Targets for drug action, #3

Target	*examples*
2.1. Receptors for physiological ligands	
Transmembrane receptors	
2.1.1. G-protein-coupled receptors	*- adrenergic receptors* *- opioid receptors*
2.1.2. ligand-gated ion channels	*- $GABA_A$ receptors*
2.1.3. kinase-linked receptors	*- insulin receptor*
Intracellular receptors	
2.1.4. nuclear receptors	*- PPARγ (peroxisome proliferator-activated receptor γ* *- pregnane X receptor*
2.2. Other targets/approaches	
2.2.1. enzymes	*- cyclo-oxygenase (in pain chapter)* *- dihydrofolate reductase* *- HIV protease* *- tyrosine kinases* *- angiotensin-converting enzyme*
2.2.2. ion channels and transporters	***- transporters in the kidney***
2.2.3. protein therapeutics	***- GLP-1 receptor agonists*** ***-TNF-α monoclonal antibodies (e.g. infliximab)***
2.2.4. gene therapy	***- Nusinersen*** ***- Tisagenlecleucel /Axicabtagene ciloleucel***

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2.2.2. ion channels and transporters

examples of targets :

transporters
- proton pumps (stomach)
- Na/K-ATPase
- GABA transporter
- **Ion transporters in the kidney**

Ion channels
- voltage-gated Calcium channels (Ca_v)
- voltage-gated Na channels (Na_v)
- epithelial K channels
- Epithelial Na channel (ENaC)
- $GABA_A$ receptors (*discussed*)

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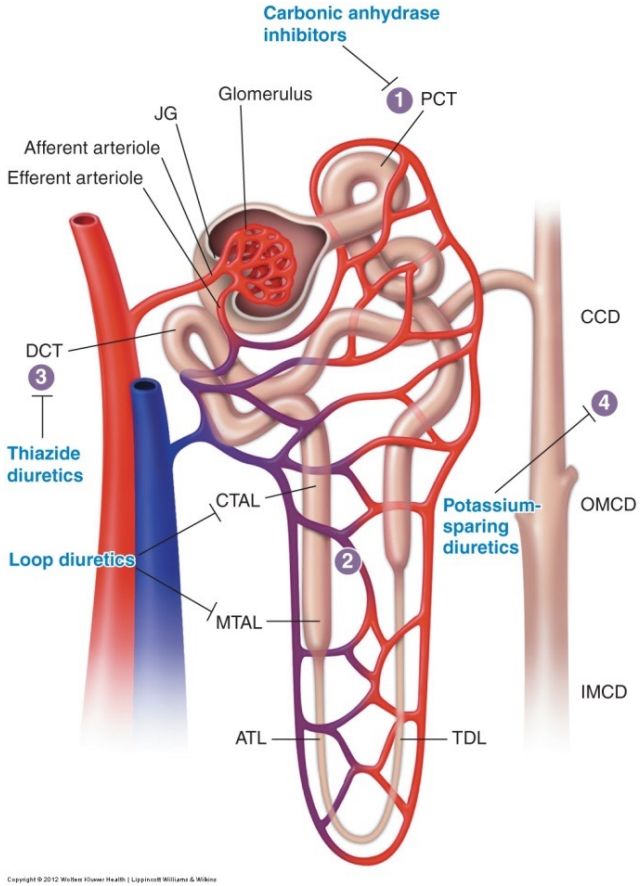
Na and Cl absorption in the nephron



1, PCT, proximal convoluted tubule
2, Loop of Henle
3, distal convoluted tubule
4, collecting duct

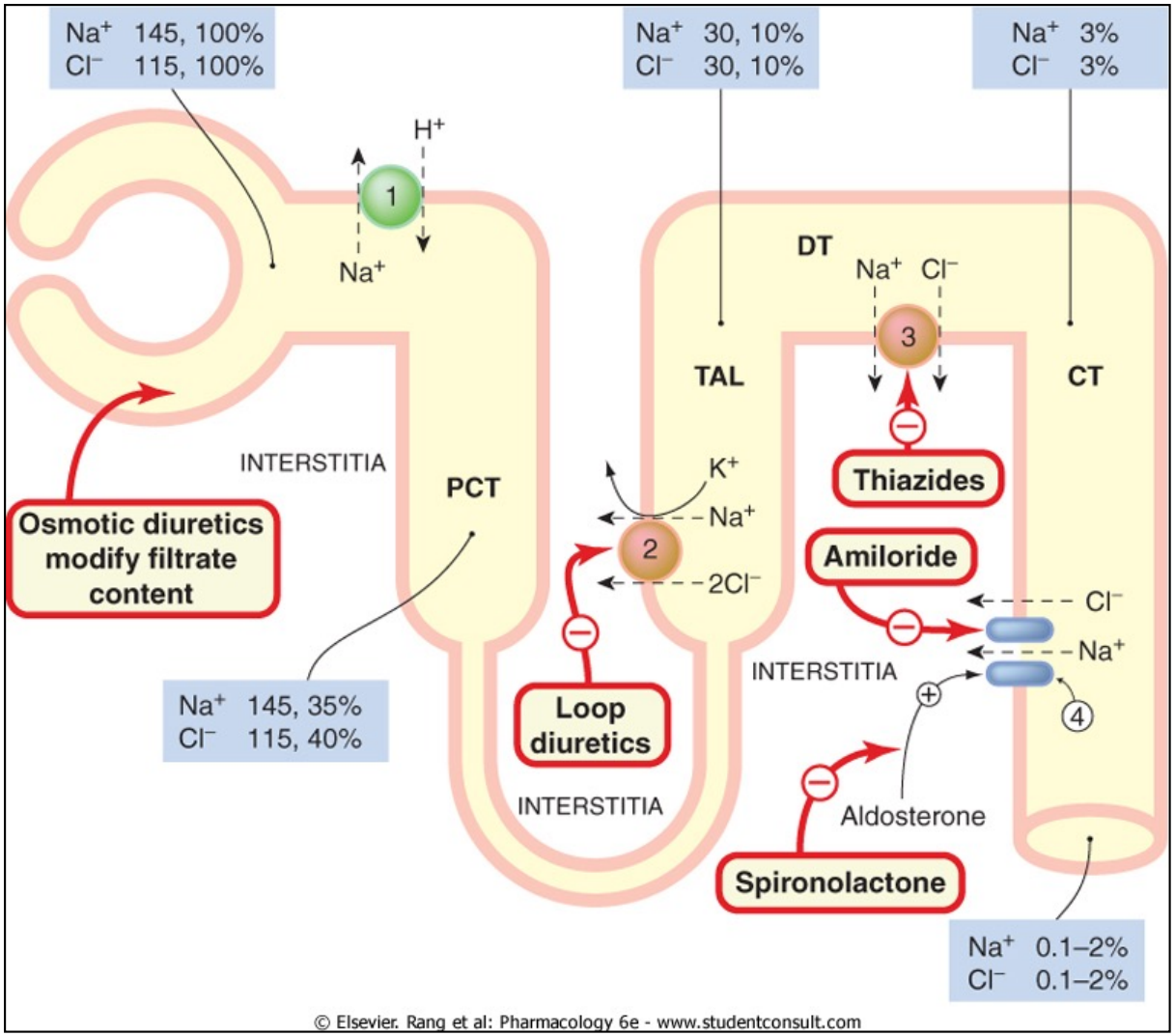


Figure 24-4 Schematic showing the absorption of sodium and chloride in the nephron and the main sites of action of drugs. Cells are depicted as an orange border round the yellow tubular lumen. Mechanisms of ion absorption at the apical margin of the tubule cell: (1) Na+/H+ exchange; (2) Na+/K+/2Cl- cotransport; (3) Na+/Cl- cotransport, (4) Na+ entry through sodium channels. Sodium is pumped out of the cells into the interstitium by the Na+/K+ ATPase in the basolateral margin of the tubular cells (not shown). The numbers in the boxes give the concentration of ions as millimoles per litre of filtrate, and the percentage of filtered ions still remaining in the tubular fluid at the sites specified. CT, collecting tubule; DT, distal tubule; PCT, proximal convoluted tubule; TAL, thick ascending loop. (Data from Greger, 2000.)

Site of action of loop diuretics

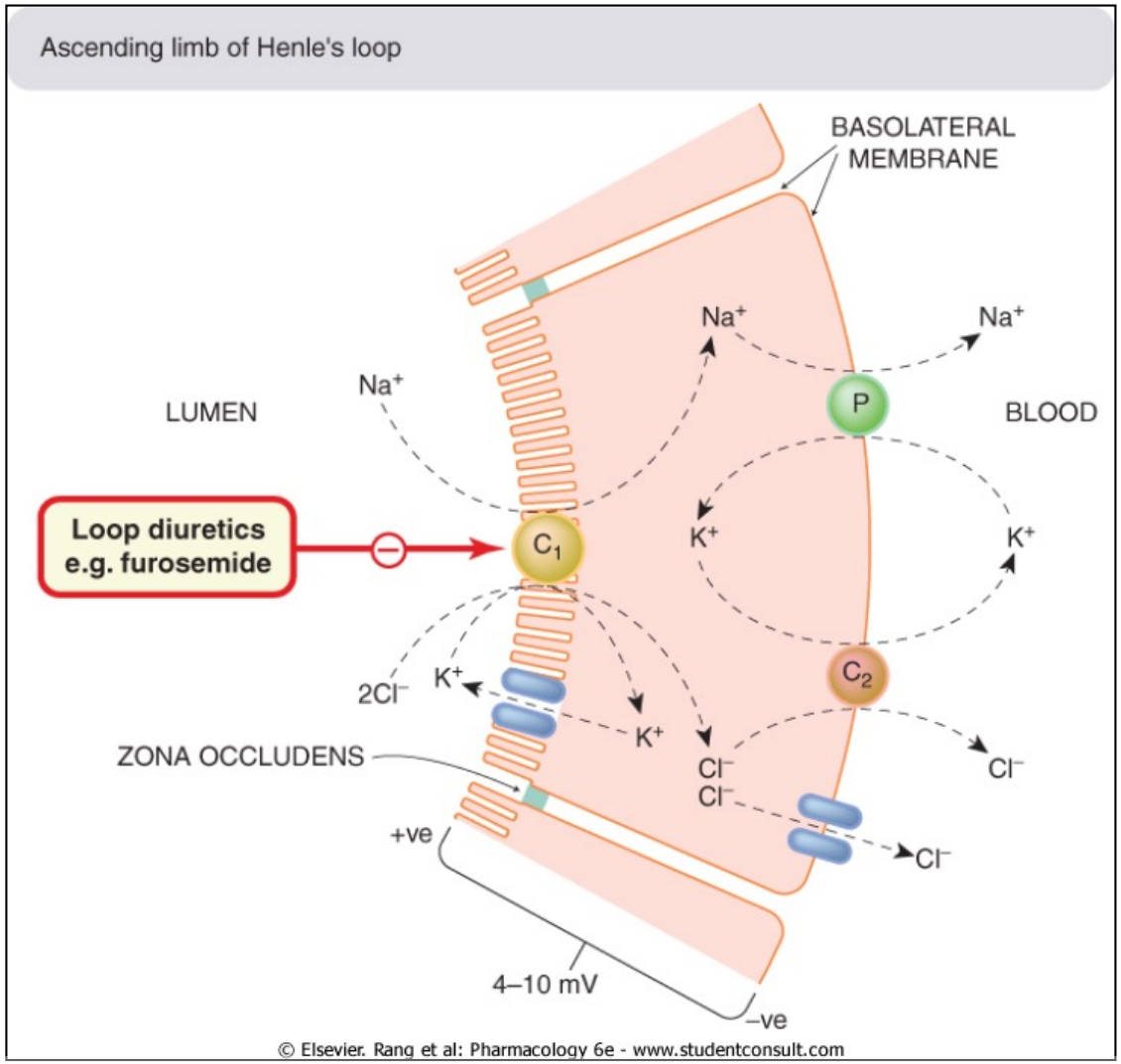
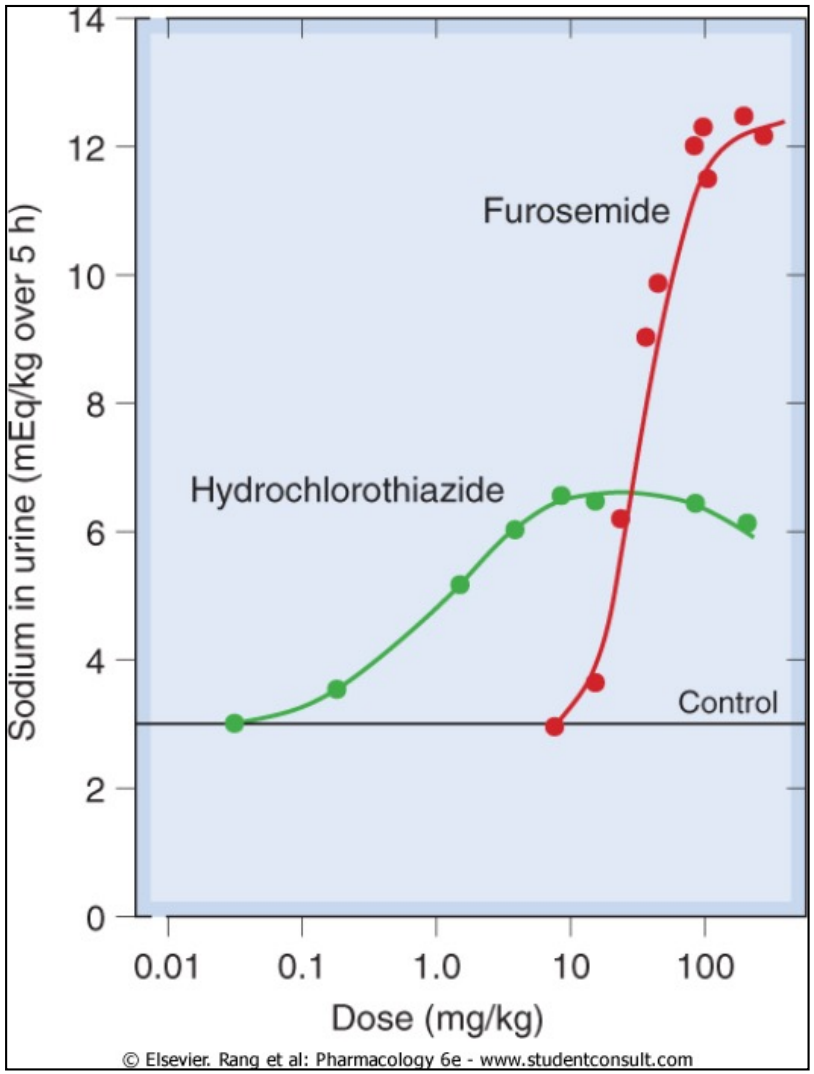


Figure 24-7 Ion transport in the thick ascending limb of Henle's loop, showing the site of action of loop diuretics. The sodium pump (P) is the main primary active transport mechanism, and Na+, K+ and Cl- enter by a cotransport system (C1). Chloride leaves the cell both through basolateral chloride channels and by an electroneutral K+/Cl- cotransport system (C2). Some K+ returns to the lumen via potassium channels in the apical membrane, and some Na+ is absorbed paracellularly through the zonula occludens. The diagram is simplified: the sodium pump exchanges 3 Na+ for 2 K+. (Based on Greger, 2000.)

Comparison of a thiazide and a loop diuretic



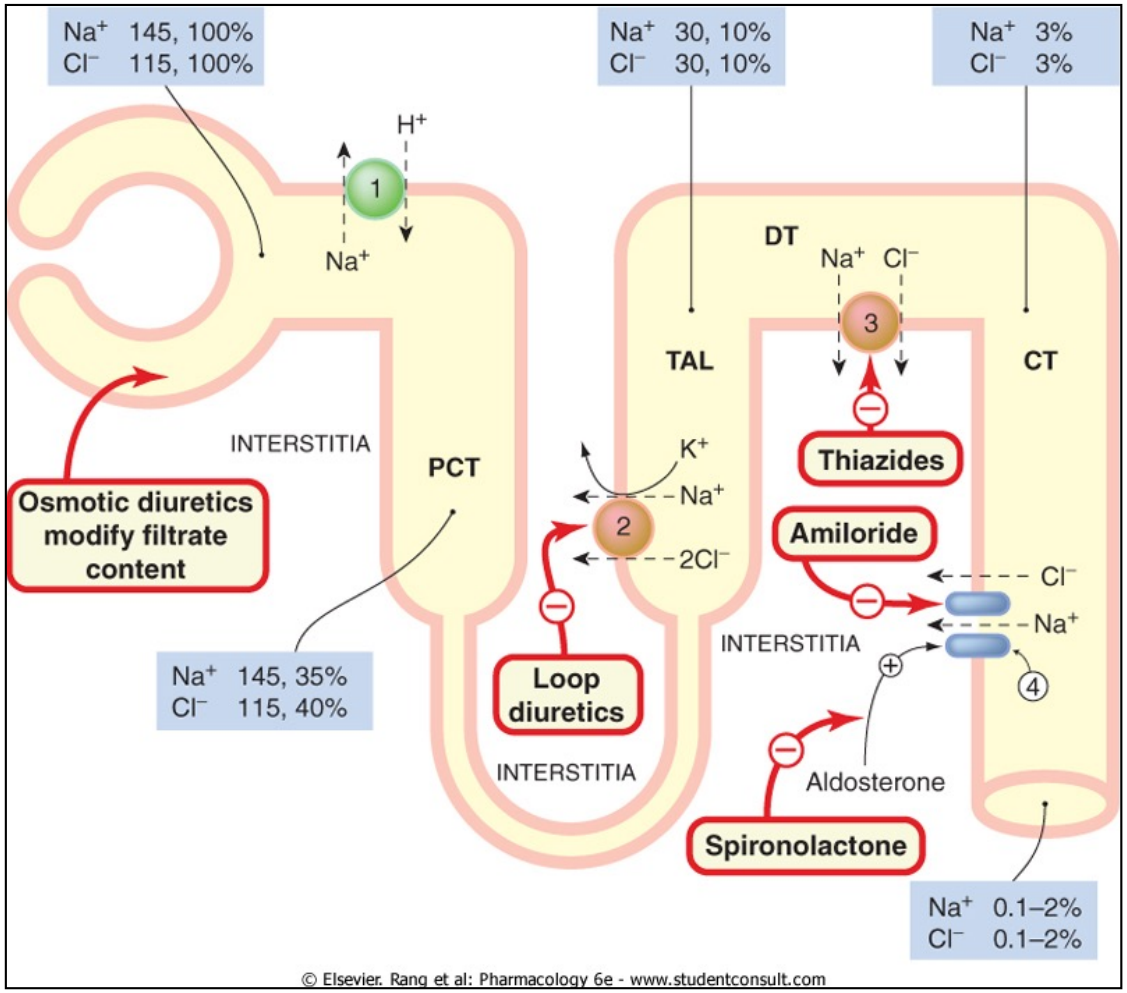


Figure 24-6 Dose-response curves for furosemide (frusemide) and hydrochlorothiazide, showing differences in potency and maximum effect 'ceiling'. Note that these doses are not used clinically. (Adapted from Timmerman R J et al. 1964 Curr Ther Res 6: 88.)

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2.2.3. Protein therapeutics

Biologics

1796 First vaccine: cowpox virus, to obtain immunization against smallpox (→ production of antibodies; Edward Jenner)

1920-1960 many other vaccines (diphtheria, tetanus, pertussis, polio, measles, mumps, rubella)

Late 19th century: antibodies that bind to poisons (venom antisera)

1921 first isolation of insulin from dog pancreas
1922 first insulin therapy on a human

Last 25 years: exponential growth of the clinical use of biologics

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Main uses of proteins in medicine:

- **Group I: Enzymes and regulatory proteins**
  - Replacing a protein that is deficient or abnormal (Insulin)
  - Augmenting an existing pathway (Erythropoietin)
  - Providing a novel function or activity (Botulinum toxin)

- **Group II: targeted proteins**
  - Interfering with a molecule or organism (→ monoclonal antibodies)

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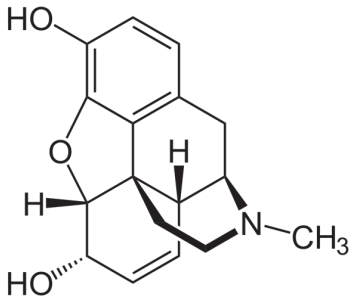
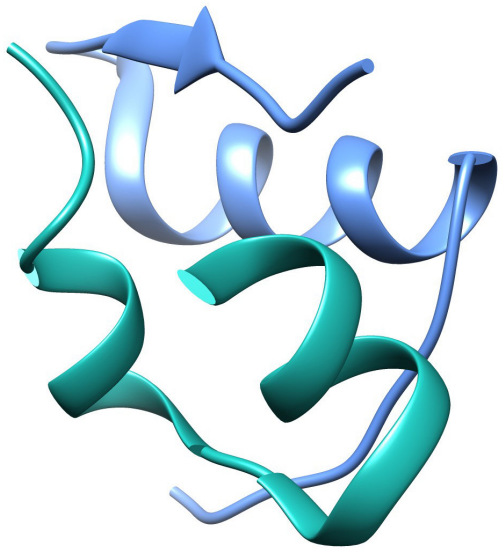
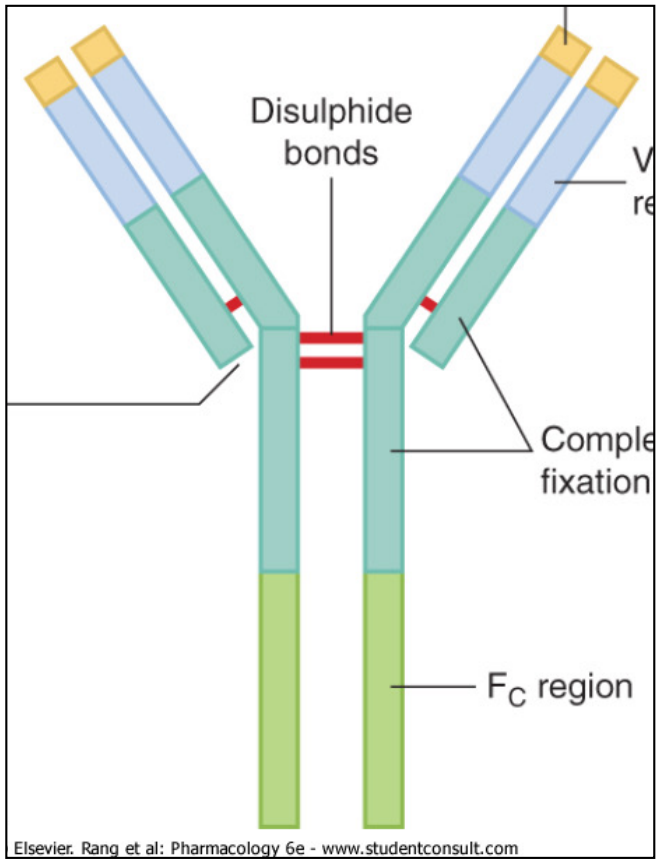
Morphine	Insulin	monoclonal Antibody
		

Molecular weight		
285 Da	5700 Da	150’000 Da
Number of amino acids		
	51	1330

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Important differences between protein therapeutics and small molecule drugs

	Small molecule drugs	Protein therapeutics
Manufacture	Chemical synthesis	Expression in cell lines, purification *(in cells that make post-translational modifications [Glysosylation, disulfide bonds])*
Storage	Room temperature (common)	Fridge or freezer
Administration	Oral (in many cases)	Subcutaneous or intravenous
Concentration dependence of effect: EC_{50} or IC_{50}	≥ nM	≤ nM
Off-target effects	common	rare
Elimination	2-step metabolism in liver / elimination by kidney	Other mechanisms ((lysosomal) proteolysis)
Elimination $t_{1/2}$	Order of hours	Some peptides: very short (min - h) mAbs: order of days
Interactions with other drugs	Common	Rare
Possible problems	Side effects	Immune response (more commonly against non-human proteins)
Costs		High (Infliximab, 20kCHF/year)

- Group I: Enzymes and regulatory proteins

  - Replacing a protein that is deficient or abnormal
    - hormone deficiencies (insulin, growth hormone)
    - hemostasis (coagulation factors)
    - metabolic enzyme deficiencies (specific deficiencies)

  - Augmenting an existing pathway: Hematopoiesis (erythropoietin) – immunoregulation (interferon) – hemostasis and thrombosis (tenecteplase) – endocrine disorders (GLP1 receptor agonists)

Erythropoietin: protein hormone secreted by the kidney, stimulates erythrocyte production in bone marrow
Tenecteplase: recombinant plasminogen activator (lysis of fibrin clot in myocardial infarction)

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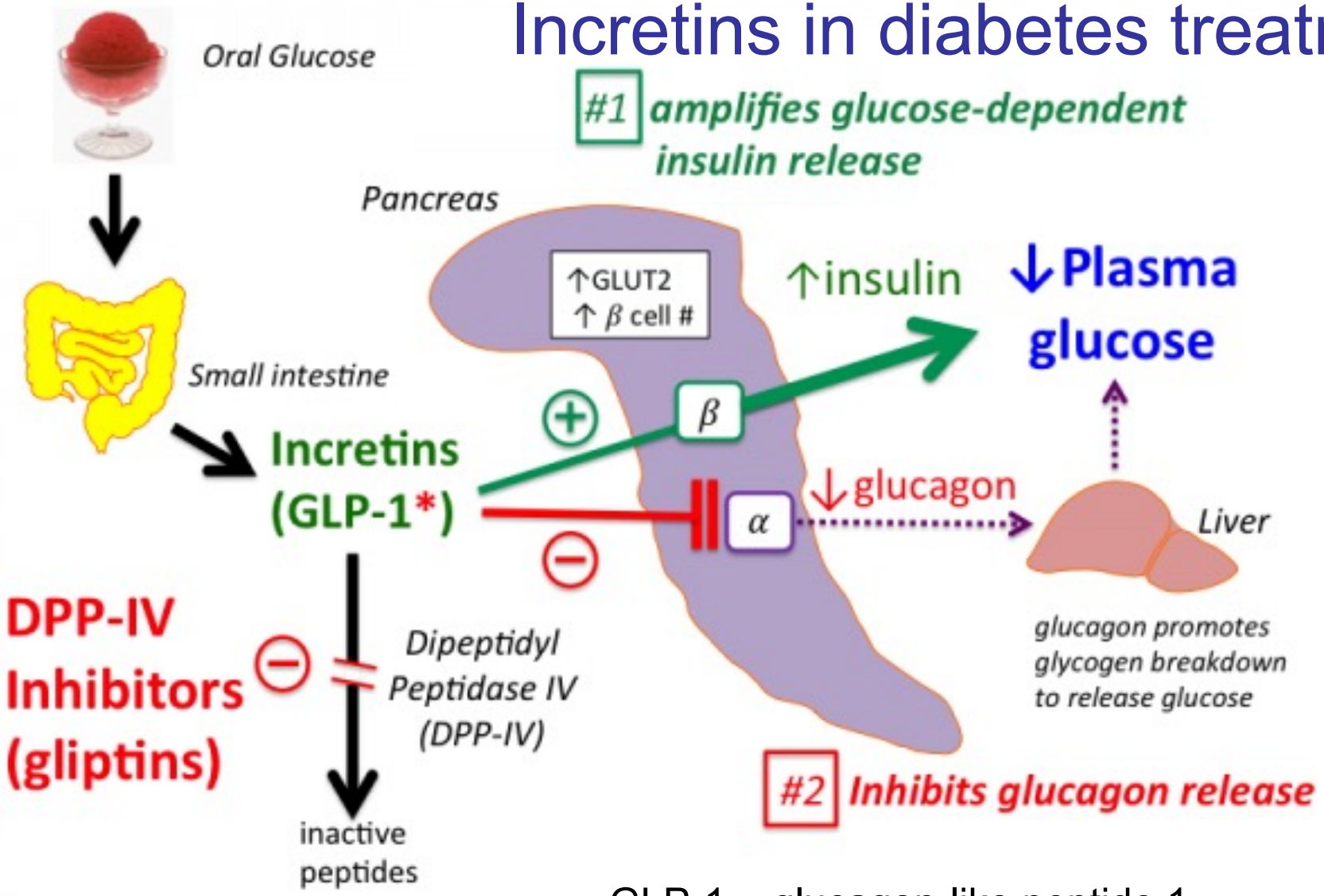
Incretins in diabetes treatment



GLP-1 = glucagon-like peptide 1

* Physiological $t_{1/2}$ ≈2 mins due to rapid inactivation by DPP-IV

Exenatide is an analog of GLP-1. It is the synthetic version of exendin-4, a peptide of the Gila monster. It has a longer $t_{1/2}$ than GLP-1 (2.4 h). It is administered subcutaneously.

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GLP-1 and GLP-1 receptor agonists (GLP-1 RAs)

- GLP-1 is primarily produced in enteroendocrine cells of distal small bowel
- Blood levels of GLP-1 are low during fasting and rise after a meal
- Act on GPCRs in the pancreas and the central and peripheral nervous system
- 2 interesting effects in the context of type 2 diabetes:
  - Increases insulin secretion in response to an increase in blood glucose levels
  - Decreases appetite (effect of the CNS), delays gastric emptying
- GLP-1 is very instable (t1/2 = 1-2 min) due to enzymatic degradation by dipeptidyl peptidase-4 (DPP-4). Inhibitors of DPP-4 are used in therapy of type 2 diabetes; they prolong the t1/2 of endogenous GLP-1 and stimulate insulin secretion at increased Glucose concentrations
- GLP-1 RAs need generally to be injected

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Sequence of some GLP-1 RAs

DPP-4 cleavage

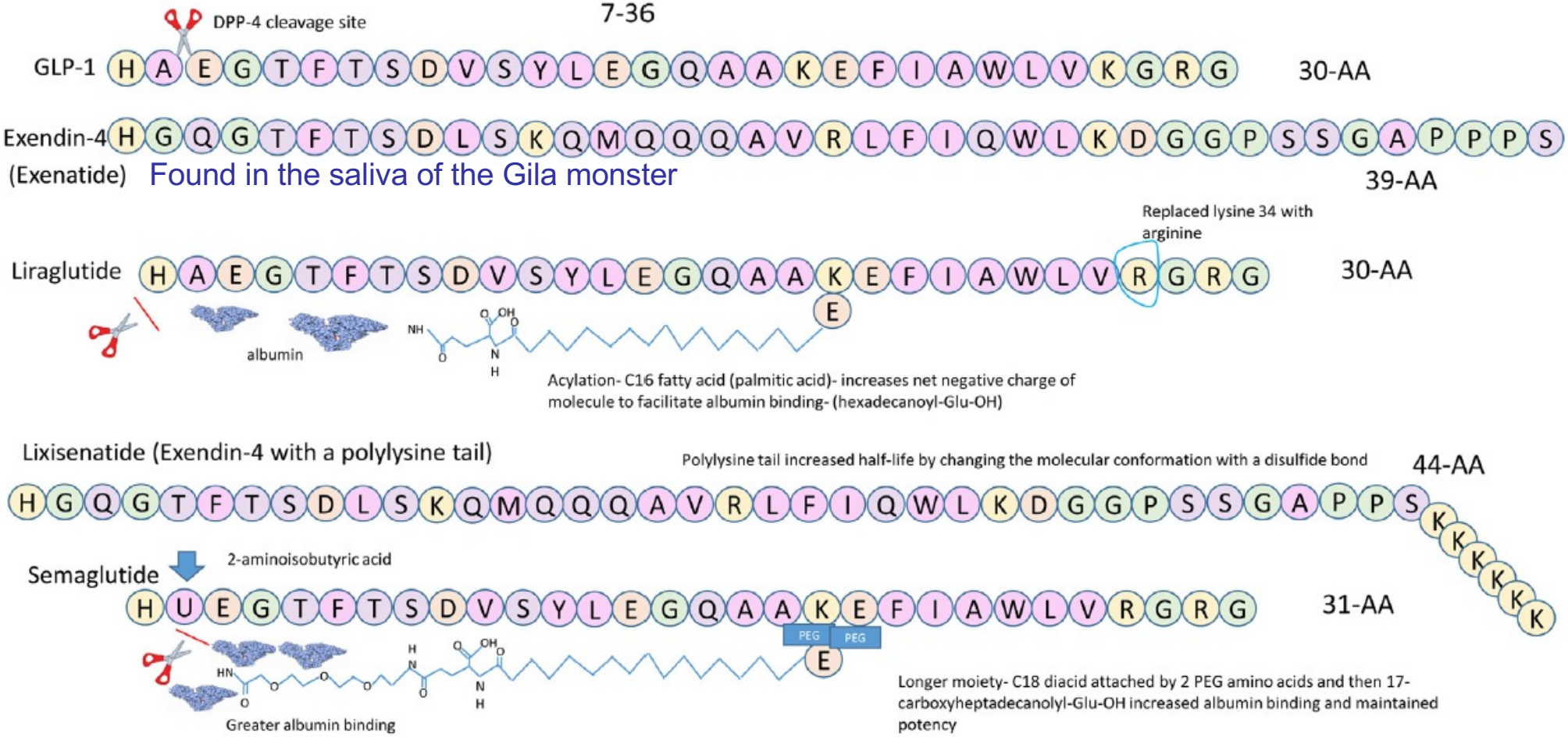

Figure 2. Primary structures of glucagon-like peptide (GLP-1), exendin-4, and synthetic analogues. Yellow circles, amino acids with positively charged side chains; pink circles, hydrophobic side chains; orange circles, negatively charged side chains; green circles, special cases; purple circles, polar uncharged side chains. Abbreviation: DPP-4, dipeptidyl peptidase type 4; PEG, polyethylamine glycolation with 8 amino-3,6-dioxaoctanoic acid.

Administration: Exenatide, 2x daily; liraglutide and lixisenatide, 1x daily; semaglutide, 1x weekly.

Development of GLP-1 RAs

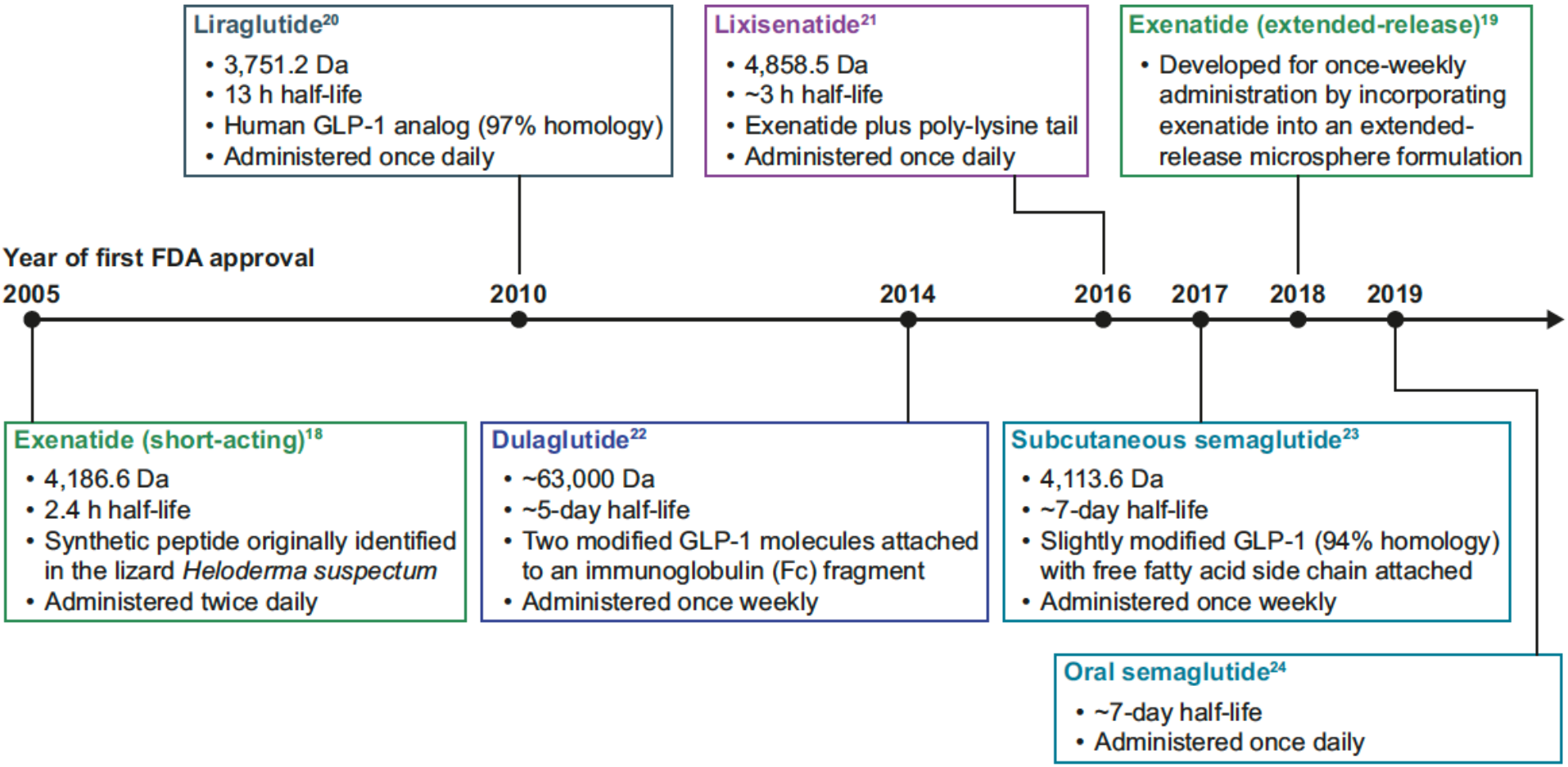

Fig. 1 Summary of GLP-1RAs approved for the treatment of type 2 diabetes. *FDA* US Food and Drug Administration, *GLP-1* glucagon-like peptide-1, *GLP-1RA* glucagon-like peptide-1 receptor agonist

Reviews in Endocrine and Metabolic Disorders (2022) 23:979–994

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https://www.science.org/content/article/breakthrough-of-the-year-2023

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GLP-1 RAs efficiently suppress appetite and induce weight loss

- March 2021: A clinical study showed that people taking semaglutide lost 15% of their body weight over a period of 16 months
- June 2021: Semaglutide is approved in the USA for the treatment of obesity. Brand names are Ozempic for diabetes and Wegovy for obesity. Doses for s.c. administration are ≤ 2mg /week for diabetes, 2.4 mg /week for obesity treatment; treatment costs > 1000 $ per week in USA.
- 2023
  - 1.7% of people in the USA have been prescribed semaglutide this year
  - A large study showed that in a population with excess weight and cardiovascular disease, people on semaglutide had a 20% lower risk of heart attacks than those on placebo
  - Semaglutide delays kidney disease progression in diabetes patients
  - Other trials under way: drug addiction, Alzheimer, Parkinsons disease
  - When semaglutide therapy is stopped, 2/3 of the lost body weight comes back within 1 year (preliminary data) → "forever" drug
- Possible side effects: Nausea, gastrointestinal problems, pancreatitis

→ A newer, more efficient drug: Tirzepatide, a GLP-1 receptor and glucose-dependent insulinotropic peptide (GIP) receptor agonist, approved in USA in 2022 for diabetes, and November 2023 for weight loss; reduced up to 21% weight.

https://www.science.org/content/article/breakthrough-of-the-year-2023#section_breakthrough

Unwanted effects of GLP-1 receptor agonists

- Gastrointestinal-related, mild to moderate: nausea (40%), diarrhea (21%), vomiting (16%)
- Gallbladder-related (cholelithiasis, 0.8%, cholecystitis, 0.5%)
- Acute pancreatitis (0.2%)
- No significant changes on cardiovascular risk parameters, on mental health, on neoplasms; decrease in mortality

Glucagon-like Receptor-1 agonists for obesity: Weight loss outcomes, tolerability, side effects, and risks

Wissam Ghusn [a,b], Maria D. Hurtado [c,*] https://doi.org/10.1016/j.obpill.2024.100127

(determined for liraglutide, semaglutide and tirzepatide)

- Any indication of changes in the bone mass?
  - A recent meta-analysis showed no change in bone turnover markers, but in an increase in a bone resorption marker upon GLP-1RA treatment (https://doi.org/10.1002/dmrr.3843)
  - A study showed that in Type 2 diabetes mellitus patients, treatment with GLP-1-RAs decreased the risk of fractures

https://doi.org/10.1016/j.bone.2024.117338

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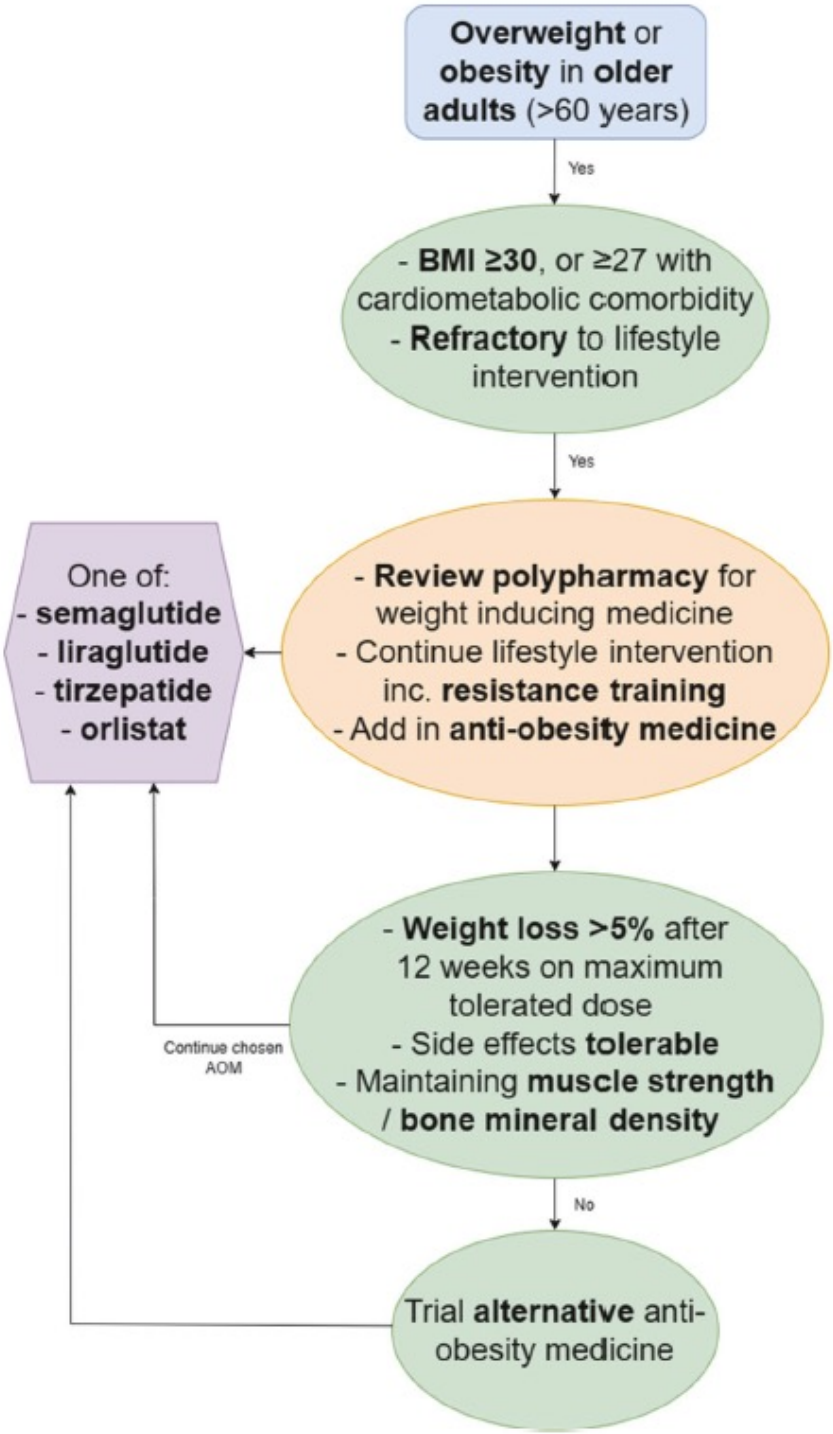

Fig. 3. An updated algorithm for the medical management of obesity using approved pharmacological agents. BMI body mass index. If in the USA, phentermine/topiramate could also be considered after semaglutide.

International Journal of Obesity; https://doi.org/10.1038/s41366-024-01529-z

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Nestlé introduces *Vital Pursuit* brand to support GLP-1 users in the US

Nestlé is introducing *Vital Pursuit*, a new line of foods intended to be a companion for GLP-1 weight loss medication users and consumers focused on weight management in the US. The products are high in protein, a good source of fiber, contain essential nutrients, and they are portion-aligned to a weight loss medication user's appetite. The new line is also well-suited to support a balanced diet for anyone on a weight management journey. *Vital Pursuit* is the first food brand from Nestlé intended for GLP-1 users with the goal of complementing the eating habits of millions of Americans who are currently prescribed a weight loss medication or actively working to manage their weight.

https://www.nestle.com/media/news/vital-pursuit-brand-glp-1-weight-support

Protein therapeutics are generally injected

Oral administration of therapeutic peptides is hindered by
- poor absorption across the gastrointestinal barrier and
- degradation by proteolytic enzymes

Strategies to increase absorption:
- Permeation enhancers
- Modulation of pH
- Direct enzyme inhibition
- Peptide cyclization (removes exposed N- and C-termini)
- Mucus-penetrating agents (to enhance the rate of passage across the mucus barrier)
- Cell-penetrating peptides that deliver the peptide via exocytosis
- Intestinal patches (protection from local degradation)
- Hydrogels facilitating prolonged retention, resisting enzymatic degredation

Some preclinical trials were successful; the products did however not pass clinical trials

→ An exception is an oral form of Semaglutide that has been approved by the FDA in 2019

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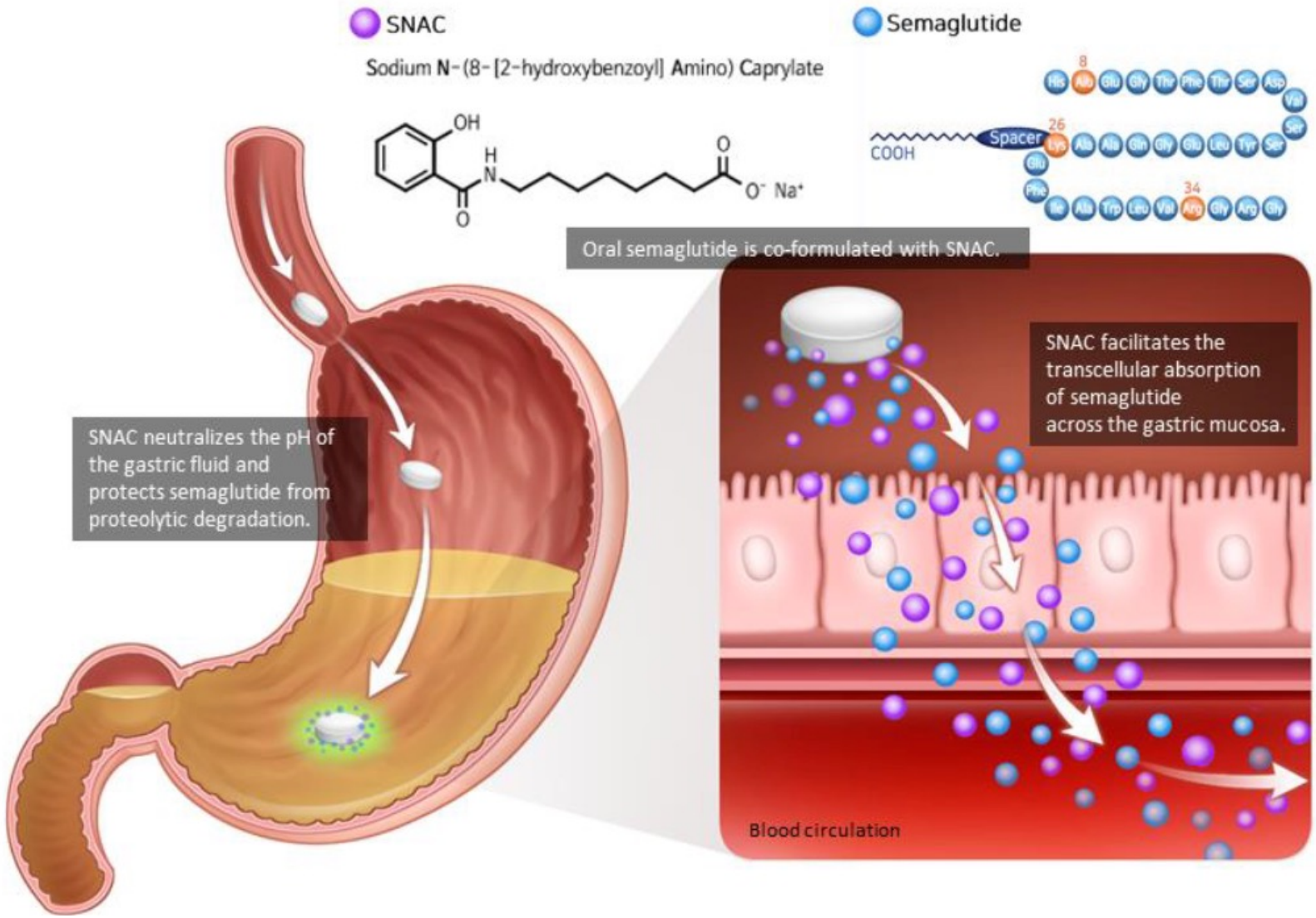
Strategy for oral administration of semaglutide



Bioavailability: 0.8-1.4%

Int. J. Mol. Sci. **2021**, *22*, 9936. https://doi.org/10.3390/ijms22189936

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Group II: targeted proteins

Interfering with a molecule or organism → monoclonal antibodies

- monoclonal antibodies are used to neutralize pathogens or other dangerous substances in the blood of the patient
- antibodies are different from conventional drugs in many aspects (they are big molecules, have a very strong and specific interaction with the target)
- Initially, monoclonal antibodies from mouse were used. Currently used mAbs were “humanized” to different degrees. This reduces the risk of an immune response, extends the plasma half-life and improves the ability of the antibody to activate human defense mechanisms
- name convention: name ending on –ximab = chimeric, -zumab = humanized antibody, -umab = human antibody

Type of mAb	Mouse part
chimeric	Variable regions
humanized	Hypervariable regions
human	-

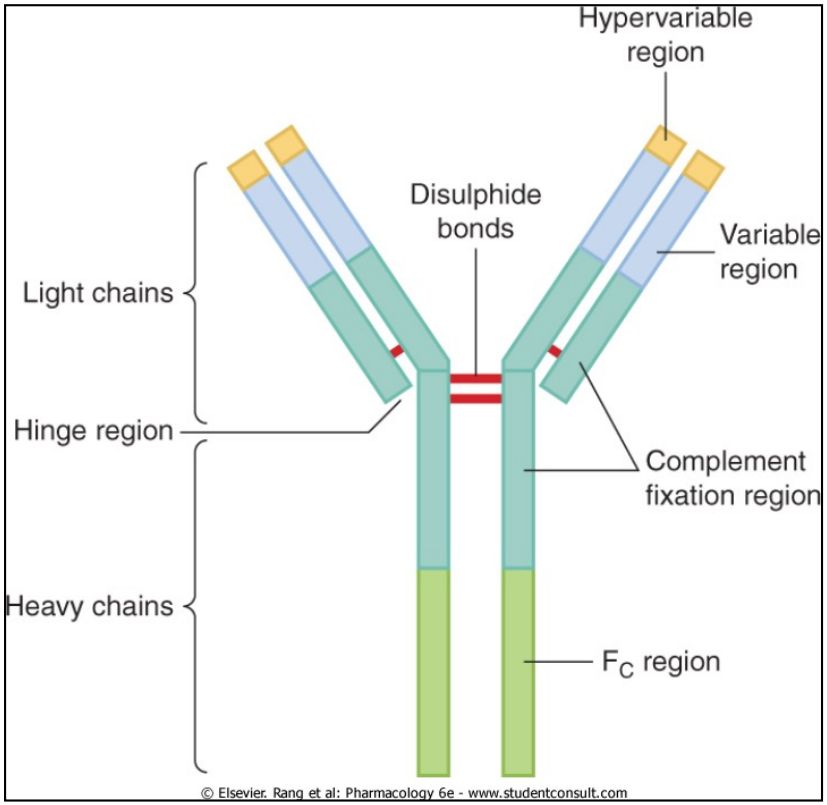

Figure 55-1 Production of engineered 'chimeric' and 'humanised' monoclonal antibodies. The Y-shaped antibody molecule consists of two main domains: the Fc (constant) domain and the Fab (antibody-binding) domain. At the tip of the Fab regions (on the arms of the 'Y') are the hypervariable regions that actually bind the antigen.. (After Walsh, 2004.)

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monoclonal antibodies

- three different ways of action
  - target specifically molecules or cells, guide the immune system to destroy them (recognition of the Fc region)
  - physical obstruction of a functionally important region of a molecule
  - Shuttle for drugs (antibody-drug conjugates)

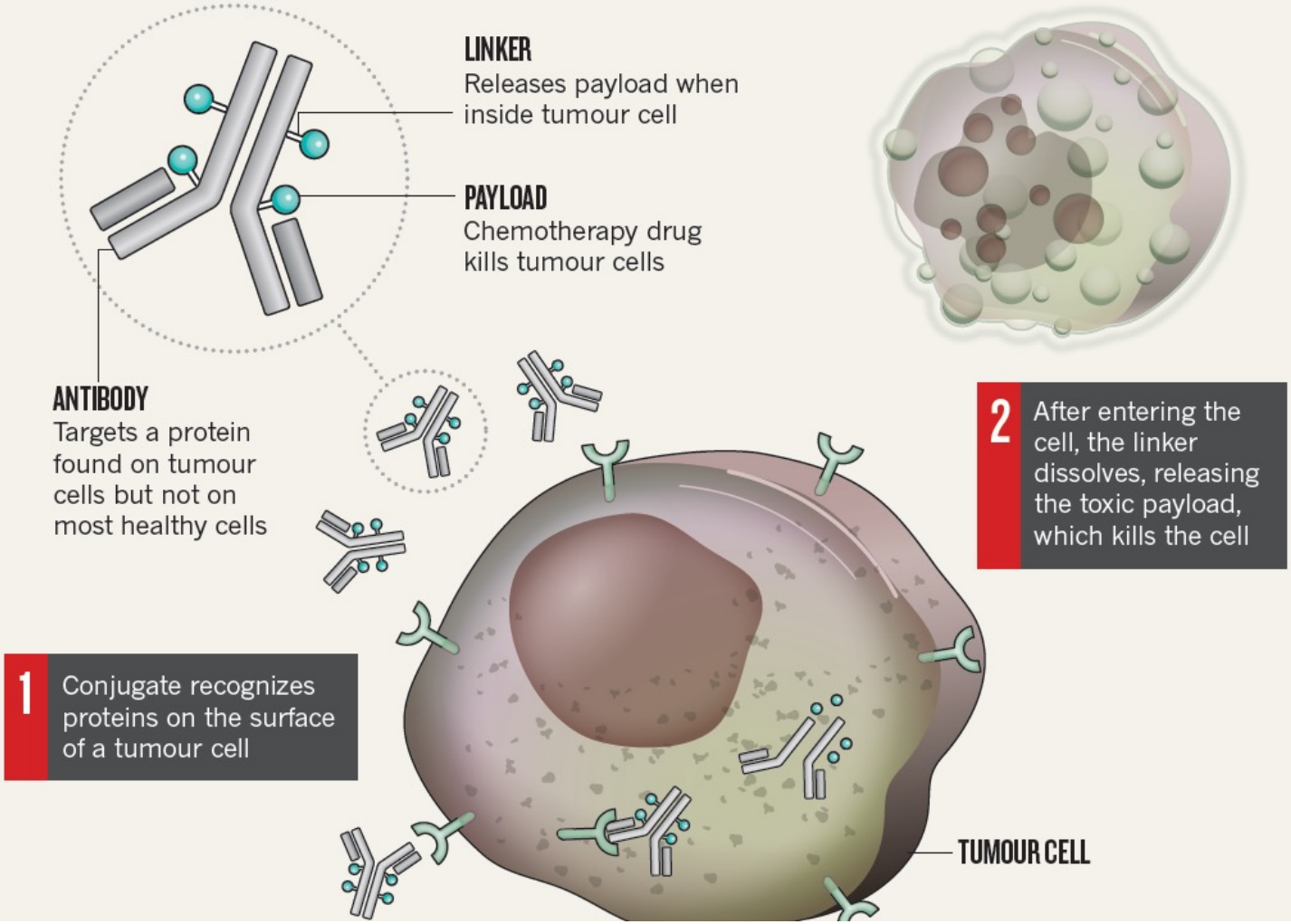
- different applications:
  - Cancer → targeting of specific signaling pathways that are increased in cancer
  - Immune-inflammatory conditions (rheumatoid arthritis, inflammatory bowel disease, psoriasis and others)
  - Transplantation (inhibition of graft rejection)
  - pulmonary disorders (asthma)
  - Hemostasis and thrombosis (ex. Inhibitor of platelet aggregation, to prevent thrombosis)

(until 2017, close to 100 monoclonal antibodies approved by EMA and FDA; http://www.actip.org/products/monoclonal-antibodies-approved-by-the-ema-and-fda-for-therapeutic-use/)

antibody drug conjugates

SEEK AND DESTROY

Scientists say that conjugates tethering a chemotherapy drug to an antibody are on the cusp of achieving clinical success for treating certain types of cancer.



A LONG TIME COMING

For nearly half a century, researchers have been trying to capture the therapeutic potential of antibody–drug conjugates, which combine the tumour-killing power of a drug with the tumour-seeking ability of an antibody.

1964
Researchers create the first antibody–drug conjugates[1].

1981
The Dana Farber Cancer Institute in Boston, Massachusetts, spins out ImmunoGen to focus on conjugates.

1986
US regulators approve the first therapeutic 'naked' antibody.

1998
Seattle Genetics is founded and focuses on conjugates.

2000
US regulators approve Pfizer's Mylotarg (gemtuzumab ozogamicin) for treatment of leukaemia.

2010
Pfizer withdraws Mylotarg after finding no significant benefit to patients.

2011
Regulators approve Seattle Genetics' Adcetris for some forms of lymphoma.

Brentuximab vedotin is an antibody-drug conjugate (ADC) comprising an anti-CD30 monoclonal antibody attached by a protease-cleavable linker to a synthetic drug, monomethyl auristatin E (MMAE) utilizing Seattle Genetics' proprietary technology. The ADC employs a linker system that is designed to be stable in the bloodstream but to release MMAE upon internalization into CD30-expressing tumor cells.

Rheumatoid arthritis and biologics

- When you have **Rheumatoid arthritis**, your immune system, which normally fights infection, attacks the lining of your joints making them inflamed. This inflammation causes your joints to be hot, swollen, stiff and painful. The small joints of your hands and feet are usually affected first. If the inflammation goes on without treatment, it can lead to damaged joints. Once the joint is damaged it cannot be repaired, so treating rheumatoid arthritis early is important

- **Biologics** in this context are a group of medications that suppress the immune system and reduce the inflammation in the joints

- **Tumor necrosis factor** (TNF) is a key regulator of inflammatory processes in immune-mediated inflammatory diseases → inactivating TNF is therefore a plausible approach in treating such conditions

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Treatment options for Rheumatoid arthritis

- Non-steroidal anti-inflammatory drugs (NSAIDs)
- Traditional DMARDs (disease-modifying anti-rheumatic drugs), e.g. methotrexate and others
- Biologic DMARDs:
  - Tumor necrosis factor inhibitors
  - Anti-CD28 therapy (abatacept)
  - Anti-IL1 therapy (anakinra)
  - Anti-B-cell therapy (rituximab)

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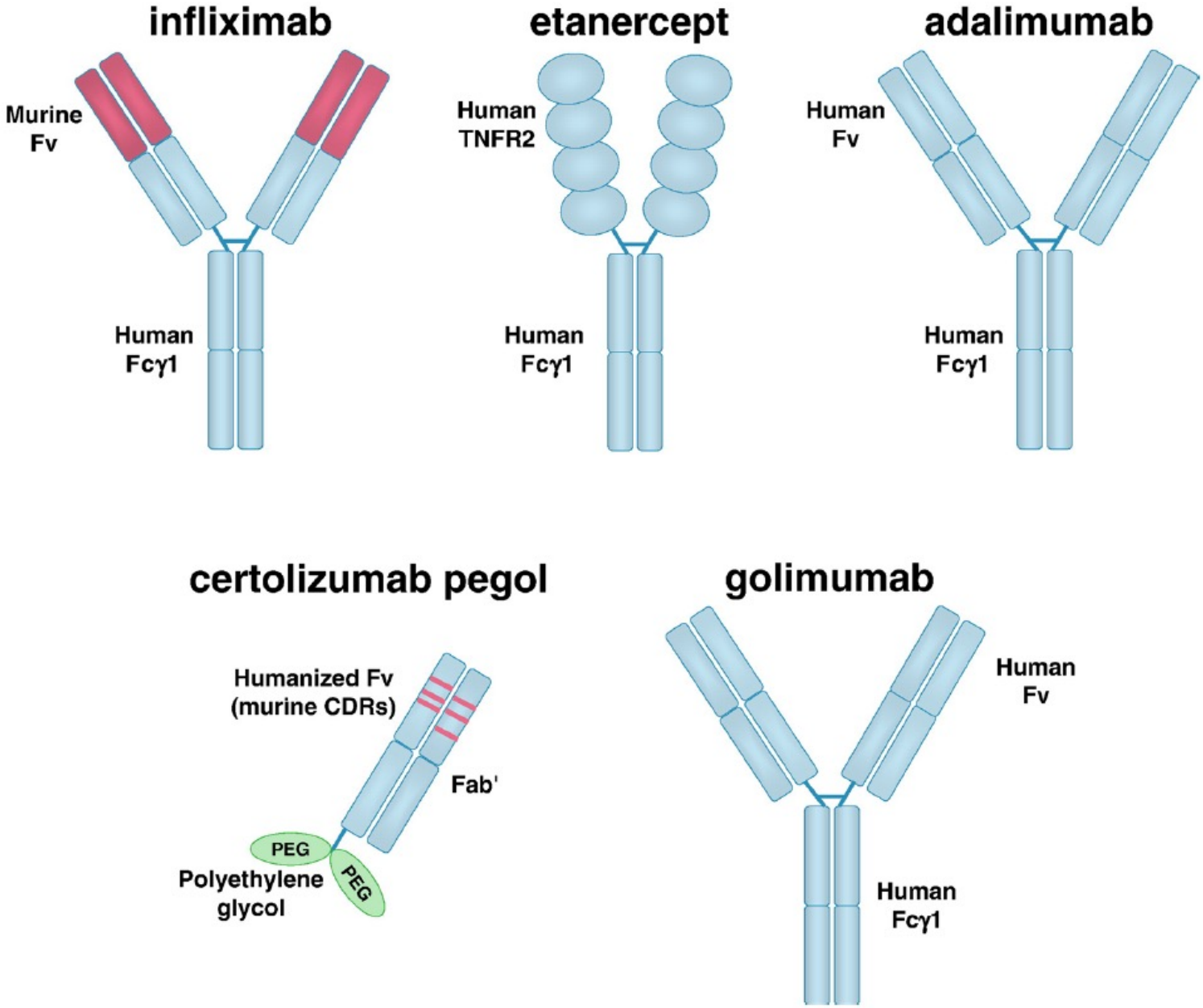
Structures of anti-TNF agents



Adalimumab, certolizumab pegol, golimumab and infliximab: mAbs: etanercept: fusion protein containing a part of the receptor. (CDR= complementarity determining region)

Jinesh, S., Inflammopharmacol DOI 10.1007/s10787-015-0229-0 (2015)

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Pharmacokinetics of TNF inhibitors

Table 1 Pharmacokinetics of TNF inhibitors (Lexicomp Online et al. 2014; Nestorov 2005; Mewar and Wilson 2011)

TNF Inhibitor	Onset time	Half-life	Time-to-peak effect	Route of administration and Frequency
Adalimumab	–	~2 weeks	~131 h	subQ, twice a week
Certolizumab pegol	–	~14 days	54–171 h	subQ
Golimumab	–	~14 days	2–6 days	subQ
Infliximab	~2 weeks, Crohn's disease	7–12 days	–	IV, every 4–8 weeks
Etanercept	~2–3 weeks; RA: 1–2 weeks	3–5 days	35–103 h	subQ, every 1–2 weeks

IV intravenous, *SubQ* subcutaneous

Jinesh, S., Inflammopharmacol DOI 10.1007/s10787-015-0229-0 (2015)

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Possible side effects of anti-TNF agents

- Viral infections (frequent)
- Local reactions (occasional-frequent)
- Heart problems (occasional)
- Tuberculosis reactivation (occasional)
- Malignancies (rare)
- Demyelination diseases (rare)

Frequent (between 1/10 and 1/100)
Occasional: between 1/100 and 1/1000
Rare: between 1/1000 and 1/10000

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Effectiveness of anti-TNF agents in RA

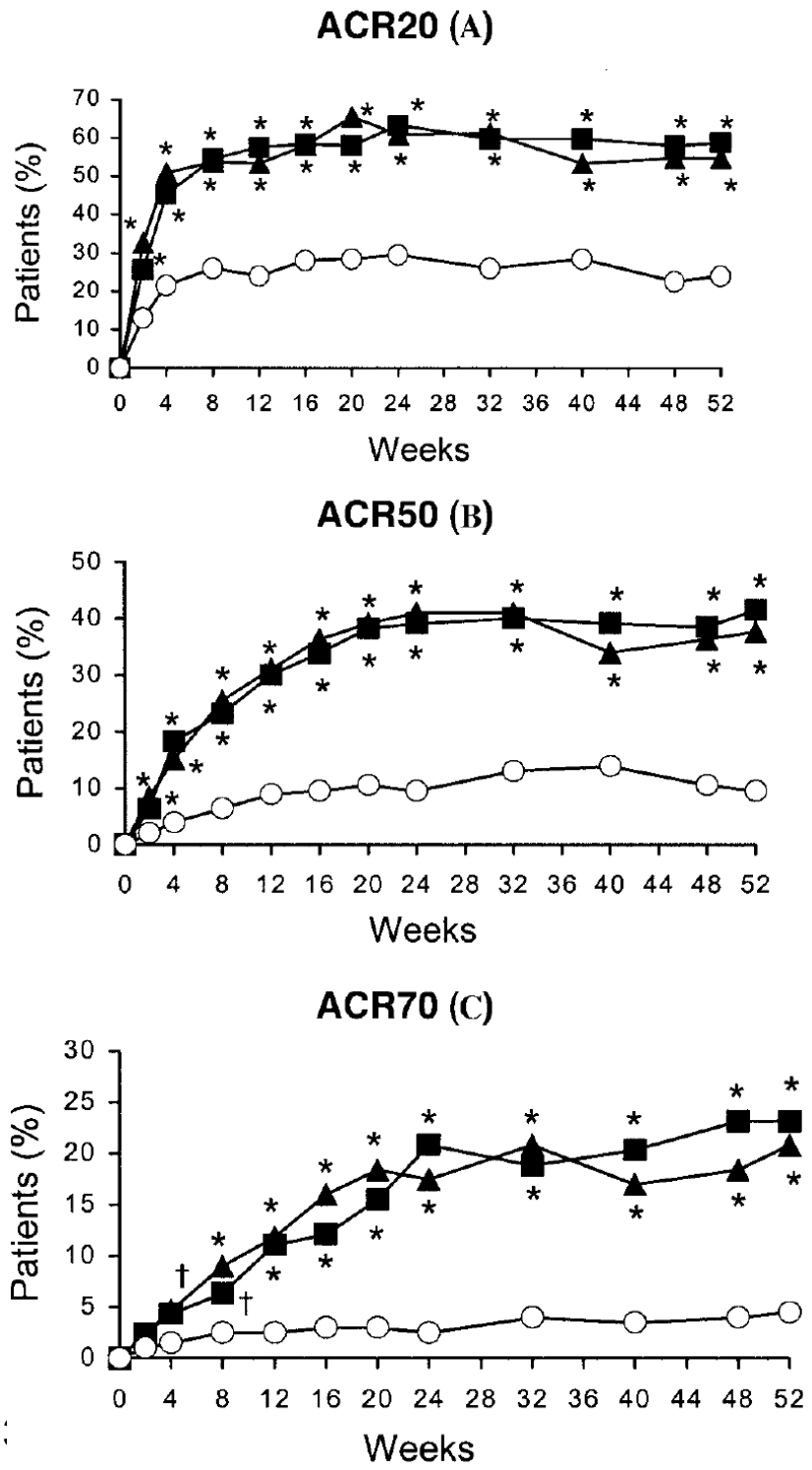


Figure 3. Distribution of patients who showed improvement in the American College of Rheumatology (ACR) criteria of at least 20%, 50%, and 70% (**A, B,** and **C,** respectively) (using nonresponder imputation), among the patients receiving adalimumab 40 mg every other week plus methotrexate (MTX) (■), adalimumab 20 mg weekly plus MTX (▲), and placebo plus MTX (○). * = $P \leq 0.001$, and † = $P \leq 0.01$ versus placebo (by Pearson's chi-square test).

This study illustrates that the treatment is highly efficient

In this study, 14 % of the patients had serious adverse effects of the treatment

ARTHRITIS & RHEUMATISM
Vol. 50, No. 5, May 2004, pp 1400–1411
DOI 10.1002/art.20217

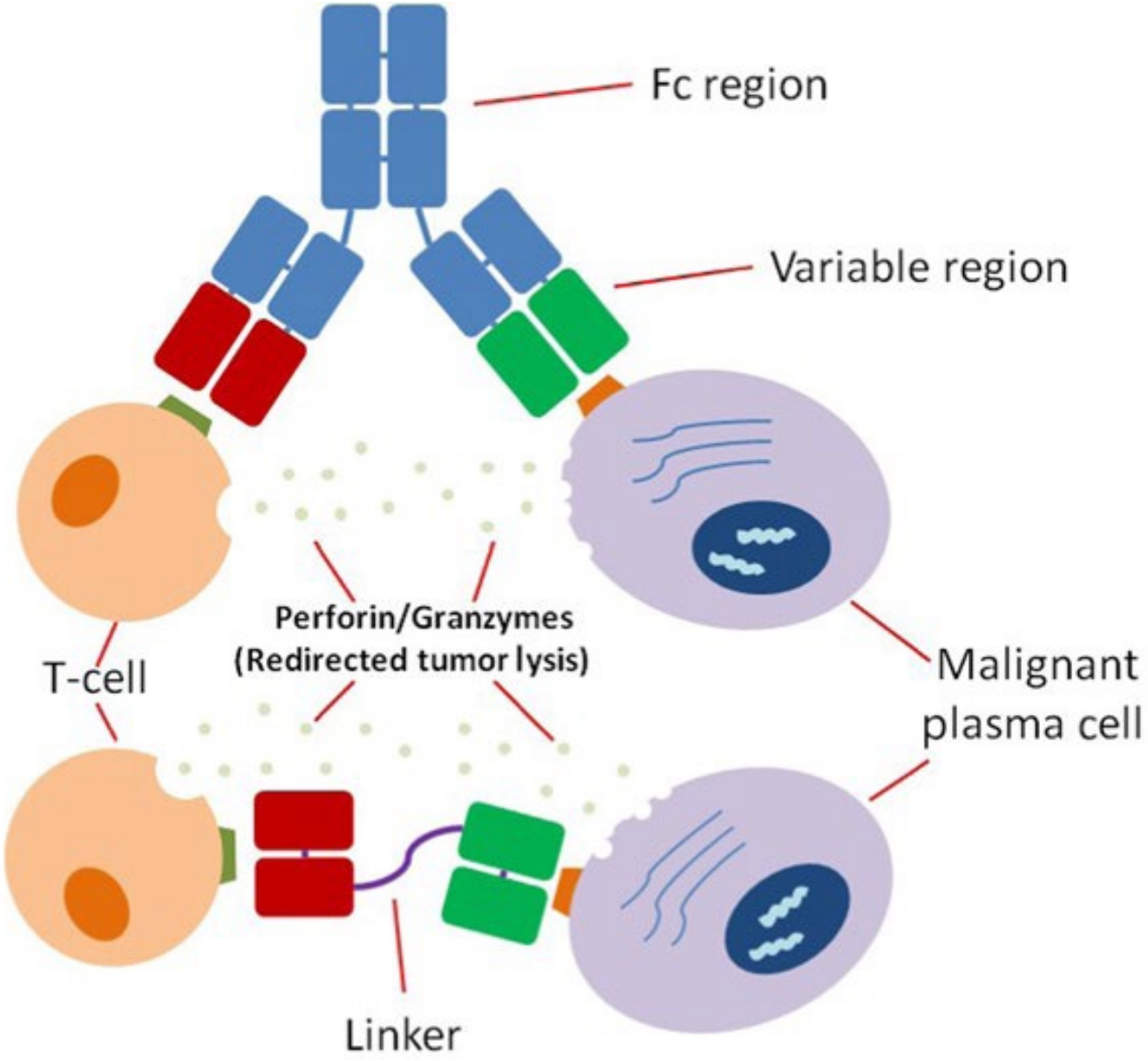
Bispecific T-cell antibodies engage the immune system directly by simultaneously binding CD3 on T cells and a target epitope

IgG-like BiAb

- Elranatamab
- REGN-5458
- Teclistamab
- CC-93269
- TNB-383B
- Cevostamab
- Talquetamab

Non-IgG-like BiAb

- AMG 420
- AMG 701 (Extended half-life)



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Biosimilars

A biosimilar is a biological agent that contains a similar version of the active substance of an already approved original biological agent (reference product), and is
intended to be used in the same manner as the reference product.

Analogous to the term “generic” for small molecule drugs *(a generic is chemically identical with the original product, a biosimilar is not)*

Biosimilars can be produced and put on the marked once the patent of the original product expires.

Before approval, biosimilars have to undergo a rigorous testing and evaluation, to make sure that they are equivalent to the original product.

Biosimilars of anti-TNF agents have been on the market since 2016, and allow a substantial cost reduction.

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Target	*examples*
2.1. Receptors for physiological ligands	
Transmembrane receptors	
2.1.1. G-protein-coupled receptors	*- adrenergic receptors* *- opioid receptors*
2.1.2. ligand-gated ion channels	*- $GABA_A$ receptors*
2.1.3. kinase-linked receptors	*- insulin receptor*
Intracellular receptors	
2.1.4. nuclear receptors	*- PPARγ (peroxisome proliferator-activated receptor γ* *- pregnane X receptor*
2.2. Other targets/approaches	
2.2.1. enzymes	*- cyclo-oxygenase (in pain chapter)* *- dihydrofolate reductase* *- HIV protease* *- tyrosine kinases* *- angiotensin-converting enzyme*
2.2.2. ion channels and transporters	*- transporters in the kidney*
2.2.3. protein therapeutics	*- GLP-1 receptor agonists* *-TNF-α monoclonal antibodies (e.g. infliximab)*
2.2.4. gene therapy	***- Nusinersen*** ***- Tisagenlecleucel /Axicabtagene ciloleucel***

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2.2.4 Gene therapy

- Gene therapy is the genetic modification of cells to prevent, alleviate or cure disease

- Points to consider:
  - Strategy (inhibit a gene, replace a gene, repair a gene…)
  - The main challenge is the **delivery** (type of vector, type of administration (*in vivo* or *ex vivo)*) and **maintenance** of new genetic information
  - Target organ(s)
  - Possible unwanted reactions
    - Related to delivery
    - Related to gene

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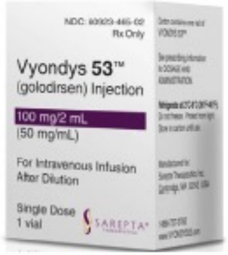
Nucleic Acids Research, 2023, Vol. 51, No. 6 2529–2573
https://doi.org/10.1093/nar/gkad067

Approved RNA therapeutics (2023)

ASO 1998
CMV Retinitis

Aptamer 2004
Age-Related Macular Degeneration (AMD)

ASO 2013
Familial Hypercholesterolemia



SSO 2016 **SSO 2019**
Duchenne Muscular Dystrophy

SSO 2016
Spinal Muscular Atrophy

Vaccine Adjuvant 2017
HBV

siRNA 2018
TTR Polyneuropathy

ASO 2018
TTR Polyneuropathy

waylivra®
(volanesorsen) injection 285 mg/1.5 mL

ASO 2019 EU
Familial Chylomicronemia Syndrome

siRNA 2019
AHP Porphyria

siRNA 2020
Primary Hyperoxaluria type 1

SSO 2020
Duchenne Muscular Dystrophy

SSO 2021

siRNA 2021
LDL-C reduction “siRNA statin”

siRNA 2022
TTR Polyneuropathy

mRNAs 2021/2022
Covid-19 Vaccines

ASO, antisense oligonucleotide; SSO, splice-switching oligonucleotide; siRNA, small interfering RNA; mRNA vaccine

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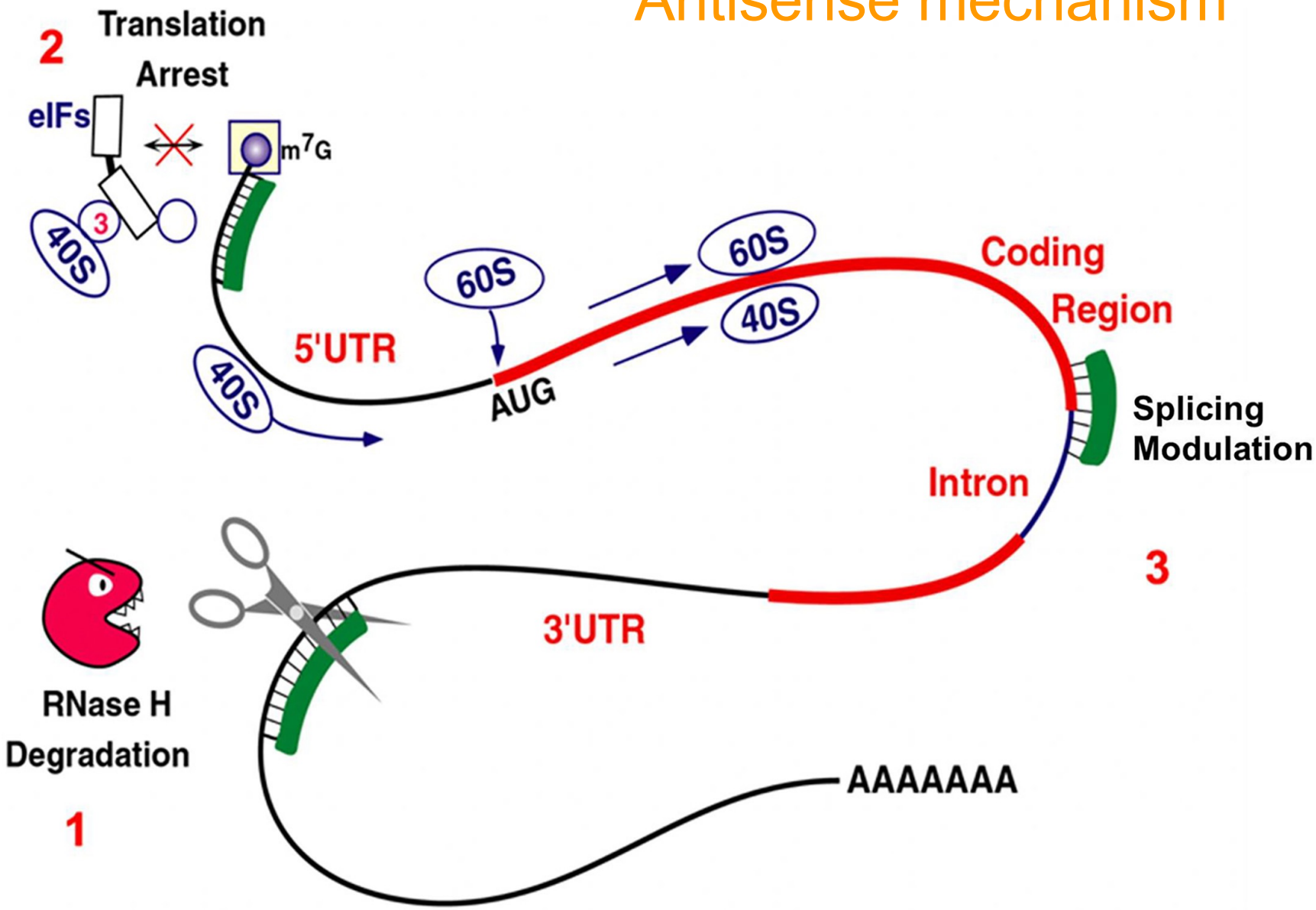
Nucleic Acids Research, 2023, Vol. 51, No. 6 2529–2573
https://doi.org/10.1093/nar/gkad067

Antisense mechanism



Cartoon of combined ASO mechanisms of action vis-`a-vis an mRNA target, either in the nucleus or cytoplasm of the cell: (1) Eliciting Rnase H with subsequent degradation of the target, (2) translation arrest, i.e. steric blockage and (3) modulation of splicing. mRNA regions include 5 -cap and 5 -untranslated region (5 -UTR), coding region, 3 -UTR and poly-A tail.

Mechanism of action of siRNA drugs

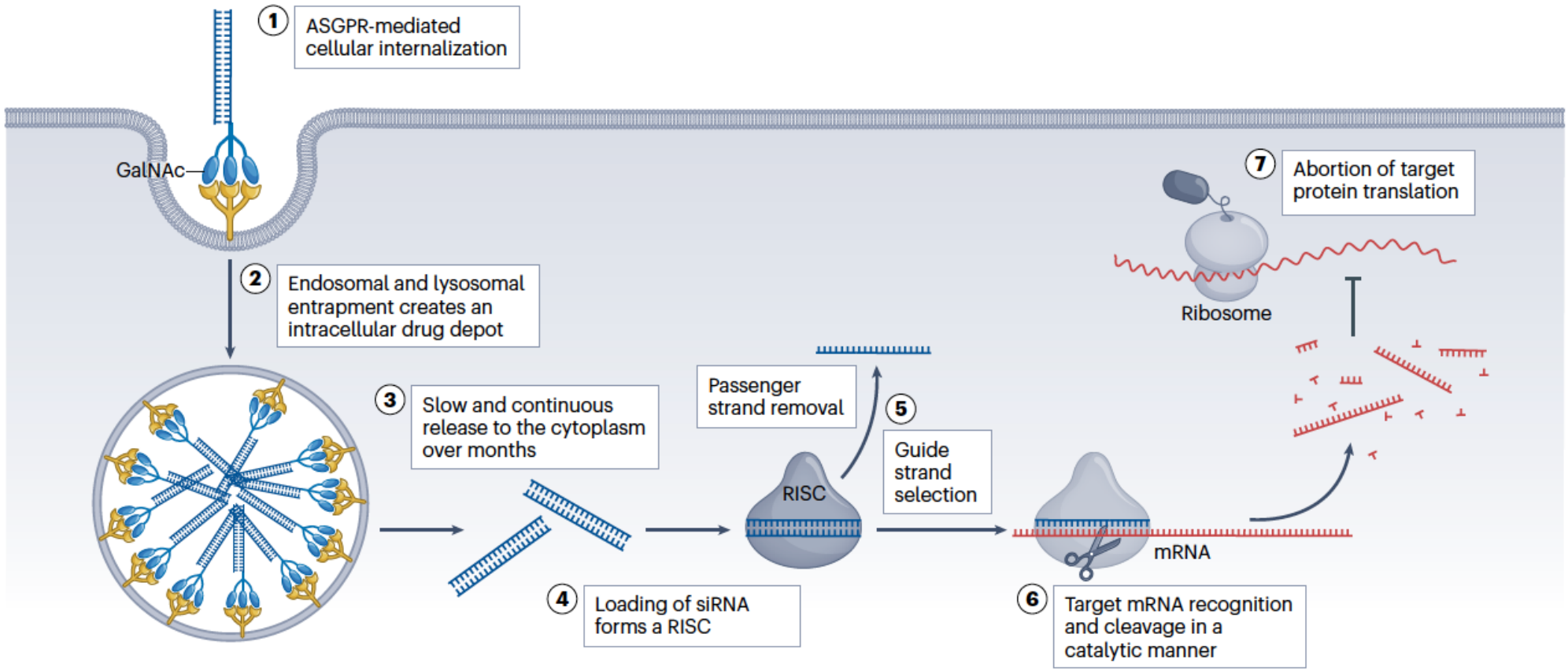


Fig. 1 | Mechanism of action of siRNA drugs. Effective gene silencing of small interfering RNA (siRNA) drugs requires efficient cellular internalization, endosomal escape, RNA-induced silencing complex (RISC) loading, target recognition and cleavage. *N*-acetylgalactosamine (GalNAc)–siRNAs exhibit multi-month durability owing to the rapid asialoglycoprotein receptor (ASGPR)-mediated membrane endocytosis and slow release from the intracellular drug depot after internalization. ASGPRs are highly expressed in hepatocytes and have a high recycling rate (minutes) for GalNAc-conjugated oligonucleotide internalization. Entrapment of chemically stabilized siRNAs in endosomal and lysosomal compartments can serve as an intracellular drug depot to support long-term durability. After being released into the cytoplasm, the siRNA needs to be assembled into a RISC to enable guide strand selection and subsequent recognition and cleavage of complementary mRNA substrates. Argonaute 2 (Ago2) protein is the catalytic component of RISC with RNA-guided endonuclease activity to mediate target cleavage. Ab, antibody.

https://doi.org/10.1038/s41573-024-00912-9

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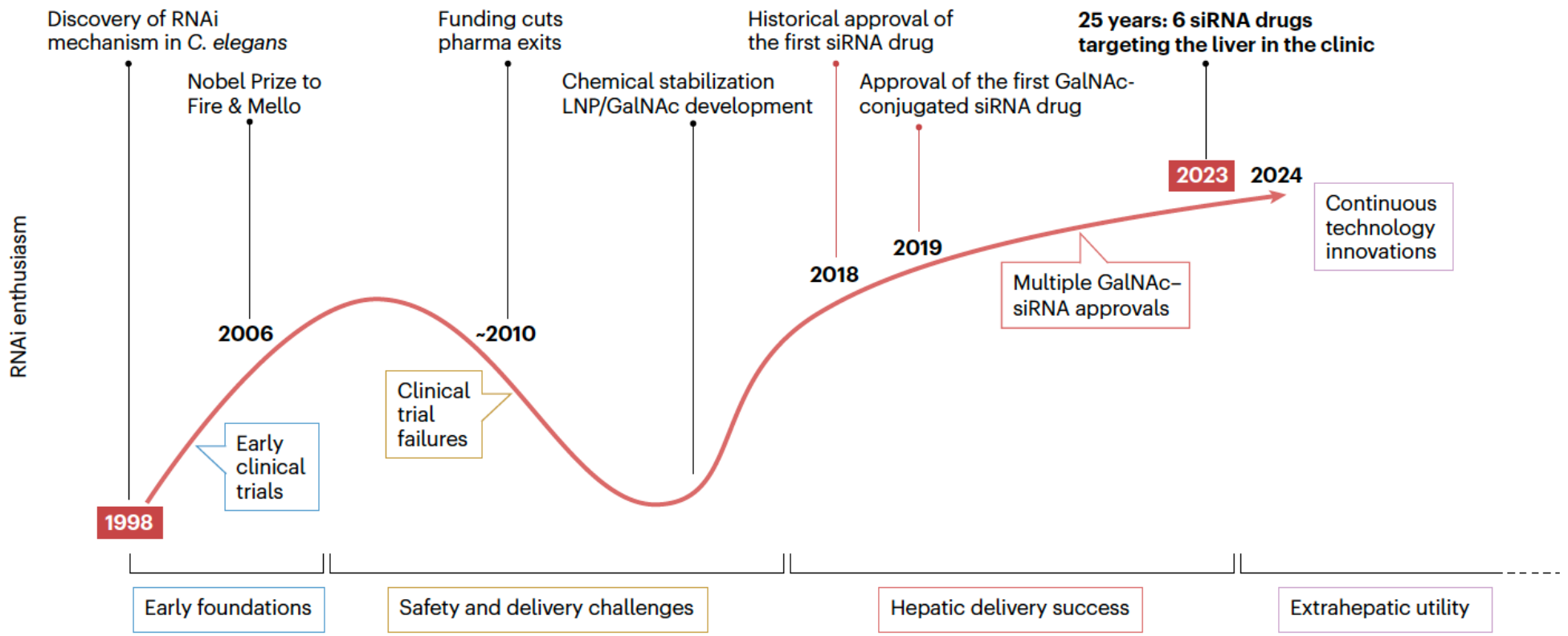
Twenty-five years of RNAi from discovery to clinic utility



https://doi.org/10.1038/s41573-024-00912-9

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Spinal muscular atrophy and Nusinersen

SMA (spinal muscular atrophy) is characterized by muscle atrophy and weakness from motor neuron degeneration in the spinal cord or brain stem. It takes away the ability to walk, eat, or breathe. It is the number one genetic cause of death for infants.

Rare disease (incidence 0.02%), autosomal recessive

SMA is caused by loss-of-function mutations in the SMN1 gene which codes for **survival motor neuron (SMN) protein**. Patients survive owing to low amounts of the SMN protein produced from the SMN2 gene, which produces a truncated version of the SMN protein.

Nusinersen is a modified anti-sense RNA. It modulates alternate splicing of the *SMN2* gene, functionally converting it into *SMN1* gene, thus increasing the level of SMN protein in the CNS.

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Pharmacokinetics of Nusinersen

- Pharmacokinetics:
  - Mean elimination half-life in the CSF: 135-177 days (plasma: 63-87 days)
  - Dosing: 12 mg, intrathecally, starting with four loading doses, 14 days interval between each of the first three administrations, 30 days between 3rd and 4th. Maintenance dose every 4 months.

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Nusinersen in adults with 5q spinal muscular atrophy: a non-interventional, multicentre, observational cohort study

Tim Hagenacker*, Claudia D Wurster*, René Günther*, Olivia Schreiber-Katz, Alma Osmanovic, Susanne Petri, Markus Weiler, Andreas Ziegler, Josua Kuttler, Jan C Koch, Ilka Schneider, Gilbert Wunderlich, Natalie Schloss, Helmar C Lehmann, Isabell Cordts, Marcus Deschauer, Paul Lingor, Christoph Kamm, Benjamin Stolte, Lena Pietruck, Andreas Totzeck, Kathrin Kizina, Christoph Mönninghoff, Otgonzul von Velsen, Claudia Ose, Heinz Reichmann, Michael Forsting, Astrid Pechmann†, Janbernd Kirschner†, Albert C Ludolph‡, Andreas Hermann, Christoph Kleinschnitz

Between July 13, 2017, and May 1, 2019, 173 patients were screened, of whom 139 (80%) were eligible for data analysis. Of these, 124 (89%) were included in the 6-month analysis, 92 (66%) in the 10-month analysis, and 57 (41%) in the 14-month analysis; patients with missing baseline HFMSE scores were excluded from these analyses. Mean HFMSE scores were significantly increased compared with baseline at 6 months (mean difference 1·73 [95% CI 1·05–2·41], p<0·0001), 10 months (2·58 [1·76–3·39], p<0·0001), and 14 months (3·12 [2·06–4·19], p<0·0001). Clinically meaningful improvements (≥3 points increase) in HFMSE scores were seen in 35 (28%) of 124 patients at 6 months, 33 (35%) of 92 at 10 months, and 23 (40%) of 57 at 14 months. To 14-month follow-up, the most frequent adverse effects among 173 patients were headache (61 [35%] patients), back pain (38 [22%]), and nausea (19 [11%]). No serious adverse events were reported.

41 *HFMSE, Hammersmith Functional Motor Scale Expanded score*

Gene therapy of monogenic diseases

- Mutations of about 1800 genes of the human genome have been identified as causing hereditary disorders
- Aspects of genetic disorders:
  - Mutation induces loss or gain of function
  - Affects or not cell survival and development
  - Tissue specificity of disease gene
- Strategy
  - Addition of a normal copy of the mutated gene (in most cases; but this is now changing)
  - Gene therapy is made of 3 components: the therapeutic gene, the vector that delivers it, and the mode of administration

Database: www.genetherapynet.com/clinical-trials.html
List of approved therapies: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products or https://alliancerm.org/available-products/

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History

- mid-1980s, first gene therapy cure in a mouse
- mid-1990s, first clinical trials with gene therapy published
- 1999, an 18-year old man dies after receiving gene therapy with an adenovirus vector infused into his liver
- Between 1990 and 2007, more than 1500 gene therapy clinical studies approved
- Since 2007, about 100 clinical studies approved per year
- 2012, first gene therapy approved in Europe: Glybera©, to treat Lipoprotein lipase deficiency
- 2017, three gene therapies approved in the USA:
  - Luxturna (voretigene neparvovec-rzyl), to treat retinal dystrophy mediated by RPE65 mutation.
  - Kymriah (tisagenlecleucel), chimeric antigen receptor T cell therapy of B cell precursor acute lymphoblastic leukemia*
  - Yescarta (axicabtagene ciloleucel), chimeric antigen receptor T cell therapy of large B-cell lymphoma*

**, only for patients who have not responded or relapsed to at least two other treatments*

Reasons for recent progress: better vector and expression technology, better understanding of targeted diseases

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Severe combined immunodeficiency disease (SCID)

"Bubble boy disease"

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Severe combined immunodeficiency disease (SCID)

- Caused by various gene deficiencies, e.g. in the γ receptor subunits for different interleukins in the case of the x-linked form SCID-X1
- Leads to impaired differentiation of T lymphocytes and other cells of the immune system
- Standard treatment is stem cell/bone marrow transplantation if possible
- 2000: Gene therapy of hematopoietic stem cells with a retrovirus containing the intact gene (ex vivo): after 1 year follow-up, the boys appeared cured.
- 2-5 year later, 4 of the 10 children in this trial (and 1 of 10 of a similar trial) developed leukemia (due to integration of the replacement gene near a T cell oncogene). This leukemia could be treated.
- After 11 years of follow-up, 18 of 20 treated boys were alive and the immunodeficiency was corrected in 17 of them.

Source: Boudes, P.F., Eur J Int Med 25, 31-36 (2014)

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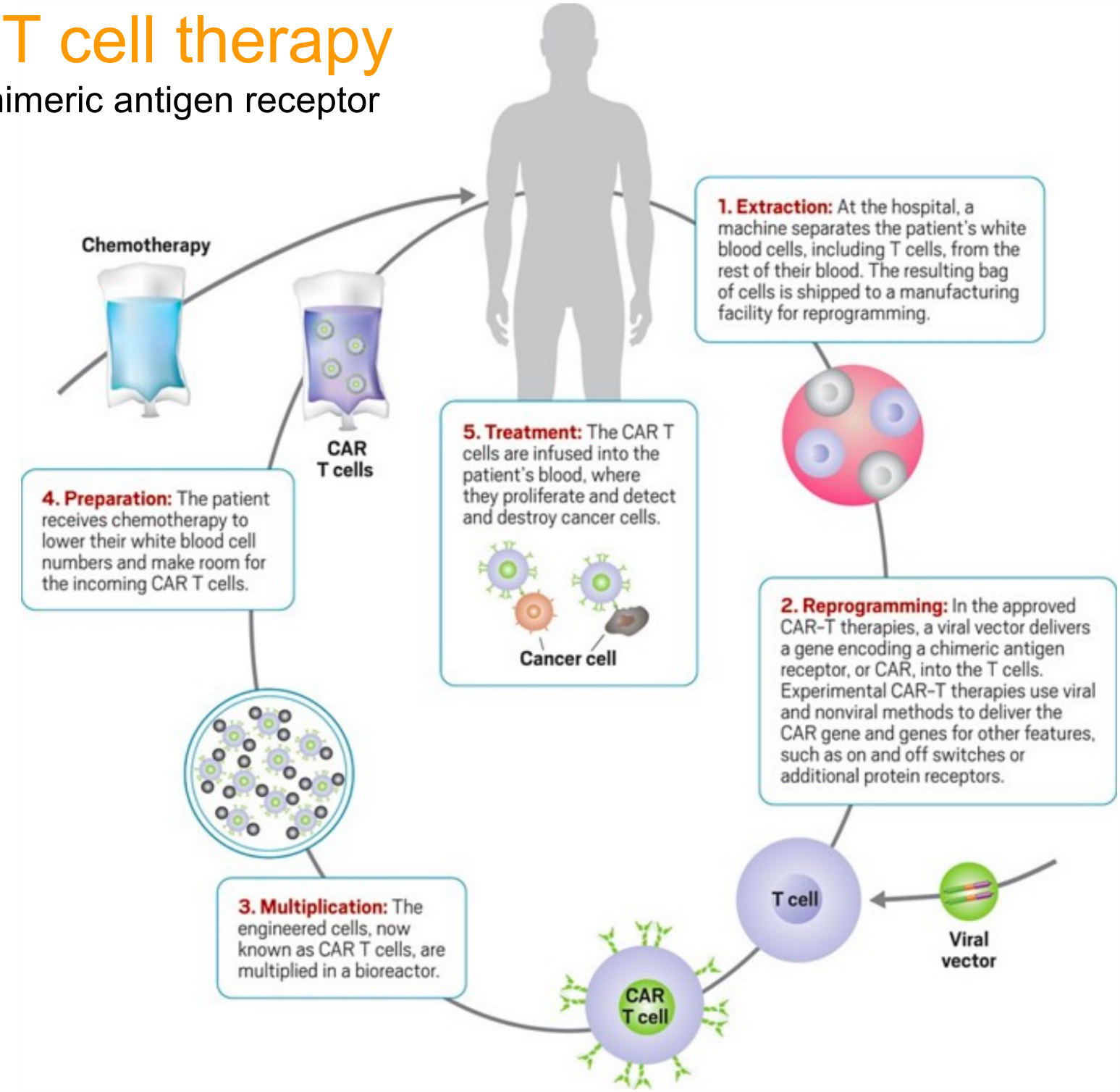
CAR T cell therapy

CAR = chimeric antigen receptor



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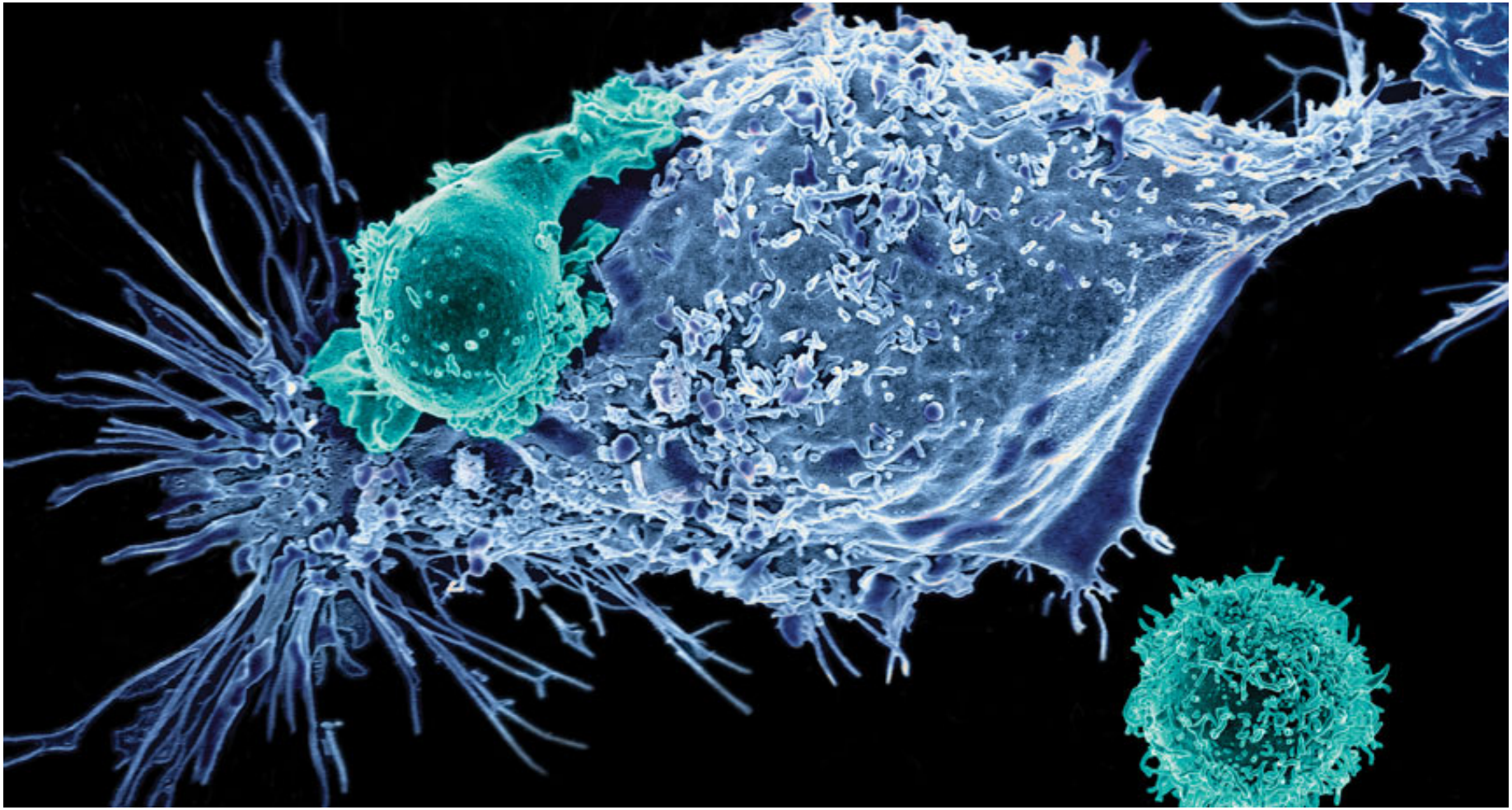

CANCER CRUSH In CAR-T cell therapy, a cancer treatment approved by the FDA this year for certain blood cancers, a patient's T cells (teal) are genetically modified to hunt down and kill cancer cells (blue). (CAR = chimeric antigen receptor)

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Tisagenlecleucel (Kymriah®, Novartis). Indications:
Adults with relapsed or refractory diffuse large B-cell lymphoma (DLBCL)
Young adult patients up to age 25 with relapsed or refractory acute lymphoblastic leukemia (ALL)

Axicabtagene ciloleucel (Yescarta®, Gilead). Indication: large B-cell lymphoma that has failed conventional treatment.

- Both therapies are directed against CD19
- B-lymphocyte antigen CD19, (Cluster of Differentiation 19), is a transmembrane protein that in humans is encoded by the gene *CD19*. Present in B cells (B lymphocytes).
- Most important side effects: Cytokine release syndrome

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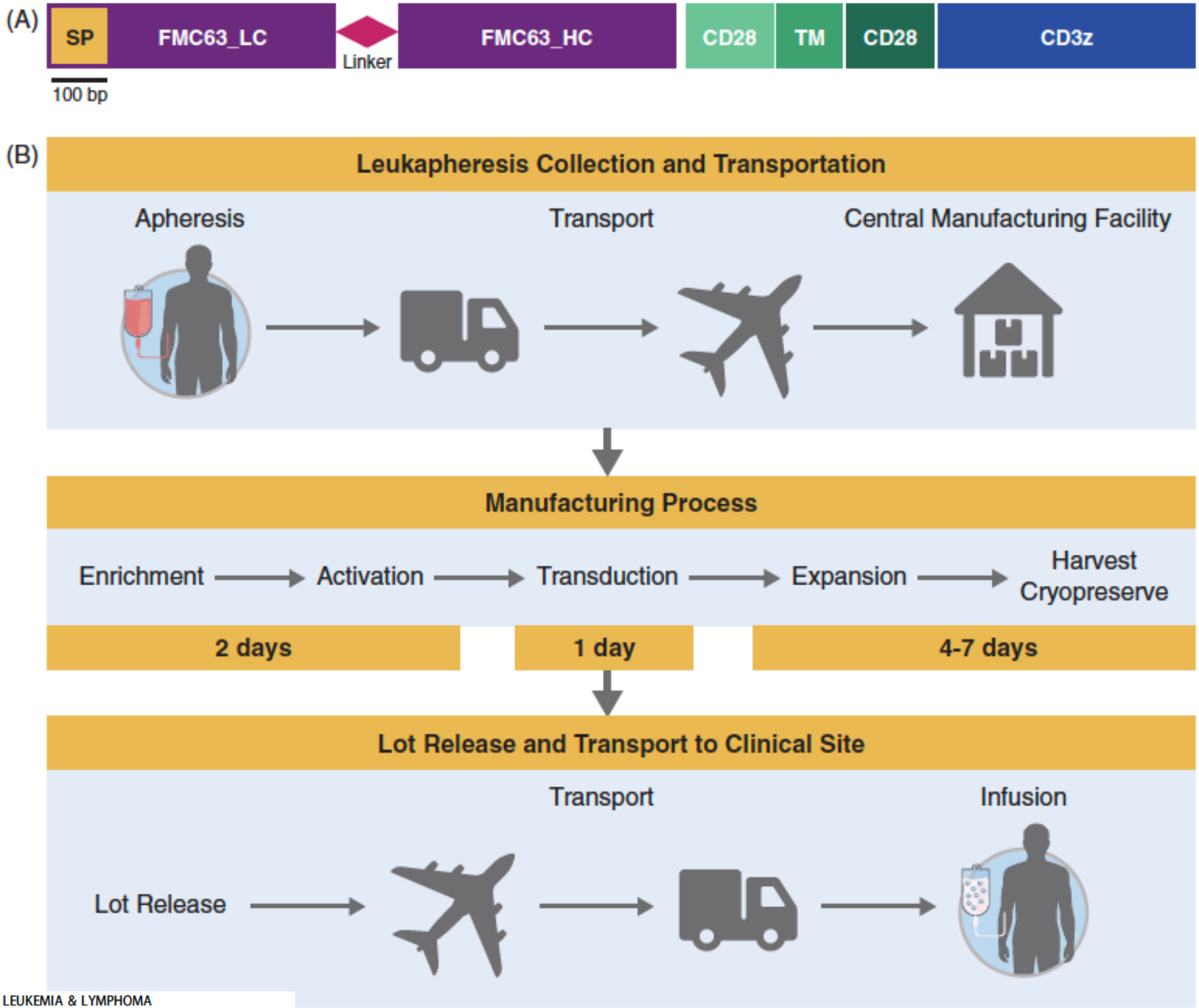


Figure 3. *CAR* gene (A) and manufacturing process (B) for axicabtagene ciloleucel. (A) Graphical representation of the *CAR* gene introduced into human T cells using a retroviral vector to make axicabtagene ciloleucel. The gene encodes a protein of ~54 kDa. SP indicates the position of the signal peptide. CD28-TM-CD28 indicates the position of the extracellular, transmembrane (TM), and intracellular regions of CD28, respectively. bp: base pairs; LC: light chain; HC: heavy chain. (b) Schematic overview of the vein to vein axicabtagene ciloleucel production process. The process begins with collection of blood cells at the clinical center. Patient material is then transported to the central manufacturing site where axicabtagene ciloleucel is produced. T cells in the incoming leukapheresis material are enriched on a closed-system density gradient. T cells are then activated with anti-CD3 antibody in the presence of IL-2 for 48 h, after which time they become receptive to transduction with a gamma-retroviral vector that encodes the anti-CD19 *CAR* gene. After transduction, cells are expanded until a target dose is achieved (2×10^6 CAR-positive cells per kg body weight). After product release, the final product is returned to the clinical center. Door-to-door turn around time is approximately 2 weeks.

LEUKEMIA & LYMPHOMA
2018, VOL. 59, NO. 8, 1785–1796
https://doi.org/10.1080/10428194.2017.1387905

Comparison gene addition versus targeted genome editing

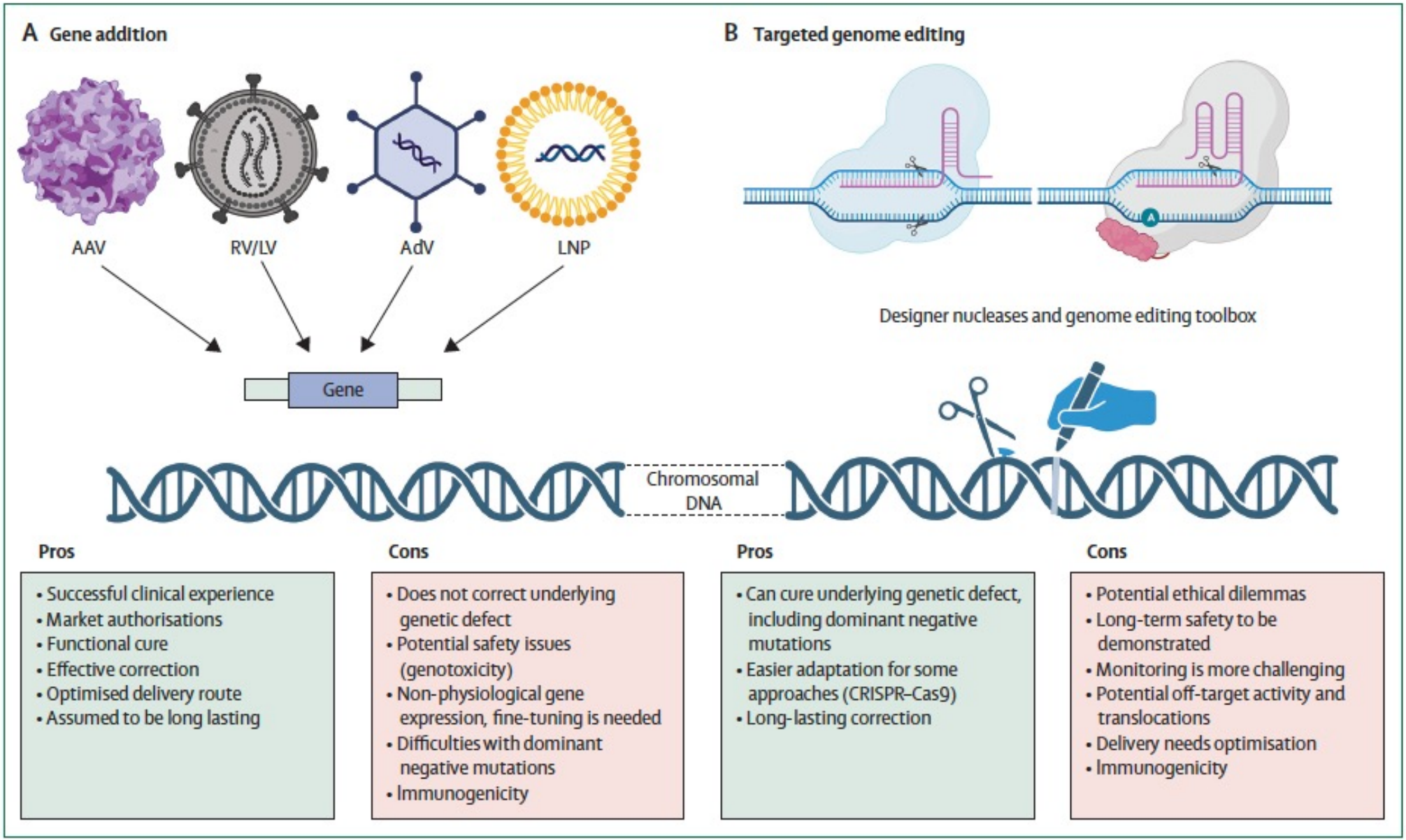
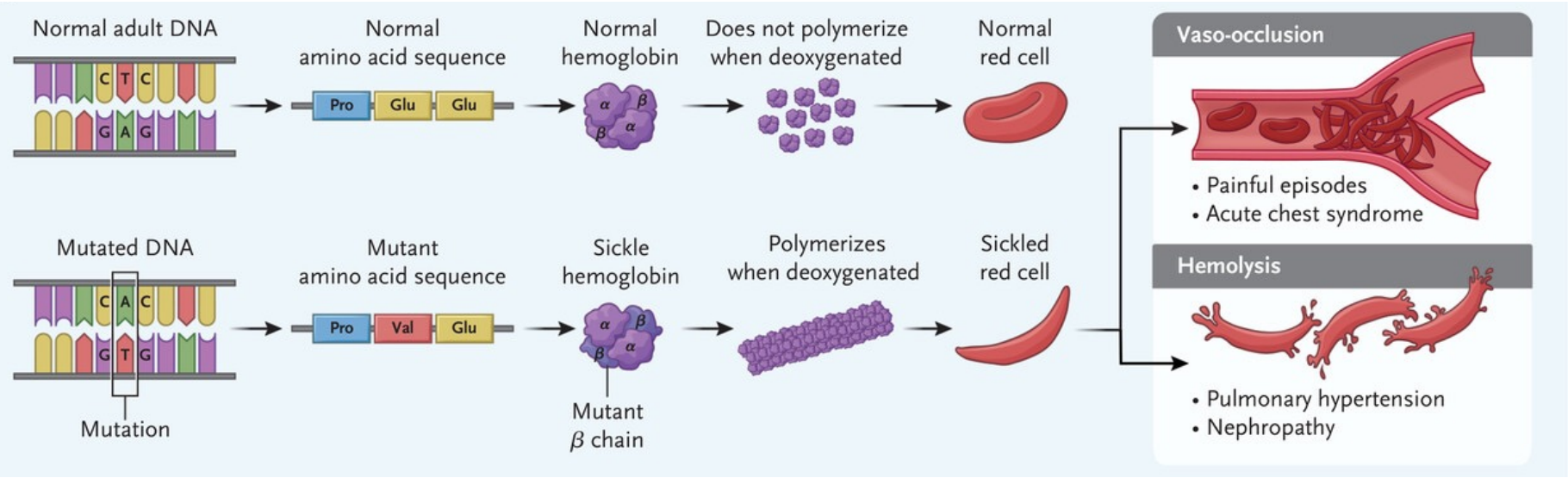
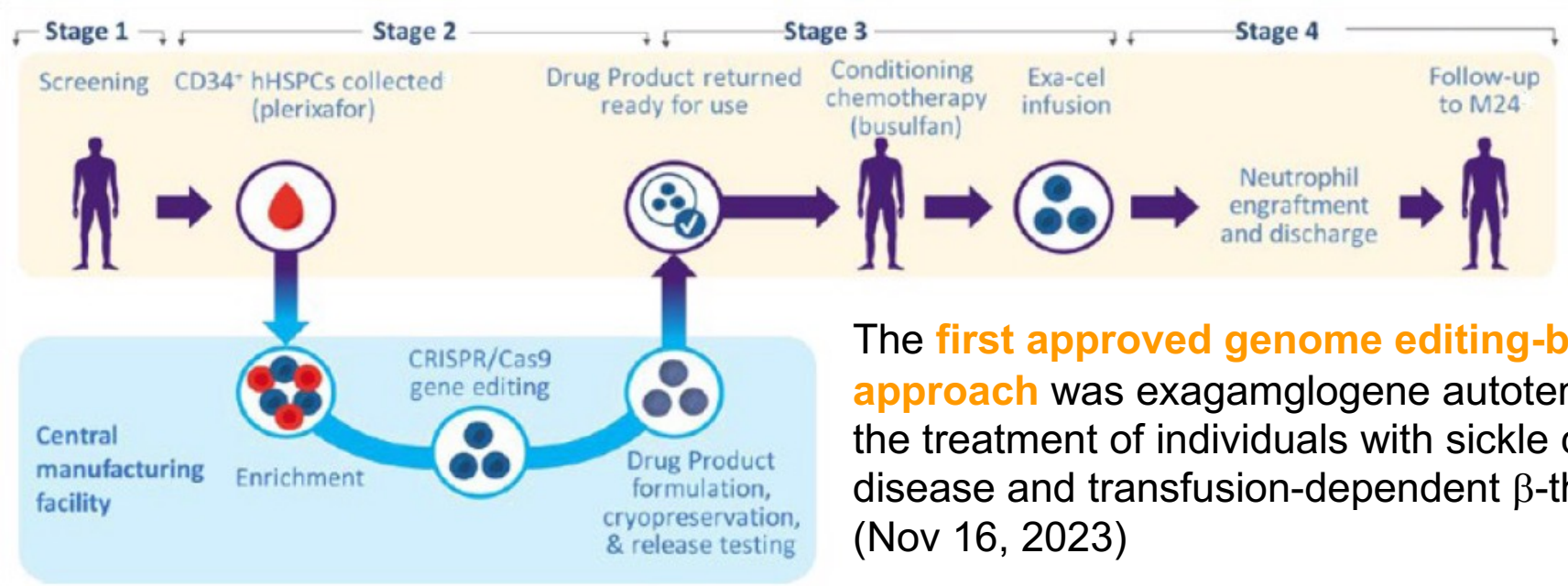


Figure 1: **Gene addition approaches versus targeted genome editing approaches**
(A) During gene addition, viral vectors, including AAV vectors, RV/LV vectors, AdV vectors, and non-viral vectors (eg, LNPs), deliver a whole gene of interest with promoter or enhancer elements and polyadenylation signals. (B) Designer nucleases and the described genome editing toolbox (eg, based on CRISPR–Cas9) lead to targeted gene editing with defined nucleotide changes in the genome. AAV=adeno-associated virus. AdV=adenoviral. Cas=CRISPR-associated protein. LNPs=lipid nanoparticles. RV/LV=lentiviral or gammaretroviral.





Source: Adapted from study 121 protocol version 6.11 US, Appendix 16.1.1

The **first approved genome editing-based approach** was exagamglogene autotemcel for the treatment of individuals with sickle cell disease and transfusion-dependent β-thalassemia (Nov 16, 2023)

Source: Steinberg Martin H. Fetal-like Hemoglobin in Sickle Cell Anemia. N Engl J Med 2022; 386:689-691
DOI:10.1056/NEJMe2119760

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