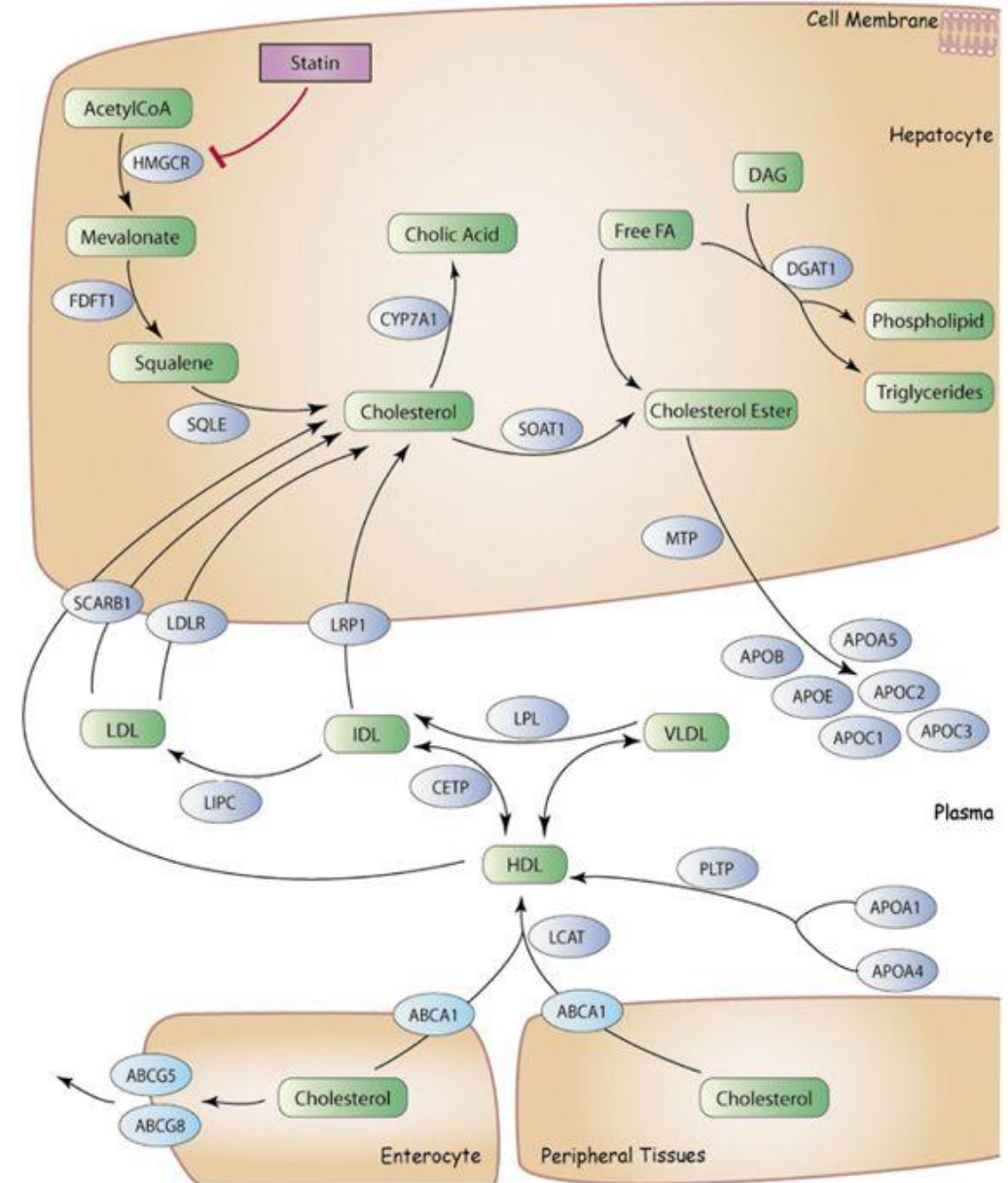
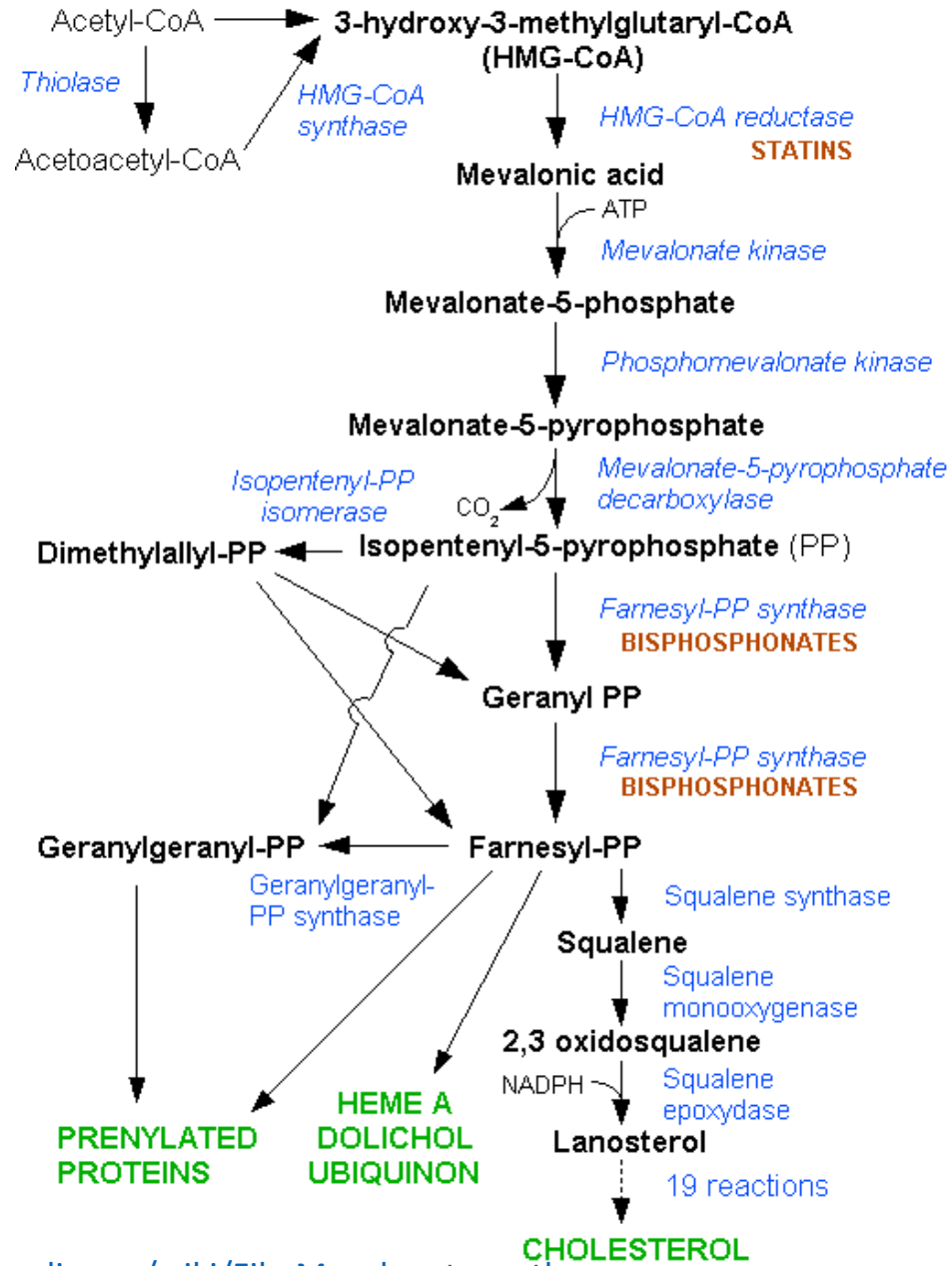
	 <b>SMALL MOLECULE DRUGS</b>	 <b>BIOLOGICS</b>	 <b>NBCDs</b>
<b>Molecular weight</b>	Low (<500)	High (range 5-900 kDa)	
<b>Structure</b>	Simple, well-defined	Complex, heterogeneous, defined by manufacturing process	
<b>Modifications</b>	Well-defined	Many options	
<b>Manufacturing</b>	Chemical synthesis	Produced in living cells or organisms	Synthetic technologies <i>(incl. nanotech)</i>
<b>Stability</b>	Stable	Generally unstable, sensitive to external conditions	
<b>Immunogenicity</b>	Mostly non-immunogenic	Mostly immunogenic	Immunogenicity varies
<b>Copy characteristics</b>	Identical copies can be made	Impossible to ensure identical copy versions	

# Monoclonal antibody-based drugs in cardiovascular medicine

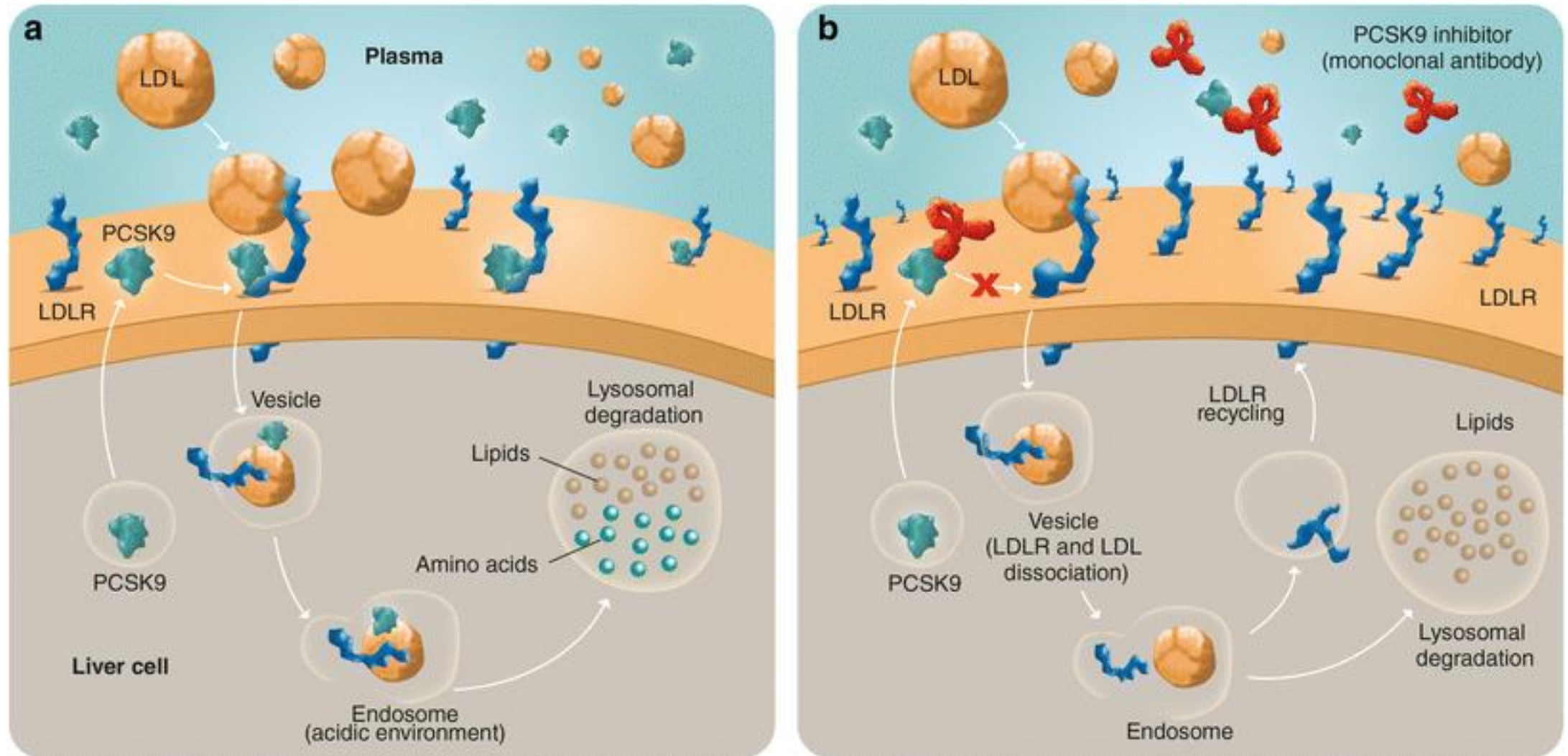


Kershaw V. Patel. Circulation. Conceptual Framework for Addressing Residual Atherosclerotic Cardiovascular Disease Risk in the Era of Precision Medicine, Volume: 137, Issue: 24, Pages: 2551-2553, DOI: (10.1161/CIRCULATIONAHA.118.035289)

# Lipid-lowering therapy (small molecule, statins)



# Lipid-lowering therapy (mAb, PCSK-9 inhibitors)



# Monoclonal antibody-based therapeutics in CVD as summarized by ChatGPT

- Alirocumab and evolocumab are monoclonal antibodies that bind to PCSK9 and prevent its interaction with the low-density lipoprotein receptor (LDLR), thereby increasing LDLR expression and reducing low-density lipoprotein cholesterol (LDL-C) levels. These antibodies have been approved by the FDA for the treatment of hypercholesterolemia in patients who are not adequately controlled by statins or who have familial hypercholesterolemia. They have also shown to reduce the risk of cardiovascular events in clinical trials.
- Canakinumab is a monoclonal antibody that inhibits interleukin-1 beta (IL-1 $\beta$ ), a pro-inflammatory cytokine that plays a role in atherosclerosis and plaque rupture. Canakinumab has been approved for the treatment of rare auto-inflammatory syndromes, and has been tested in a large randomized trial (CANTOS) for the prevention of recurrent cardiovascular events in patients with a history of myocardial infarction and elevated C-reactive protein levels. The trial showed a significant reduction in cardiovascular mortality and non-fatal myocardial infarction, but also an increased risk of fatal infections.
- Inclisiran is a monoclonal antibody that targets apolipoprotein B (apoB), the main protein component of LDL-C and other atherogenic lipoproteins. Inclisiran inhibits the synthesis of apoB by binding to its mRNA and inducing its degradation. Inclisiran has been shown to lower LDL-C levels by up to 50% in phase 2 trials, and is currently being evaluated in phase 3 trials for cardiovascular outcomes.
- Other monoclonal antibodies that target inflammatory cytokines, such as tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), interleukin-17 (IL-17), and interleukin-12/23 (IL-12/23), have been used for the treatment of autoimmune diseases, such as rheumatoid arthritis, psoriasis, and Crohn's disease. Some of these antibodies may also have beneficial effects on cardiovascular diseases, as inflammation is a key driver of atherosclerosis and its complications. However, the evidence for their efficacy and safety in cardiovascular settings is limited and inconsistent, and further studies are needed to determine their role in this field.