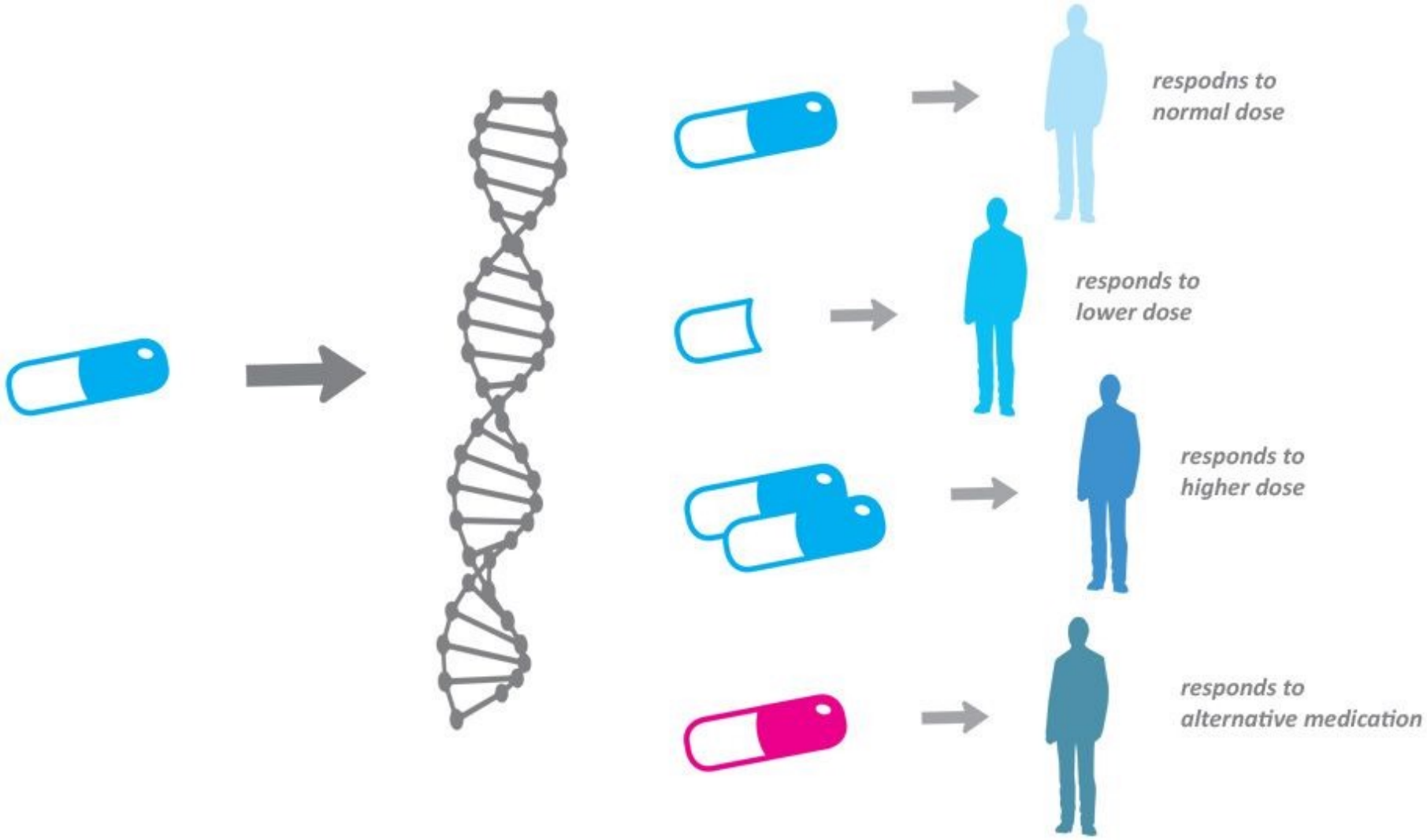
Chapter 4: Personalized/Precision medicine



People differ biologically — medicine should reflect that

- Individuals vary in genetics, metabolism, environment, lifestyle, and microbiome.

These differences strongly influence:

- how well a drug works,
- risk of side effects,
- likelihood of developing certain diseases.

Precision medicine aligns treatments with each person's unique biology, increasing effectiveness and reducing harm. Traditional medicine assumes uniformity that simply doesn't exist.

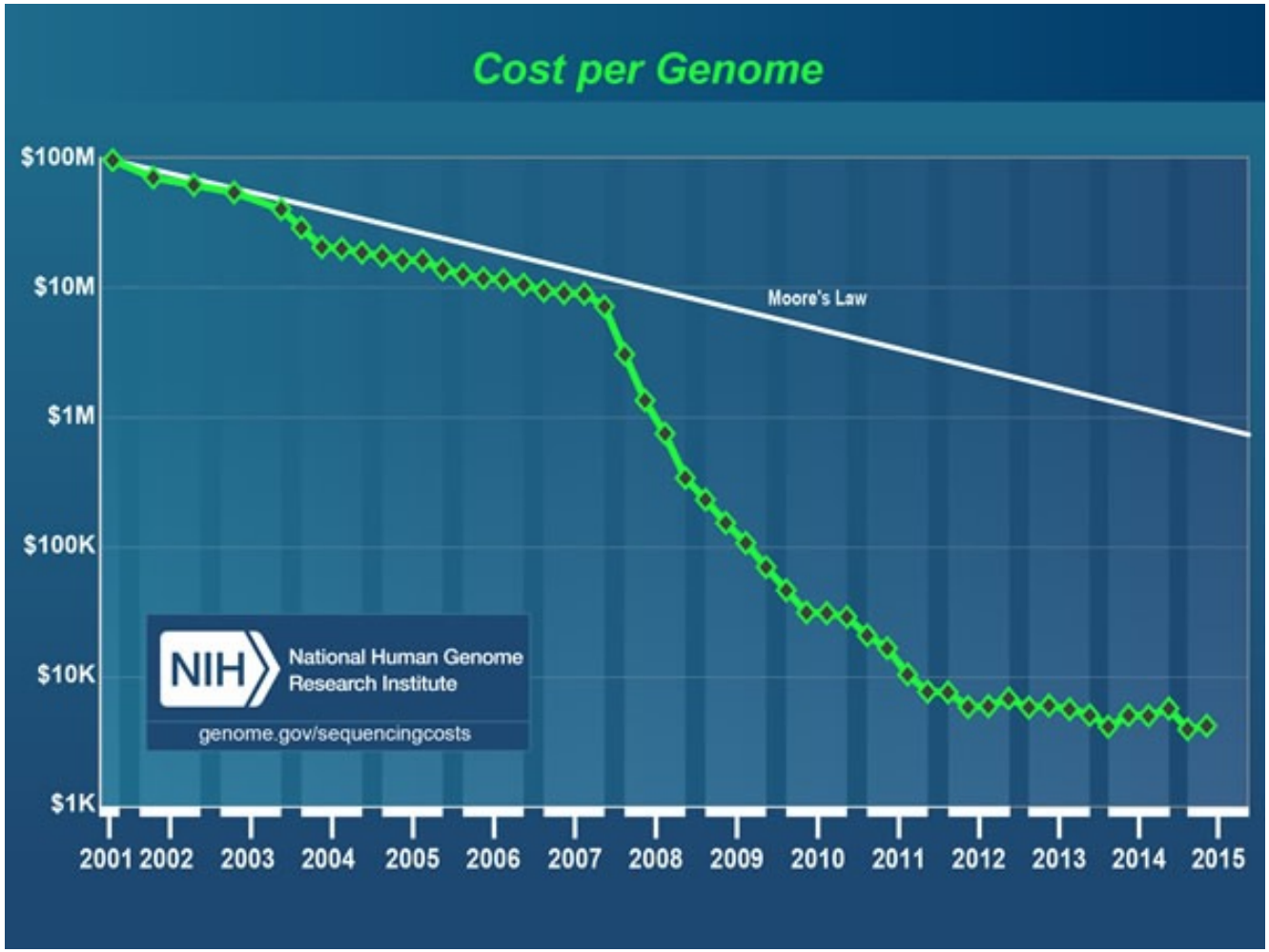
Precision medicine improves treatment effectiveness

- Some cancer therapies help only people with specific genetic mutations in their tumors.
- By matching therapies to biological markers, precision medicine turns trial-and-error prescribing into targeted, evidence-based treatment.

Beginning of Precision medicine

- **1990–2003 – Human Genome Project:**
  The sequencing of the human genome is often considered the **true beginning of modern precision medicine**.
  It provided a complete reference map of human DNA and made gene-based diagnostics feasible.

The cost per genome sequencing



Basically, the first human genome cost nearly $3 billion to sequence

Databases relevant for precision medicine

Category	What it stores	Examples
Genetic / genomic databases	DNA variants, allele frequencies, genome annotations	gnomAD, ClinVar, dbSNP, Ensembl
Multi-omics databases	Genomics + RNA + protein + epigenetics	TCGA, ICGC, GEO
Clinical / health databases	Patients medical history, lifestyle, imaging, outcomes	UK Biobank, All of Us
Specialized databases	Drug–gene interactions, tumor mutations, protein pathways	COSMIC, PharmGKB, KEGG

Swiss databases
Swiss Personalized Health Network (SPHN)

- As of mid-2024, SPHN's network contained **consented data from over 700,000 patients** across Switzerland.
- SPHN covers a wide variety of medical domains: from general clinical care data to specialized domains such as **oncology**, **intensive care**, **pediatrics**, **infectious diseases**, etc.

SNP stands for **Single Nucleotide Polymorphism**.

- It is a **variation at a single position in the DNA sequence** among individuals.
- A SNP occurs when **one person's DNA has a different nucleotide at a specific position** compared to another person.
- Reference sequence: A T C G A T G C
- Individual's sequence: A T T G A T G C

Key Features of SNPs

Mostly harmless: Many SNPs don't affect health or function. They may be in non-coding regions.

Sometimes functional: Some SNPs can affect:

- Gene function or protein structure
- Disease susceptibility (risk factors)
- Drug response (pharmacogenomics)

SNPs are a **cornerstone of precision medicine** because they:

1. Help **identify diseases** or predict **disease risk**
2. Guide **drug selection and dosing**
3. Enable **targeted therapies**

Databases of disease-associated SNPs

Databases of disease-associated SNPs

- **dbSNP (NCBI)** – comprehensive SNP repository.
- **ClinVar (NCBI)** – clinical significance of variants.
- **GWAS Catalog** – SNPs linked to complex disease risk in genome-wide association studies.

Five well-known single nucleotide changes that drive cancer

Gene	Mutation (single nucleotide)	Amino Acid Change	Cancer Type(s)	Effect on Protein/Cell
KRAS	G12D: GGT → GAT	Glycine → Aspartic acid	Pancreatic, colorectal, lung	Constitutive activation of KRAS → uncontrolled cell growth
BRAF	V600E: GTG → GAG	Valine → Glutamic acid	Melanoma, thyroid, colorectal	Constitutive kinase activation → continuous MAPK signaling
TP53	R175H: CGC → CAC	Arginine → Histidine	Li-Fraumeni syndrome, many solid tumors	Loss of tumor suppressor function → failure of DNA damage response
EGFR	L858R: CTG → CGG	Leucine → Arginine	Non-small cell lung cancer	Constitutive receptor tyrosine kinase activation → uncontrolled proliferation
IDH1	R132H: CGT → CAT	Arginine → Histidine	Gliomas	Produces oncometabolite 2-HG → epigenetic dysregulation and tumor growth

World Health Organization

Monogenic diseases (inherited)

- Monogenic diseases: result from mutations in a single gene in all cells of the body.
- Dominant
- Recessive

Monogenic diseases

- Example: **Sickle cell disease**, **recessive disease**
- Occurs only when maternal and paternal copies of the HBB (hemoglobin subunit beta) gene are defective.
- Hemoglobin is the protein in red blood cells that transports oxygen from the lungs to the body's tissues and returns carbon dioxide to the lungs.
- The atypical hemoglobin molecules called hemoglobin S, which can distort red blood **cells** into a **sickle**, or crescent, shape. Sickle shaped blood cells have a short lifetime. They can block blood flow and cause pain.
- Higher rate of infections, fatigue, pain
- Frequency 1-5 /10 000 people

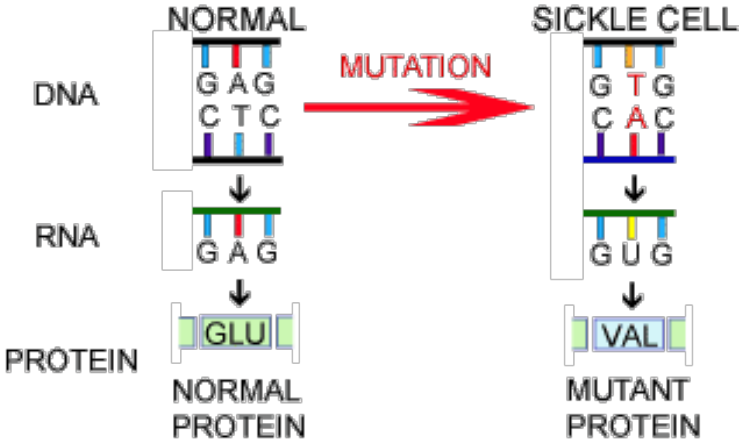
"Sickle"

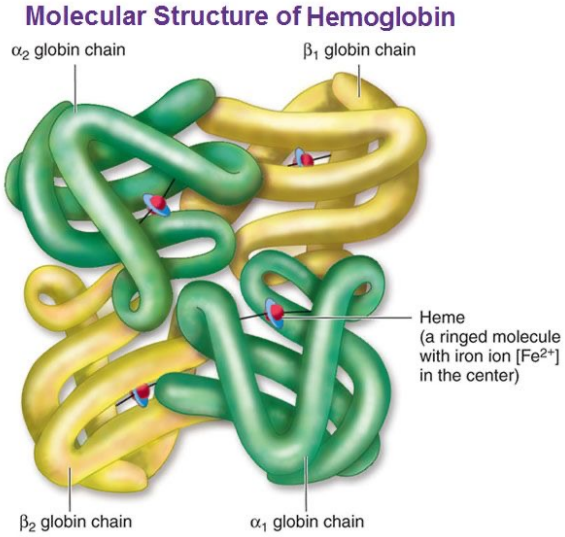
Normal Red Blood cell

Sickle cell disease

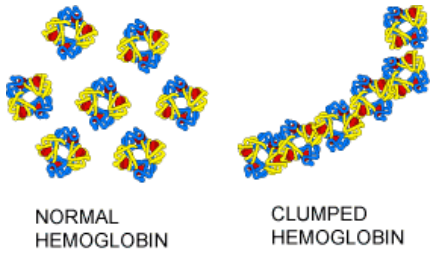
In 2021, an estimated 7.74 million people were living with sickle-cell disease globally, primarily in sub-Saharan Africa, which accounts for nearly 80% of global cases.

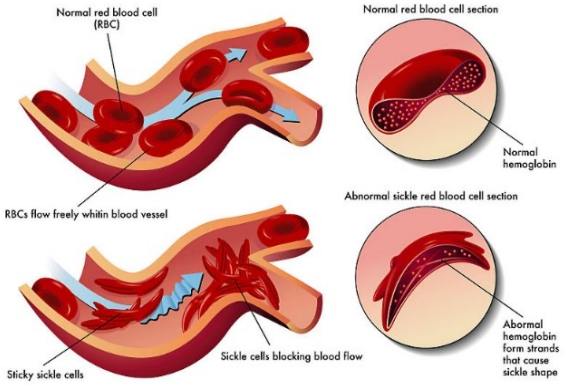
- Effects at the DNA level





- Effects at the protein level





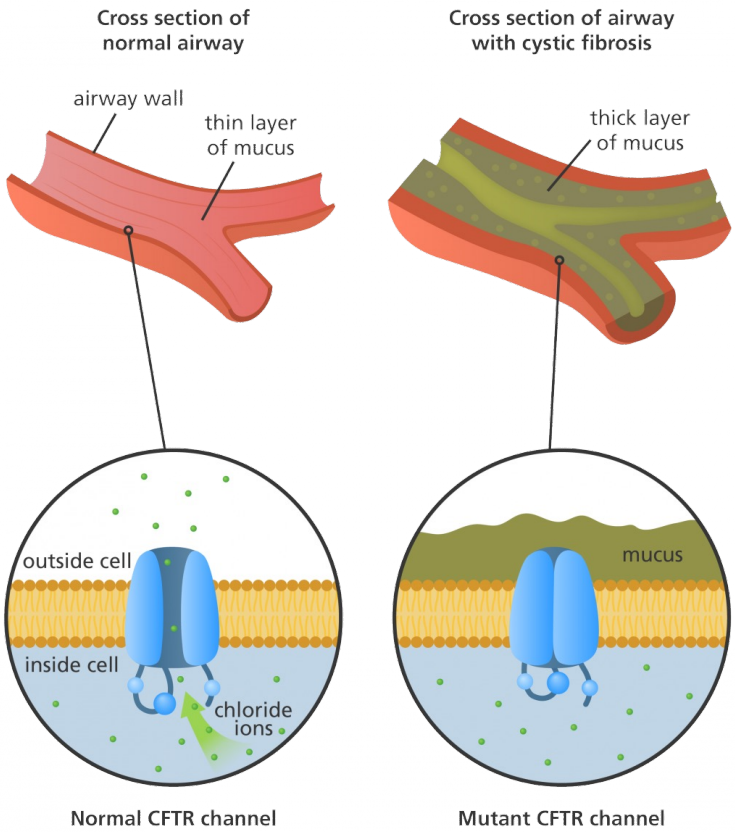
Monogenic diseases

- **Cystic fibrosis (CF), recessive**
- Cystic Fibrosis is a genetic disorder that affects the respiratory, digestive and reproductive systems involving the production of abnormally thick mucus linings in the lungs and can lead to fatal lung infections.
- CF frequency in EU: 1 in 2000/3000 newborns

Cystic fibrosis

Mutation in CFTR: Cystic fibrosis transmembrane conductance regulator.

- Mutation disrupts the function of the CFTR gene encoding a chloride channel, inhibiting the flow of chloride ions and water in and out of the cells.

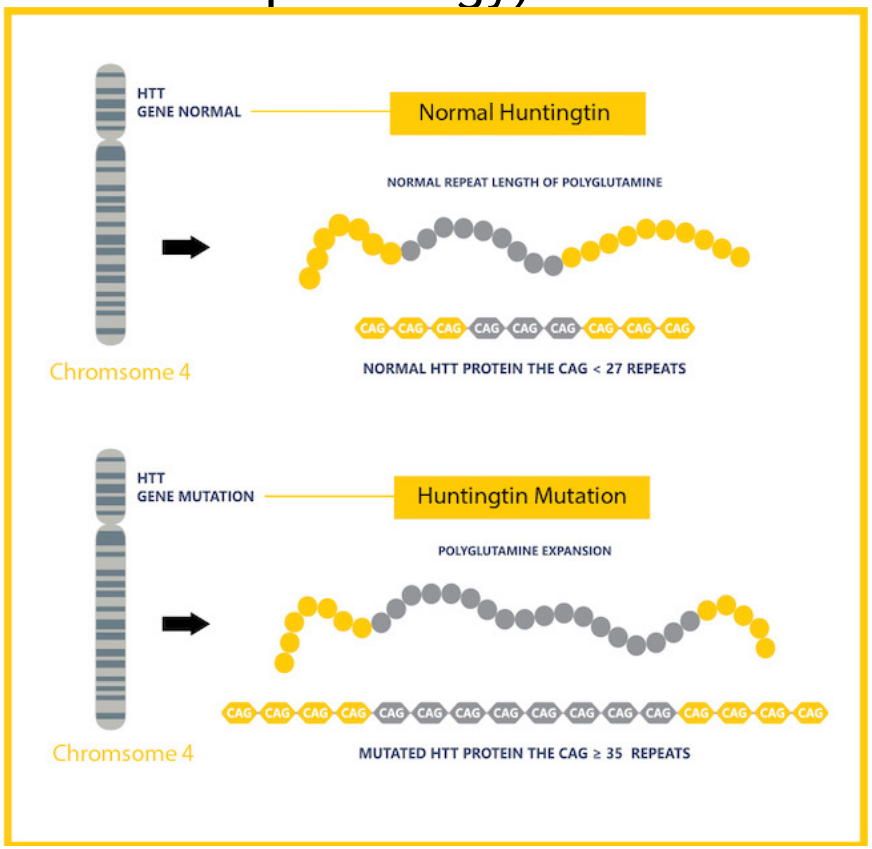


Monogenic diseases

- **Huntington disease**
- **Progressive breakdown (degeneration) of nerve cells in the brain** that causes uncontrolled movements, emotional problems, and loss of thinking ability (cognition).
- **Dominant** having a change (mutation) in only one of the 2 copies of the **HTT gene** is enough to cause the condition.
- Frequency: 3-7 in 100 000 people

Huntington disease

- The extra “CAG” nucleotides in the gene lead to an instability that causes the resulting huntington protein to be extra-long and difficult for the body to maintain and remove from brain cells.
- Over many years, the mutant protein forms clumps in brain cells, which causes them to become damaged and die (similar to those seen in Alzheimer disease pathology).



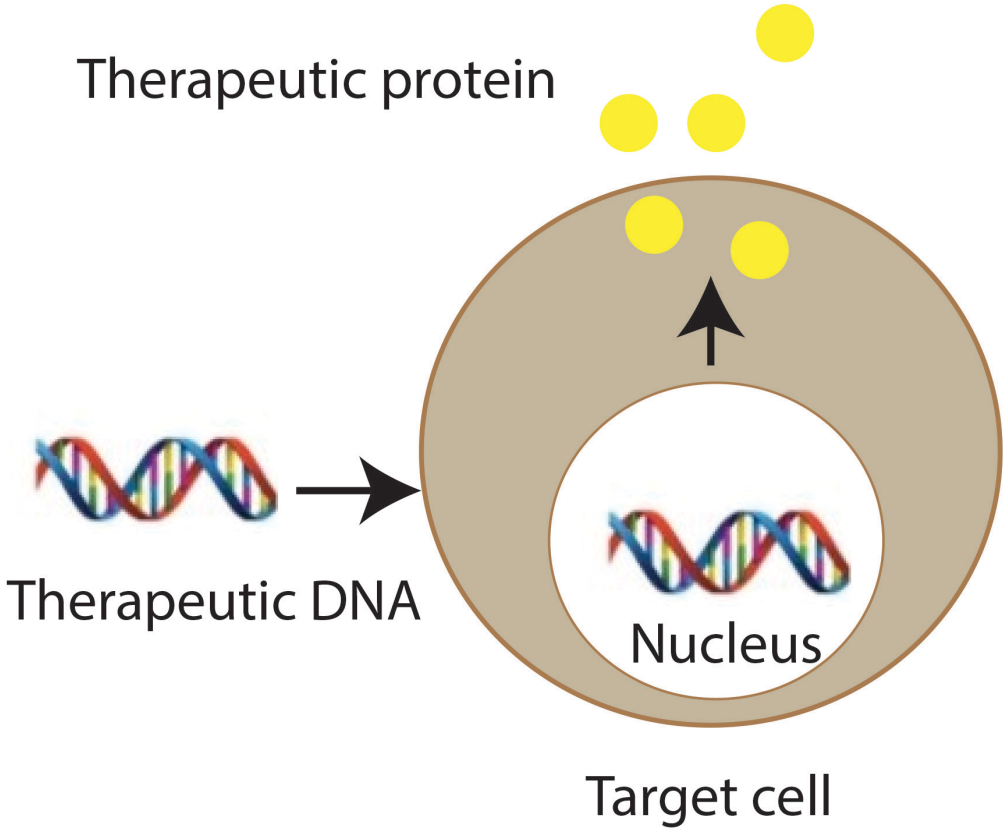
Polygenic diseases (inherited)

- Caused by **mutations in many genes** combined with environmental factors.
- Polygenic diseases are **more frequent** than monogenic diseases. Examples: Hypertension, Cancers, Obesity, Atherosclerosis, Diabetes, Autoimmune diseases, Osteoporosis, Asthma, Schizophrenia
- *Nature Medicine* **22**, 1065–1066 (2016)

Genetic predisposition

- Most diseases involve mutations in many genes in complex interactions, in addition to environmental influences.
- An individual may not be born with a disease but may be at high risk of acquiring it. This is called as genetic **predisposition** or **susceptibility**.

Principle of Gene Therapy



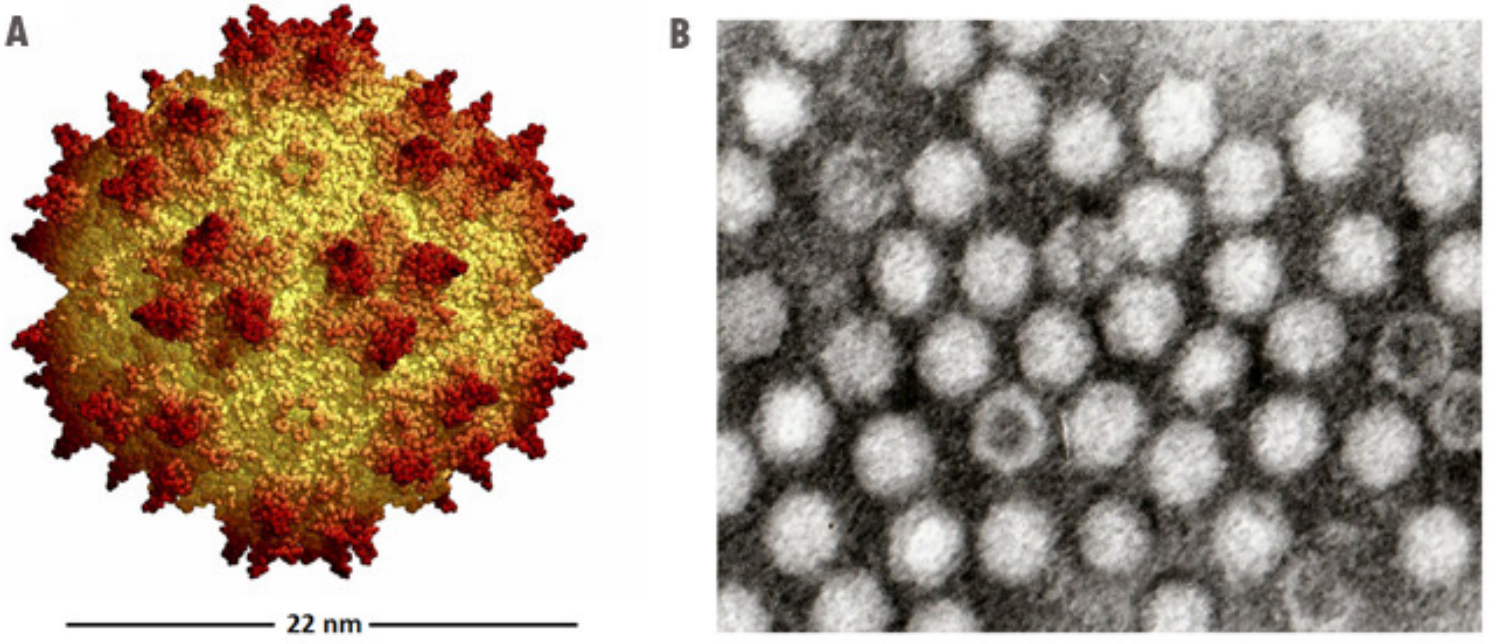
Current gene therapies deliver the functional version of a gene that can produce the missing or defective protein.

“Adeno-associated virus (AAV) vectors are the leading platform for gene delivery for the treatment of a variety of human diseases”

Wang et al., *Nature Reviews Drug Discovery* **volume 18**, pages358–378(2019)

Adeno-associated viruses (AAV)

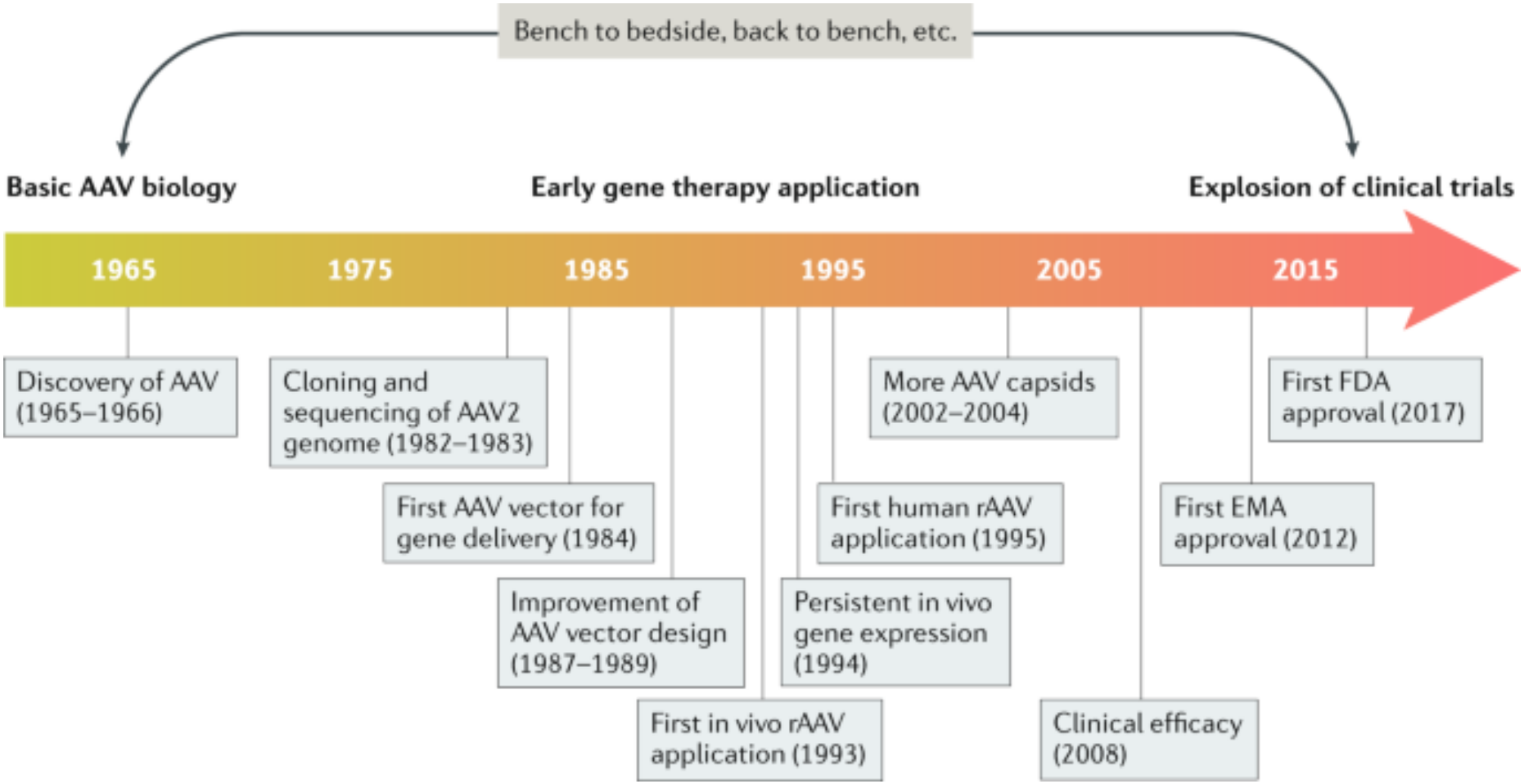
- **AAVs are not known to cause any disease** in humans. They are considered **non-pathogenic**, which is why they are widely used as vectors in gene therapy.



- **Figure 1. Adeno-Associated Virus (AAV) Structure and Genome map. A: A cartoon showing simulated AAV size and 3-D structure; B: A picture showing electron microscopy of purified AAV vector.**

SignaGen® Laboratories

Timeline: key milestones in adeno-associated virus (AAV) gene therapy development



Wang et al., *Nature Reviews Drug Discovery* **volume 18**, pages358–378(2019)

AAV-Therapy

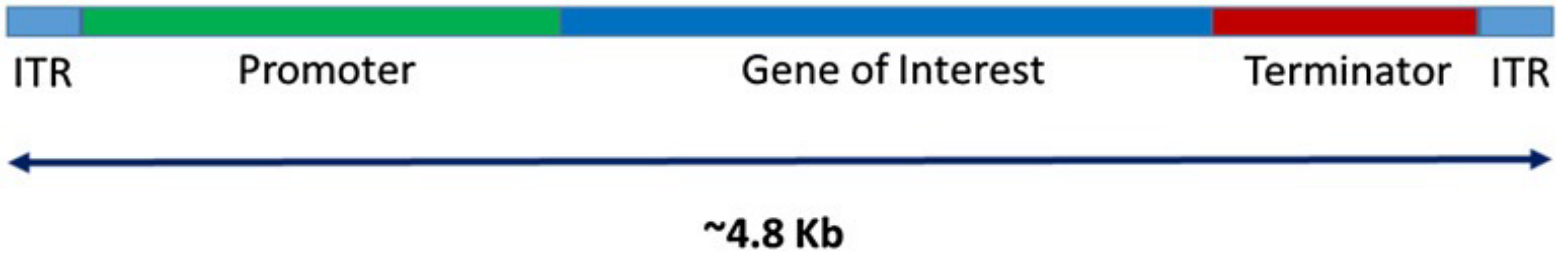
- AAV therapy delivers a functional copy of a gene to target cells using an adeno-associated virus vector, allowing cells to produce the correct protein and compensate for a defective or missing
- Works best for **monogenic, loss-of-function diseases**.

Some characteristics of AAVs

- Small (20 nm capsid diameter), non-enveloped virus
- Single stranded DNA genome, ~4.8 kb
- Dependovirus: They replicate only in the presence of a helper virus that can be Adenovirus (AdV) or Herpes simplex (HSV)

Recombinant AAV (rAAV) with therapeutic gene

- Small packaging size (~4.8 kb, including ITRs) compared with other viral vectors. (No big transgenes can be packed into the virus)
- ITR (inverted terminal repeats) ~145 bp long
- Hairpin ITRs mediate circularization → episome formation



Schematic representation of the basic components of a gene insert packaged inside recombinant AAV gene transfer vector. *AAV* adeno-associated virus, *ITR* inverted terminal repeat

Naso et al., BioDrugs. 2017; 31(4): 317–334

Different **AAV serotypes** are used to target different organs because **each serotype (strain) has a unique capsid protein** that determines which cells it can enter efficiently.

Capsid determines cell entry:

•The viral capsid binds to specific **cell surface receptors**.

•Different serotypes recognize different receptors on different cell types.

Tissue specificity:

- **AAV1 & AAV9:** Good for **muscle and heart**.
- **AAV8 & AAV9:** Efficient in **liver**.
- **AAV2:** Preferentially targets **retina and CNS (Central nervous system)**.

Therapy optimization:

Choosing the right serotype (strain) improves **gene delivery efficiency**, reduces required dose, and **minimizes off-target expression**.

Wu et al., (2006) Molecular therapy, 14: 316-327

AAV therapy

- **AAV therapy is generally long-lasting in non-dividing tissues** but less permanent in rapidly dividing cells because episomes do not replicate with the genome.
- **Non-dividing cells (e.g., neurons, retinal cells, hepatocytes in adults):**
- Episomes can persist for **years to decades**, sometimes effectively for the lifetime of the cell.
- This is why therapies like **Luxturna (RPE65)** show long-term effects after a single injection.

- (However it can also stably integrate at a specific position in human chromosome 19)

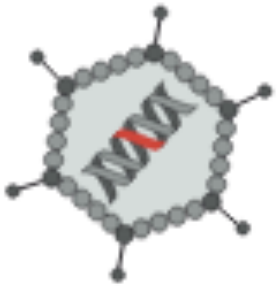
Limitations of rAAV

1. **AAV gene size limitation: Maximum packaging capacity: ~4.8 kilobases (kb)** of DNA (including the promoter and regulatory elements)

2. **AAV therapy is generally long-lasting in non-dividing tissues** but less permanent in rapidly dividing cells because episomes do not replicate with the genome.

3. A patient who had already an infection with “natural” AAVs, might have **developed immunity** against certain AAVs.

Gene Therapy



Zolgensma (Novartis gene therapies)

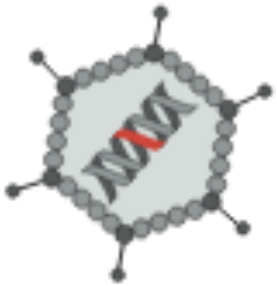
Gene defect SMN1 (Survival motor neuron protein 1)

Disease: **Spinal muscular atrophy**

FDA approved 2019

EMA approved: 2020

Symptoms: Weakness in muscles used for movement (skeletal muscles)



Gene Therapy

- **Zolgensma** (Novartis gene therapies)

Mechanism:

1.Uses **AAV9 vector** to deliver a **functional copy of SMN1** to motor neurons.

2.Motor neurons begin producing **SMN protein**, which is essential for survival and function of these cells.

Administration: Single **intravenous infusion**.

Effect:

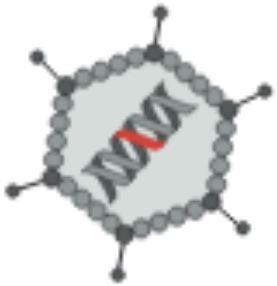
•Improves motor function, survival, and reduces the need for ventilatory support in infants.

•Long-lasting because **AAV9 delivers the gene to non-dividing motor neurons**, where the DNA persists as episomes.

How many patients have been treated

- As of August 2024, over **4,000 patients worldwide** have received Zolgensma.
- Because Zolgensma's cost is extremely high (≈ 2 million USD / 1.9–2 million Euro per dose)
- Some insurers or health systems insist on evidence of "added benefit" compared with existing treatment before reimbursement or may negotiate price and payment models before agreeing.

Gene Therapy



Luxturna (Spark Therapeutics, USA)

Defect in gene RPE65/blindness

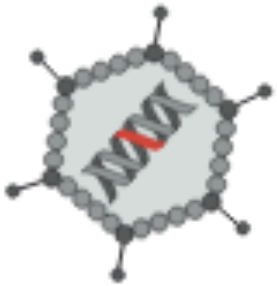
Enzyme in visual pigment regeneration

Cost of treatment: 850 000 Dollar

FDA approved: 2017

EMA approved: 2018

Gene Therapy



Luxturna (Spark Therapeutics, USA)

Mechanism:

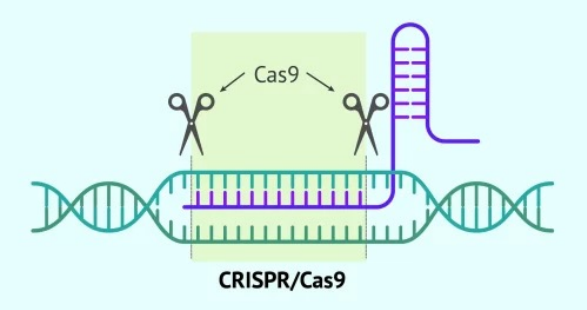
1. Uses **AAV2 vector** to deliver a **functional copy of the RPE65 gene** directly to retinal cells (subretinal injection).
2. Retinal pigment epithelium (RPE) cells start producing **functional RPE65 enzyme**, restoring the visual cycle.
3. Improves the ability of photoreceptors to respond to light.
4. Long-lasting because **retinal cells are non-dividing**, so the episomal AAV DNA persists for years.

CRISPR-Cas therapy **has been approved** for treating Sickle cell disease (SCD)

By FDA Dec. 2023

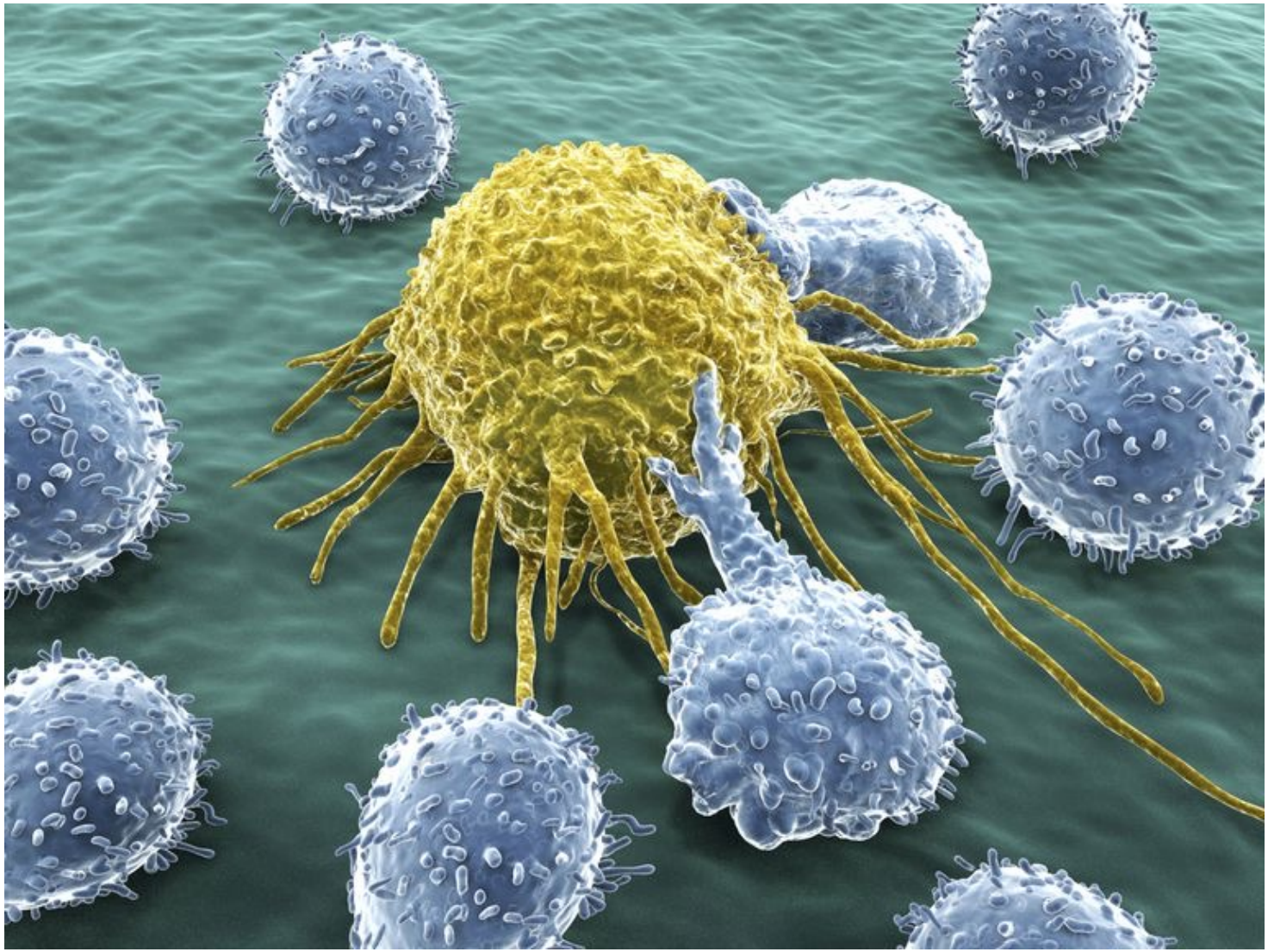
By EMA Feb. 2024

Genome editing using CRISPR/Cas9



- **Target gene:** CASGEVY uses CRISPR/Cas9 to edit the **BCL11A enhancer** in the patient's **hematopoietic stem cells**.
- **BCL11A** is a regulator that normally **switches off fetal hemoglobin (HbF)** after birth.
- **Result of editing:** By disabling this enhancer in stem cells, the cells **reactivate fetal hemoglobin production**.
- **Why this helps Sickle cell disease:**
- Fetal hemoglobin (HbF) **does not sickle**, even in the presence of the sickle mutation.
- Red blood cells with HbF remain flexible and live longer, reducing anemia, pain crises, and organ damage.

T cell engineering to fight cancer



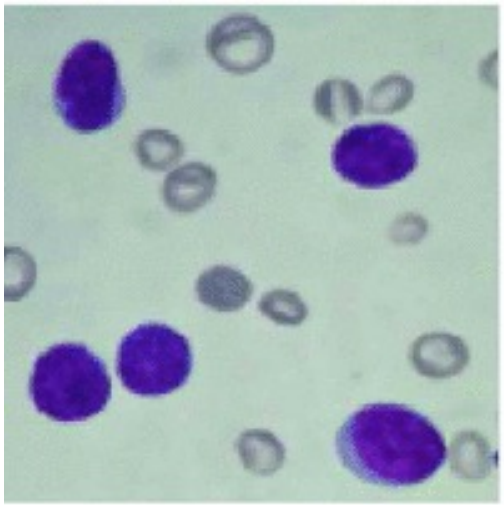
https://www.verywell.com/t-cells-2252171

CAR T-cell therapy

- **Chimeric antigen receptor** (CAR) T-cell therapy is a type of immunotherapy that uses a patient's own genetically modified T cells to find and kill cancer cells.

Acute Lymphoblastic Leukemia (ALL)

- ALL occurs at an annual rate of approximately 41 cases per 1 million people aged 0 to 14 years and approximately 17 cases per 1 million people aged 15 to 19 years



High number of lymphoblasts (precursor B cells)

Neural Computing and Applications (2014) Volume 24 Issue 7-8, Pages 1887-1904

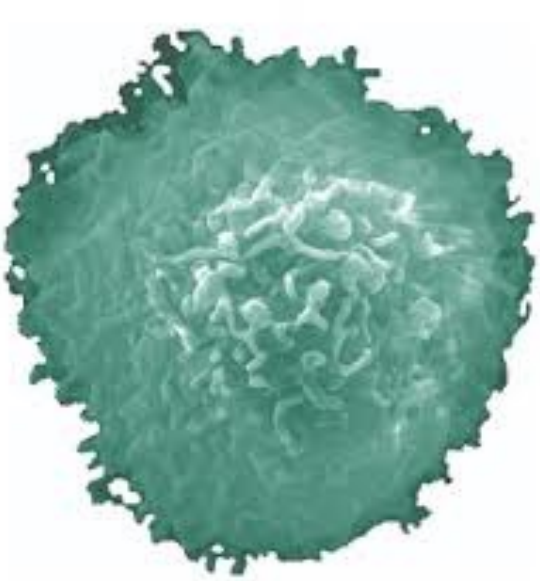
T cells

- T cells from our immune system can identify and kill cancer cells however cancer cells have developed strategies do evade the immune system, making T cells ineffective and a tumors can develop

NOVARTIS

FDA approval, July 13, 2017

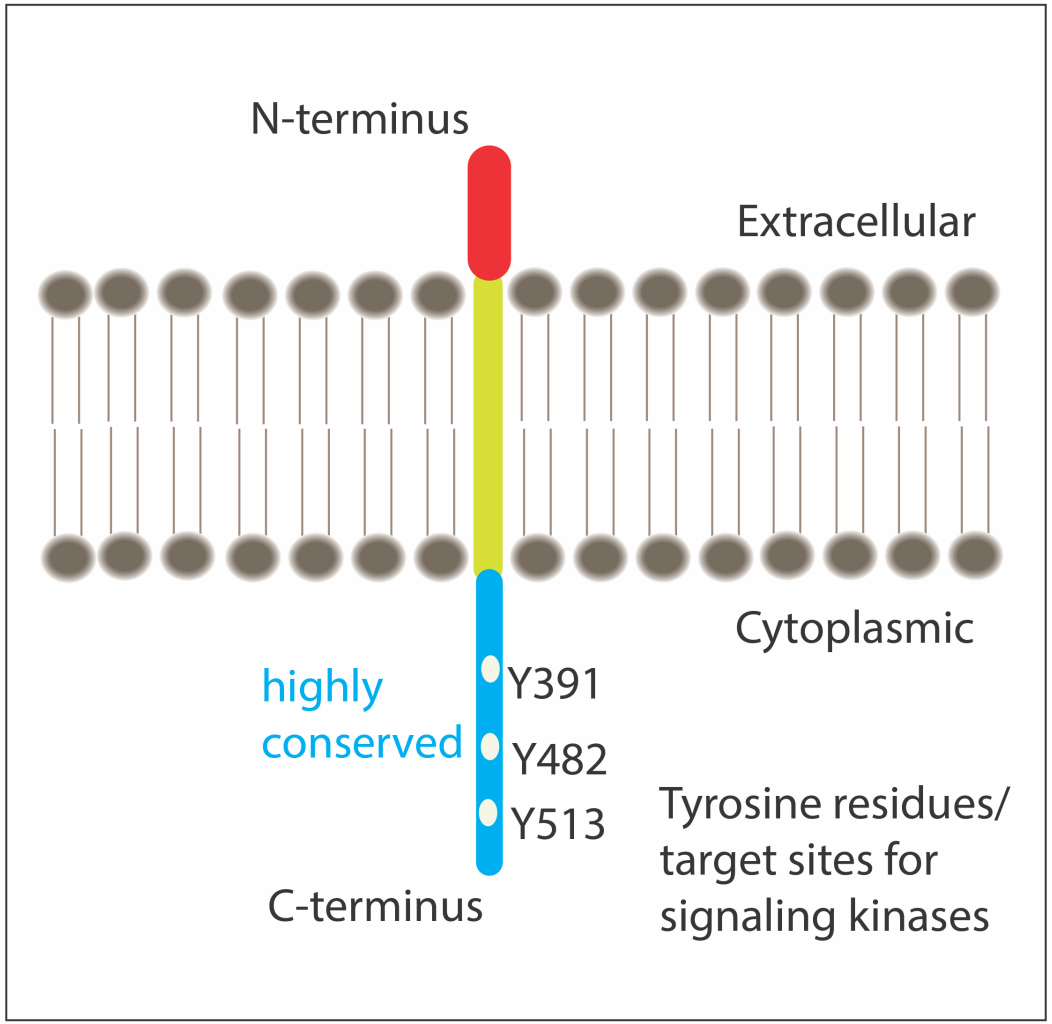
- CAR T-cell therapy CTL019 (Kymriah)



New York Times, July 12, 2017

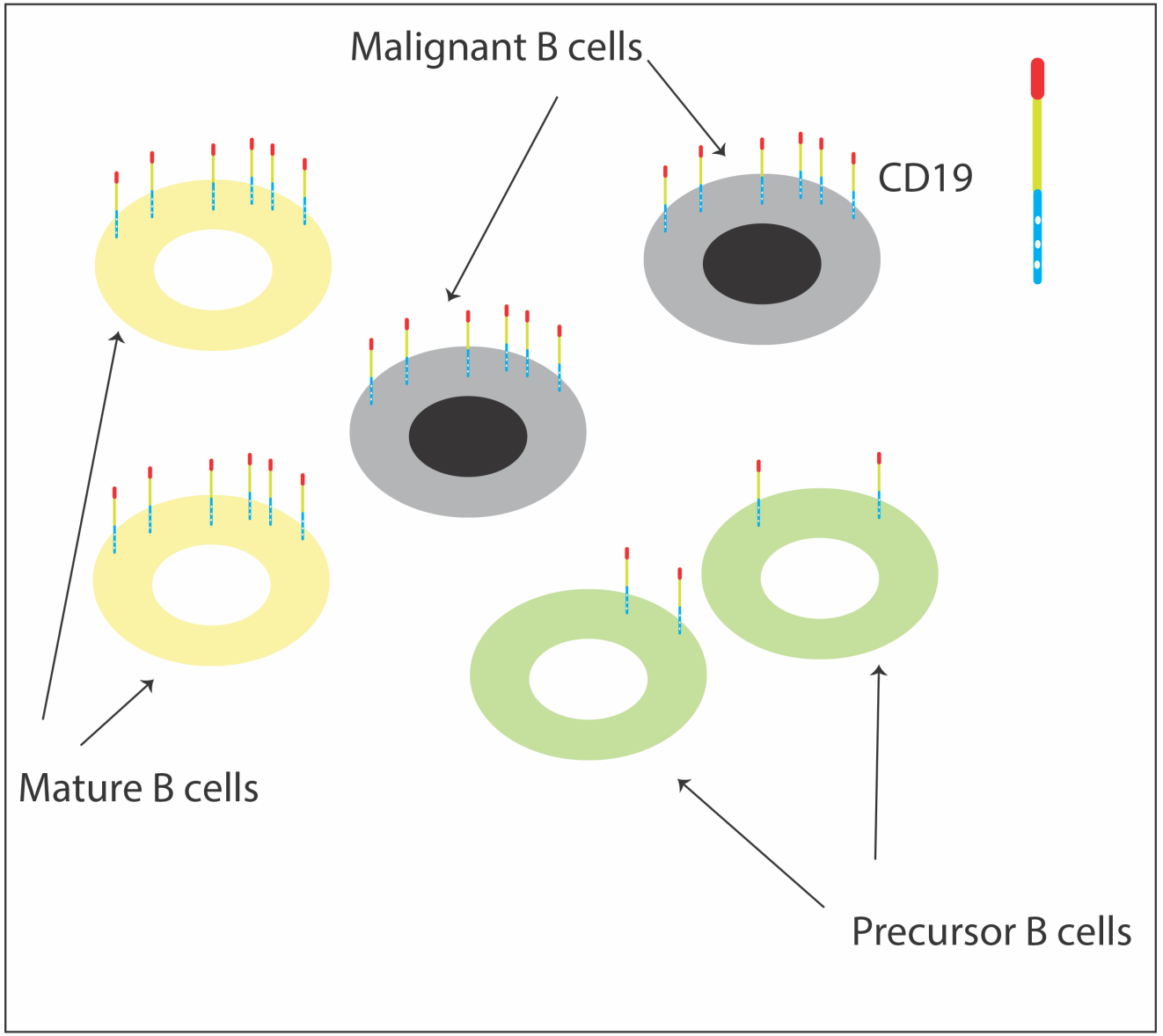
- Emily Whitehead, 12, and her parents, Tom and Kari Whitehead, appeared at an F.D.A. hearing on Tuesday about a treatment for leukemia that had saved Emily's life

CD19 structure

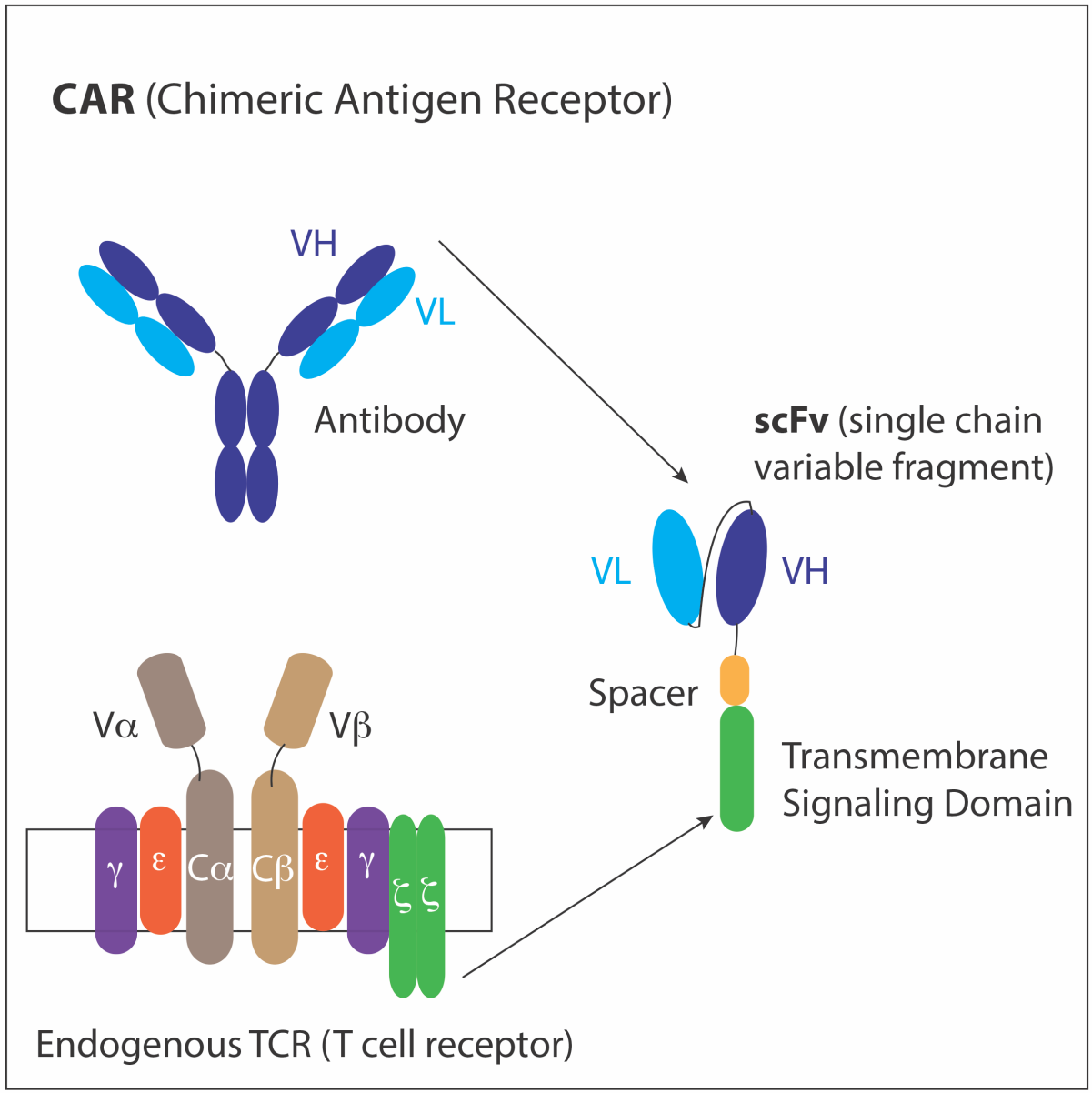


CD19 is a cell surface molecule that assembles with the antigen receptor of B lymphocytes in order to decrease the threshold for antigen receptor-dependent stimulation

Targeting malignant B cells via CD19



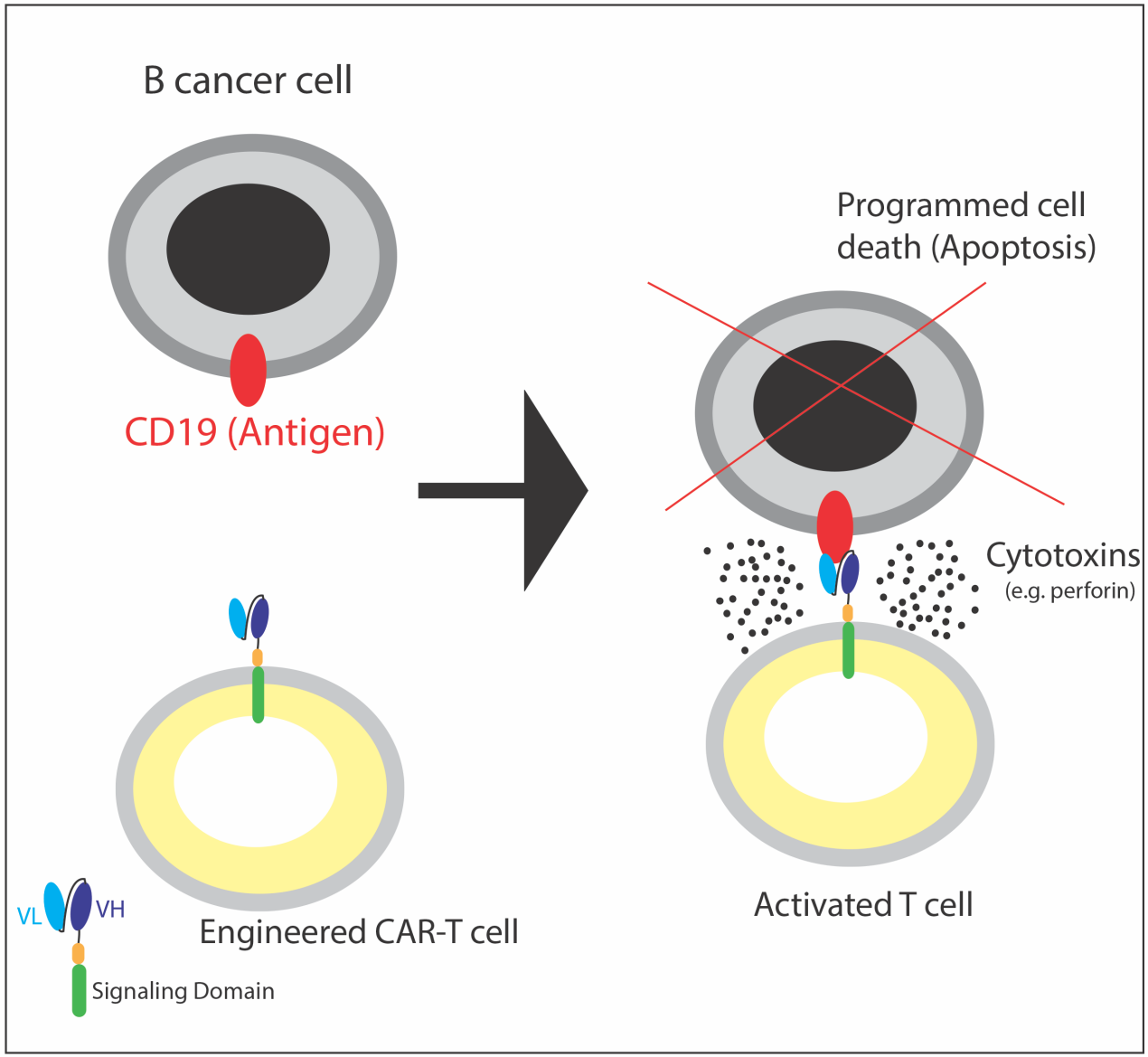
1st generation CAR



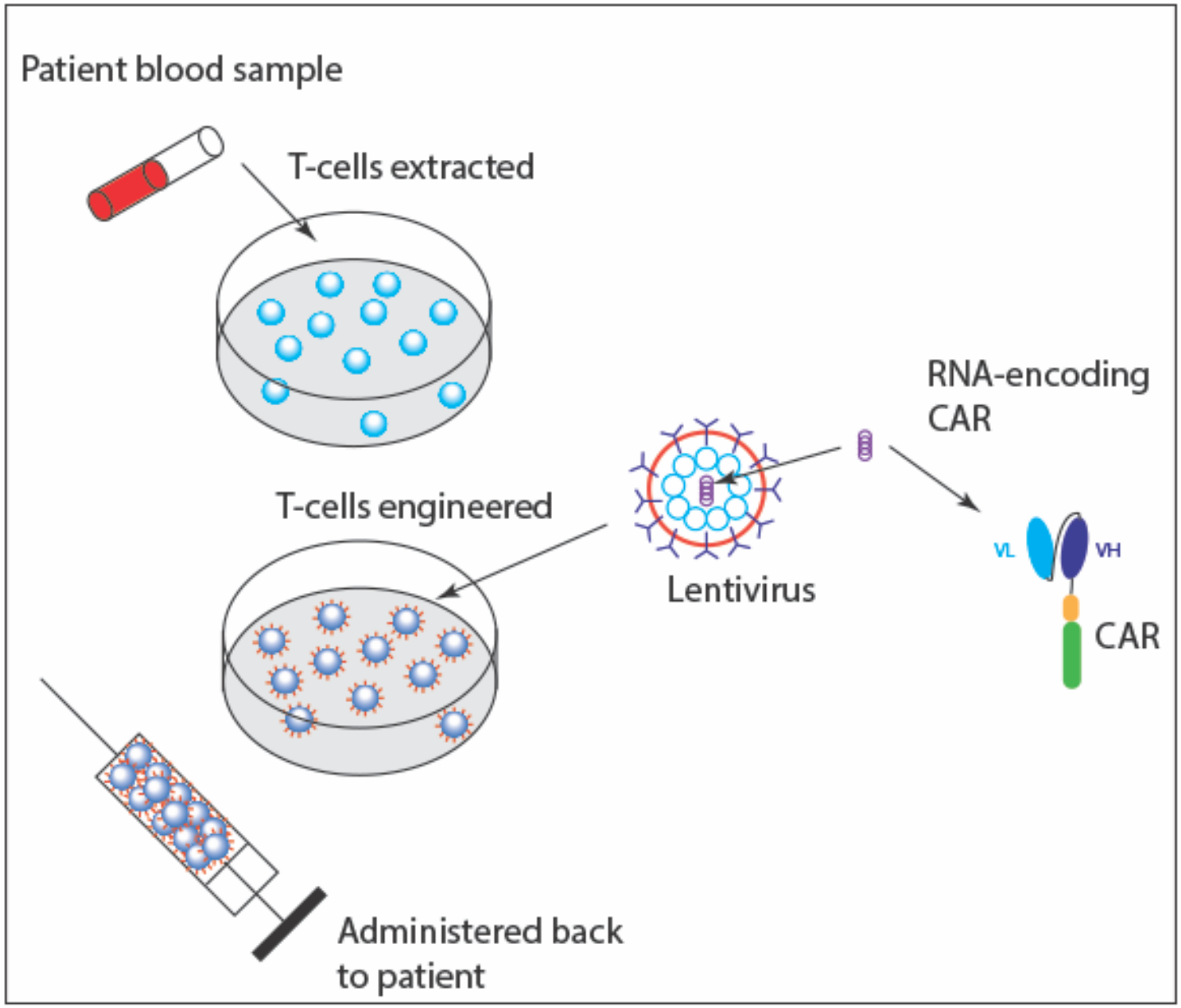
Recognizes and binds to CD19

Induces T-cell proliferation

Activated CAR T-cells killing B cancer cells



CAR T-cell therapy

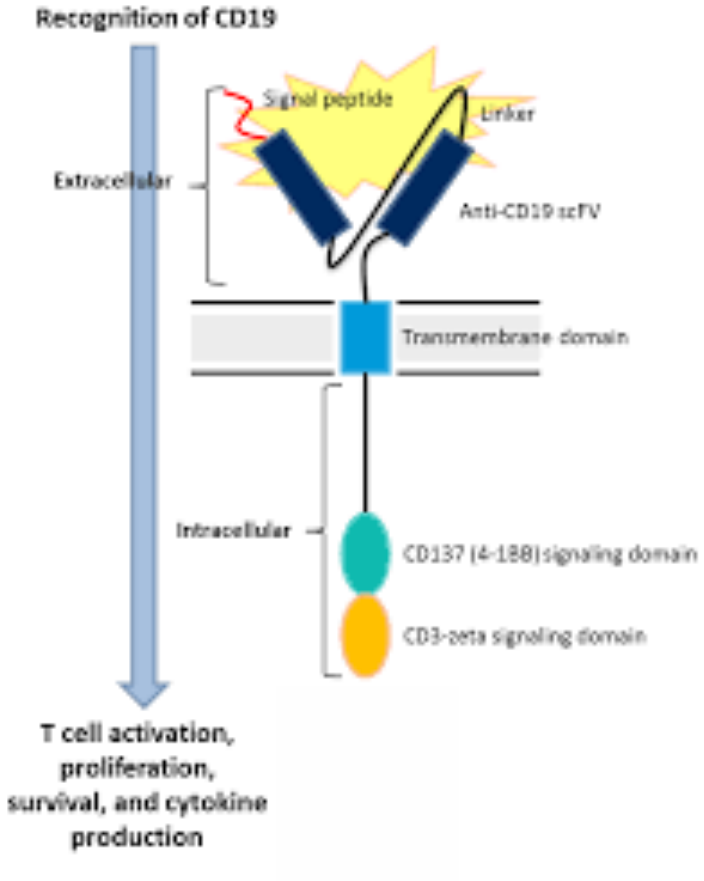


CAR T-cell multistep process

- **Evaluation**: Tests if CAR T-cell is an appropriate therapy
- **Collection**: T cells are collected from patients
- **Engineering**: T cells are genetically engineered to express chimeric antigen receptors (CARs) on their surface
- **Multiplication**: The genetically modified T-cells are "expanded" by growing cells in the laboratory until there are millions of them
- **Conditioning**: Chemotherapy: killing of other immune cells to “create space” for the infused CAR T-cells to expand.
- **Infusion**: Engineered CAR T-cells are infused. Patients need to stay in hospital for a few days up to few weeks depending on side effects.
- **Recovery**: 2-3 months

“CTL019” CAR T-cell therapy

- Novartis



Pharma Intelligence “modified”

Why is it essential to improve intracellular signalling domains of a CAR

- Proper signalling is essential for:
  - **Activation:** triggering T-cell cytotoxic functions.
  - **Proliferation:** expanding the CAR-T population after infusion.
  - **Persistence:** ensuring long-term anti-tumor activity.
- If the signalling is **too weak**, CAR-T cells may fail to kill cancer cells effectively.
  If it is **too strong**, it can cause overactivation, or severe toxicity.

Clinical trial results

- Novartis-sponsored ELIANA study (NCT02435849)
- the first global CAR-T cell trial involving 25 centers in the US, EU, Canada, Australia and Japan. In the Phase II study, 82% (41 of 50) of patients infused with CAR-T cells achieved complete remission.

CAR-T cell therapy costs (estimations)

- In 2017, Novartis' Kymriah became the first CAR-T therapy approved by the FDA
- Costs for one T cell treatment course: $475,000 per patient
- Existing treatment options: Chemotherapy + stem cell transplantation: $100,000 to $200,000

Downsides

Severe side effects

- High fewer (due to cytokine release)
- Dramatic decrease in blood pressure (hypotension)
- Respiratory and renal problems
- B cell aplasia (complete removal of B cells)

(Maude et al., N Engl J Med. 2014)

- It may be argued however that antibody deficiency is an acceptable price to pay for an effective new treatment for otherwise untreatable malignancy, particularly since it can be corrected with immunoglobulin replacement therapy.

Emily Whitehead was the **first** child to be enrolled in a clinical trial for CAR-T cell therapy

- 2012 (age 7) 2021

2022: Celebrating 10 years cancer free

2023: Celebrating 11 years cancer free

2024 Cured

5 FDA-approved CAR T-cell Therapies (Hematological cancers)

- KYMRIAH™
- ABECMA®
- BREYANZI®
- TECARTUS™
- YESCARTA™

Indications

- Diffuse large B-cell lymphoma (DLBCL)
- Primary mediastinal B-cell lymphoma
- High grade B-cell lymphoma
- DLBCL that results from follicular lymphoma
- Follicular lymphoma