New translational perspectives in cardiovascular medicine

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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.

www.nobelprize.org

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

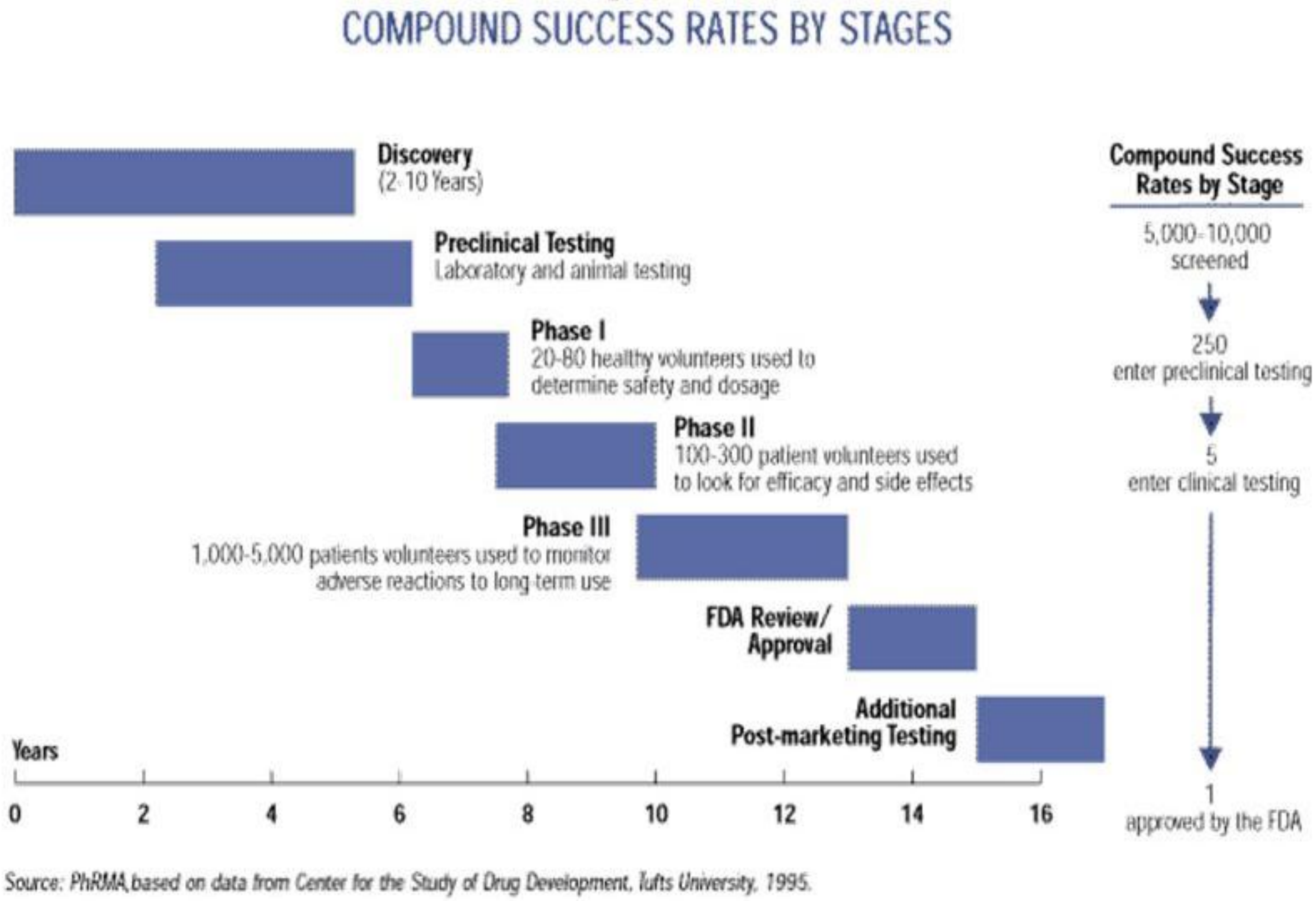
Phase 1 (T1): move basic discovery to clinical application

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.

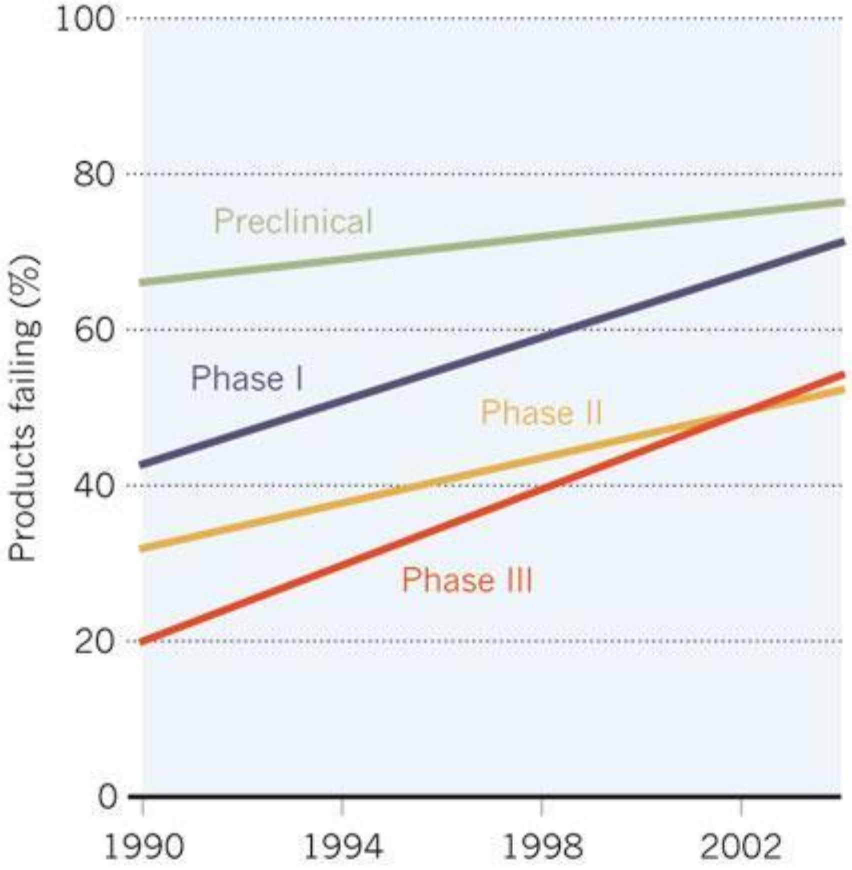
Drug development



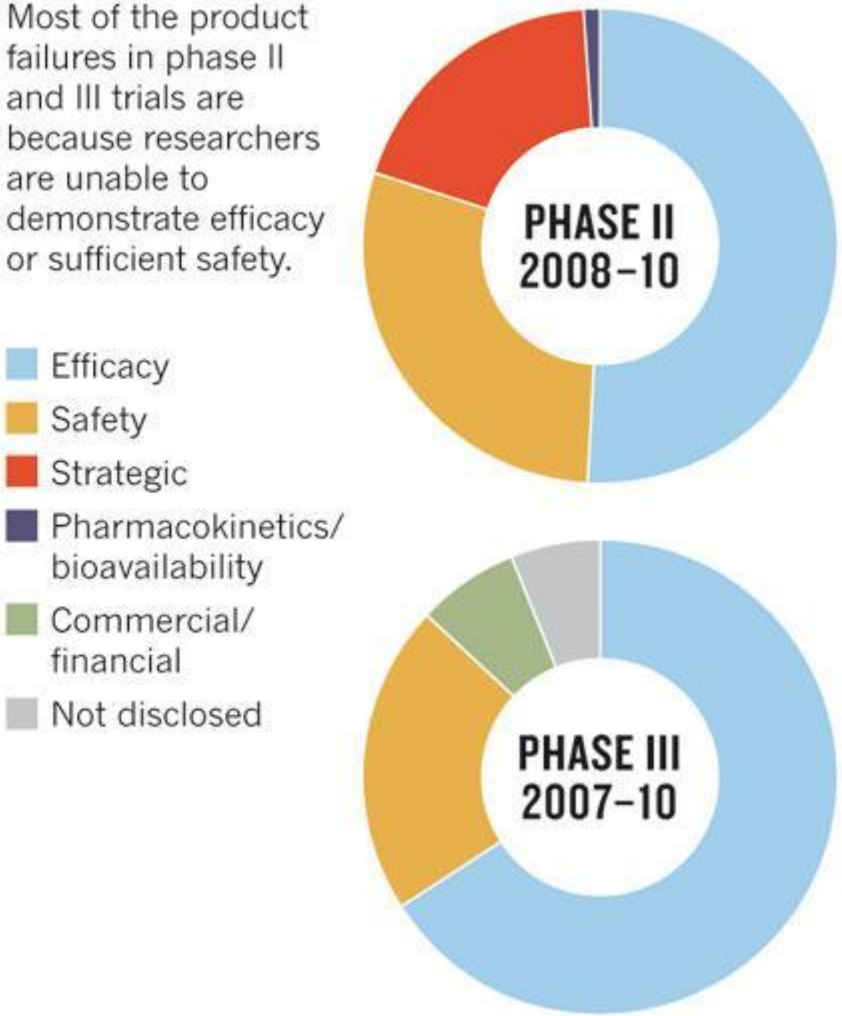
THE CLINICAL-TRIAL CLIFF

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.

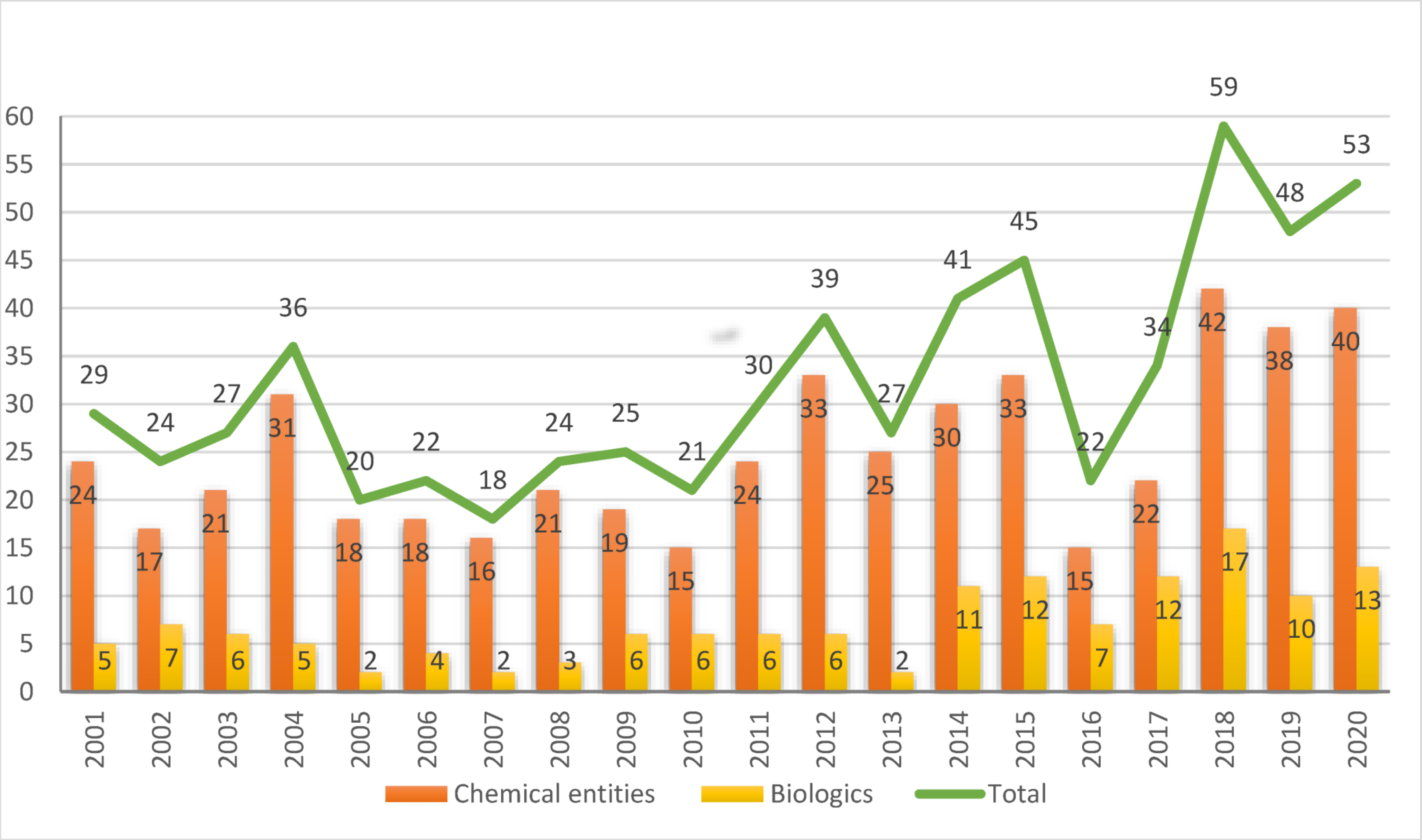


Nature 477, 526-528 (2011)

Modern pharmacology

Properties	**Small molecules**	**Protein-based drugs**	**siRNA/miRNA-based drugs**
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

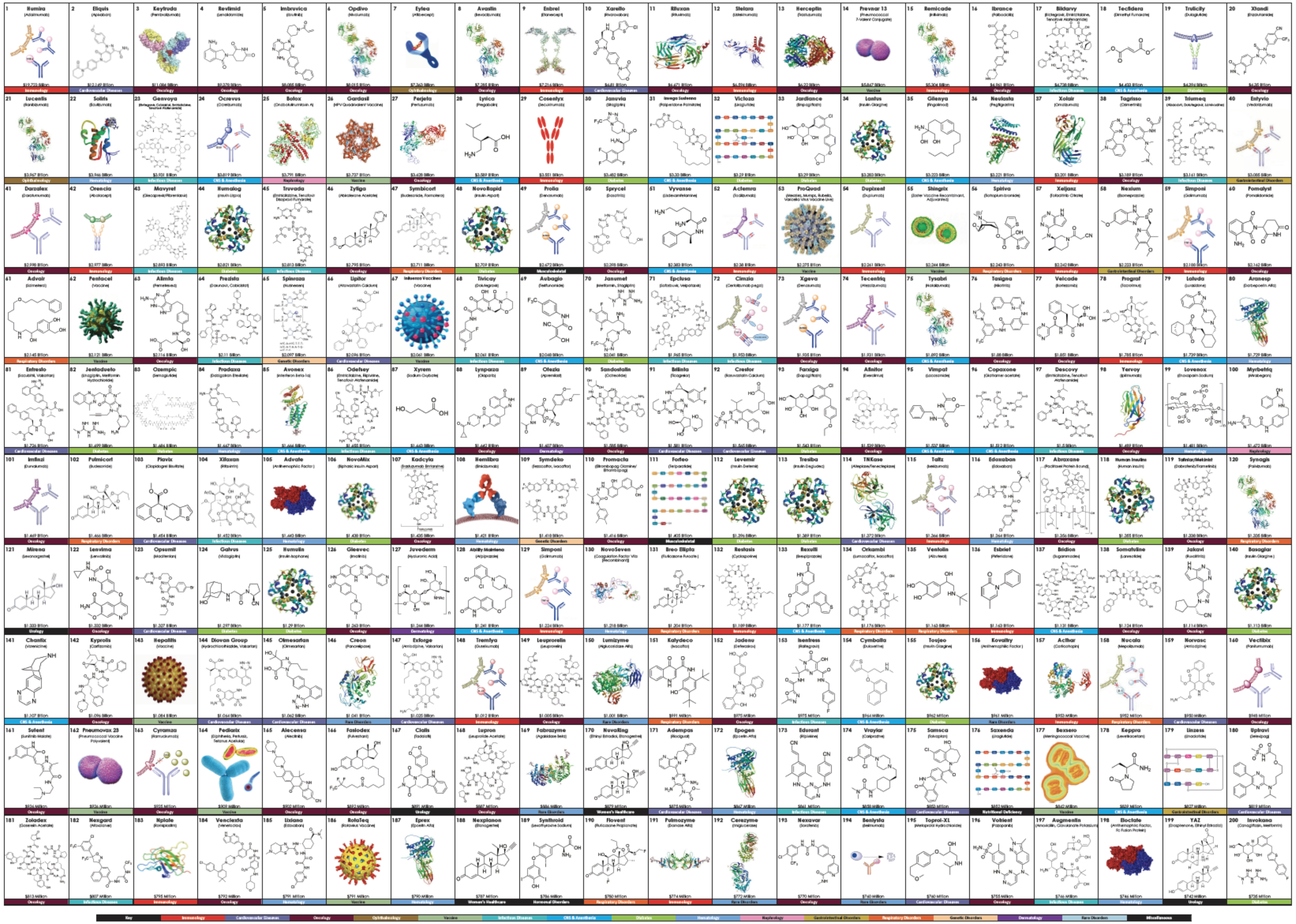
Small molecules - biologicals



Molecules **2021**, *26*(3), 627; https://doi.org/10.3390/molecules26030627

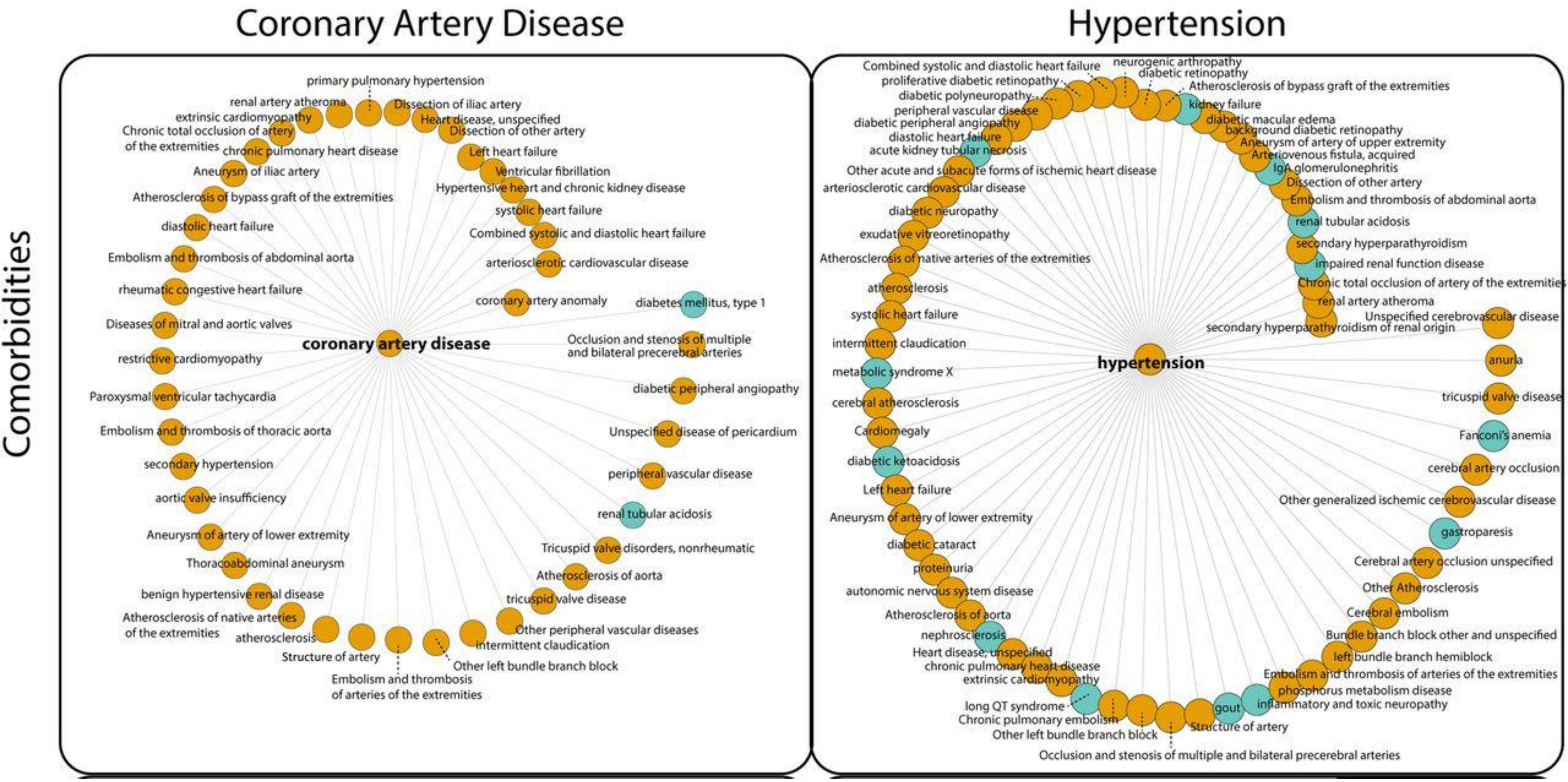
Top 200 Pharmaceuticals by Retail Sales in 2019

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

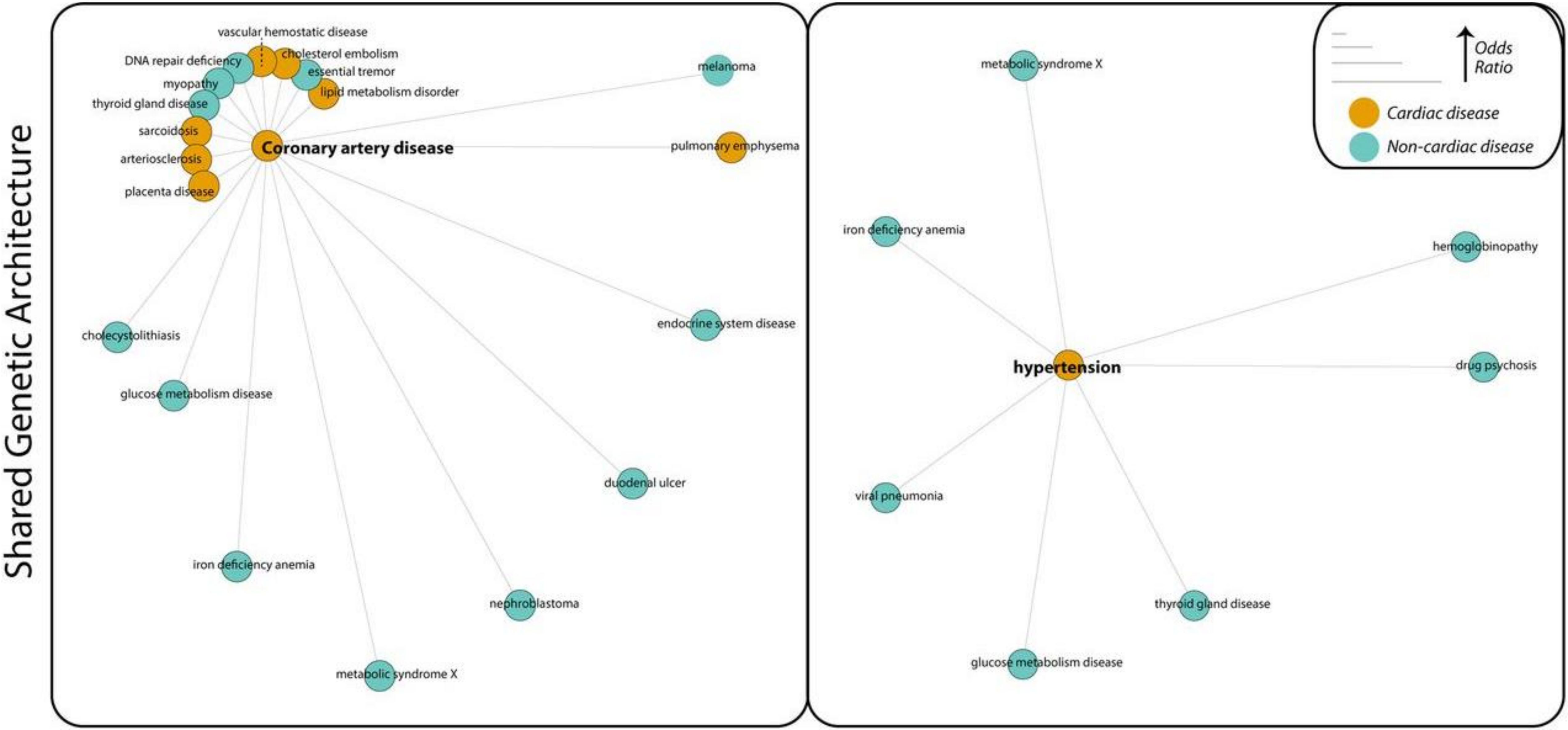


https://www.pharmaexcipients.com/news/top-200-drugs-2019/

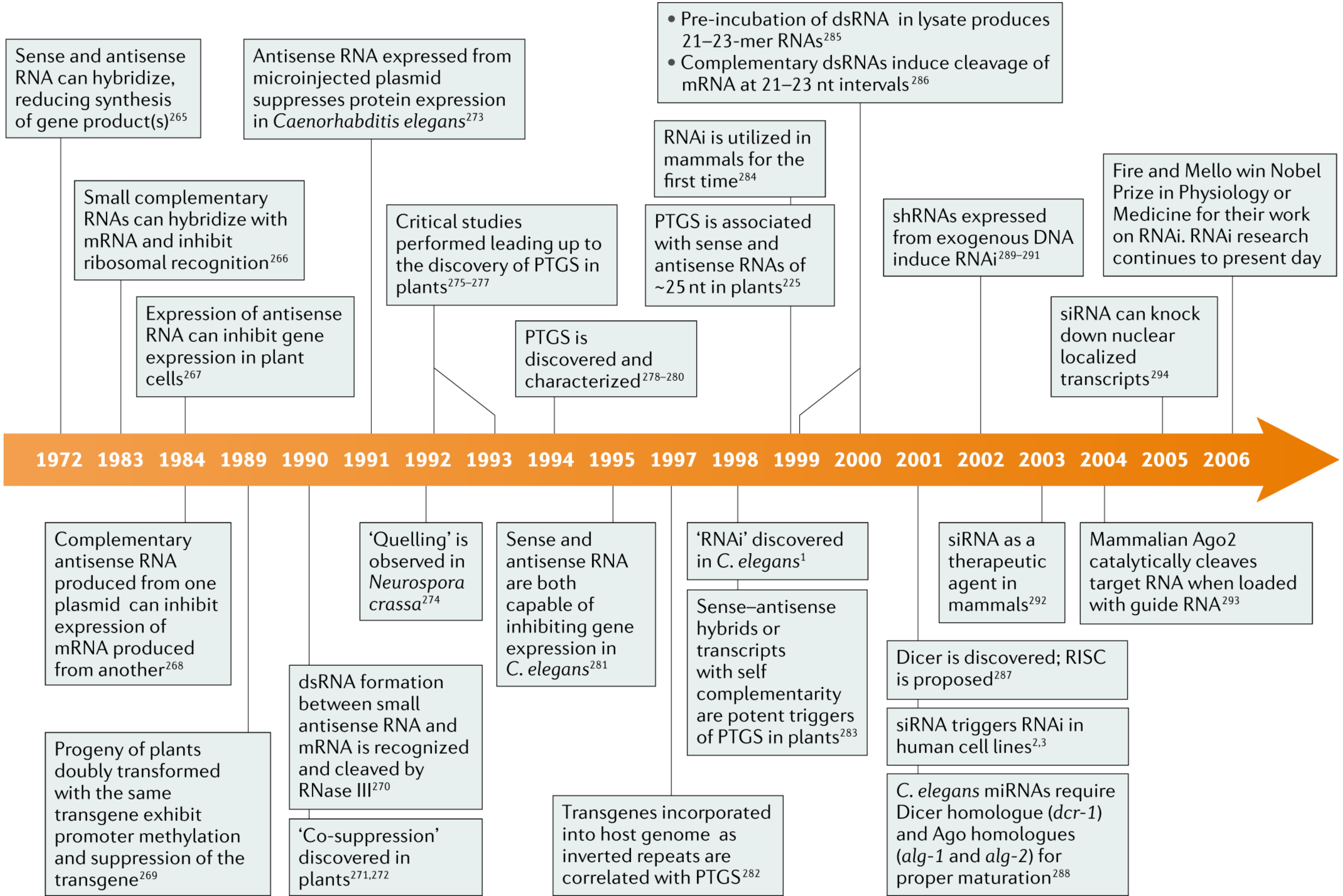
The complex picture of cardiovascular diseases



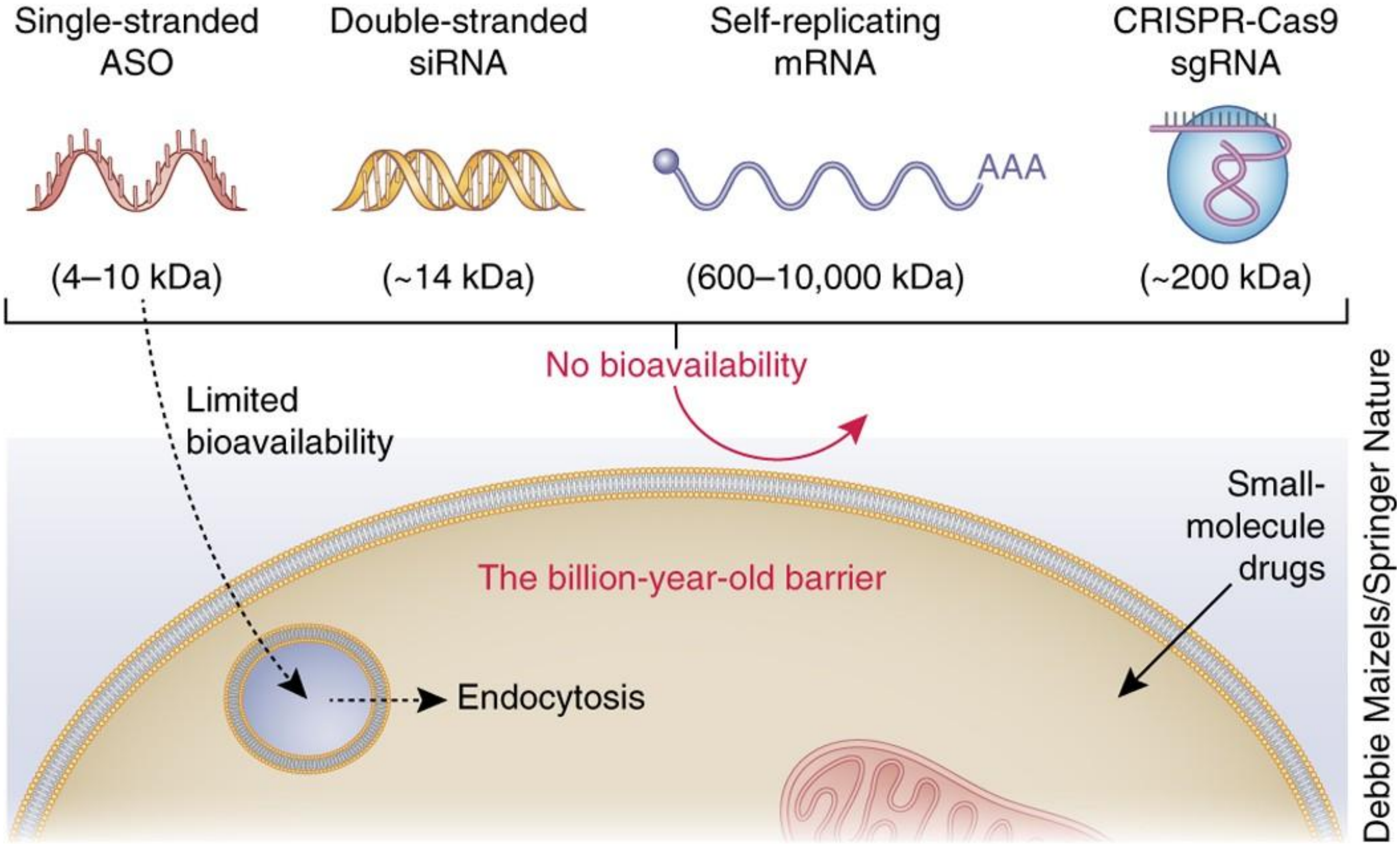
The complex picture of cardiovascular diseases

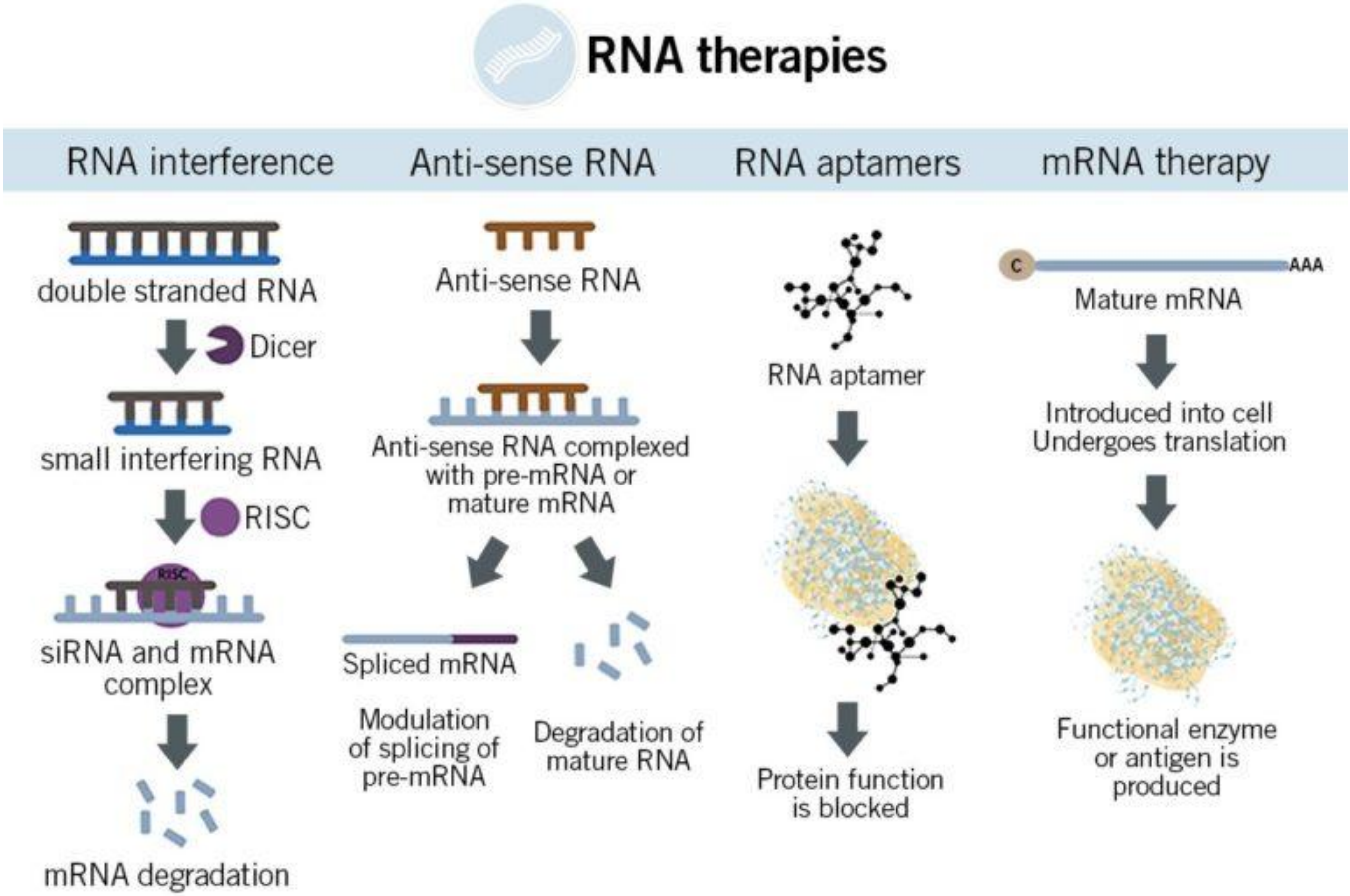


RNA-based medications – translational view



RNA based therapeutical approaches

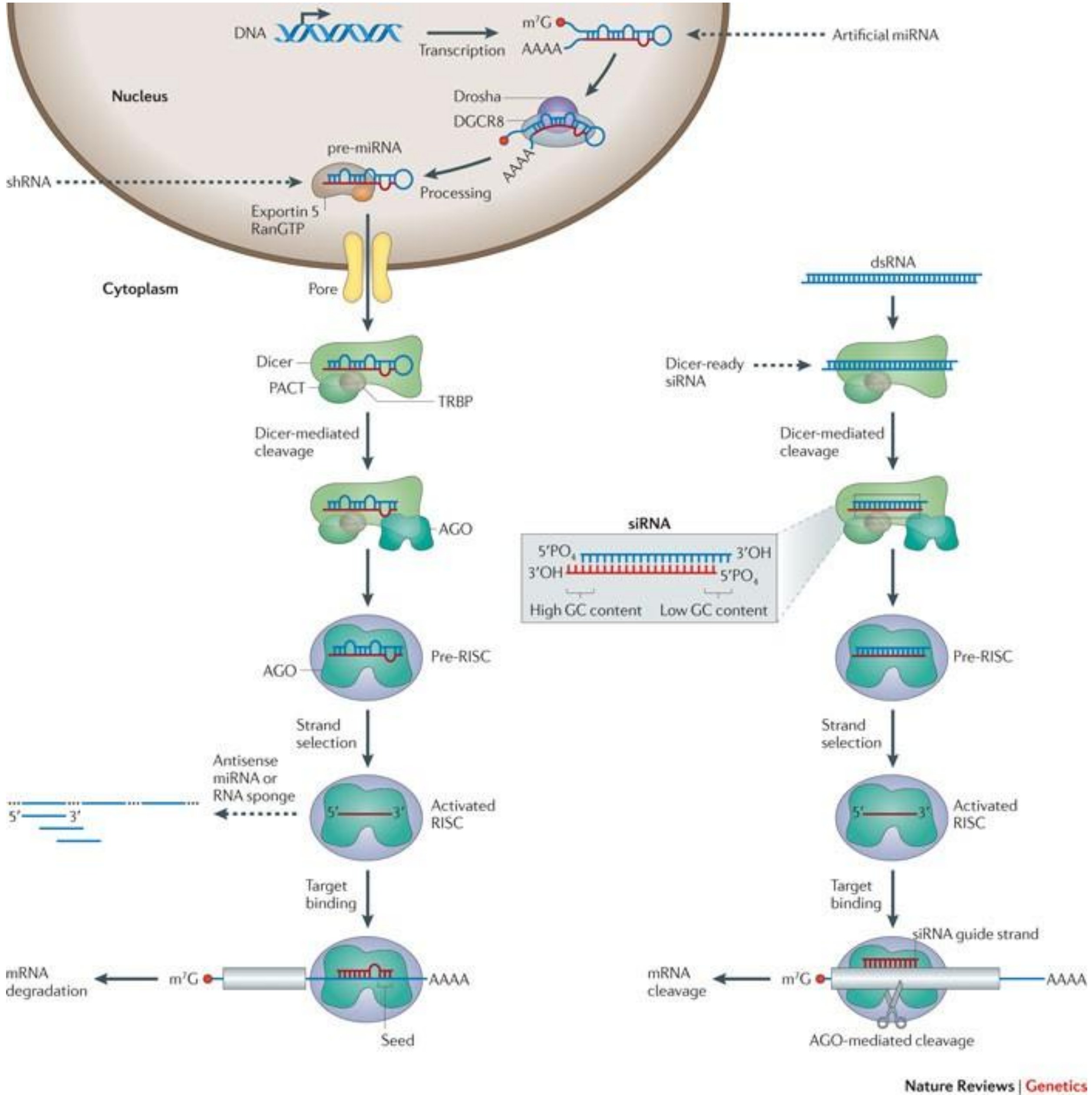



RNA therapies
RNA interference
Anti-sense RNA
RNA aptamers
mRNA therapy
double stranded RNA
Dicer
small interfering RNA
RISC
RISC
siRNA and mRNA complex
mRNA degradation
Anti-sense RNA
Anti-sense RNA complexed with pre-mRNA or mature mRNA
Spliced mRNA
Modulation of splicing of pre-mRNA
Degradation of mature RNA
RNA aptamer
Protein function is blocked
C
AAA
Mature mRNA
Introduced into cell
Undergoes translation
Functional enzyme or antigen is produced

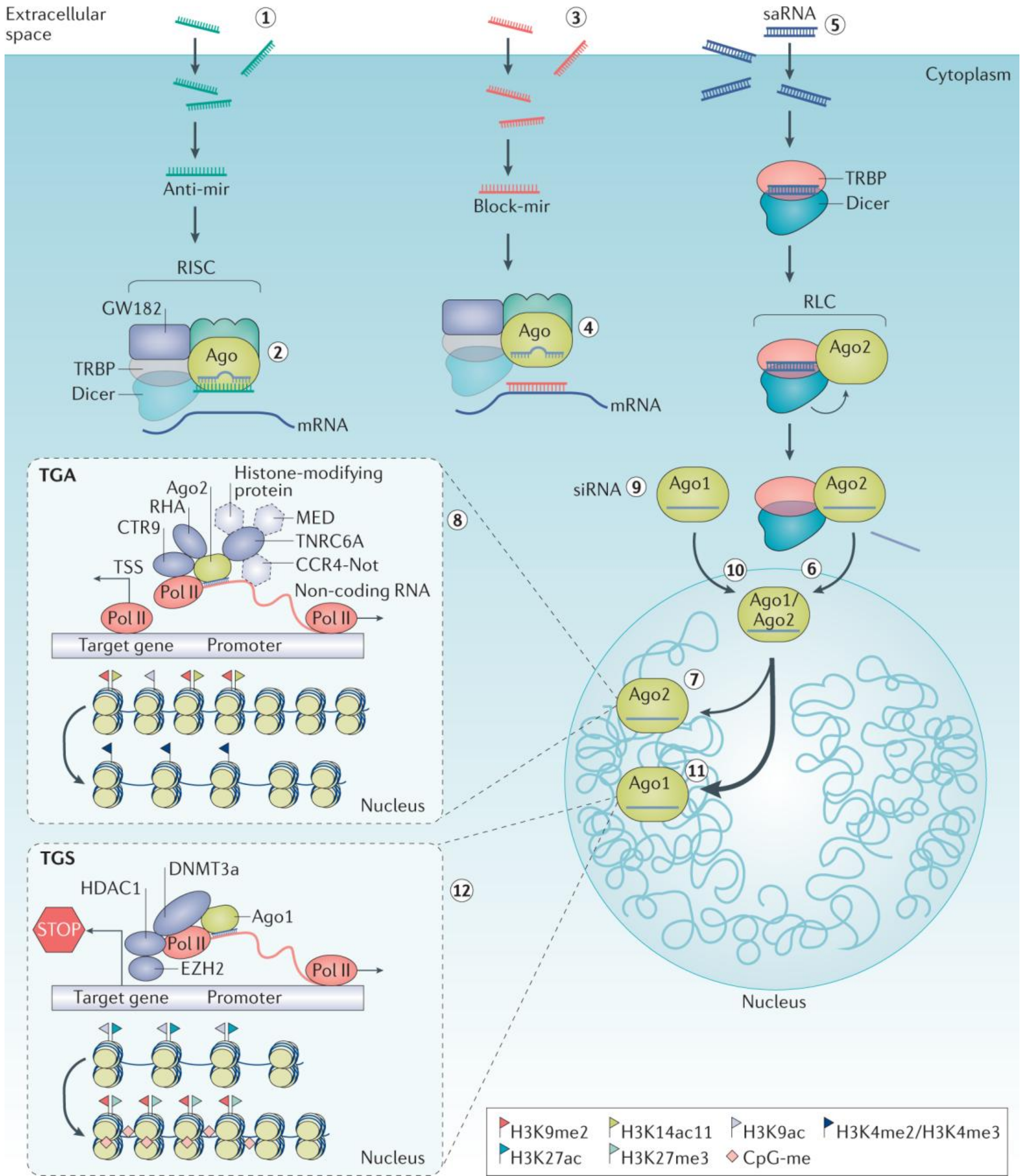
Short therapeutic RNA types

	siRNA	miRNA
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**)

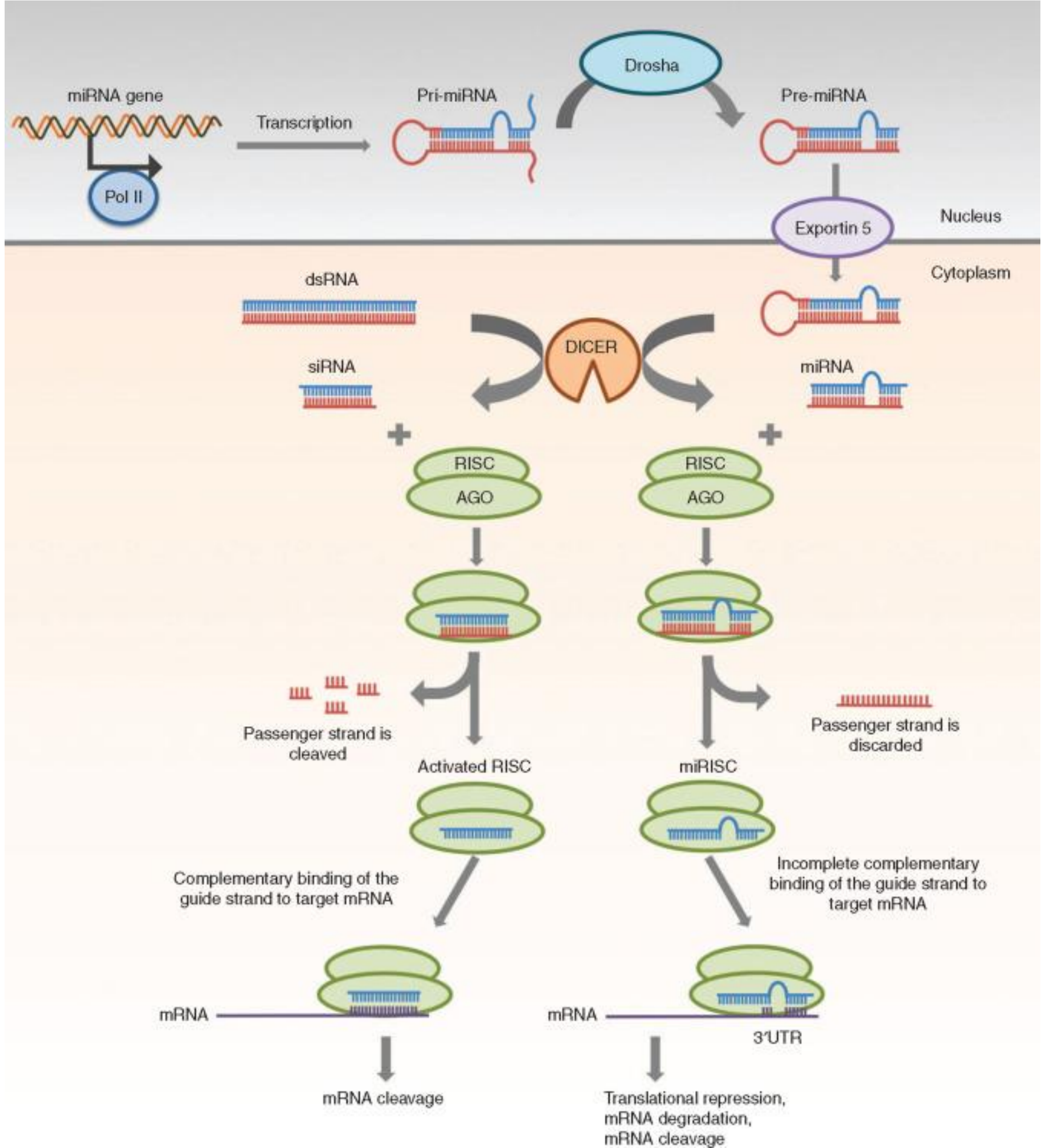


microRNA (miRNA; 19-25 mer)

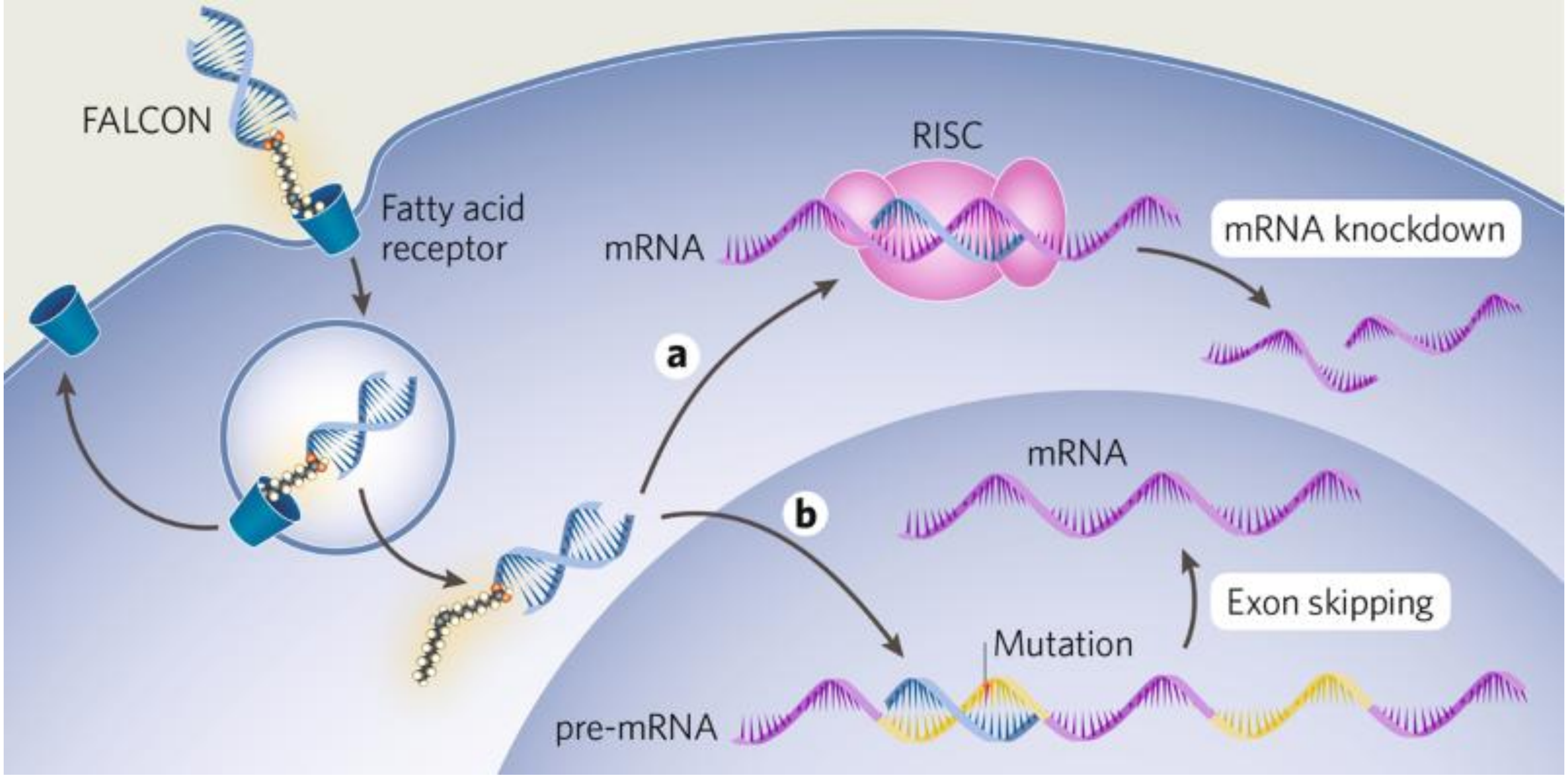


Nature Reviews Genetics **volume 12**, pages329–340 (2011)

Therapeutic short RNA – simplified mechanism



Therapeutic RNA application – exon skipping



RNA therapeutics – formulation and pharmacokinetics

Species/formulation	Packaging capacity	Applications and considerations
		Viral vector
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
		Bacterial vector species [‡]
Escherichia coli, S. Typhymurium[§]		Delivery of short hairpin RNA or small interfering RNA to gut tissue
		Non-viral formulations [||]
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.

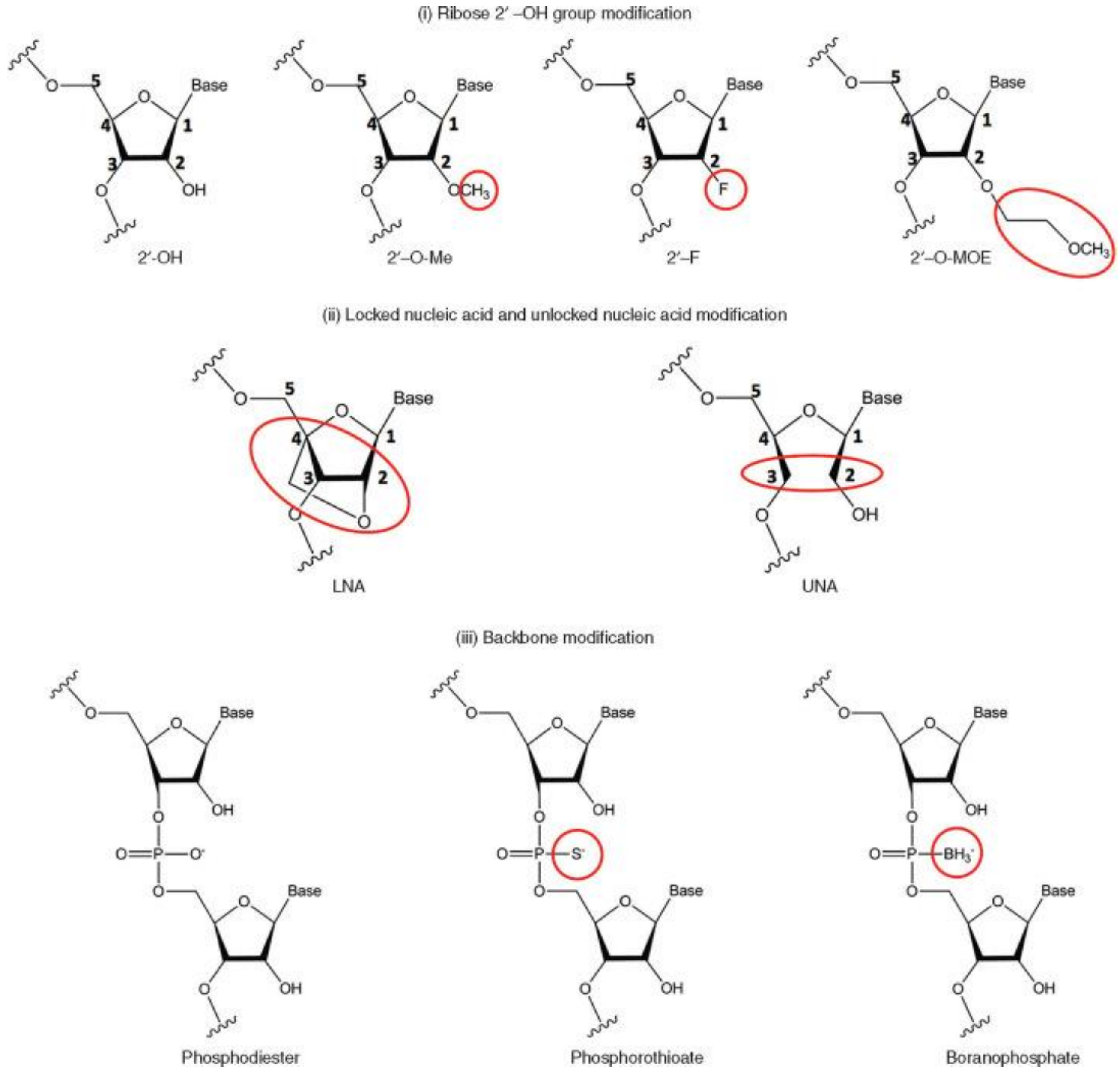
[‡]Bacterial minicells can carry plasmids, short interfering RNAs or drugs.

[§]*Salmonella enterica* subsp. *enterica* serovar Typhimurium.

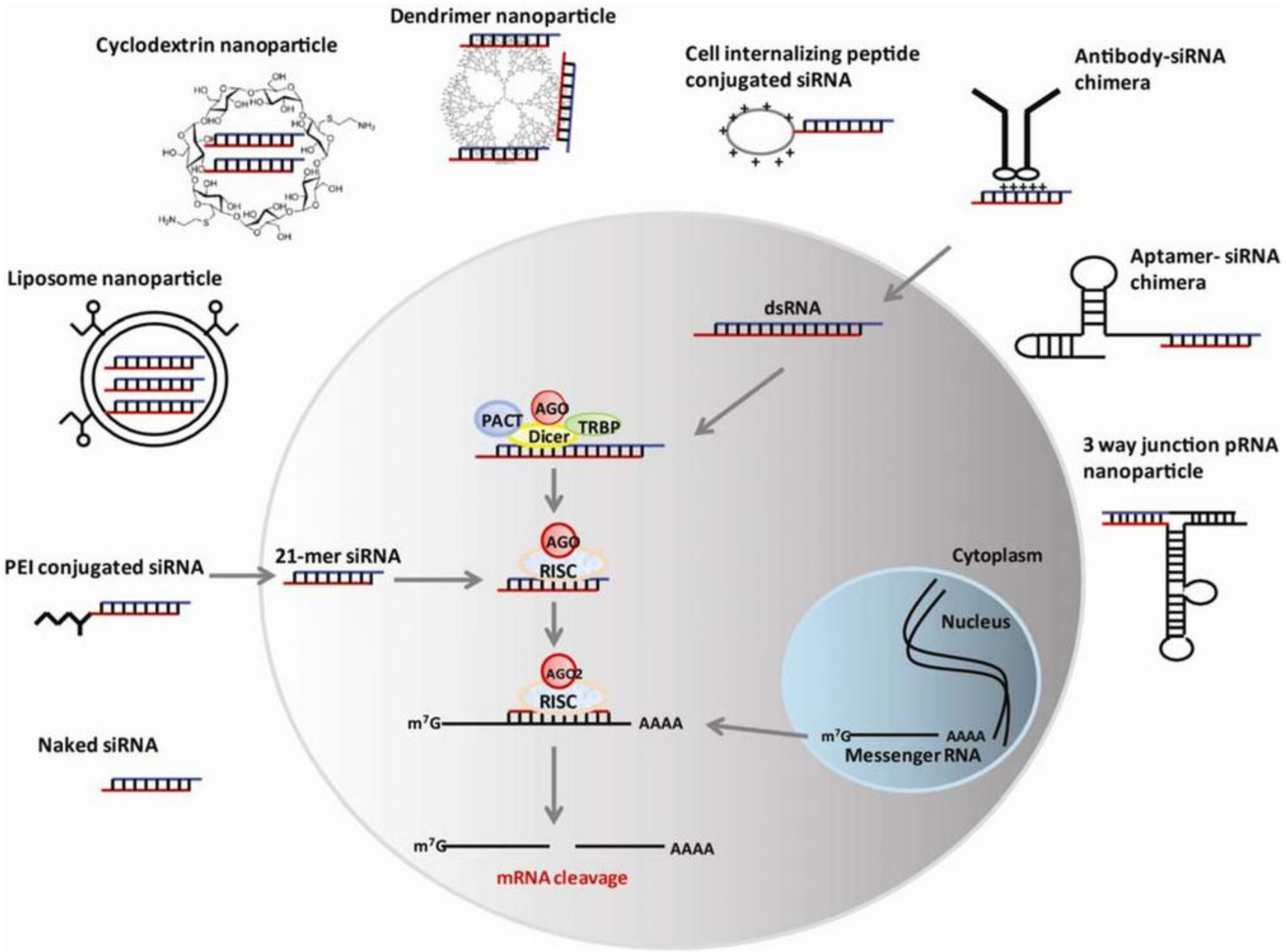
[||]The nucleic acids in non-viral carriers can be any size from small oligonucleotides to large artificial chromosomes.

Nature Reviews Genetics **volume 12**, pages329–340 (2011)

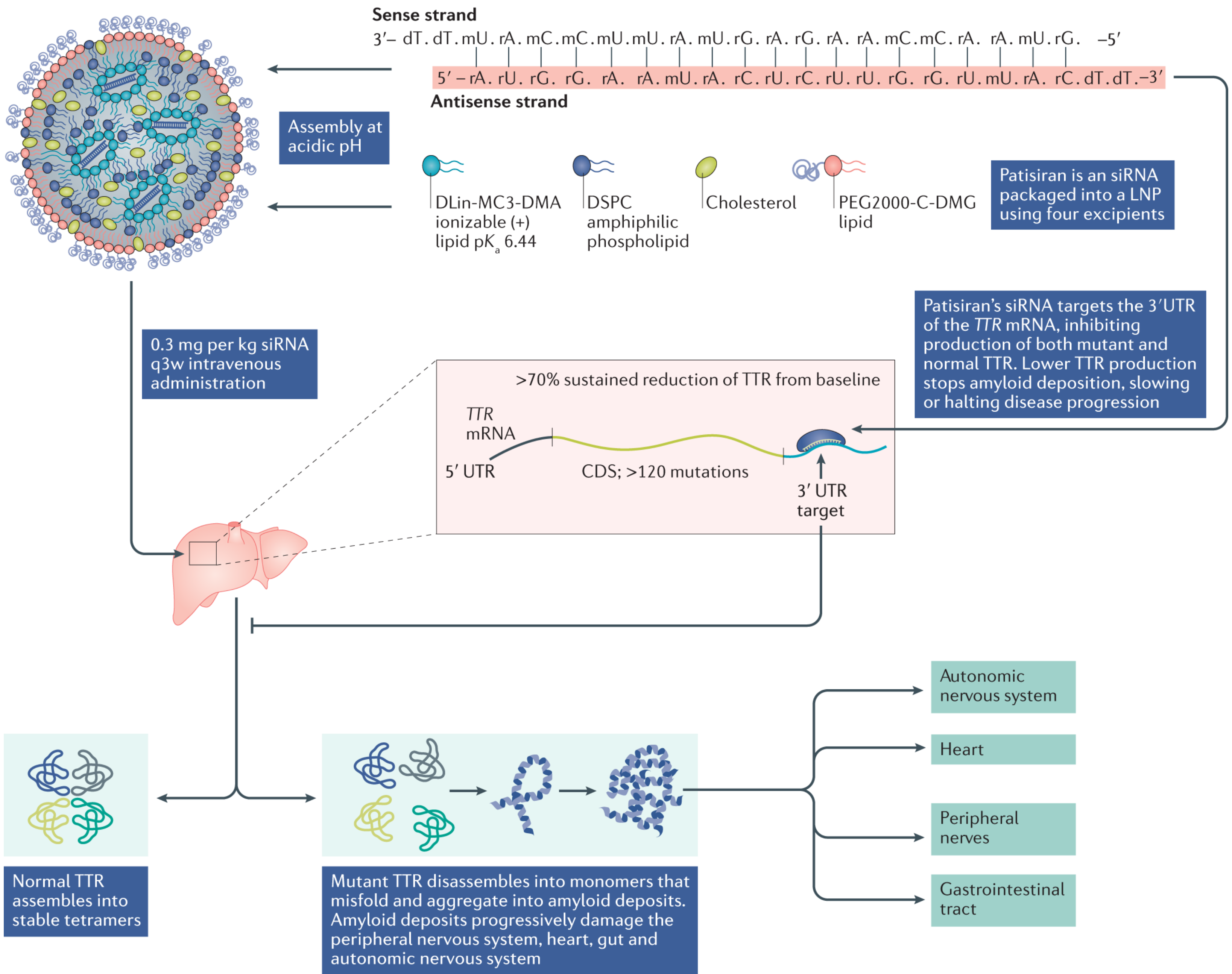
RNA therapeutics – formulation and pharmacokinetics



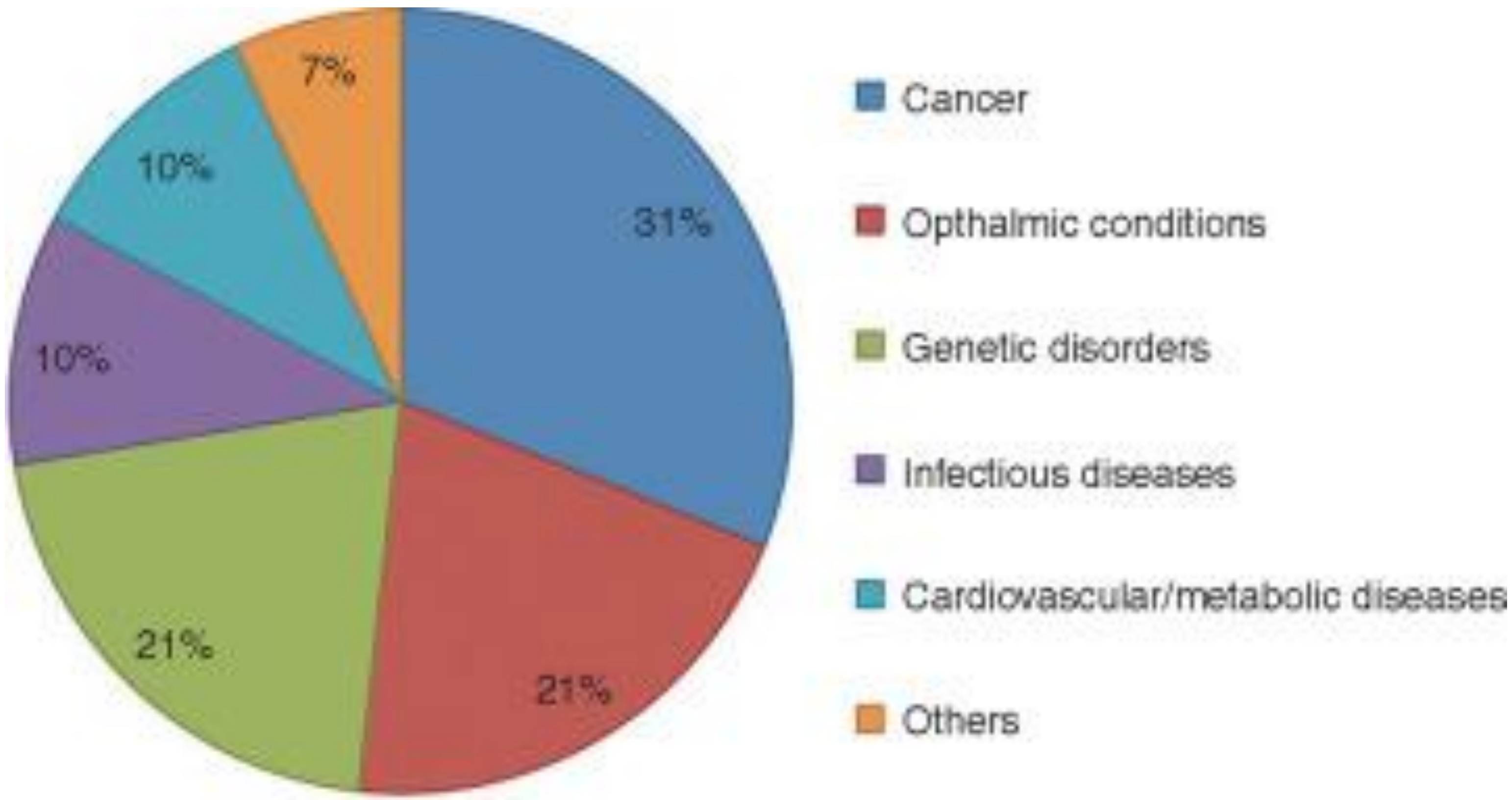
RNA therapeutics – formulation and pharmacokinetics



RNA therapeutics – formulation and pharmacokinetics



RNA therapeutics –applications



RNA therapeutics in CVD as summarized by ChatGPT

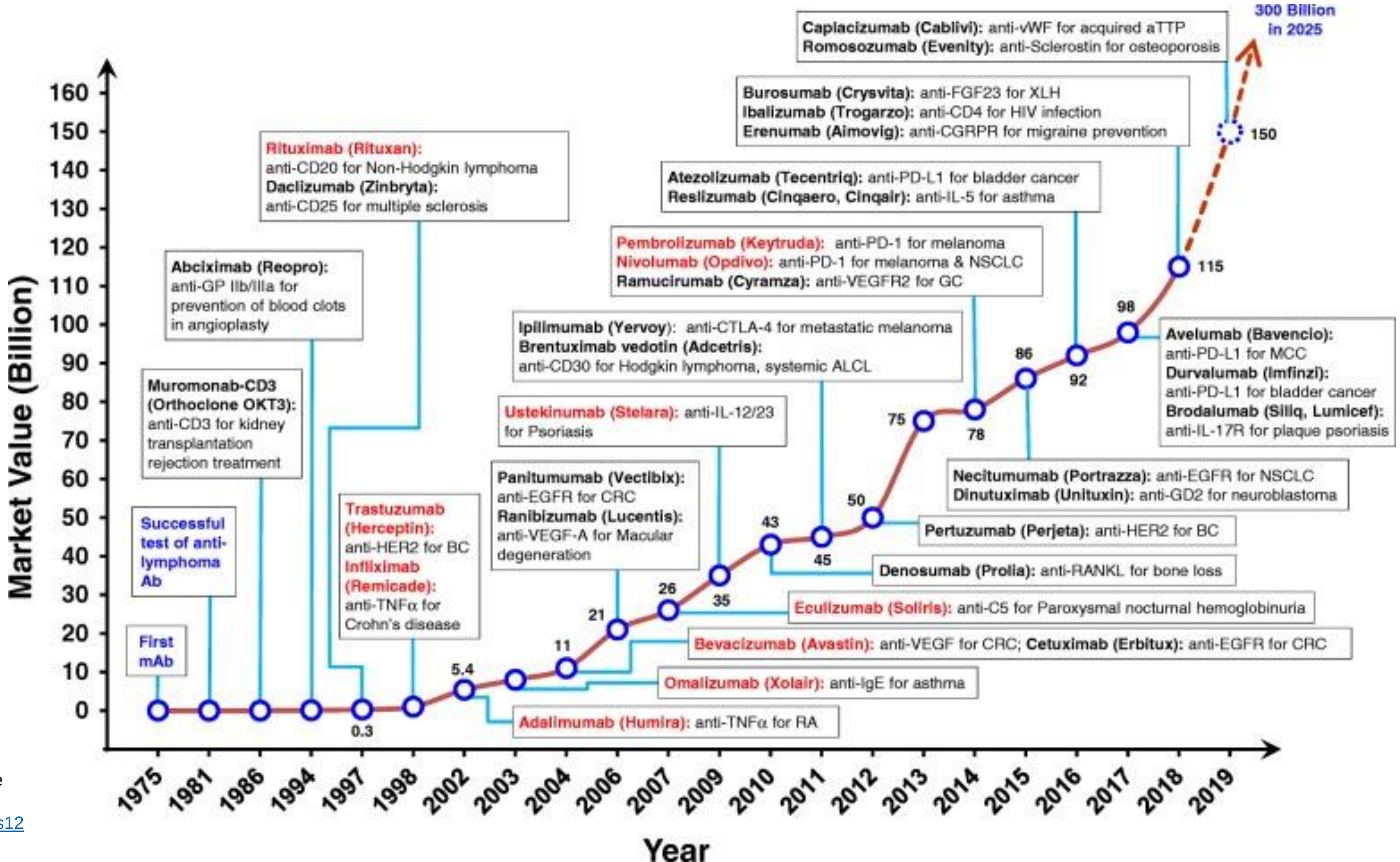
- Antisense oligonucleotides (ASOs): These are single-stranded RNA molecules that bind to complementary sequences of target RNAs and inhibit their function by various mechanisms, such as degradation, splicing modulation, or translation inhibition. ASOs have been used to treat CVD by targeting genes involved 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).

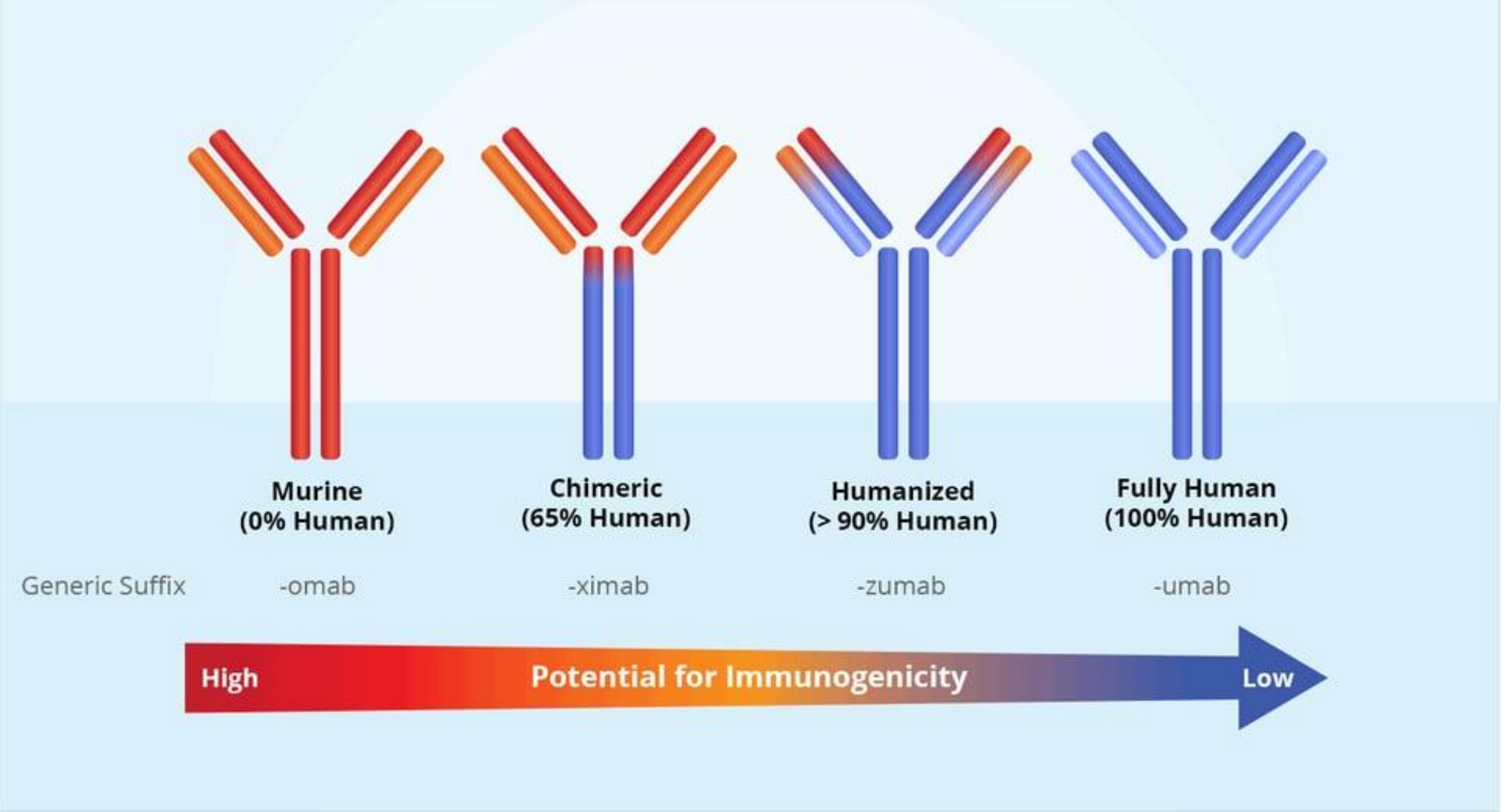
- Messenger RNAs (mRNAs): These are single-stranded RNA molecules that encode proteins and are translated by ribosomes. mRNAs have been used to treat CVD by delivering therapeutic proteins or antigens to cells or tissues. mRNAs have been used to induce angiogenesis, cardiomyocyte regeneration, immunomodulation, and vaccination. Examples of approved mRNAs for CVD are none yet, but several candidates are in clinical trials.

Monoclonal antibody-based drugs

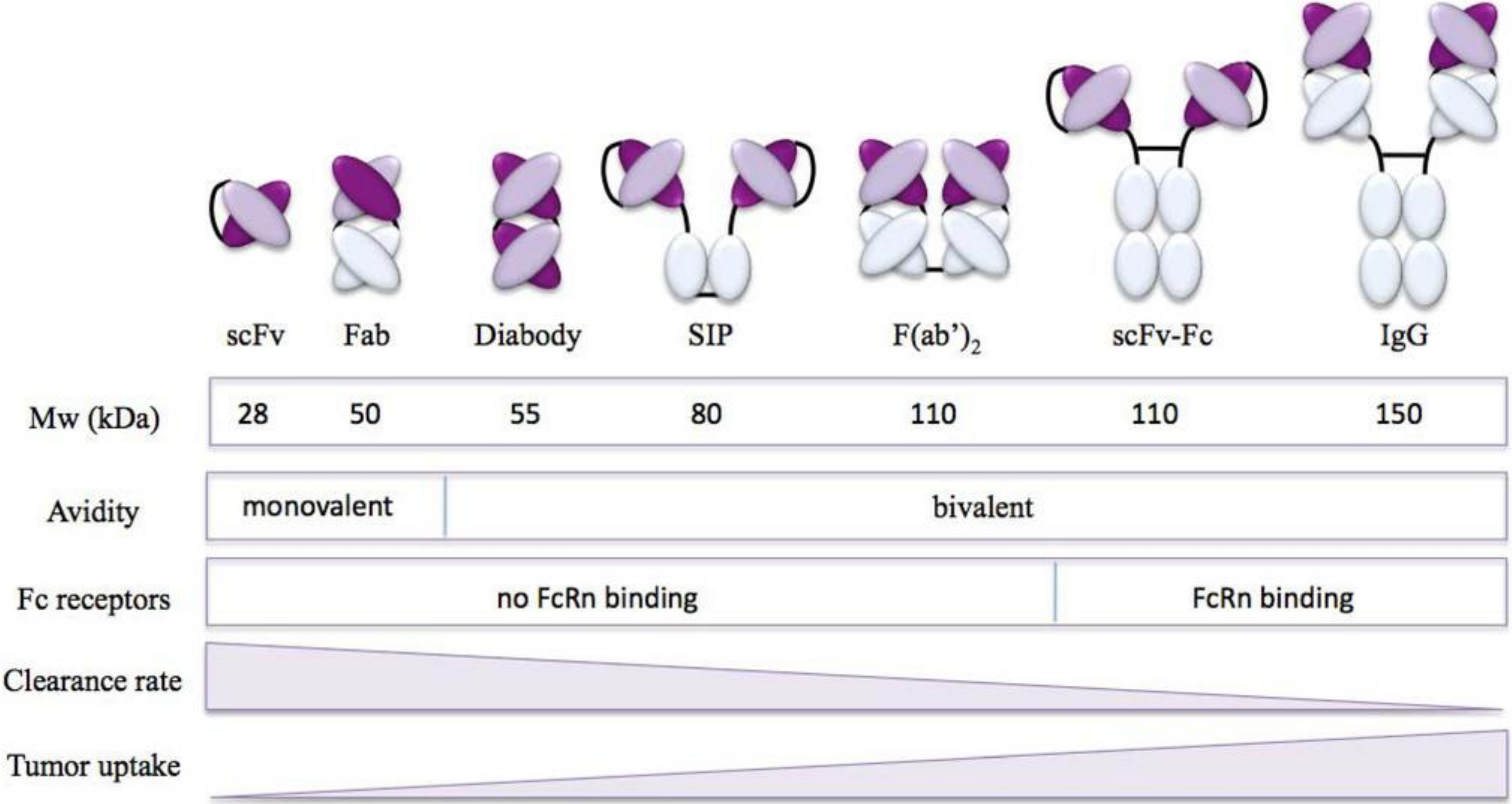


J Biomed Sci. 2020; 27: 1.
Published online 2020 Jan 2. doi: 10.1186/s12929-019-0592-z

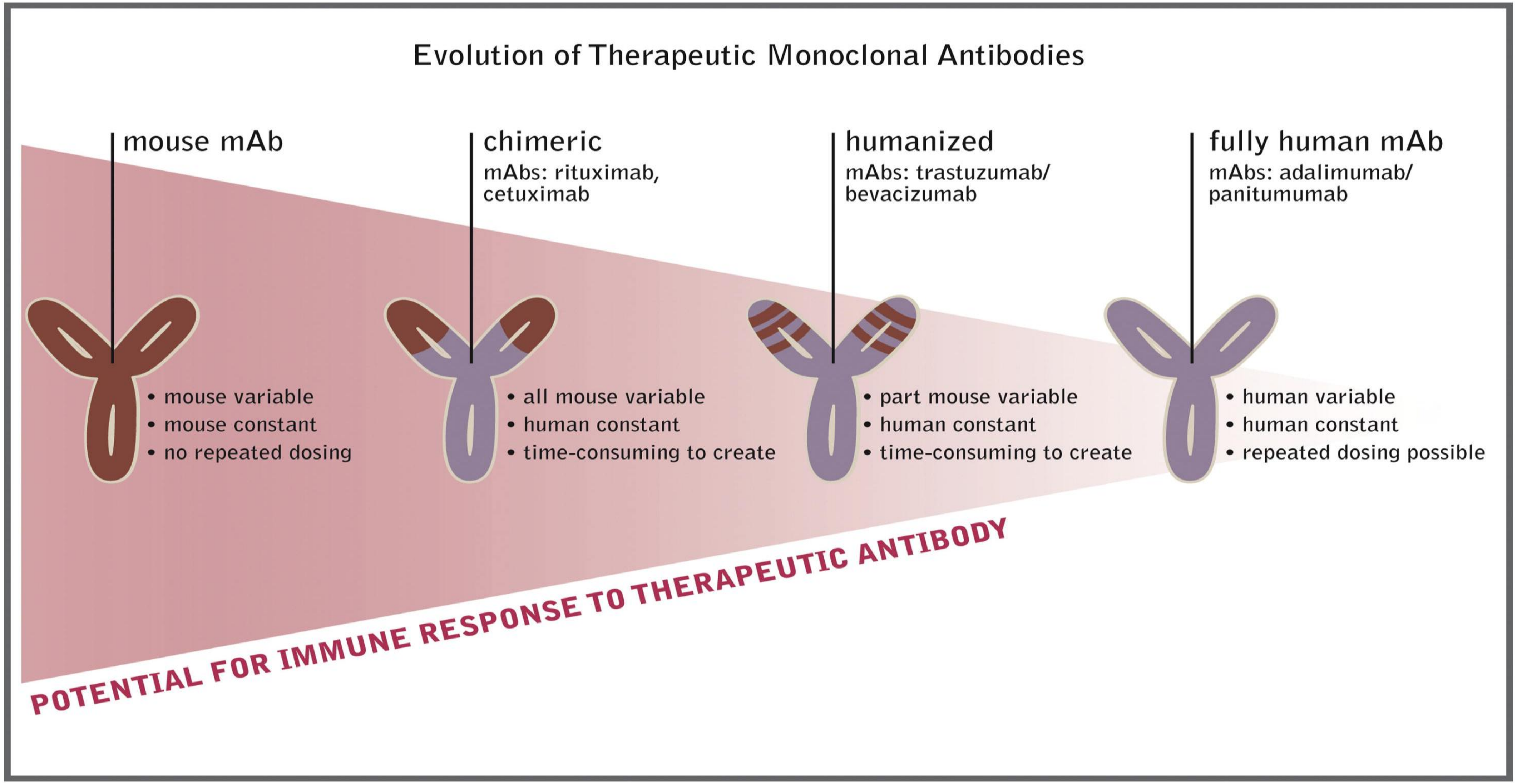
Monoclonal antibody-based drugs



Monoclonal antibody-based drugs

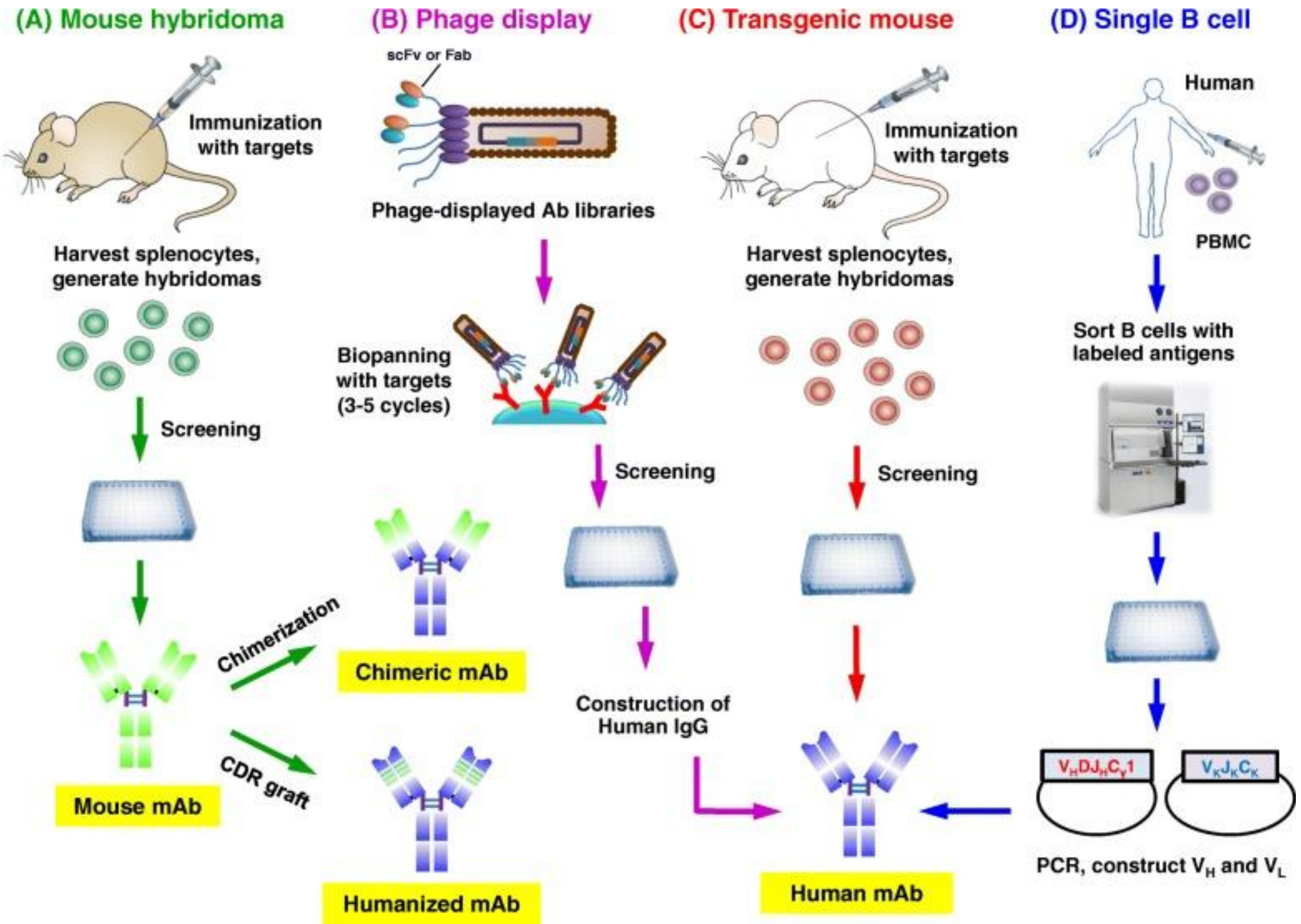


Monoclonal antibody-based drugs



Atherosclerosis 2013 22818-28DOI: (10.1016/j.atherosclerosis.2013.01.044)

Monoclonal antibody-based drugs – development strategies



J Biomed Sci. 2020; 27: 1.
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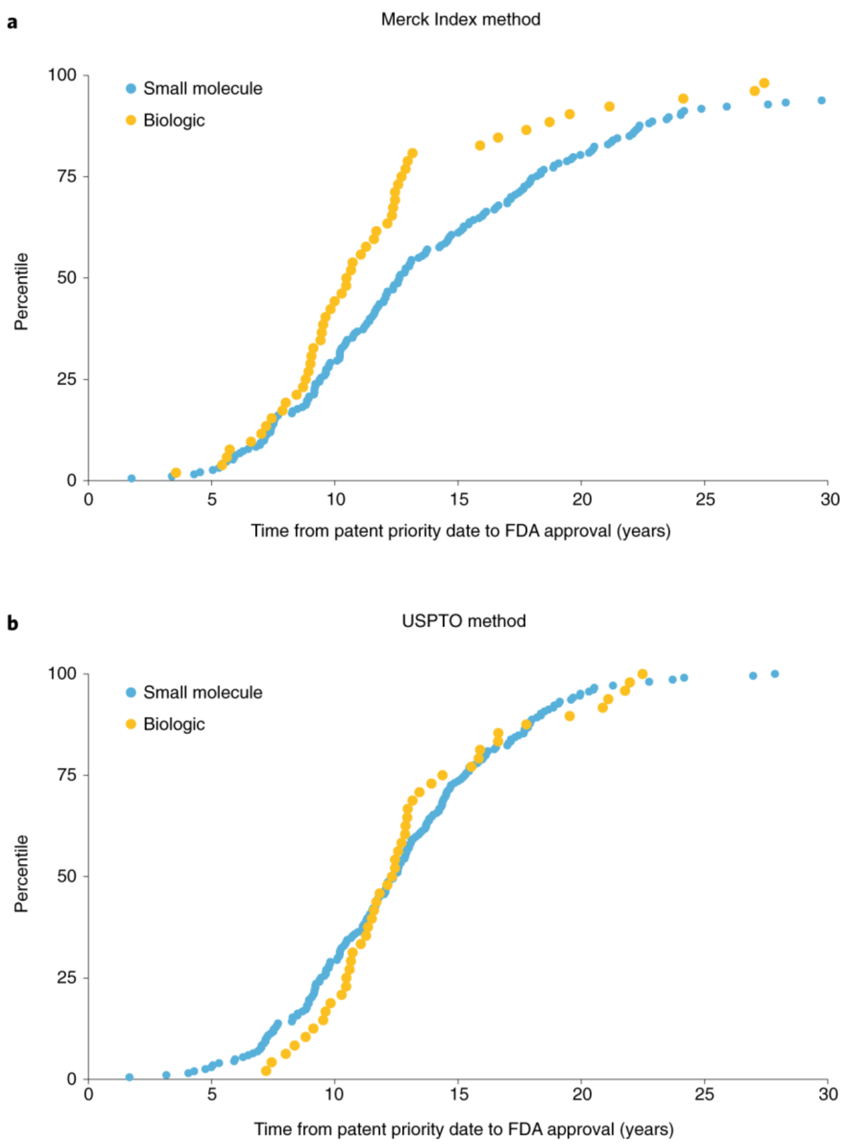
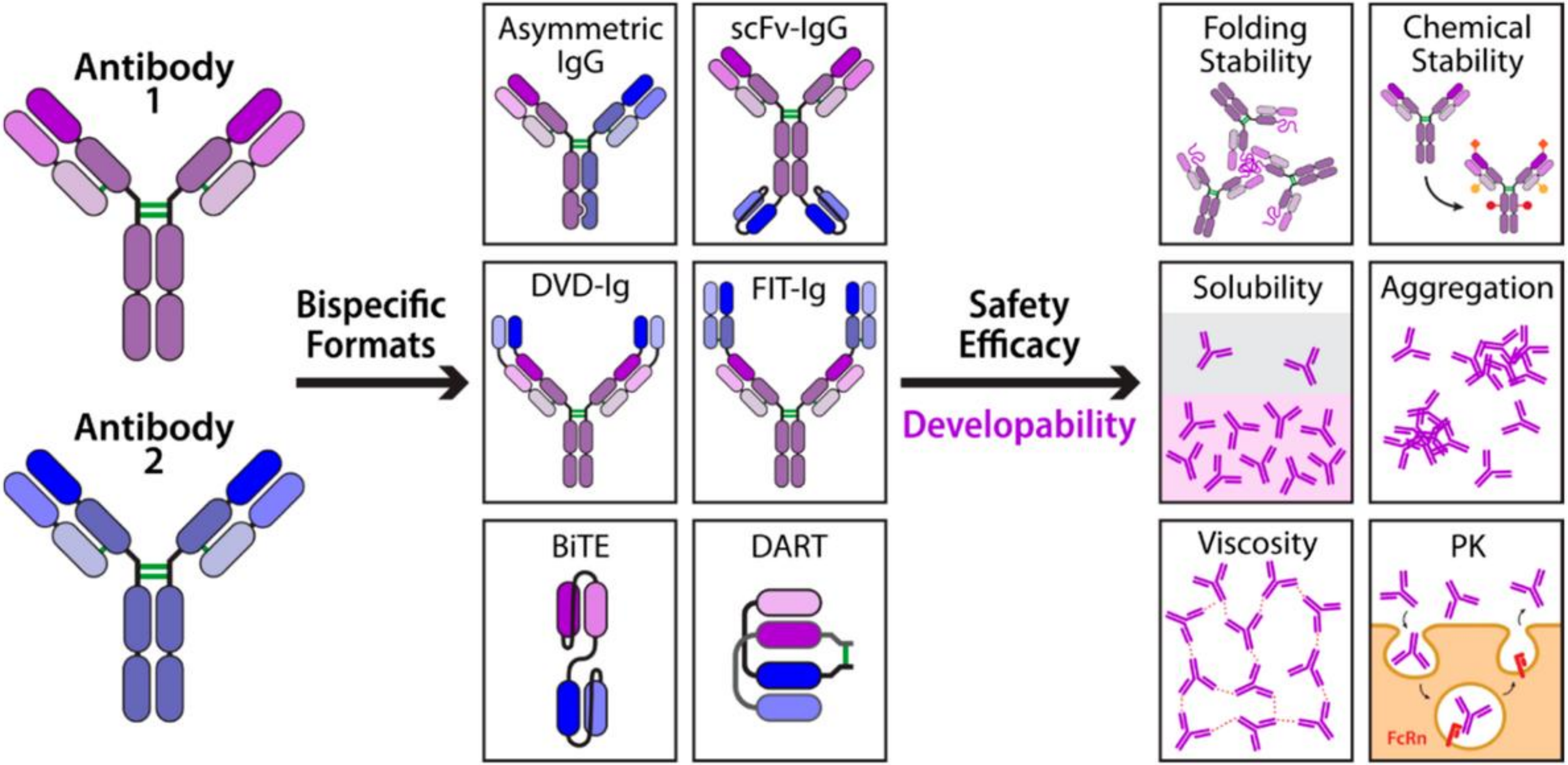
Monoclonal antibody-based drugs – development time



Nature Biotechnology **volume 37**, pages708–711 (2019)

Monoclonal antibody-based drugs – pharmacokinetics



Monoclonal antibody-based drugs – risks

B. Flühmann et al. European Journal of Pharmaceutical Sciences 128 (2019) 73–80

<table>
<tr><th></th><th>SMALL MOLECULE DRUGS</th><th>BIOLOGICS</th><th>NBCDs</th></tr>
<tr><td>Molecular weight</td><td>Low (<500)</td><td colspan="2">High (range 5-900 kDa)</td></tr>
<tr><td>Structure</td><td>Simple, well-defined</td><td colspan="2">Complex, heterogeneous, defined by manufacturing process</td></tr>
<tr><td>Modifications</td><td>Well-defined</td><td colspan="2">Many options</td></tr>
<tr><td>Manufacturing</td><td>Chemical synthesis</td><td>Produced in living cells or organisms</td><td>Synthetic technologies (incl. nanotech)</td></tr>
<tr><td>Stability</td><td>Stable</td><td colspan="2">Generally unstable, sensitive to external conditions</td></tr>
<tr><td>Immunogenicity</td><td>Mostly non-immunogenic</td><td>Mostly immunogenic</td><td>Immunogenicity varies</td></tr>
<tr><td>Copy characteristics</td><td>Identical copies can be made</td><td colspan="2">Impossible to ensure identical copy versions</td></tr>
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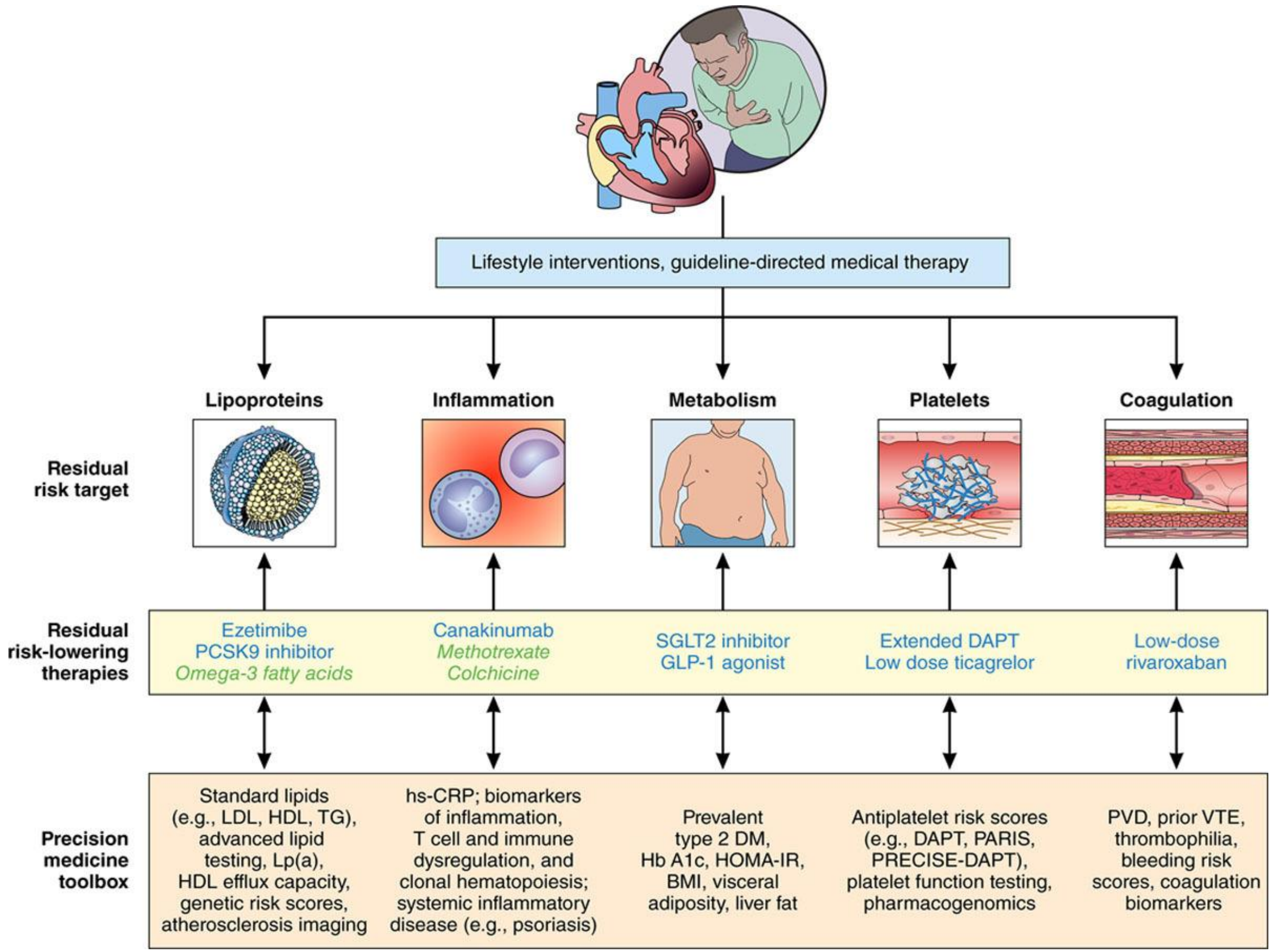
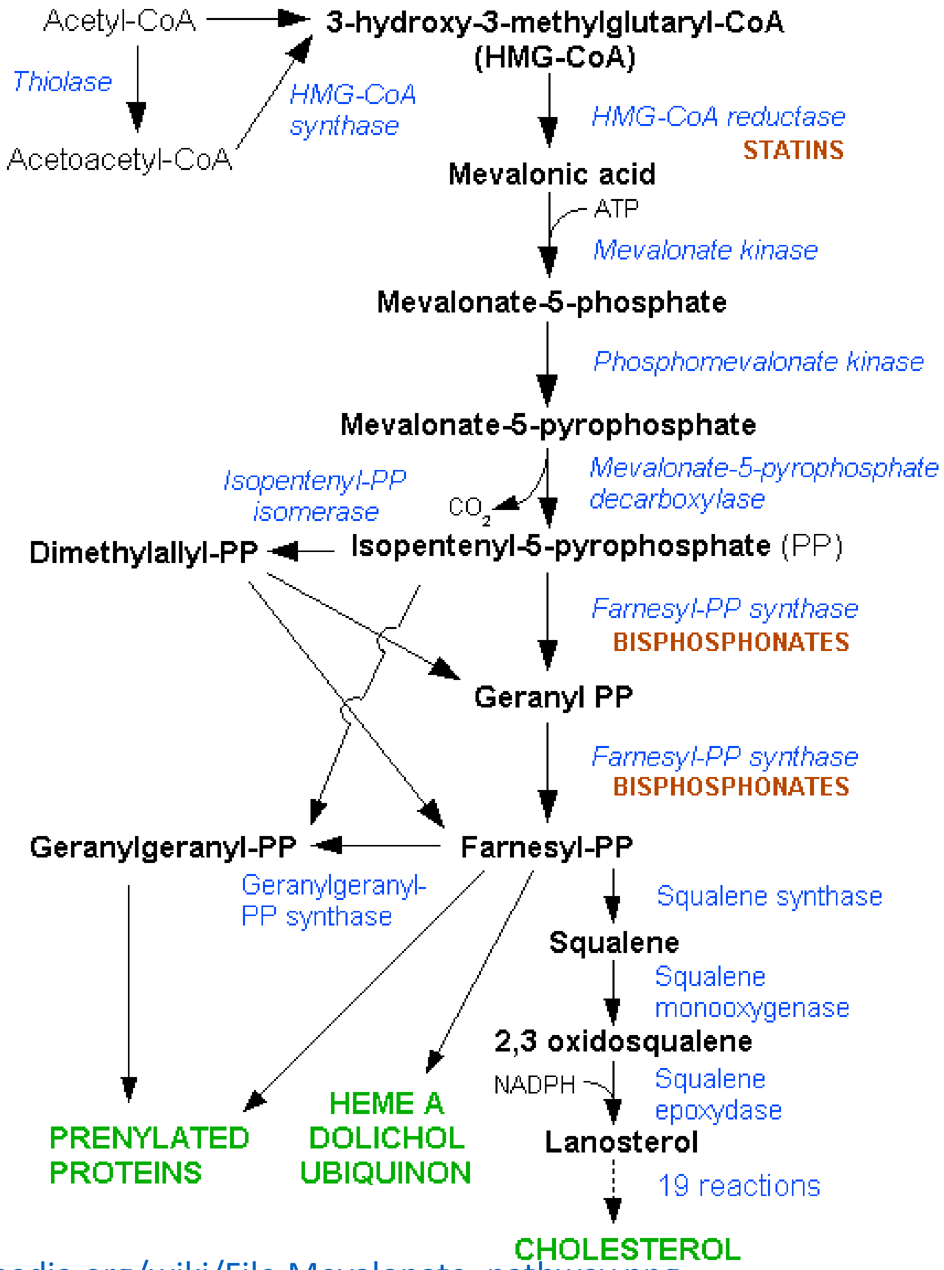
Monoclonal antibody-based drugs in cardiovascular medicine

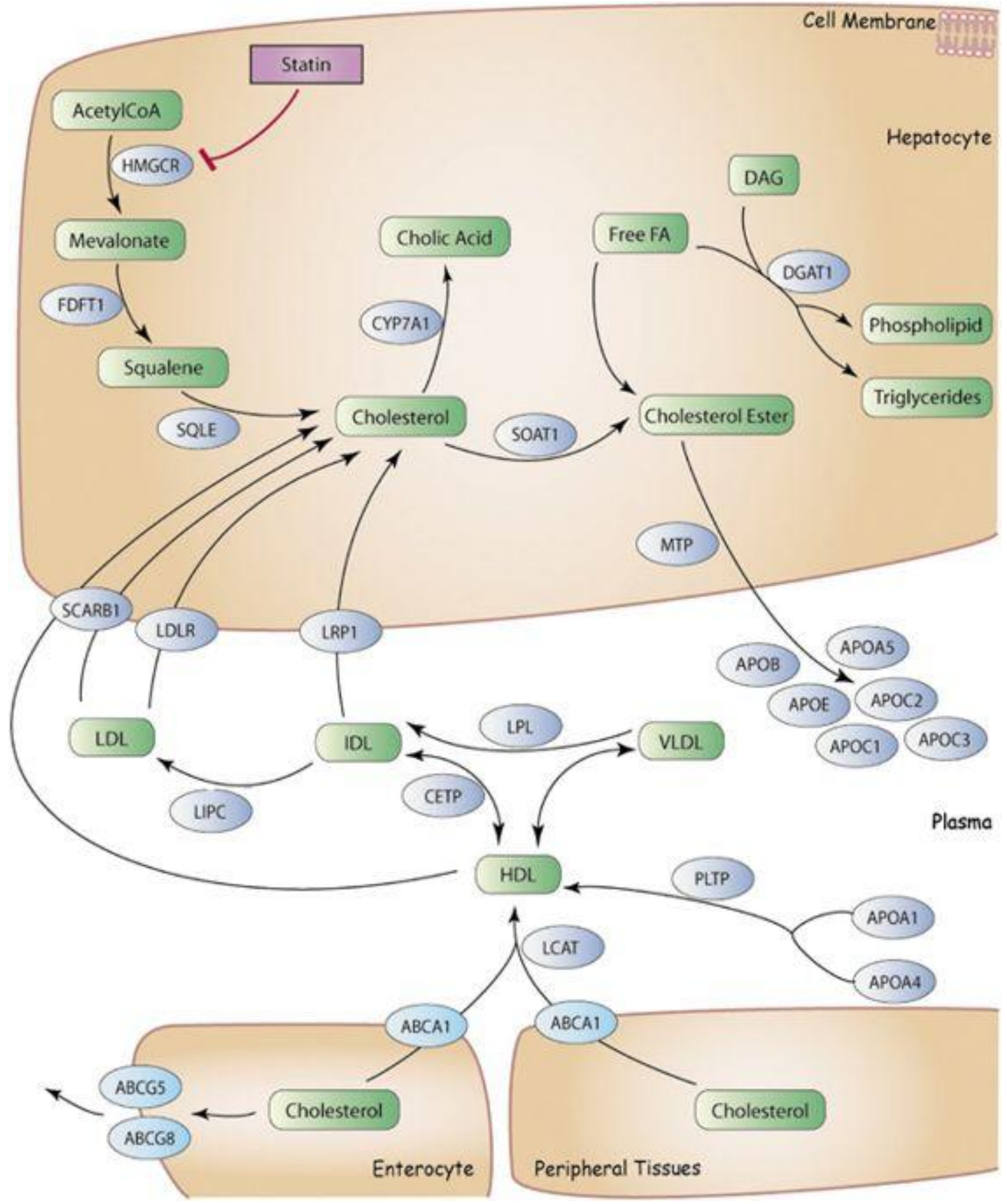


Kershaw V. Patel. Circulation. Conceptual Framework for Addressing Residual Atherosclerotic Cardiovascular Disease Risk in the Era of Precision Medicine, Volume: 137, Issue: 24, Pages: 2551-2553, DOI: (10.1161/CIRCULATIONAHA.118.035289)

Lipid-lowering therapy (small molecule, statins)

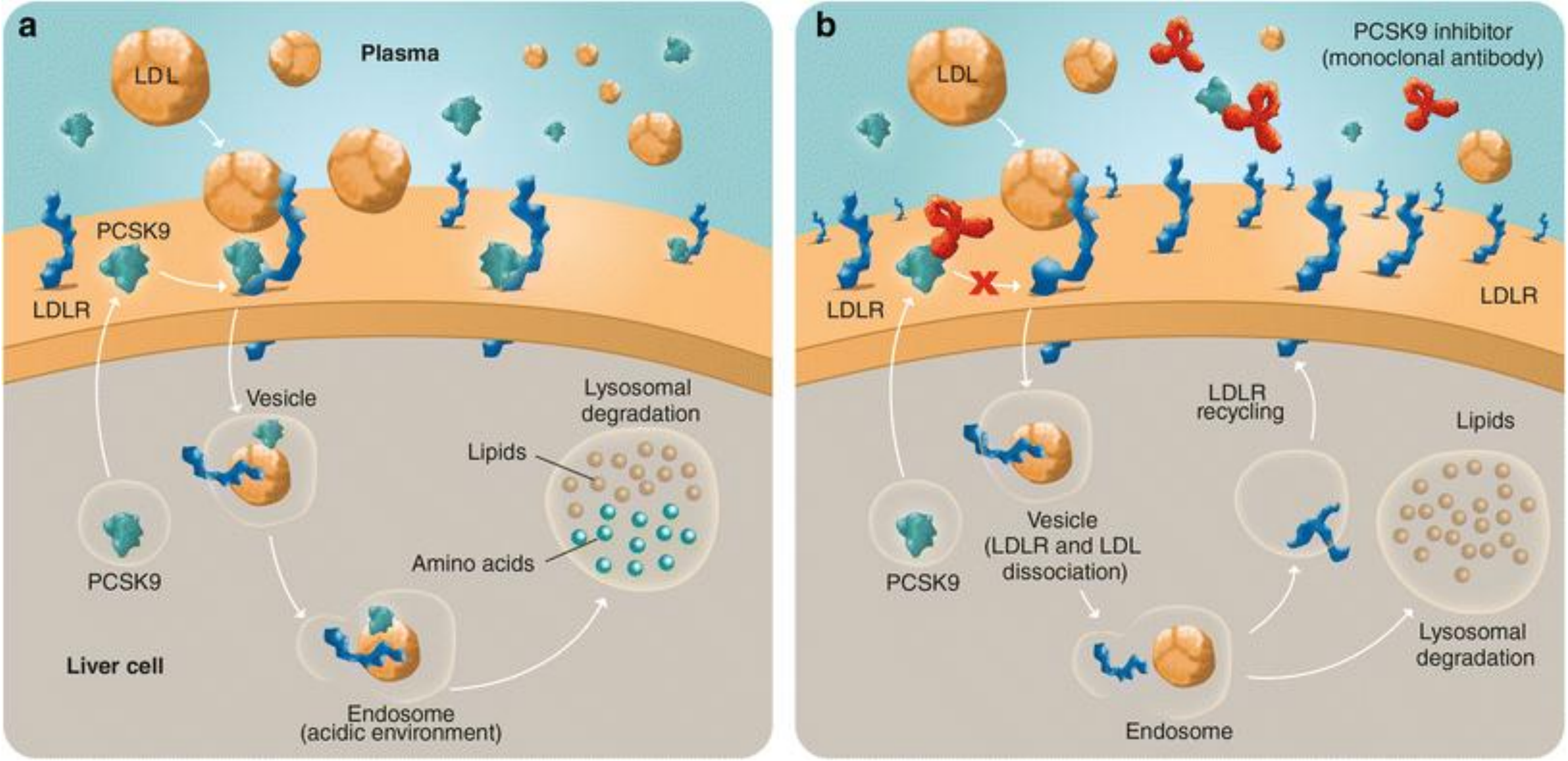


http://en.wikipedia.org/wiki/File:Mevalonate_pathway.png



Mangravite et al, The Pharmacogenomics Journal **volume 6**, pages 360–374 (2006)

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



Krähenbühl, S., Pavik-Mezzour, I. & von Eckardstein, A. Drugs (2016) 76: 1175. https://doi.org/10.1007/s40265-016-0613-0

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-1β), 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-α), 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.