Polymers in Biotechnology

Wedding Invites, Meals, and Genes

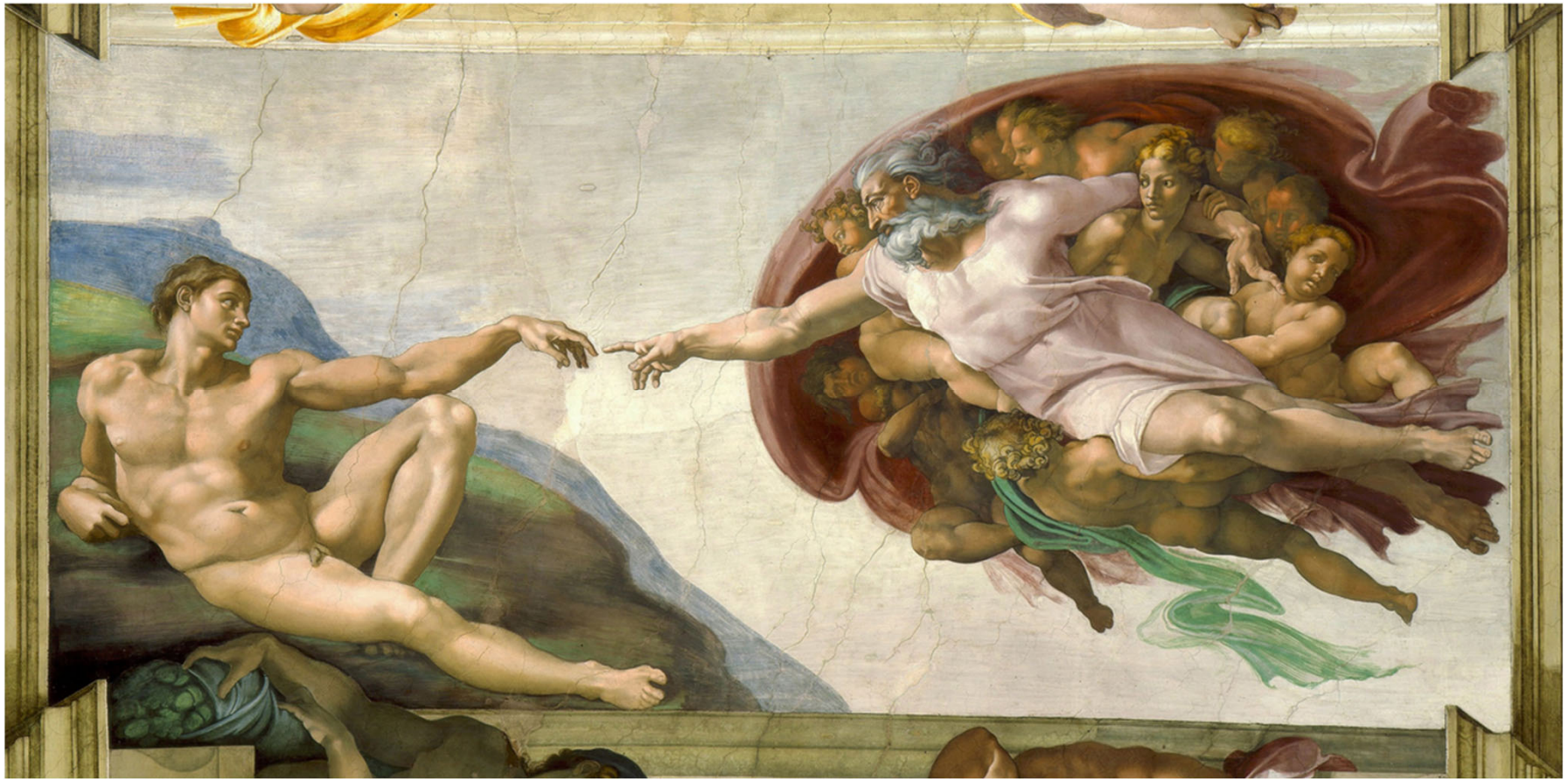
Spreadsheets of genetic failure #RonnyChieng

DNA & The Human Genome

Imagine the world without genetic engineering.

DNA: The Code of Life

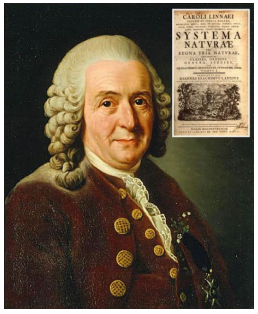
The Creation of Adam by Michelangelo Buonarroti. Sistine Chapel



The Enormity of a Billion Years

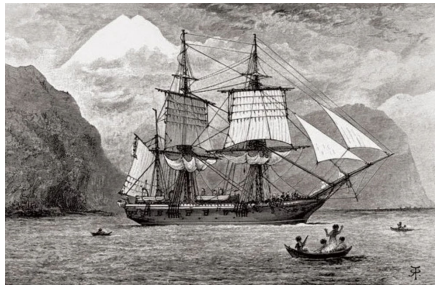
Darwin's Evolutionary Theory: 150 Years Later

610-546 B.C.: Anaximander (Greek) suggests that all life-forms evolved from fish in the seas and went through a process of modification once they were established on land.

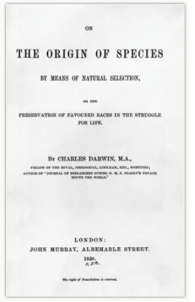


1735: Carl Linnaeus' book Systema Naturae, the foundations for taxonomy. Later he suggested that plants descend from a common ancestor.

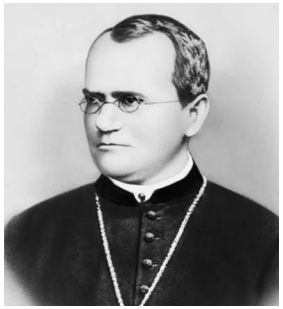
1830: Charles Lyell's Principles of Geology. Darwin's thinking about the gradualism of natural processes can be witnessed in the Grand Canyon.



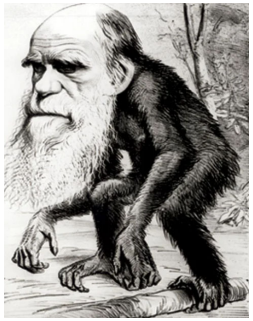
1831: Darwin leaves on a five-year around-the-world journey on the HMS Beagle.



1838: Charles Darwin's theory of natural selection printed in 1858.

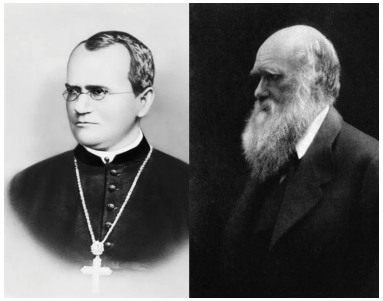


1865: Czech monk Gregor Mendel publishes his research on inheritance, but the importance of his work is not recognized for 35 more years.

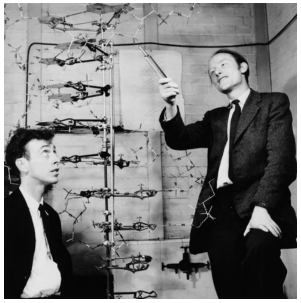


1871: In The Descent of Man, Darwin ties the human lineage to primate ancestors, provoking outrage in some quarters and the caricaturing of his image.

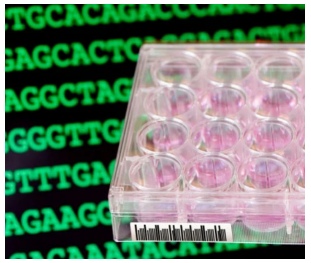
1925: The Scopes Monkey trial in Tennessee tries to make it illegal to teach any theory that denies divine creation.



1936-1947: The modern synthesis combines Darwin's (right) evolutionary theory with Mendelian genetics.



1953: Watson and Crick discover the structure of DNA, opening the door for the molecular biology of evolution.



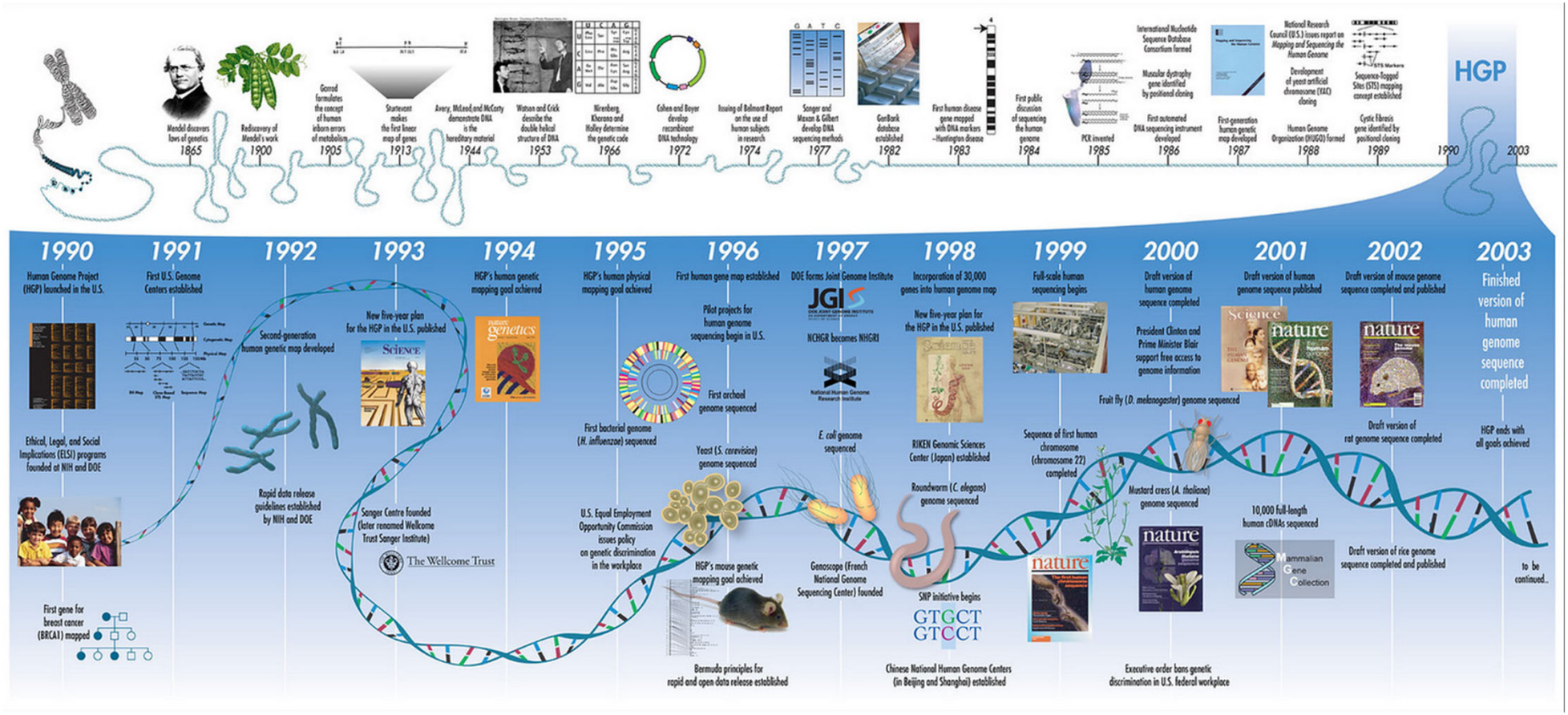
Mid-2000s: Relatively recent human evolution - dating back several thousand years.

2009: Darwin Day marks the naturalist's birthday on February 12.

https://www.scientificamerican.com/slideshow/darwins-living-legacy/#slide-3

The Human Genome Project

The Human Genome Project (HGP) was one of the great feats of exploration in history. Rather than an outward exploration of the planet or the cosmos, the HGP was an inward voyage of discovery led by an international team of researchers looking to sequence and map all of the genes -- together known as the genome -- of members of our species, Homo sapiens. Beginning on October 1, 1990 and completed in April 2003, the HGP gave us the ability, for the first time, to read nature's complete genetic blueprint for building a human being. (https://www.genome.gov/human-genome-project)

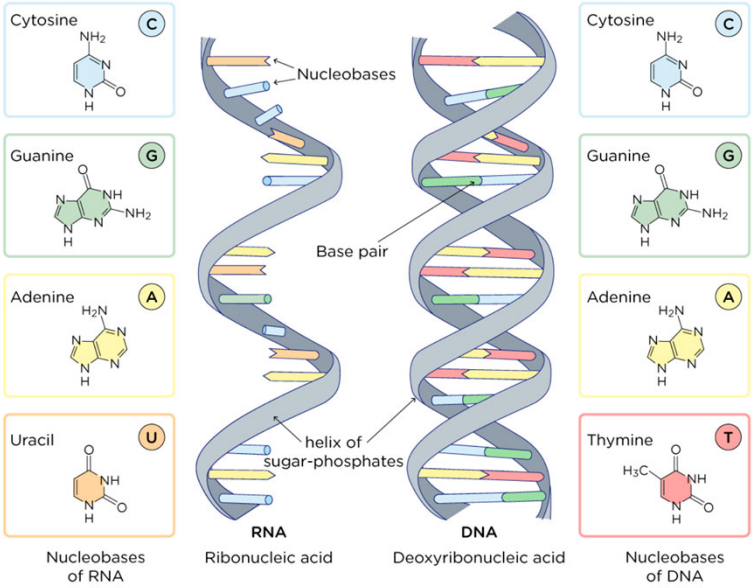


From DNA to Precision Medicine

What is DNA and Why Do We Need It?

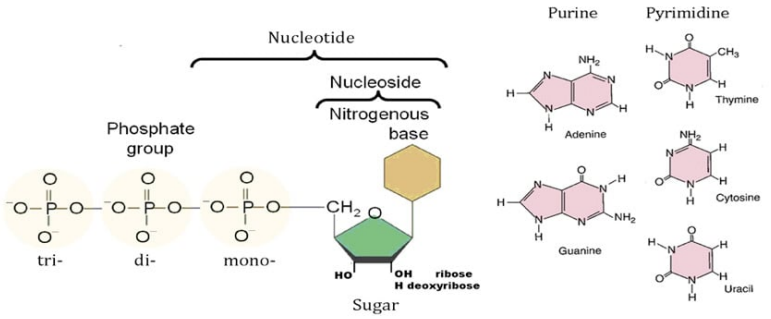
DNA is like a large instruction book, approximately 800 Bibles long, written in the strange language "genish", which consists of only four letters (A, C, T, and G). This book of life contains everything needed to know about building and maintaining a living organism and it directs all the events performed by a cell.

In our cells the DNA is located in the nucleus and packed into 46 chromosomes, 23 from the mother and 23 from the father which combine to form a unique individual. This book of life, the DNA, inherited from one cell to its daughter cells and from one generation to another through replication.



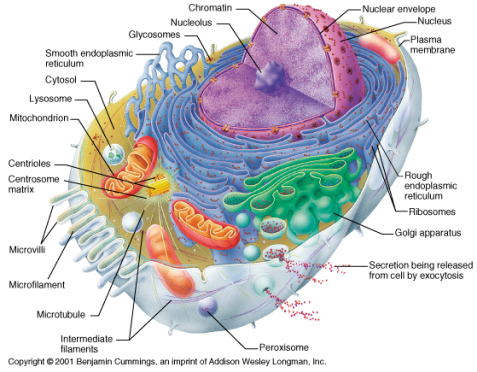
DNA Base Pair

Purine	Pyrimidine
T	A
C	G



Nucleotides (Building blocks)

Nucleic acids are polymers of nucleotides.
Polymers store instructions.
Monomers cannot store instructions.



Professor Tamara Minko <minko@pharmacy.rutgers.edu>

https://jackwestin.com/resources/mcat-content/nucleic-acid-structure-and-function/deoxyribonucleic-acid-dna-double-helix-watson-crick-model-of-dna-structure
https://microbenotes.com/nucleic-acids-nucleosides-and-nucleotides/

https://www.aceorganicchem.com/blog/what-is-a-cell/

Polymeric Artificial Cells

Artificial cells that emulate the structure and function of living systems have attracted significant research attention. Recent advances have led to the creation of synthetic cells that mimic key characteristics of living cells. Polymer-based systems attract enormous interest due to their chemical versatility, robustness, and programmability. These attributes enable the construction of artificial cells with precise control over physicochemical properties, architecture, and functionality. Representative polymeric artificial cells have demonstrated essential hallmarks of life, including membranization, integration of suborganelles, and formation of cytoskeletal frameworks.

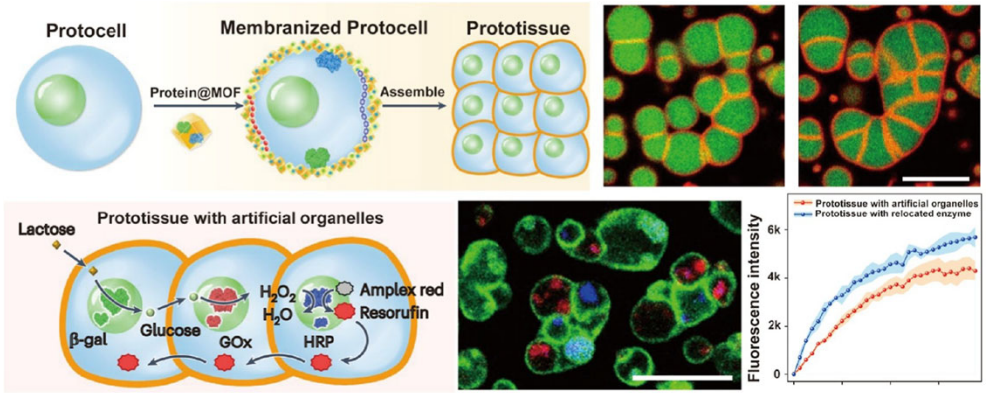


Figure 7. Metal−organic framework (MOF)-stabilized coacervate protoplast model capable of integrating proteins and constructing multicompartmental artificial cellular tissue-like structures, enabling retrograde communication and enzymatic cascade reactions.

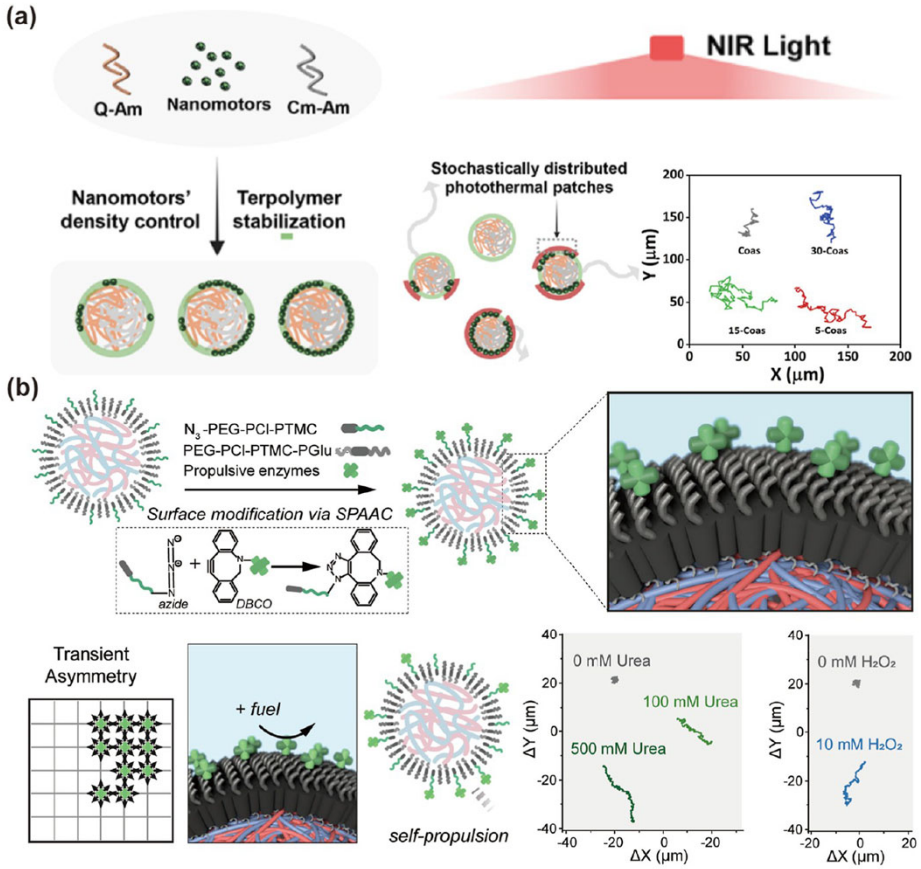
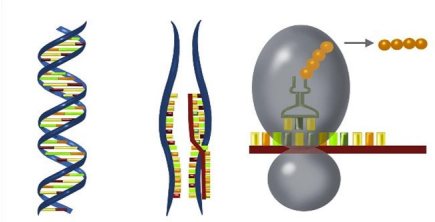


Figure 9. (a) Nanomotors and terpolymer-stabilized coacervate protocells exhibit light-driven mobility capabilities. (b) Enzyme motor-driven motility of polymer-membraned coacervate.

Song 2021, Engineering transient dynamics of artificial cells by stochastic distribution of enzymes
Sun 2025, Engineering motile coacervate droplets via nanomotor stabilization.
Ji 2025, Interfacial assembly of biomimetic MOF-based porous membranes on coacervates to build complex protocells and prototissues.
Han 2026, Polymeric artificial cells- From interfacial membranization to cytomimetic architecture engineering

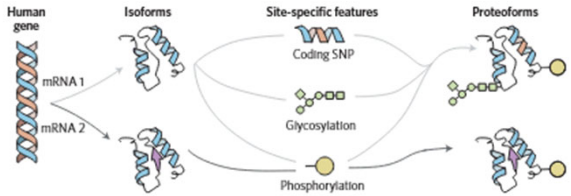
Genetics by the Numbers

3.2 billion base pairs of DNA - or sets of genetic "letters" - make up the human genome. If you were to sing the genetic code to the tune of the “ABCs,” you’d be singing nonstop for over 585 years! However, humans are by no means the species with the most base pairs. The marbled lungfish (Protopterus aethiopicus) has about 133 billion of them in its genome, which is>40 times larger than humans'.



Approximately 20,000 genes in the human genome provide cells with the information they need to make proteins. Genes are transcribed into RNA molecules, which are then translated into proteins. These protein-coding genes make up less than 2 percent of the entire genome. Other genes encode RNA molecules that don’t directly code for a protein, which are sometimes called RNA genes.

The human genome contains approximately 19,500 to 20,500 protein-coding genes. Due to processes like alternative splicing and post-translational modifications, these genes produce a much more diverse proteome, estimated at 80,000 to over 400,000 different protein types (proteoforms) throughout the body (Aebersold 2018, How many human proteoforms are there?)



The DNA of any two people on Earth is 99.9 percent identical. But that **0.1 percent variation represents about 3.2 million base pairs**, which can explain many of the differences between individuals, especially if the changes lie in key genes. Each gene has about 1-3 base pairs that differ from person to person. Some of these differences have no effect on how we look or feel, some of them lead to changes in how we look or grow, and some of these variants put us at risk for some diseases. Our environment also contributes to our individuality.

More than 98 percent of our genome is noncoding DNA—DNA that doesn't encode proteins. Scientists have yet to identify functions for some of this noncoding DNA, but they’ve learned that other segments have specific jobs like regulating gene expression—when and where genes are turned on and off - or protecting the ends of chromosomes (long strings of DNA) from being degraded during copying.

37 genes in our mitochondrial DNA. Mitochondria are organelles that fuel the cell by converting food and oxygen into ATP (adenosine triphosphate), and many of their genes are involved in the production of cellular energy. You inherit mitochondria directly from your biological mother, so your mitochondrial DNA is identical to hers and can be used to trace maternal ancestry for generations!

Rachel Crowley. https://nigms.nih.gov/biobeat/2024/04/genetics-by-the-numbers

Number of Protein-Coding Genes

Human DNA encodes approximately 20,000-25,000 protein-coding genes.

Total Proteins: Due to mechanisms such as alternative splicing, where a single gene can encode multiple proteins, the human body can produce over 100,000 proteins.

Coding Capacity Percentage: The percentage of the human genome that actually codes for proteins is indeed very small, estimated at approximately 1-2%.

Non-coding DNA: The remaining 98-99% of the genome does not code for proteins but is not necessarily "junk." Much of it is responsible for regulating gene activity (turning genes on/off).

The human genome is highly efficient, using a small, specialized portion of DNA to create a massive variety of proteins

Dave Cox, 2023
https://www.bbc.com/future/article/20230412-the-mystery-of-the-human-genomes-dark-matter

The mystery of the human genome's dark matter

When human genome project was complete, the prevailing belief was that the vast majority of the human genome would consist of instructions for making proteins, the building blocks of all living organisms that perform a bewildering range of roles within and between our cells. With over 200 different types of cells in the human body, it seemed to make sense that each would need its own genes to carry out its necessary functions. The appearance of unique sets of proteins was thought to have been vital in the evolution of our species and our cognitive powers. (We are, after all, the only species capable of sequencing our own genome.)

Instead, it transpired that less than 2% of the three billion letters of the human genome are dedicated to proteins. Only around 20,000 distinct protein-coding genes were found to exist in the long lines of molecules known as base pairs that make up our DNA sequences.

The remaining 98% of our DNA became known as dark matter, or the dark genome, a mysterious melee of letters with no obvious meaning or purpose. Initially some geneticists suggested that the dark genome was simply junk DNA or the rubbish bin of human evolution – the remnants of broken genes which had long ceased to be relevant.

Now, two decades on, we have the first inklings of the role of the dark genome. Its primary function appears to be regulating the decoding process, or expression, of protein-making genes. It helps to control how our genes behave in response to all the environmental pressures our bodies face throughout our lives, ranging from diet to stress, pollution, exercise, and how much we sleep, a field known as epigenetics.

As scientists first began sifting through the book of life in the mid 2000s, one of the biggest challenges was that the non-protein coding regions of the human genome appeared to be littered with sequences of repetitive DNA known as **transposons**. These repetitive sequences are so ubiquitous that they comprise nearly half the genome in all living mammals.

Polymorphism

- **Polymorphism**: The occurrence of two or more distinct variations in a specific DNA sequence among individuals or populations. Applied to many situations ranging from genetic traits or disorders in a population to the variation in the sequence of DNA or proteins. Genetic variations occurring in ≥ 1% of a population would be considered useful polymorphisms for genetic linkage analysis.

- A polymorphism has been defined as the least common allele occurring in 1% or greater of the population, whereas **mutations** are rare differences that occur in less than 1% of the population (usually much less than 1%).

Single Nucleotide Polymorphism (SNP)

- DNA in the human genome is made up of about three billion nucleotides, or chemical letters, which code for all the macromolecules needed to build and sustain a human being.

- About 99.9% of the letters are the same in all human beings, and that one in every thousand nucleotides differs from one person to another.

- Three million SNPs account for variations in height, eye color and other such visible characteristics. More importantly for medicine, they also account for variations in susceptibility to disease and in the way individuals respond to therapy.

Professor Tamara Minko <minko@pharmacy.rutgers.edu>

Mutation

A mutation is a change in a DNA sequence.

Mutations can result from DNA copying mistakes made during cell division, exposure to ionizing radiation, exposure to chemicals called mutagens, or infection by viruses.
Germline mutations occur in the eggs and sperm and can be passed on to offspring, while somatic mutations occur in body cells and are not passed on.
https://www.genome.gov/genetics-glossary/Mutation

Human genome contains 3.2×10^9 base pairs.

Human chromosomes range in size from about 50,000,000 to 300,000,000 base pairs. Because the bases exist as pairs, and the identity of one of the bases in the pair determines the other member of the pair, scientists do not have to report both bases of the pair.
https://www.genome.gov/human-genome-project/Completion-FAQ

Phenotype: An organism with respect to a particular character or group of characters (physical, biochemical, and physiological), as a result of the interaction of its genotype and its environment. Often used to define the consequences of a particular mutation. Types of mutations include point mutations, deletions, insertions, and changes in number and structure of chromosomes.

Point Mutation:

Wild - AATGATGCT

↓

Mutated - AATGGTGCT

Insertion:

Wild – AATG TGCT

↓

Mutated – AATGATTTGCT

Deletion:

Wild - AATGATGCT

↓

Mutated – AATG TGCT

The human germline mutation rate per basepair per generation ($\sim 1.2 \times 10^{-8}$) = 40 basepair/generation

Lindsay 2019, Similarities and differences in patterns of germline mutation between mice and humans

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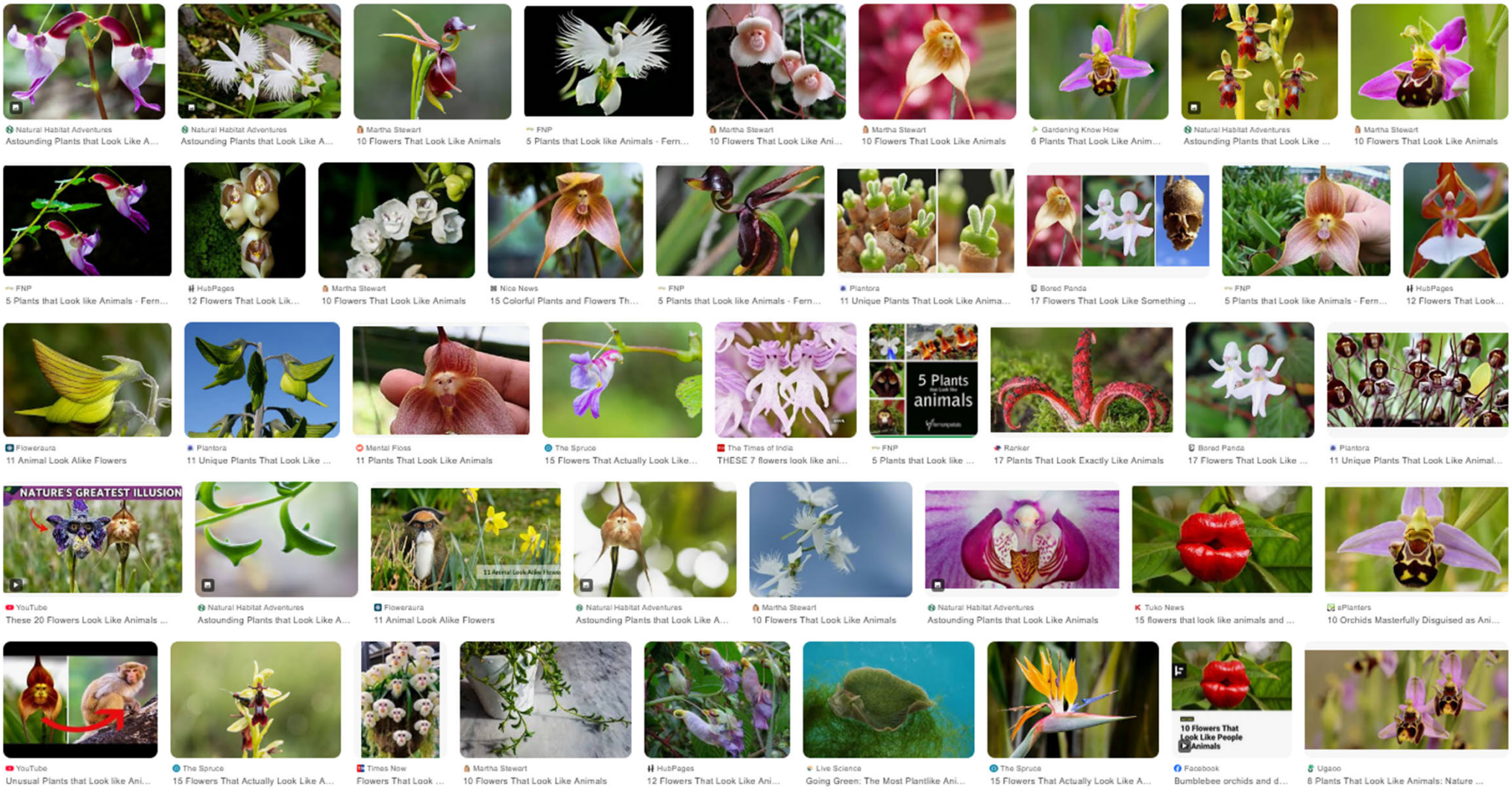
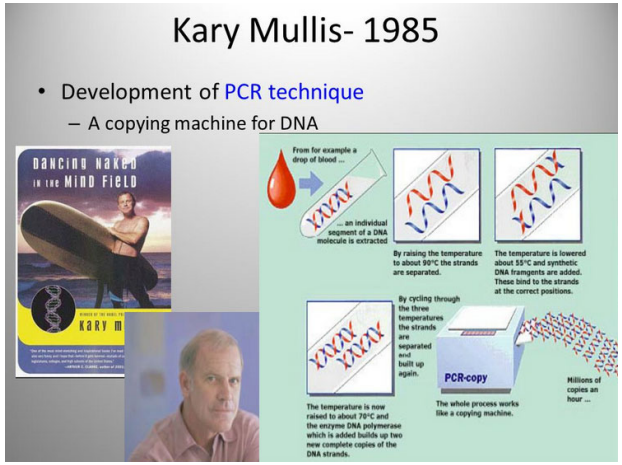
Plants That Look Like Animals



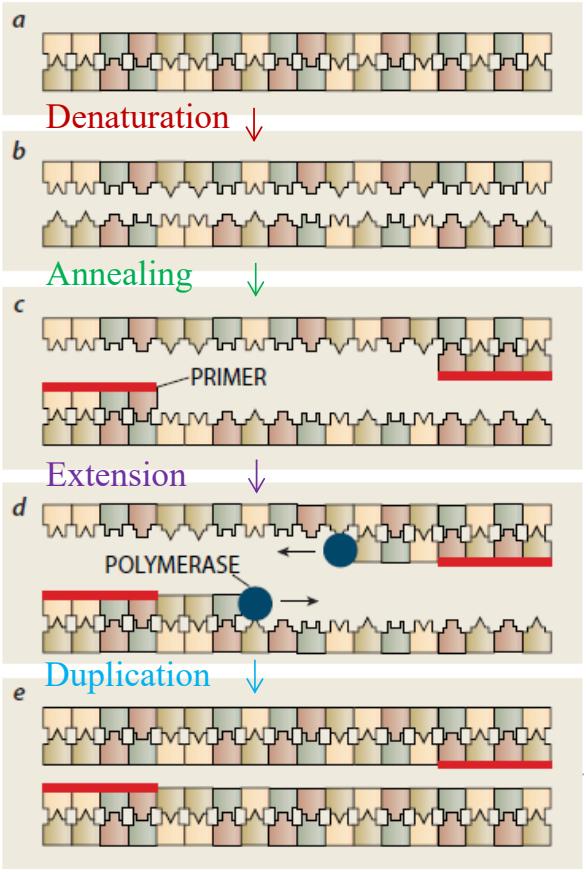
Polymerase Chain Reaction (PCR)



PCR is a biochemistry and molecular biology technique for isolating and exponentially amplifying a fragment of DNA, via enzymatic replication, without using a living organism (such as E. coli or yeast). As PCR is an in vitro technique, it can be performed without restrictions on the form of DNA, and it can be extensively modified to perform a wide array of genetic manipulations.

Invented in 1983 by Kary Mullis (while driving at night while his wife was sleeping), PCR is now a common technique used in medical and biological research labs for a variety of tasks, such as the sequencing of genes and the diagnosis of hereditary diseases, the identification of genetic fingerprints (used in forensics and paternity testing), the detection and diagnosis of infectious diseases, and the creation of transgenic organisms.

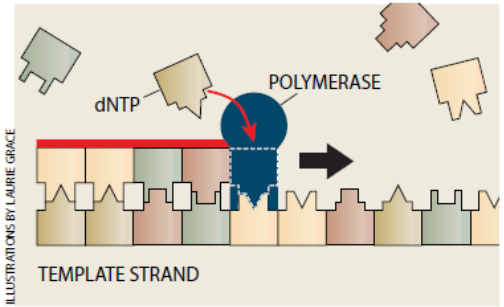
Mullis 1990, The unusual origin of the polymerase chain reaction
http://slideplayer.com/slide/4737320/



This cyclic reaction takes only minutes or less and can be repeated indefinitely.

Dragon 1998, Polymerase chain reaction, Sci. Am. May 1998, p. 112.

(a) DUPLICATING DNA begins with a double-stranded stretch of DNA to be amplified, or copied.
(b) In a solution heated to 95 degrees Celsius (203 degrees Fahrenheit), hydrogen bonds between the strands break, leaving two single strands.
(c) When the mixture is cooled to between 50 and 65 degrees C, specially manufactured DNA primers bind complementarily to each strand at points flanking the region to be copied.
(d) At 72 degrees C, polymerase enzymes extend the bound primers in one direction, using the original DNA as a template.
(e) The products are two new double strands of DNA, both identical to the original.



POLYMERASE ENZYME extends a bound primer. From the surrounding medium, it extracts a free-floating deoxynucleotide triphosphate (dNTP) that will complement the next unpaired position in the template strand of DNA. The enzyme then joins the dNTP to the end of the primer and moves on to the next position

Polymerase Chain Reaction (PCR)

The Unusual Origin of the Polymerase Chain Reaction

A surprisingly simple method for making unlimited copies of DNA fragments was conceived under unlikely circumstances—during a moonlit drive through the mountains of California

by Kary B. Mullis

One Friday evening late in the spring I was driving to Mendocino County with a chemist friend. She was asleep. U.S. 101 was undemanding. I liked night driving; every weekend I went north to my cabin and sat still for three hours in the car, my hands occupied, my mind free. On that particular night I was thinking about my proposed DNA-sequencing experiment. My plans were straightforward.

First I would separate a DNA target into single strands by heating it. Then I would hybridize an oligonucleotide to a complementary sequence on one of the strands. I would place portions of this DNA mixture into four different tubes. Each tube would contain all four types of dideoxynucleotide triphosphates (ddNTP's), but in each tube a different type of ddNTP would be radioactively labeled. Next I would add DNA polymerase, which would extend the hybridized oligonucleotides in each tube by a single ddNTP. By electrophoresis I could separate the extended oligonucleotides from the residual ddNTP's; by identifying which radioactively labeled ddNTP had been incorporated into the oligonucleotide, I could determine the corresponding complementary base in the target strand. Simple.

In the spring of 1984, while working on the patent, I presented a poster describing the PCR at the annual Cetus Scientific Meeting. These meetings were always fun, because Cetus had some first-rate scientific advisers, and I was looking forward to talking with them about my invention. Yet nobody seemed to be interested in my poster, and I felt increasingly anxious. People would glance at it and keep walking. Finally, I noticed Joshua Lederberg, president of the Rockefeller University, nearby, and I snared him into looking at my results. Josh looked the poster over carefully and then turned his enormous head, the Nobel-laureated head, the head that had deduced in 1946 that bacteria could have sexual intercourse. "Does it work ? " He seemed amused.

Mullis 1990, The unusual origin of the polymerase chain reaction, Sci. Am. April 1990, p.56.

Pharmacogenetics & Pharmacogenomics

Pharmacogenetics studies how a single gene affects drug response, while pharmacogenomics is the broader field looking at how the entire genome (multiple genes, interactions) influences how a person reacts to medications, aiming for personalized treatment by considering the complex genetic landscape. Think of pharmacogenetics as a specific tool (one gene), and pharmacogenomics as the whole toolkit (all genes), with the latter incorporating the former for a comprehensive view of drug efficacy and safety.

Pharmacogenetics

- Focus: Single gene-drug interactions.
- Scope: Narrower, looking at how variations in specific genes (like CYP2C9) affect metabolism of a particular drug (like warfarin).
- Goal: Understand the genetic basis of variability in drug response.

Pharmacogenomics

- Focus: The influence of the entire genetic makeup (genome) on drug response.
- Scope: Broader, encompassing multiple genes, gene-gene interactions, and other "-omics" (proteomics, metabolomics).
- Goal: Develop comprehensive, personalized medicine strategies by understanding the complex genetic profile for tailored drug choices and dosing.

Key Distinction

- Pharmacogenetics: "One gene, one drug".
- Pharmacogenomics: "Whole genome, whole patient".

Usage

- The terms are often used interchangeably, but pharmacogenomics represents a more modern, comprehensive approach that integrates pharmacogenetics findings

Pharmacogenetics & Pharmacogenomics

Pharmacogenetics

The study of variability in drug response (in particular drug metabolism) due to single genes.

Relationship between genetic variation and drug response (from the perspective of inherited and ethnic differences).

Variation among individual genotypes means that many drugs work for only 60% of the population at best.

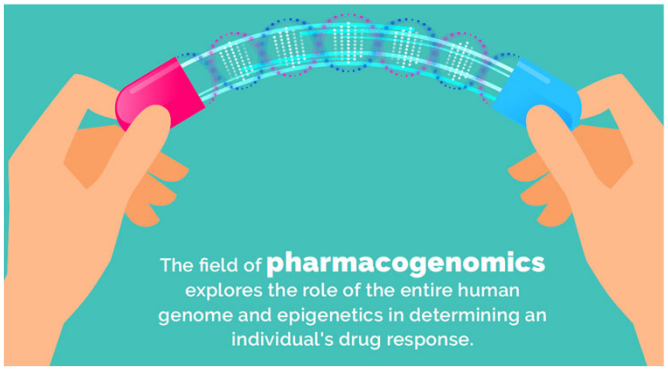
Pharmacogenomics

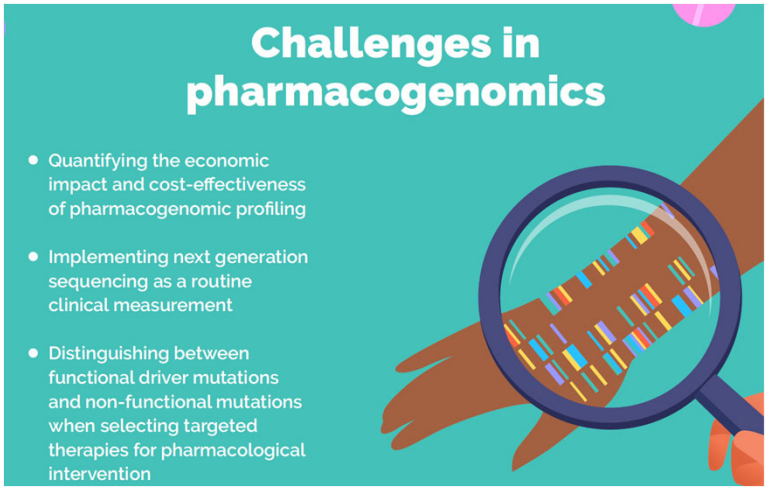
The controls of most drug responses are multifactorial, different groups of genes.

Relationship between genome (all genes) and drug response or disease (from the perspective of non-inherited genetic traits (e.g., single nucleotide polymorphisms).

The genetic factors determining the drug efficacy and toxicity.

If an SNP occurs in the coding region of the genome then there can be significant structural and functional alterations to the protein that is subsequently produced.





Pharmacogenomics is a part of precision medicine. Pharmacogenomics is the study of how genes affect a person's response to particular drugs. This relatively new field combines pharmacology (the science of drugs) and genomics (the study of genes and their functions) to develop effective, safe medications and doses that are tailored to variations in a person's genes.

Pirmohamed 2001, Pharmacogenetics and pharmacogenomics. Br. J. Clin. Pharmacol. 52: 345-347.

Kalow 2006, Pharmacogenetics and pharmacogenomics. The Pharmacogenomics Journal 6,162–165.

Technology Networks 2020, Pharmacogenomics - Infographic

https://medlineplus.gov/genetics/understanding/precisionmedicine/precisionvspersonalized/

Personalized Medicine / Precision Medicine

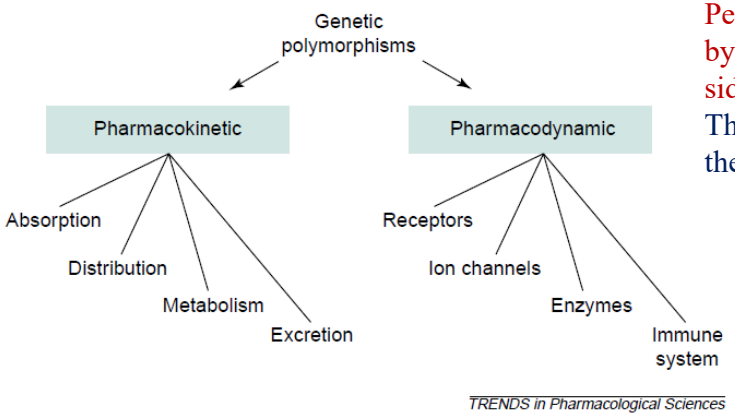


Fig. 1. Genetic variability leading to susceptibility to adverse drug reactions can affect both pharmacokinetic and pharmacodynamics pathways. (Pirmohamed 2001, Genetic susceptibility to adverse drug reactions. Trends in Pharmacological Sciences 22, 298-305).

Drug action studies focus on two major determinants. Scientists rely on pharmacokinetic and pharmacodynamic considerations when assessing genetic polymorphisms in drug action studies. Pharmacokinetics describes how much of a drug is needed to reach its target in the body, and encompasses four processes: absorption, distribution, metabolism, and excretion. Pharmacodynamics describes how well the target cells, such as heart tissue or neurons, respond to the drug. Target cells include receptors, ion channels, enzymes, and immune system components. (J. Adams, Pharmacogenomics and Personalized medicine, Nature Education 1(1):194, 2008).

Personalized medicine is based on using an individual's genetic profile to make the best therapeutic choice by facilitating predictions about whether that person will benefit from a particular medicine or suffer serious side effects. Drugs are generally tested on a large population of people and the average response is reported. This sort of evidence-based medicine (that is, medical decision making based on empirical data) relies on the law of averages; personalized medicine, on the other hand, recognizes that no two patients are alike.

Pharmacokinetics is the drug concentration as a function of time. Right after taking a drug, the drug concentration increases due to absorption, and the absorption is balanced by distribution throughout the body, metabolism into different chemical species, and excretion from the body. **Pharmacodynamics** is the study on the pharmacological effect.

If one takes aspirin for high temperature, we can measure the aspirin concentration over time. This is pharmacokinetics. But if we measure the temperature, instead of the aspirin concentration, it is pharmacodynamics.

Genetic polymorphisms and adverse drug reactions

A gene can be defined as exhibiting a genetic polymorphism if the variant allele exists in the normal population at a frequency of at least 1% (Meyer, U.A. (2000) Pharmacogenetics and adverse drug reactions. Lancet 356, 1667–1671). Genetic polymorphisms are a source of variation of drug response in the human body. In relation to adverse drug reactions (ADRs), most interest has centered on the involvement of pharmacokinetic factors and, in particular, drug metabolism. However, there is now increasing realization that genetic variation in drug targets (pharmacodynamic factors) might also predispose to ADRs, although research into this area is in its infancy (Fig. 1). It is important to note that although the focus of this review is genetic sources of variation, environmental factors such as disease, alcohol, smoking and diet might also be significant sources of variability and might predominate. Indeed, the environment might interact with the genetic factors and either increase or decrease the risk of an ADR.

Engineering Precision Medicine Technologies

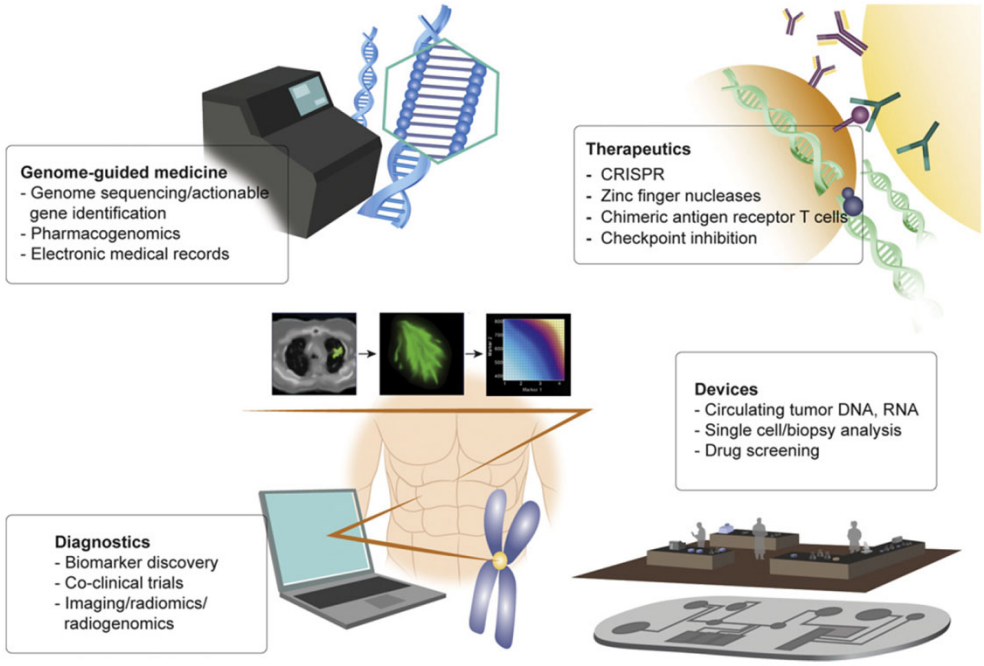


Figure 1. Engineering Precision Medicine Technology Platforms. From genome-guided medicine to clustered regularly interspaced short palindromic repeats (CRISPR), a broad spectrum of technology platforms that bridge engineering with precision medicine are poised to impact clinical outcomes.

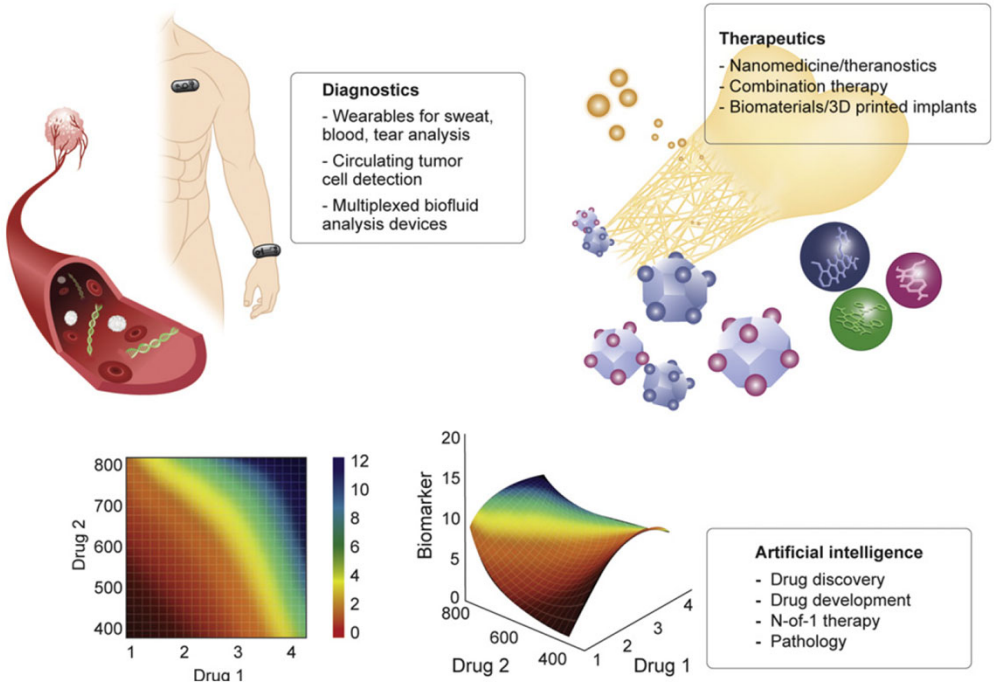


Figure 2. Engineering Personalized Medicine Technology Platforms. By bridging wearable technologies with artificial intelligence and other engineering platforms, marked enhancements in the development of individualized treatment and monitoring may be realized.

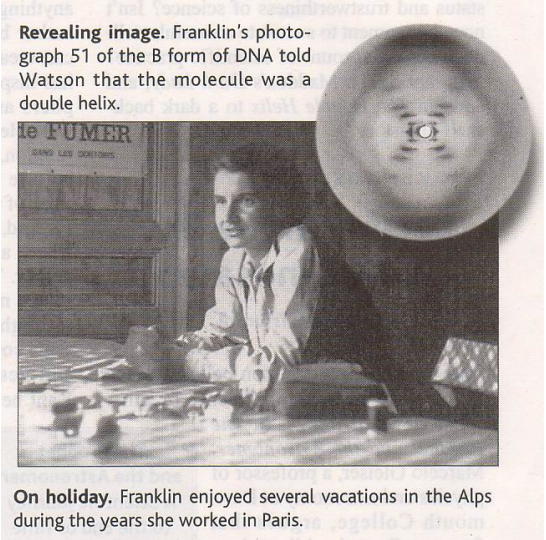
Ho 2019, Enabling technologies for personalized and precision medicine

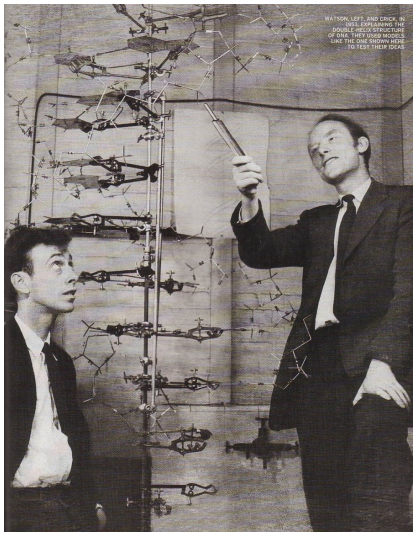
The Dawning of the Age of Genetic Engineering

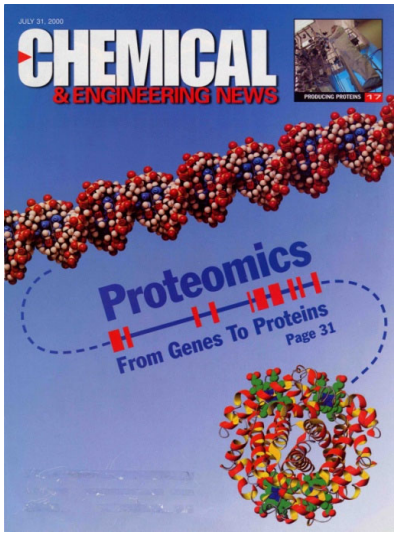
Discovery of the DNA Structure

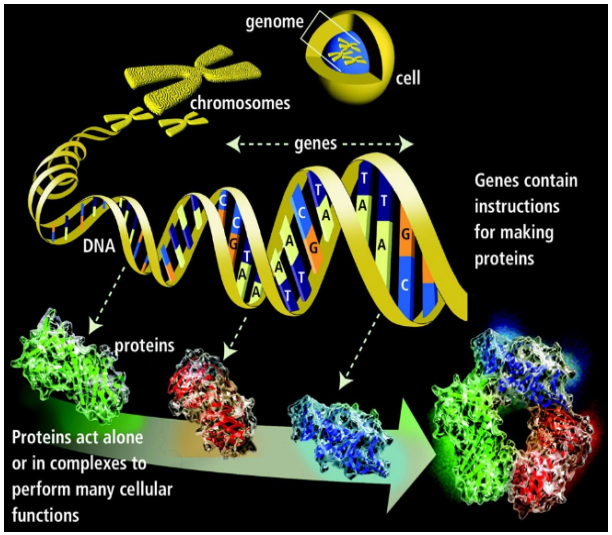
All living things — including the fruits, vegetables and meat that we eat — contain genes that provide the instructions that tell the cells how to function. That information and many important traits are passed from generation to generation through genes, which are made of a large molecule called DNA, shaped much like a spiral staircase or "double helix."

Rosalind Franklin worked with Maurice Wilkins. Her x-ray crystal diffraction micrographs provided positive proof of DNA' helical form. She was such perfectionist and published her findings only after completing her painstaking analysis. If she published earlier, she would have received the Nobel prize while she was alive (William Moran).





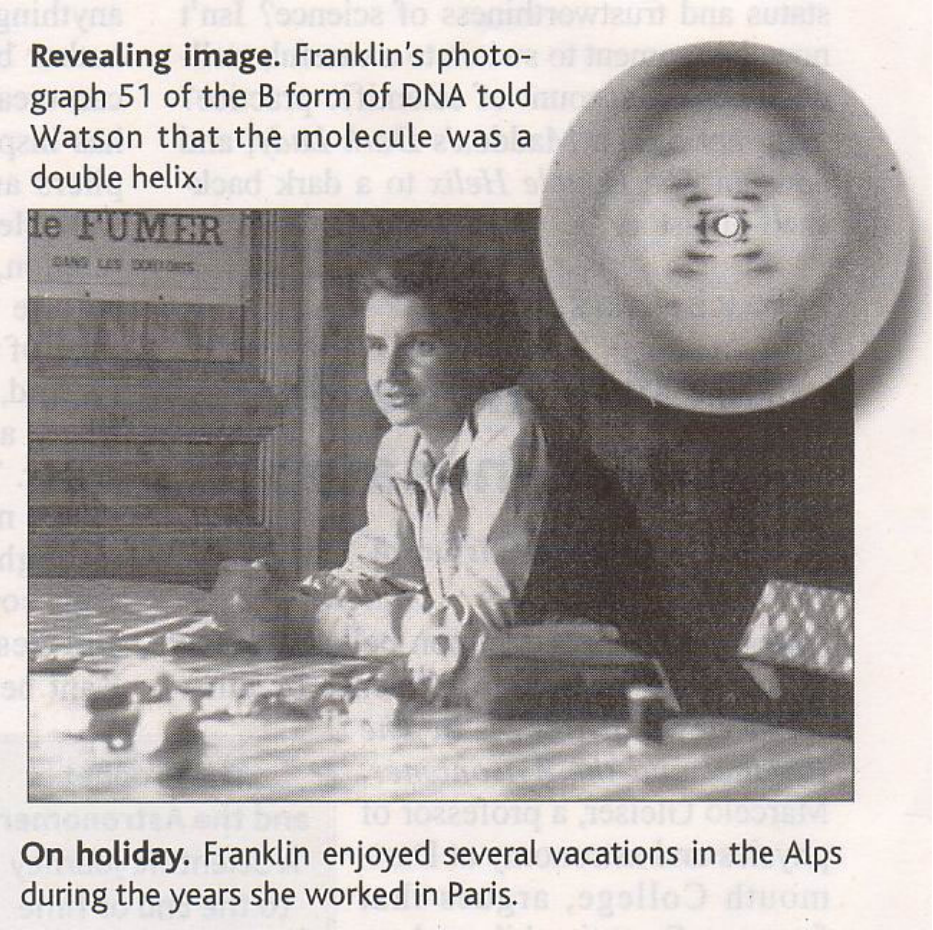




William Moran. https://www.quora.com/In-what-ways-did-Rosalind-Franklin-contribute-to-the-understanding-of-DNA-and-x-ray-diffraction

http://blogs.cornell.edu/gmodialogue/what-is-genetic-engineering-2/

Discovery of the DNA Structure



William Moran. https://www.quora.com/In-what-ways-did-Rosalind-Franklin-contribute-to-the-understanding-of-DNA-and-x-ray-diffraction

Biotechnology & Genetic Engineering

Discovery of the DNA Structure (1953) started the age of genetic engineering. Genetic engineering allows introduction of new traits to an organism to produce genetically modified organisms.

Scientists do genetic engineering by cutting and moving snippets of DNA from one plant, animal or microbe to another in a process called gene splicing. Genetic engineering can also include changing the expressing of a gene in a plant. Unlike traditional breeding techniques that simultaneously introduce many genes (including unwanted genes), genetic engineering is considered more precise since it introduces just the gene for a specific desirable trait.

Biotechnology

1. The use of biological processes or organisms for the production of materials and services of benefit to humankind. Biotechnology includes the use of techniques to improve the characteristics of economically important plants and animals, and to develop micro-organisms to act on the environment.
2. The scientific manipulation of living organisms, especially at the molecular genetic level, to produce new products, such as hormones, vaccines or monoclonal antibodies.

Genetic engineering

Changes in the genetic constitution of cells (apart from selective breeding) resulting from the introduction or elimination of specific genes through modern molecular biology techniques. This technology uses a vector to transfer useful genetic information from a donor organism into a cell or organism that does not possess it.

A broader definition of genetic engineering also includes selective breeding and other means of **artificial selection**.

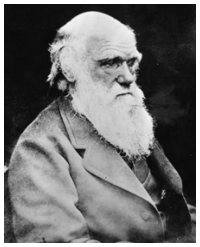
Zaid 1999, Glossary of biotechnology and genetic engineering.
http://repositorio.conicyt.cl/bitstream/handle/10533/171497/GLOSSARY_OF_BIOTECHNOLOGY_AND_GENETIC_ENGINEERING.pdf.pdf?sequence=1

Scientists Who Revolutionized The World

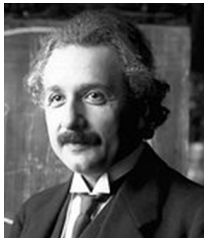

Nicolaus Copernicus

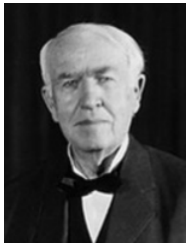

Galileo Galilei

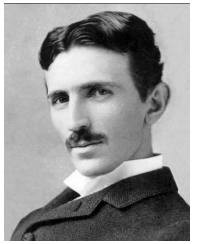

Isaac Newton

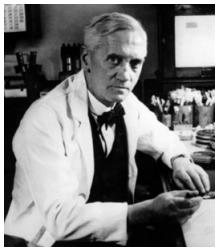

Charles Darwin


Marie Curie


Albert Einstein


Thomas Edison


Nikola Tesla


Alexander Fleming


Louis Pasteur


Crawford Long

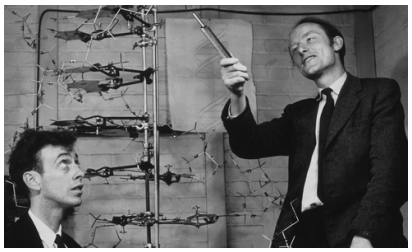

Wilhelm Röntgen

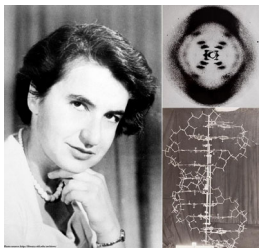

Felix Hoffmann

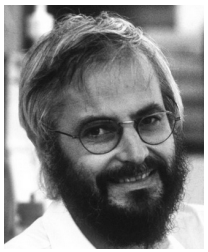

Frederick Banting

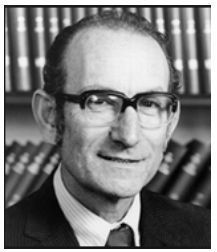

John Leal


Marie Tharp


James Watson & Francis Crick


Rosalind Franklin


Georges Köhler


César Milstein


Herbert Boyer


Stanley Cohen


Emmanuelle Charpentier & Jennifer Doudna

Pioneers of Biotechnology

A Boyer-Cohen Collaboration

Recombinant-DNA (rDNA) technology—the way in which genetic material from one organism is artificially introduced into the genome of another organism and then replicated and expressed by that other organism—was invented largely through the work of Herbert W. Boyer, Stanley N. Cohen, and Paul Berg, although many other scientists made important contributions to the new technology as well.

Herbert Boyer

Stanley Cohen

Boyer's Work with rDNA and Bacteria

After Paul Berg's 1971 landmark gene-splicing experiment, the next landmark in the development of modern biotechnology was the insertion of rDNA into bacteria in such a way that the foreign DNA would replicate naturally (see Figure). This step was taken in 1972 by Boyer at the University of California, San Francisco (UCSF), in collaboration with Cohen of Stanford University.

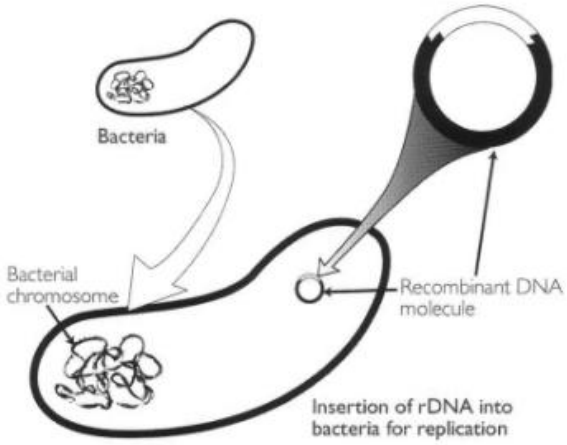


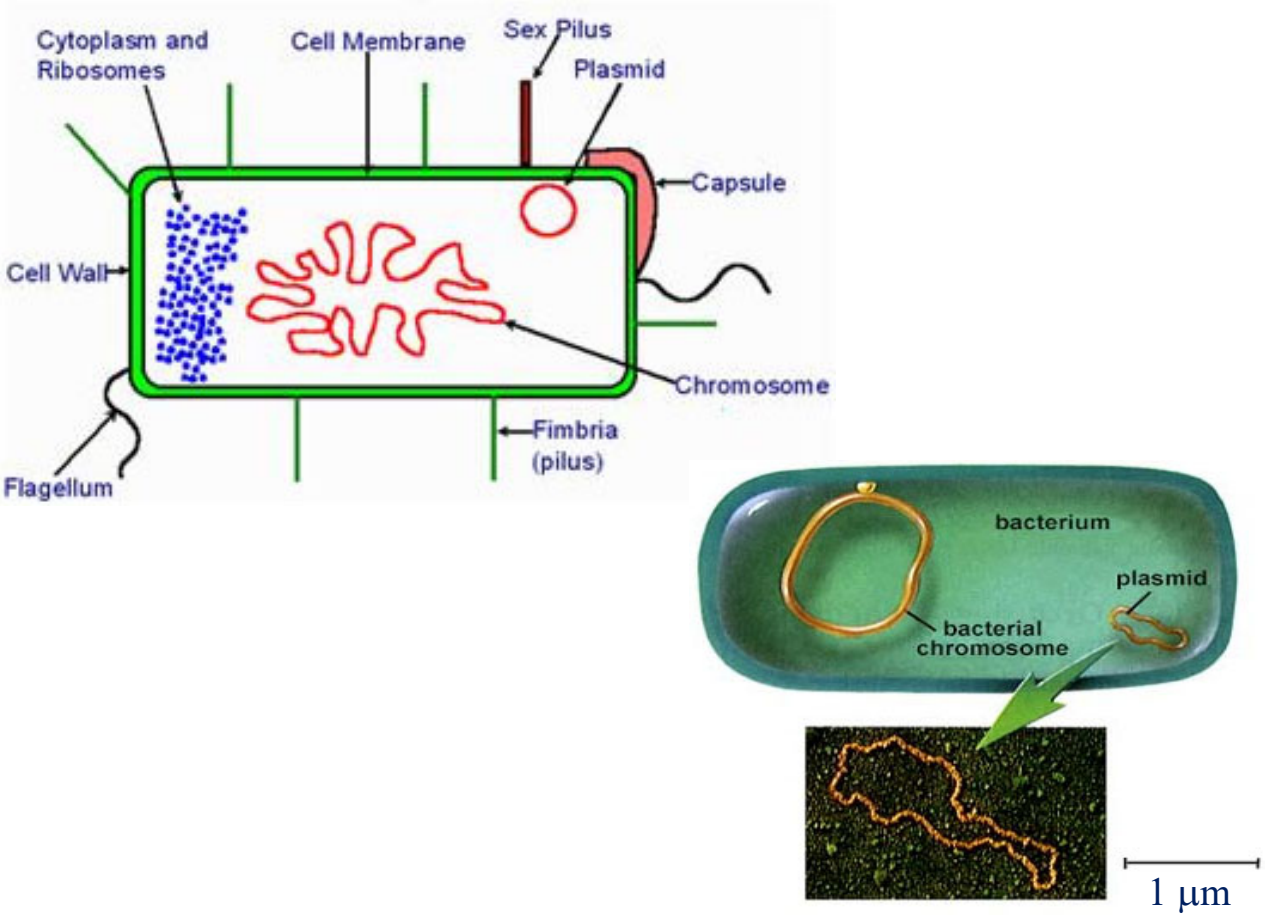
Figure. The insertion of recombinant DNA so that the foreign DNA will replicate naturally, as pioneered by Herbert Boyer and Stanley Cohen.

A Boyer-Cohen Collaboration

November 1972 found both Boyer and Cohen in Hawaii giving papers at a U.S.-Japan joint meeting on plasmids. A plasmid is DNA, found especially in bacteria, that is physically separate from and can replicate independently of the bacterium's chromosomal DNA. While Boyer was describing his data showing the nature of the DNA ends generated by EcoRI cleavage, Cohen was reporting on a procedure recently discovered in his laboratory that enabled bacteria to take up plasmid DNA and produce offspring that contained self-replicating plasmids identical to the original implant—clones. Over sandwiches late one night at the conference, the two men laid plans for a collaborative project to discover what genes are present on plasmids and how they are arranged.

https://www.chemheritage.org/historical-profile/herbert-w-boyer-and-stanley-n-cohen

Bacterial Chromosome & Plasmid



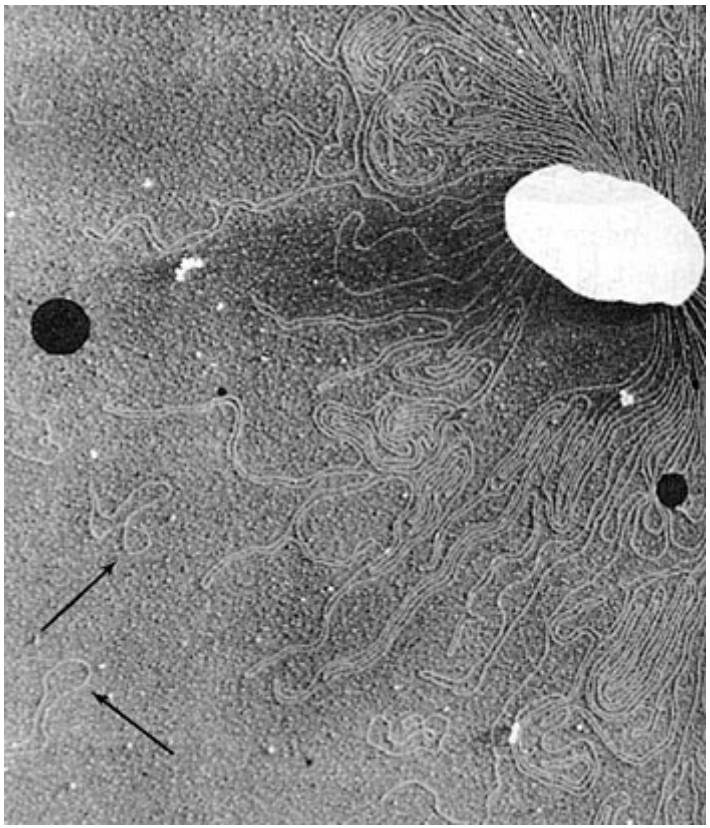
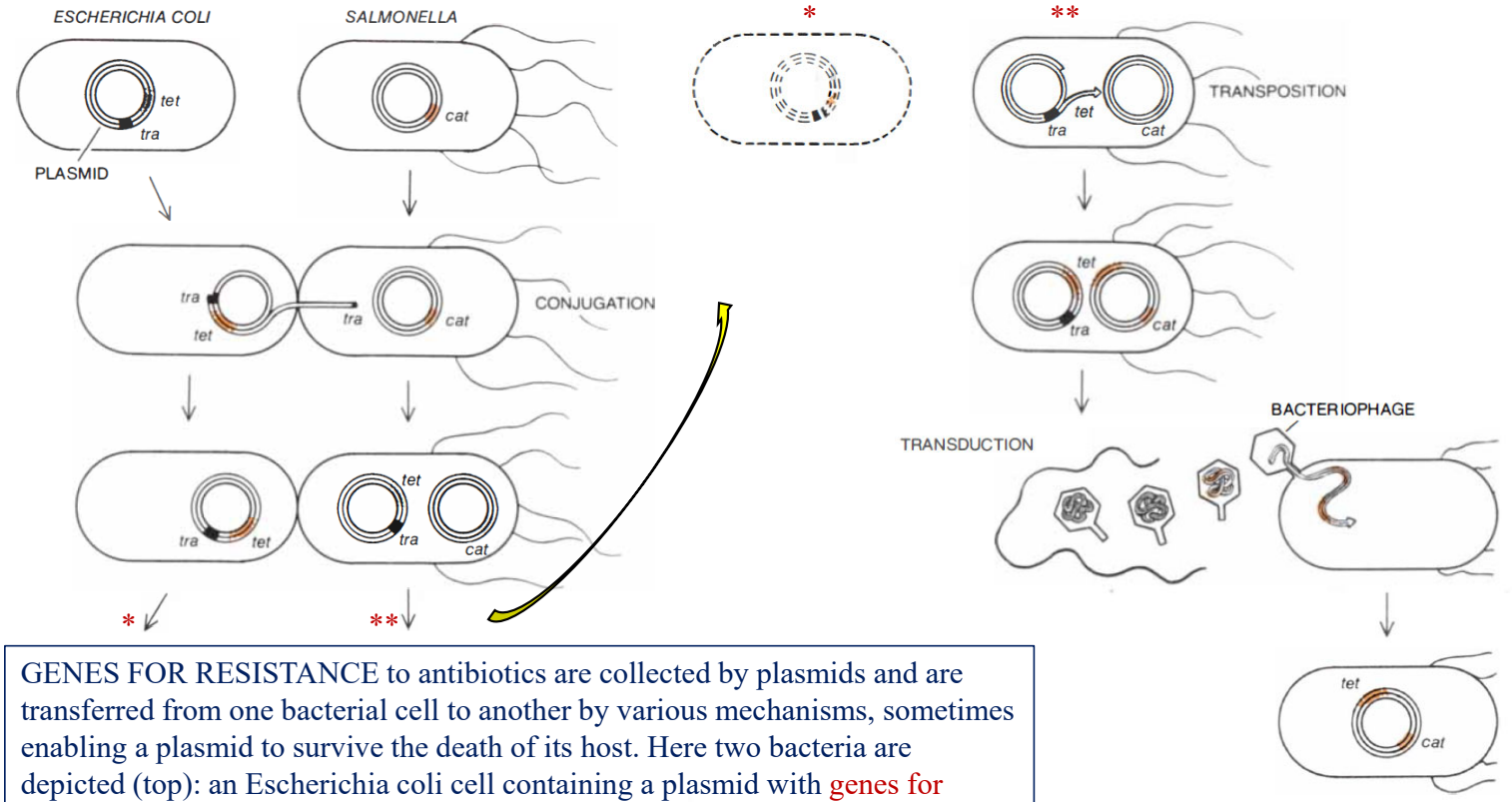


Figure 4. Release of chromosomal and plasmid (arrows) DNA from an unidentified bacterium. (Courtesy H. Potter and D. Dressler, from *Brock Biology of Microorganisms*, 9th edition, used by permission of M. T. Madigan)

http://sandwalk.blogspot.com/2009/03/on-evolution-of-bacterial-chromosomes.html
http://www.tutorvista.com/content/biology/biology-iii/chromosomes/bacterial-chromosome.php
http://www.apsnet.org/edcenter/K-12/TeachersGuide/DNA_Easy/Pages/Background.aspx

Plasmid

These accessory genetic elements in bacteria, best known as carriers of resistance to antibiotics and as vehicles for genetic engineering, are actually subcellular organisms poised on the threshold of life.

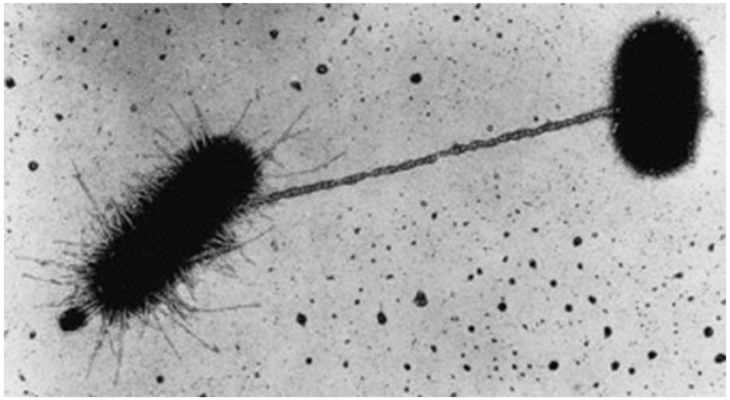


In an environment containing both antibiotics the E. coli die, but their plasmid survives in the successful host. The tet gene is on a **transposon** that subsequently moves from one plasmid to the other, which then carries genes for resistance to both antibiotics. Finally the double-resistance plasmid may be transferred again, by transduction. A bacterial virus infects the salmonella and proliferates, killing the cell; one phage particle incorporates the plasmid instead of viral DNA and transfers it to new cell.

GENES FOR RESISTANCE to antibiotics are collected by plasmids and are transferred from one bacterial cell to another by various mechanisms, sometimes enabling a plasmid to survive the death of its host. Here two bacteria are depicted (top): an Escherichia coli cell containing a plasmid with genes for transmission by conjugation (tra) and for tetracycline resistance (tet) and a Salmonella cell with a plasmid carrying a gene for resistance to chloramphenicol (cat). The two cells conjugate and the tet-carrying plasmid is transferred to the salmonella, rendering it resistant to tetracycline as well as to chloramphenicol.

Novick 1980, Plasmids

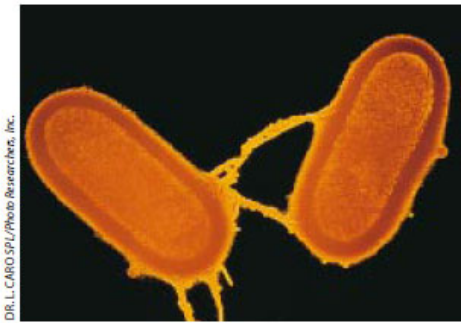
Horizontal Gene Transfer (HGT) in Biofilms: Reach Out and Touch Someone

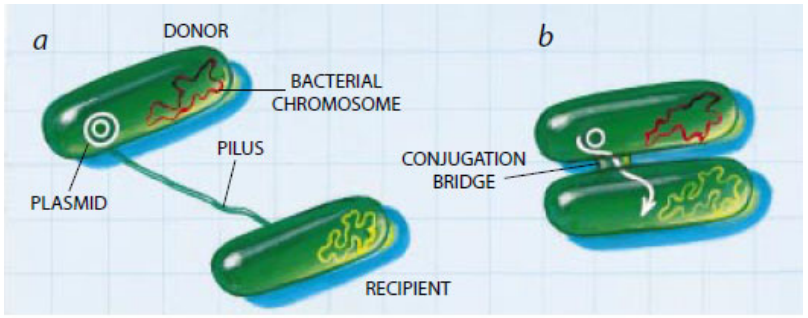


(Electron microscopic image by Charles C. Brinton, Jr., of a mating pair initially brought together by means of an F pilus)

The horizontal transfer of genes from individual to individual by conjugation, or via extracellular DNA by transformation, is a remarkable and prevalent phenomenon in bacterial communities, where the spatial arrangement of donor and recipient is obviously important.

http://www.birmingham.ac.uk/schools/biosciences/staff/profile.aspx?ReferenceId=6059&Name=dr-jan-ulrich-kreft





BACTERIA CAN TRANSFER PLASMIDS, circles of DNA, through conjugation. In gram-negative bacteria, a donor cell extends one or more projections—pili—that attach to a recipient cell and pull the two bacteria together (*micrograph* and *a*). Next a bridge (essentially a pore) forms between the cells. Then one strand of plasmid DNA passes into the recipient bacterium (*b*), and each single strand becomes double-stranded again (*c*). With the transfer complete, the bacteria separate (*d*). Conjugation in gram-positive bacteria (*not shown*) is similar, but the cells are drawn together by chemical signaling instead of by a pilus.

BACTERIA CAN TRANSFER PLASMIDS, circles of DNA, through conjugation. In gram-negative bacteria, a donor cell extends one or more projections – pili - that attach to a recipient cell and pull the two bacteria together (micrograph and a). Next a bridge (essentially a pore) forms between the cells. Then one strand of plasmid DNA passes into the recipient bacterium (b), and each single strand becomes double-stranded again. With the transfer complete, the bacteria separate. Conjugation in gram-positive bacteria (not shown) is similar, but the cells are drawn together by chemical signaling instead of by a pilus.

Bacterial Gene Swapping in Nature by Robert Miller. Sci. Am. January 1998.
Watanabe 1967, Infectious drug resistance (Sci. Am. 217(6): 19, 1967, December).

Construction of Biologically Functional Bacterial Plasmids In Vitro

Proc. Nat. Acad. Sci. USA
Vol. 70, No. 11, pp. 3240–3244, November 1973

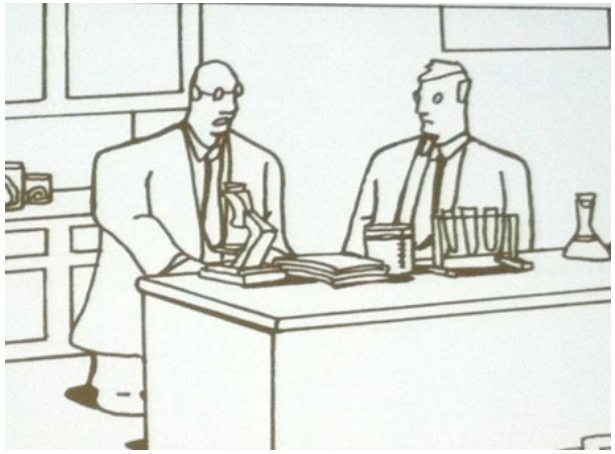
Construction of Biologically Functional Bacterial Plasmids *In Vitro*

(R factor/restriction enzyme/transformation/endonuclease/antibiotic resistance)

STANLEY N. COHEN*, ANNIE C. Y. CHANG*, HERBERT W. BOYER†, AND ROBERT B. HELLING†

* Department of Medicine, Stanford University School of Medicine, Stanford, California 94305; and † Department of Microbiology, University of California at San Francisco, San Francisco, Calif. 94122

ABSTRACT **The construction of new plasmid DNA species by *in vitro* joining of restriction endonuclease-generated fragments of separate plasmids is described. Newly constructed plasmids that are inserted into *Escherichia coli* by transformation are shown to be biologically functional replicons that possess genetic properties and nucleotide base sequences from both of the parent DNA molecules. Functional plasmids can be obtained by reassociation of endonuclease-generated fragments of larger replicons, as well as by joining of plasmid DNA molecules of entirely different origins.**



Stan, there's no way for it to work! Species barriers will prevent genetic exchange between unrelated bacteria.

Process for Producing Biologically Functional Molecular Chimeras

United States Patent 4,237,224. Dec. 2, 1980.
Inventors: Stanley N. Cohen, Herbert W. Boyer
Filed: Jan. 4, 1979.

[57] **ABSTRACT**

Method and compositions are provided for replication and expression of exogenous genes in microorganisms. Plasmids or virus DNA are cleaved to provide linear DNA having ligatable termini to which is inserted a gene having complementary termini, to provide a biologically functional replicon with a desired phenotypical property. The replicon is inserted into a microorganism cell by transformation. Isolation of the transformants provides cells for replication and expression of the DNA molecules present in the modified plasmid. The method provides a convenient and efficient way to introduce genetic capability into microorganisms for the production of nucleic acids and proteins, such as medically or commercially useful enzymes, which may have direct usefulness, or may find expression in the production of drugs, such as hormones, antibiotics, or the like, fixation of nitrogen, fermentation, utilization of specific feedstocks, or the like.

We claim:

1. A method for replicating a biologically functional DNA, which comprises:

transforming under transforming conditions compatible unicellular organisms with biologically functional DNA to form transformants; said biologically functional DNA prepared in vitro by the method of:

(a) cleaving a viral or circular plasmid DNA compatible with said unicellular organism to provide a first linear segment having an intact replicon and termini of a predetermined character;

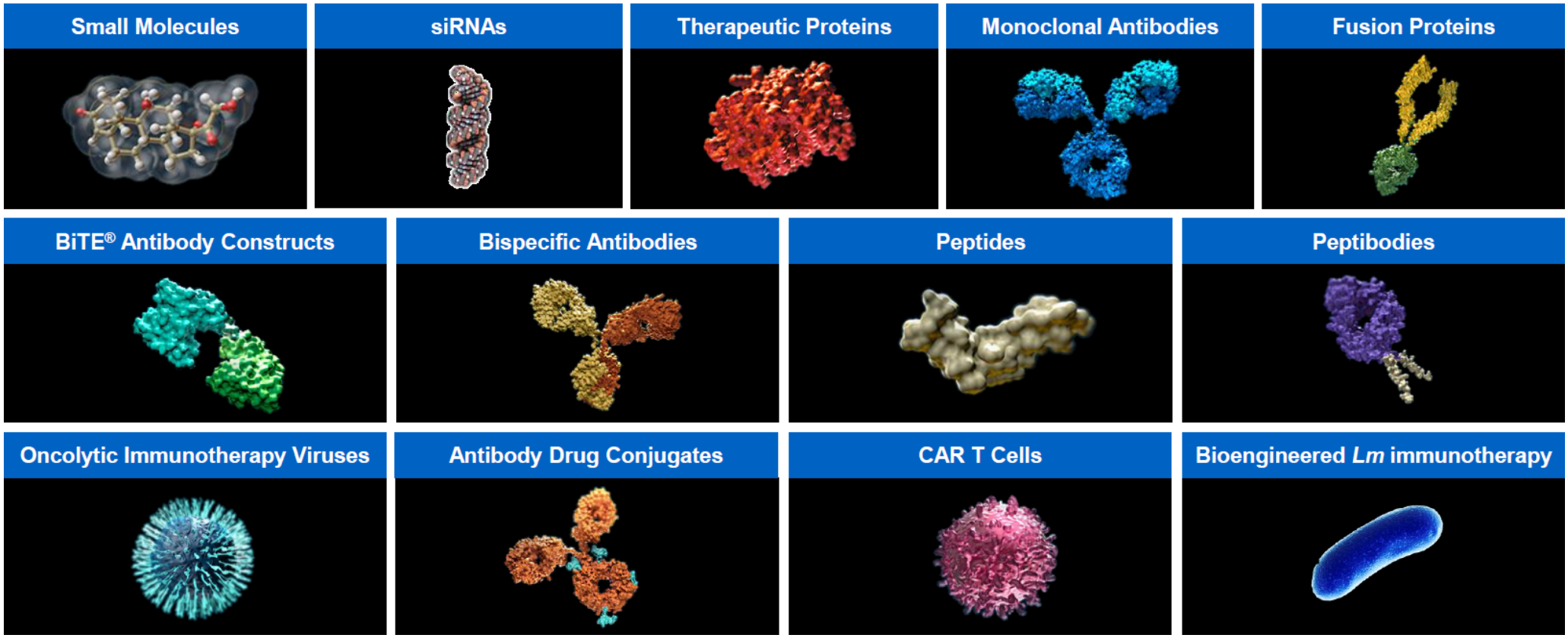
(b) combining said first linear segment with a second linear DNA segment, having at least one intact gene and foreign to said unicellular organism and having termini ligatable to said termini of said first linear segment, wherein at least one of said first and second linear DNA segments has a gene for a phenotypical trait, under joining conditions where the termini of said first and second segments join to provide a functional DNA capable of replication and transcription in said unicellular organism;

growing said unicellular organisms under appropriate nutrient conditions; and

isolating said transformants from parent unicellular organisms by means of said phenotypical trait imparted by said biologically functional DNA.

Biotechnology: Peptide & Protein Drugs

Drug Modalities



BiTE: Bispecific T-cell Engager
CAR = Chimeric Antigen Receptor
Lm = Listeria Monocytogenes

Sai Prasanth Chamarthy, Ph.D. AMGEN®
(Nov. 2, 2022 at Purdue University)

Biotechnology

Biotechnologically manufactured pharmaceuticals

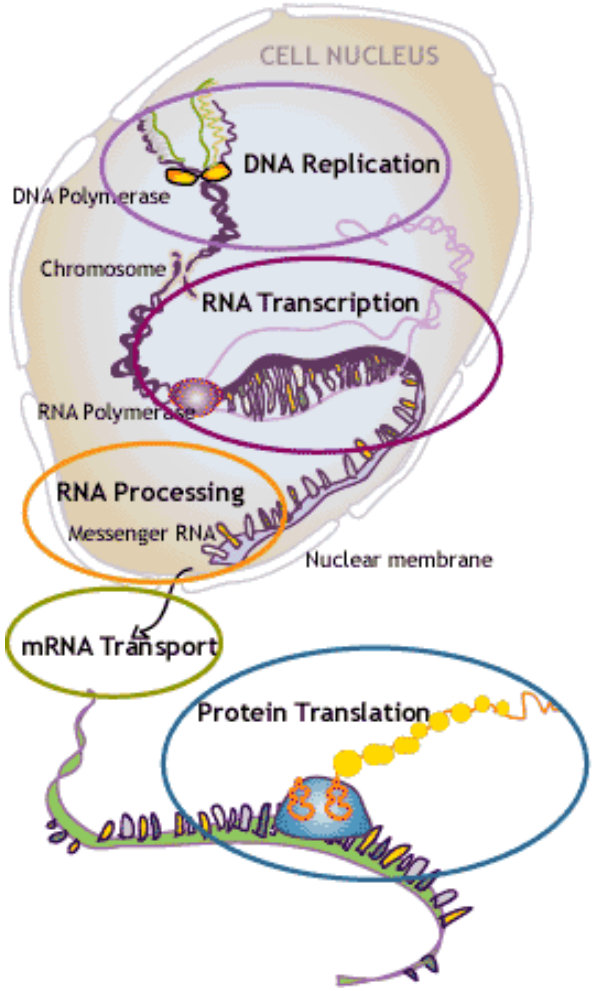
- Conversion of the genetic information into **protein drugs**
- Appropriate selection, design, and cultivation of cells and microorganisms harboring the corresponding biosynthetic pathways and physiological properties.

(Frank-Ranier Schmidt in Handbook of Pharmaceutical Biotechnology, Shayne C Gad, Ed. 2007).

One of the great outcomes of biotechnology is to produce protein drugs (such as insulin, and many other important proteins) in large quantities using recombinant DNA technology.

Many protein drugs have very short half-lives in the blood, and sometimes they are chemically modified to introduce poly(ethylene glycol) (PEG) to increase their half-lives. This process is called PEGylation.

From DNA to Protein



http://www.nobel.se/medicine/educational/dna/index.html

Every cell must contain the genetic information and the DNA is therefore duplicated before a cell divides (replication).

When proteins are needed, the corresponding genes are transcribed into RNA (transcription).

The RNA is first processed so that non-coding parts are removed (processing) and is then transported out of the nucleus (transport).

Outside the nucleus, the proteins are built based upon the code in the RNA (translation).

Therapeutic Antibodies & AI

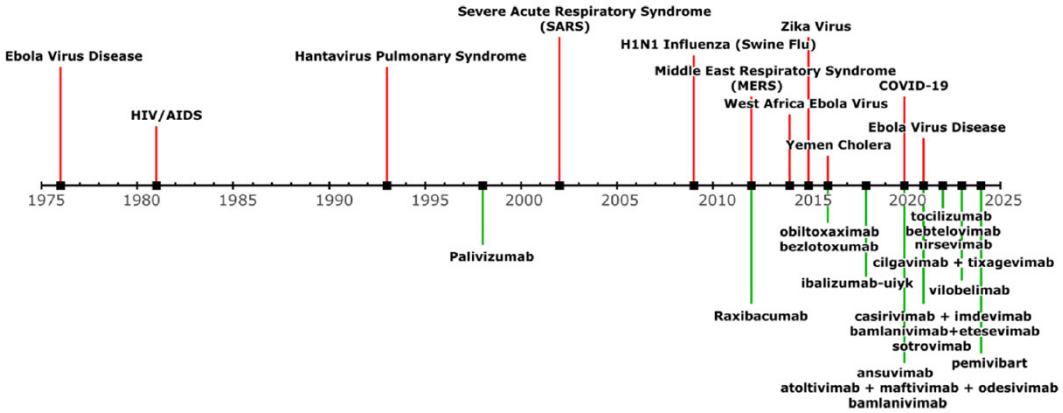
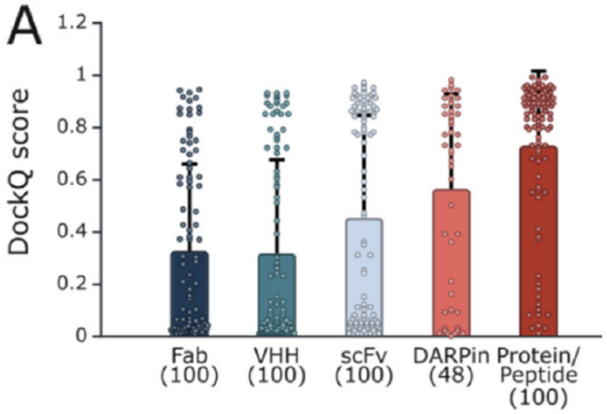


Figure 1. Timeline of the emergence of Pandemics (top, red)2 and the initial FDA approval of therapeutic antibodies for infectious disease (bottom, green) over the past 50 years. FDA Emergency Use Authorizations (EUAs) of therapeutics for infectious disease treatment are included in this timeline.14 Nearly all antibodies for infectious diseases have been approved within the last 10 years.



A summary of the trends in prediction accuracy is presented in Figure 4A. For the prediction of complexes with antibody-based moieties (Fabs, scFvs, and VHHs), the resulting DockQ scores show a wide range of variability in prediction success, with many that are far from the experimentally observed structures of the complexes. These results demonstrate the known challenges that even the state-of-the-art prediction tool, AlphaFold3, faces in predicting antibody−antigen complexes.[48,49] Among the antibody types, scFv predictions slightly outperformed VHHs and Fabs, though not in a statistically significant manner, suggesting comparable performance across all three binder types. AlphaFold3 performs better with DARPin complexes, but we observed the best performance with the prediction of natural protein and peptide complexes. In this latter class, binding typically is mediated via regular structural elements such as α-helices and β-strands, in contrast to the flexible complementarity-determining region (CDR) loops involved in the binding of antibodies, providing a plausible structural explanation for the differences in prediction accuracy.

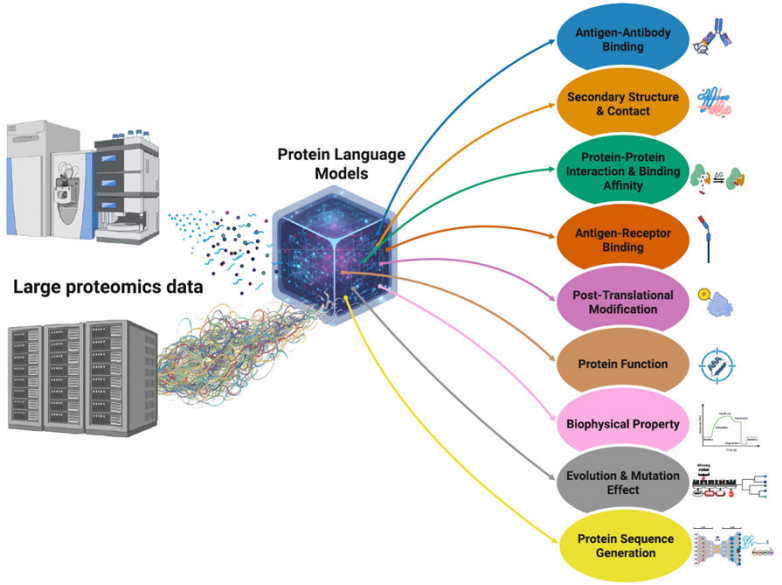
FUTURE PERSPECTIVE

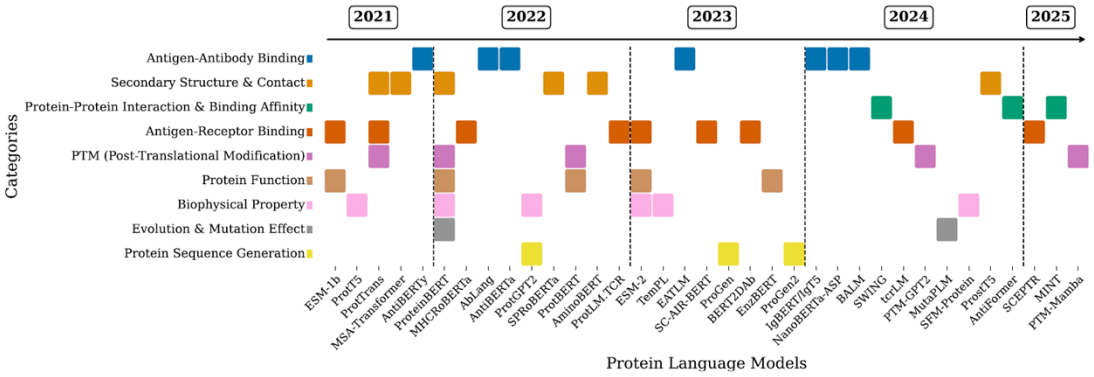
Treatment of infectious diseases with therapeutic antibodies, especially in the absence of a vaccine, could be an important tool in our arsenal to treat infectious diseases. Integration of next-generation sequencing, display technologies, and AI-based methods could reduce the time required for development of the antibodies and enhance the efficacy. The generation of bispecific antibodies, which can engage more than one target and/or influence multiple immune pathways also presents new therapeutic possibilities. However, traditional antibody discovery workflows and timelines are inherently reactive and not conducive to proactively developing therapies in response to a pandemic. To prepare for future pandemics, it will be essential to develop effective proactive approaches that enable a rapid response. In all likelihood, the next pandemic will likely arise from a known virus family; therefore, the generation of a large libraries of neutralizing antibodies against each family could be a good strategy to roll out therapeutics rapidly. However, one of the greatest challenges during the COVID-19 pandemic was the rapid rate at which escape mutations emerged, resulting in a quick loss of efficacy of vaccines and antibody therapies over the course of months. As the success of AI-driven predictive strategies improves, in a few years, it may be possible to generate antibodies "on-the-fly" by effectively leveraging existing databases of structures of related antigens. An even more ambitious goal would be to extend this further and use AI-based methods to anticipate viral evolution and preemptively design an arsenal of potential therapeutic antibody- or protein-based therapeutics to enable health care providers to switch to a proactive response for viral outbreaks. We do not know when the next pandemic will strike, but our success in managing future pandemics will depend on having a multiplicity of both prophylactic and therapeutic strategies readily available.

Subramaniam 2025, Therapeutic antibodies for infectious diseases- Recent past, present, and future

Protein Language Models: Applications and Perspectives

ABSTRACT: Large language models (LLMs) originally developed for human text have been adapted to proteomics as protein language models (pLMs). These models treat amino acid sequences like sentences, and they learn patterns from millions of sequences. pLMs are used for several key tasks, including the prediction of protein structures, annotating protein functions, designing novel protein sequences with specific characteristics, and mapping the interactions between proteins and other molecules. Compared with traditional approaches, pLMs deliver insights more quickly but demand large computing resources and careful data management. Developers are focused on decreasing prediction inaccuracies and biases by exploring more efficient training techniques and smaller models to decrease the resources required. As sequence databases continue to grow, pLMs will improve to uncover links between proteins and disease pathways, speeding drug development and basic research while offering new proteome-scale insights that support experimental design and validation.





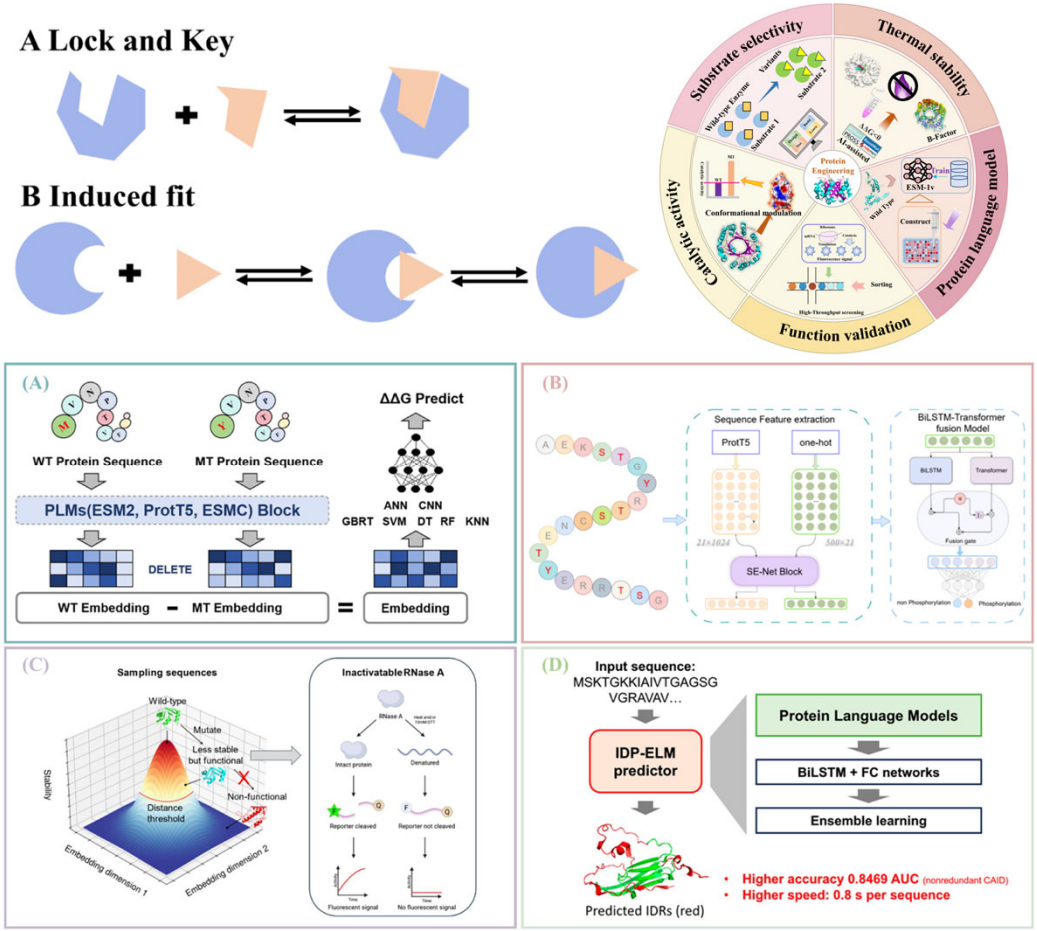
By replacing manual feature engineering with self-supervised pretraining on millions of sequences, pLMs models offer rapid, proteome-wide inference. Yet important hurdles must be overcome before protein language models become routine tools. **The opacity of their internal representations** makes it difficult to trace predictions back to biophysical principles, and **biases in training databases** can skew outputs toward over-represented species or folds.

The quick pace of new pLMs and changing protein data can make it **hard to choose the right tool**, so shared benchmarks and up-to-date model lists are essential.

Figure 1. Timeline of standalone pLMs arranged by release date and color-coded by primary prediction category. Only standalone pLMs are shown; derivative methods that build on embeddings from existing pLMs are excluded.

Leclercq 2026, Protein language models- Applications and perspectives

Modification of Enzymatic Properties by Protein Engineering



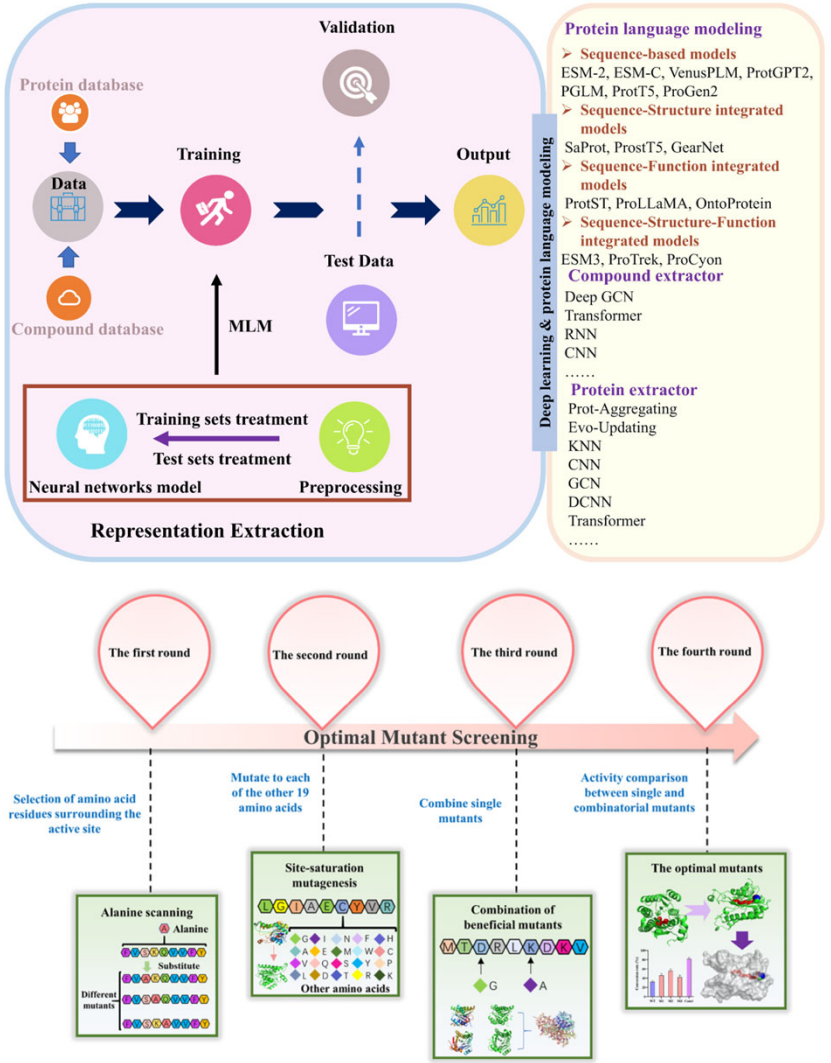


Figure 3. An overview of the different functions of the PLMs. (A) Prediction of protein−protein binding affinity changes. (B) Determination of protein phosphorylation sites. (C) Design of a Labile RNase A. (D) Accurate prediction of intrinsically disordered proteins.

Huang 2026, Advances in the modification of enzymatic properties based on protein engineering strategies

Biosimilars

https://www.fda.gov/drugs/biosimilars/biosimilar-and-interchangeable-products#biological

What is a biological product?

Biological products are regulated by the Food and Drug Administration (FDA) and are used to diagnose, prevent, treat, and cure diseases and medical conditions. Biological products are a diverse category of products and are generally large, complex molecules. These products may be produced through biotechnology in a living system, such as a microorganism, plant cell, or animal cell, and are often more difficult to characterize than small molecule drugs. There are many types of biological products approved for use in the United States, including **therapeutic proteins** (such as filgrastim), **monoclonal antibodies** (such as adalimumab), and **vaccines** (such as those for influenza and tetanus).

The nature of biological products, including the inherent variations that can result from the manufacturing process, can present challenges in **characterizing and manufacturing these products that often do not exist in the development of small molecule drugs**. Slight differences between manufactured lots of the same biological product (i.e., acceptable within-product variations) are normal and expected within the manufacturing process. As part of its review, FDA assesses the manufacturing process and the manufacturer's strategy to control within-product variations. These control strategies are put in place to help ensure that manufacturers produce biological products with consistent clinical performance.

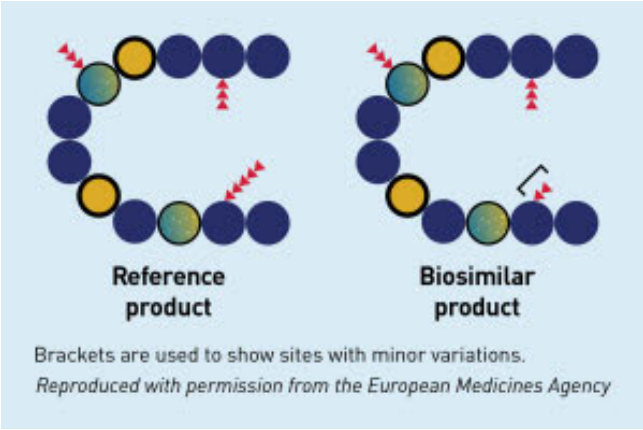
What is a biosimilar product?

A biosimilar is a biological product that is highly similar to and has no clinically meaningful differences from an existing FDA-approved reference product. These two standards are described further below.

What does it mean to be "highly similar"?

A manufacturer developing a proposed biosimilar demonstrates that its product is highly similar to the reference product by extensively analyzing (i.e., characterizing) the structure and function of both the reference product and the proposed biosimilar. State-of-the-art technology is used to compare characteristics of the products, such as purity, chemical identity, and bioactivity. The manufacturer uses results from these comparative tests, along with other information, to demonstrate that the biosimilar is highly similar to the reference product.

Minor differences between the reference product and the proposed biosimilar product in clinically inactive components are acceptable. For example, these could include minor differences in the stabilizer or buffer compared to what is used in the reference product. Any differences between the proposed biosimilar product and the reference product are carefully evaluated by FDA to ensure the biosimilar meets FDA's high approval standards.



Minor differences between the references product and the proposed biosimilar product in clinically inactive components are acceptable.

FDA-Approved Biosimilar Products: The number increases each year.

Biosimilar Name	Approval Date	Reference Product
Avsola (infliximab-axxq)	December 2019	Remicade (infliximab)
Abrilada (adalimumab-afzb)	November 2019	Humira (adalimumab)
Ziextenzo (pegfilgrastim-bmez)	November 2019	Neluasta (pegfilgrastim)
Hadlima (adalimumab-bwwd)	July 2019	Humira (adalimumab)
Ruxience (rituximab-pvvr)	July 2019	Rituxan (rituximab)
Zirabev (bevacizumab-bvzr)	June 2019	Avastin (bevacizumab)
Kanjinti (trastuzumab-anns)	June 2019	Herceptin (trastuzumab)
Eticovo (etanercept-ykro)	April 2019	Enbrel (etanercept)
Trazimera (trastuzumab-qyyp)	March 2019	Herceptin (trastuzumab)
Ontruzant (trastuzumab-dttb)	January 2019	Herceptin (trastuzumab)
Herzuma (trastuzumab-pkrb)	December 2018	Herceptin (trastuzumab)
Truxima (rituximab-abbs)	November 2018	Rituxan (rituximab)
Udenyca (pegfilgrastim-cbqv)	November 2018	Neulasta (pegfilgrastim)

Biosimilar Name	Approval Date	Reference Product
Hyrimoz (adalimumab-adaz)	October 2018	Humira (adalimumab)
Nivestym (filgrastim-aafi)	July 2018	Neupogen (filgrastim)
Fulphila (pegfilgrastim-jmdb)	June 2018	Neluasta (pegfilgrastim)
Retacrit (epoetin alfa-epbx)	May 2018	Epogen (epoetin-alfa)
Ixifi (infliximab-qbtx)	December 2017	Remicade (infliximab)
Ogivri (trastuzumab-dkst)	December 2017	Herceptin (trastuzumab)
Mvasi (Bevacizumab-awwb)	September 2017	Avastin (bevacizumab)
Cyltezo (Adalimumab-adbm)	August 2017	Humira (adalimumab)
Renflexis (Infliximab-abda)	May 2017	Remicade (infliximab)
Amjevita (Adalimumab -atto)	September 2016	Humira (adalimumab)
Erelzi (Etanercept-szzs)	August 2016	Enbrel (etanercept)
Inflectra (Infliximab-dyyb)	April 2016	Remicade (infliximab)
Zarxio (Filgrastim-sndz)	March 2015	Neupogen (filgrastim)

FDA-Approved Biosimilar Products

Biosimilar Name	Approval Date	Reference Product
Abrilada (adalimumab-afzb)	November 2019	Humira (adalimumab)
Hadlima (adalimumab-bwwd)	July 2019	Humira (adalimumab)
Hyrimoz (adalimumab-adaz)	October 2018	Humira (adalimumab)
Cyltezo (Adalimumab-adbm)	August 2017	Humira (adalimumab)
Amjevita (Adalimumab -atto)	September 2016	Humira (adalimumab)

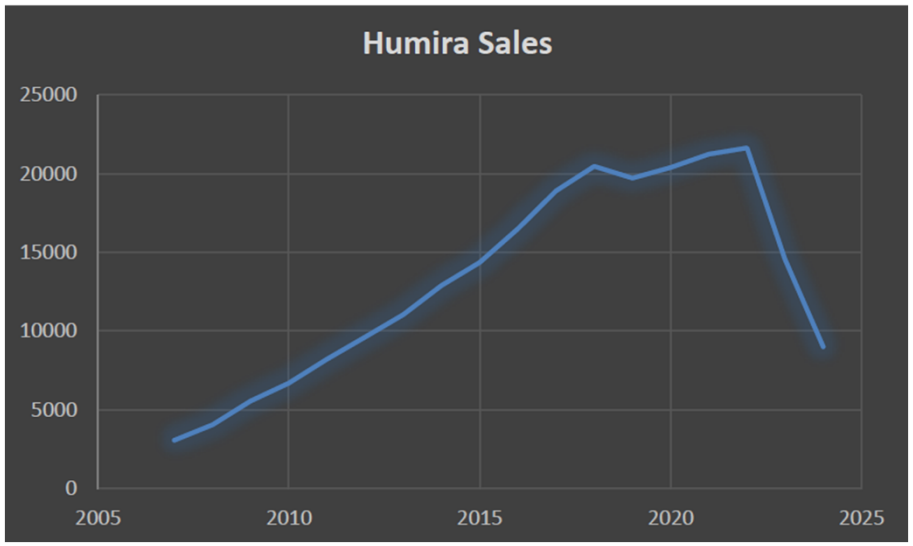


Table 1: Key Biologics Sales After Biosimilar Approval

Product	Peak Sales ($Billion)	Sales 2024 ($Billion)
Humira	21.6	9.0
Stelara	11.3	10.8
Eylea	10.2	8.9
Remicade	9.9	1.9
Enbrel	9.2	4.0
Rituxan	7.6	1.4
Herceptin	7.2	1.6
Avastin	7.0	1.4
Neulasta	4.8	0.6
Lucentis	4/3	1.2

FDA-Approved Biosimilar Products in 2023

2023 Full Year FDA Approvals Summary.BLA and NDA Approvals. A Short Report
PharmaCircle™ LLC. 2024-01 v1.0

Application Number	Product Name	Molecule	Route	Dosage Form	Company Name	Approval Date	Indication
125771	Altuviiio	efanesoctocog alfa	Injection	Lyophilized Powder For Solution	Bioverativ Therapeutics	2023-02-22	Hemophilia
125738	Omisirge	omidubicel-onlv	Injection	Suspension	Gamida Cell	2023-04-17	Bone Marrow Transplantation
125757	Vowst	fecal microbiota spores,live-brpk	Oral	Capsule	Seres Therapeutics	2023-04-26	Infections, Clostridioides difficile
125775	Arexvy	Respiratory Syncytial Virus Vaccine,Adjuvanted	Injection	Lyophilized Powder For Solution	GlaxoSmith Kline	2023-05-03	Infections, RSV
125774	Vyjuvek	beremagenegeperpavec-svdt	Topical	Gel	Krystal Biotech	2023-05-19	Epidermolysis Bullosa
125769/8	Abrysvo	Respiratory Syncytial Virus Vaccine	Injection	Lyophilized Powder For Solution	Pfizer	2023-05-31	Infections, RSV
125781	Elevidys	delandistrogenemoxeparvovec-rokl	Injection	Suspension	Sarepta Therapeutics	2023-06-22	Duchenne Muscular Dystrophy
125734	Lantidra	donislecel-jujn	Injection	Suspension	CellTrans	2023-06-28	Diabetes, Type 1
125720	Roctavian	Valoctocogene roxaparvovec-rvox	Injection	Suspension	BioMarin	2023-06-29	Hemophilia A
125761	Cyfendus	Anthrax Vaccine Adsorbed, Adjuvanted	Injection	Suspension	EmergentBio Solutions	2023-07-20	Infections, Anthrax
125776	Balfaxar	prothrombin complex concentrate, human-lans	Injection	Suspension	Octapharma	2023-07-21	Coagulopathy
125770	Penbraya	Meningococcal Groups A, B, C, W, and Y Vaccine	Injection	Lyophilized Powder For Suspension	Pfizer	2023-10-20	Infections, Meningitis
125777	Ixchiq	Chikungunya Vaccine, Live	Injection	Lyophilized Powder For Solution	Valneva	2023-11-09	Infections, Chikungunya
125795	Adzynma	ADAMTS13, recombinant-krhn	Injection	Lyophilized Powder For Solution	Takeda	2023-11-09	Thrombotic Thrombocytopenic Purpura

CBER BLA Emergency Use Authorization

Application Number	Product Name	Molecule	Route	Dosage Form	Company Name	Approval Date	Indication
Emergency	Moderna COVID-19 Vaccine (2023-2024 Formula)	andusomeran	Injection	Suspension	Moderna	2023-09-11	Infections, COVID-19
Emergency	Pfizer-BioNTech COVID-19 Vaccine (2023-2024 Formula)	raxtozinameran	Injection	Suspension	BioNTech	2023-09-11	Infections, COVID-19
Emergency	Novavax COVID-19 Vaccine, Adjuvanted (2023-2024 Formula)	NVX-CoV2601	Injection	Suspension	Novavax	2023-10-03	Infections, COVID-19

CDER BLA 351(a), Type 1, NME

Application Number	Product Name	Molecule	Route	Dosage Form	Company Name	Approval Date	Indication
761269A	Leqembi	lecanemab-irmb	Injection	Solution	Eisai	2023-01-06	Alzheimer's
761278	Lamzede	velmanase alfa-tycv	Injection	Lyophilized Powder For Solution	Chiesi Farmaceutici	2023-02-16	Lysosomal Storage Disease
761334A	Zynyz	retifanlimab-dlwr	Injection	Solution	Incyte	2023-03-22	Cancer, Merkel Cell Carcinoma Metastatic
761161	Elfabrio	pegunigalsidase alfa-iwxj	Single-Use	Solution	Chiesi Farmaceutici	2023-05-09	Fabry Disease
761324A	Epkinly	epcoritamab- bysp	Subcutaneous	Solution	Genmab	2023-05-19	Cancer, B-Cell Lymphomas, DLBCL
761309A	Columvi	glofitamab-gxbm	Injection	Solution	Genentech	2023-06-15	Cancer, DLBCL
761286	Rystiggo	Rozanolixizumab-noli	Subcutaneous	Solution	Ucb	2023-06-26	Myasthenia Gravis
761184	Ngenla	somatrogon-ghla	Injection	Solution	Pfizer	2023-06-27	GH Deficiency, Child
761328	Beyfortus	nirsevimab-alip	Injection	Solution	Astrazeneca	2023-07-17	Infections, RSV
761342A	Talvey	talquetamab-tgvs	Injection	Solution	Janssen Biotech	2023-08-09	Cancer, Multiple Myeloma
761345A	Elrexfio	elranatamab	Injection	Solution	Pfizer	2023-08-14	Cancer, Multiple Myeloma
761339	Veopoz	pozelimab-bbfg	Injectable	Solution	Regeneron	2023-08-18	Gastrointestinal Diseases

FDA-Approved Biosimilar Products in 2024-2025

The 19 FDA Approvals in 2024 (Chronologically)

- Simlandi (adalimumab-ryvk)
- Jubbonti (denosumab-bddz)
- Wyost (denosumab-bddz)
- Tyenne (tocilizumab-aazg)
- Selarsdi (ustekinumab-aekn)
- Hercessi (trastuzumab-strf)
- Opuviz (aflibercept-yszy)
- Yesafili (aflibercept-jbvf)
- Bkemv (eculizumab-aeeb)
- Pyzchiva (ustekinumab-ttwe)
- Nypozi (filgrastim-txid)
- Ahzantive (aflibercept-mrbb)
- Epysqli (eculizumab-aagh)
- Enzeevu (aflibercept-abzv)
- Pavblu (aflibercept-ayyh)
- Otulfi (ustekinumab-aauz)
- Imuldosa (ustekinumab-srlf)
- Yesintek (ustekinumab-kfce)
- Stequeyma (ustekinumab-stba)

https://www.centerforbiosimilars.com/view/a-banner-year-for-biosimilars-the-18-fda-approvals-from-2024

Biosimilars Approved and on the Market Approval Date Reference Product More Information

Biosimilar Name	Approval Date	Reference Product	More Information
Osvyrti and Jubereq (denosumab-desu)	October 2025	Prolia and Xgeva (denosumab)	
Eydenzelt (aflibercept-boav)	October 2025	Eylea (aflibercept)	Eydenzelt Information
Enoby and Xtrenbo (denosumab-qbde)	September 2025	Prolia and Xgeva (denosumab)	Enoby and Xtrenbo Information
Aukelso and Bosaya (denosumab-kyqq)	September 2025	Prolia and Xgeva (denosumab)	Aukelso and Bosaya Information
Bildyos and Bilprevda (denosumab-nxxp)	August 2025	Prolia and Xgeva (denosumab)	Bildyos and Bilprevda Information
Kirsty (insulin aspart-xjhz)	July 2025	Novolog (insulin aspart)	Kirsty Information
Starjemza (ustekinumab-hmny)	May 2025	Stelara (ustekinumab)	Starjemza Information
Jobevne (bevacizumab-nwgd)	April 2025	Avastin (bevacizumab)	Jobevne Information
Bomyntra and Conexxence (denosumab-bnht)	March 2025	Prolia and Xgeva (denosumab)	Bomyntra and Conexxence Information
Omlyclo (omalizumab-igec)	March 2025	Xolair (omalizumab)	Omlyclo Information
Stoboclo and Osenvelt (denosumab-bmwo)	February 2025	Prolia and Xgeva (denosumab)	Stoboclo and Osenvelt Information
Merilog (insulin aspart-szjj)	February 2025	Novolog (insulin aspart)	Merilog Information
Ospomyv and Xbryk (denosumab-dssb)	February 2025	Prolia and Xgeva (denosumab)	Ospomyv and Xbryk Information

https://biosimilarsforum.org/approved-biosimilars/

In 2025 alone, the FDA approved 18 biosimilars, including aflibercept (1), bevacizumab (1), denosumab (8), insulin aspart (2), omalizumab (1), pegfilgrastim (1), pertuzumab (1), ranibizumab (1), tociluzumab (1), and ustekinumab (1), covering a variety of treatments, with Shanghai Henlius's Poherdy (pertuzumab-dpzb) standing out as the only approved interchangeable biosimilar that year.

https://gabionline.net/biosimilars/general/biosimilars-approved-in-the-us#:~:text=In%202025%20alone%2C%20the%20FDA,Henlius's%20Poherdy%20(pertuzumab%2Ddpzb)

Strategies to Enhance Protein Delivery

There has been unprecedented development of therapeutic proteins in the past three decades. More than 200 proteins have been approved by the FDA for treating various diseases, including hemophilia A and B, cancers, diabetes, growth hormone deficiency, autoimmune diseases, chronic inflammatory diseases, etc. However, the efficacy of therapeutic proteins is sometimes limited by their short circulation in the blood. This short circulation necessitates repeated dosing and high treatment concentrations, as maintaining certain protein levels is indispensable for effective treatment. In addition, therapeutic proteins are often recognized as "foreign" by the immune system, inducing the production of anti-drug antibodies in patients. The presence of these antibodies expedites the clearance of therapeutic proteins from the human body. The short circulation half-life of the proteins and their immune responses not only reduce their therapeutic efficacy but may also cause side effects, for example, hypersensitivity reactions. Owing to extensive research, various strategies to extend the circulation half-lives of therapeutic proteins have been developed. The FDA-approved strategies, including PEGylation, XTENylation, Fc fusion, and albumin attachment, are the focus of this Review, along with other emerging approaches under development.

STRATEGIES TO EXTEND THE CIRCULATION HALF-LIFE OF A PROTEIN

- PEGylation
- XTENylation: XTEN is a class of non-immunogenic polypeptides composed of hydrophilic amino acids A (alanine), E (glutamic acid, glutamate), G (glycine), P (proline), S (serine), and T)(threonine).
- Fc Fusion: Some endogenous proteins like immunoglobulin G (IgG) have a circulation half-life of 2−3 weeks. Fc region is combined to a protein of interest.

MECHANISMS OF PROTEIN CLEARANCE IN THE BODY

- Renal clearance
- Metabolism
- Enzymatic degradation

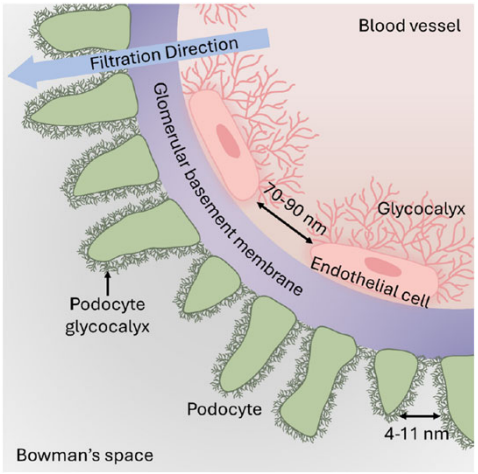


Figure 1. Protein clearance in the kidneys by filtration membranes under increased blood pressure. The membranes are composed of an endothelial cell layer, a glomerular basement membrane (GBM), and podocytes.

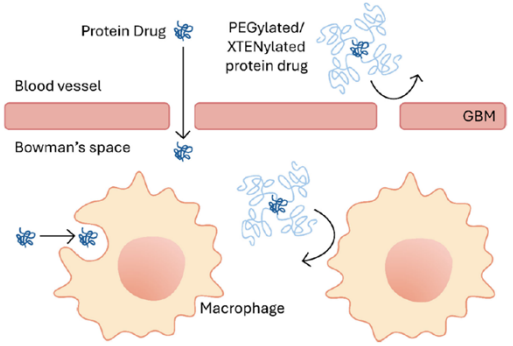


Figure 3. PEGylation and XTENylation reduce renal clearance and uptake by macrophages.

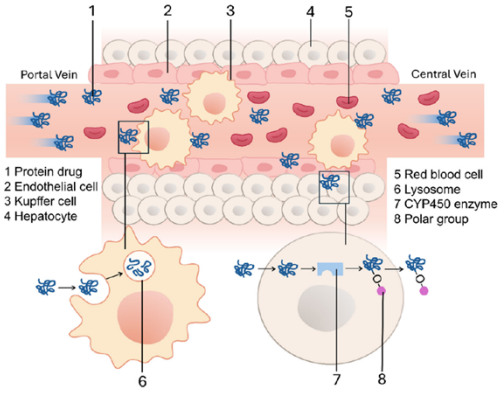


Figure 2. Protein clearance in the liver by Kupffer cells and hepatocytes.

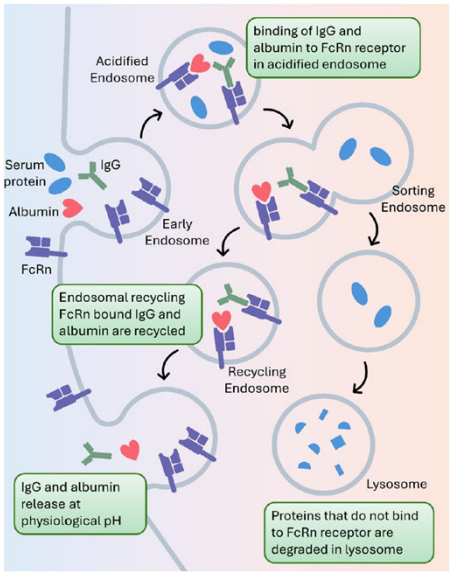
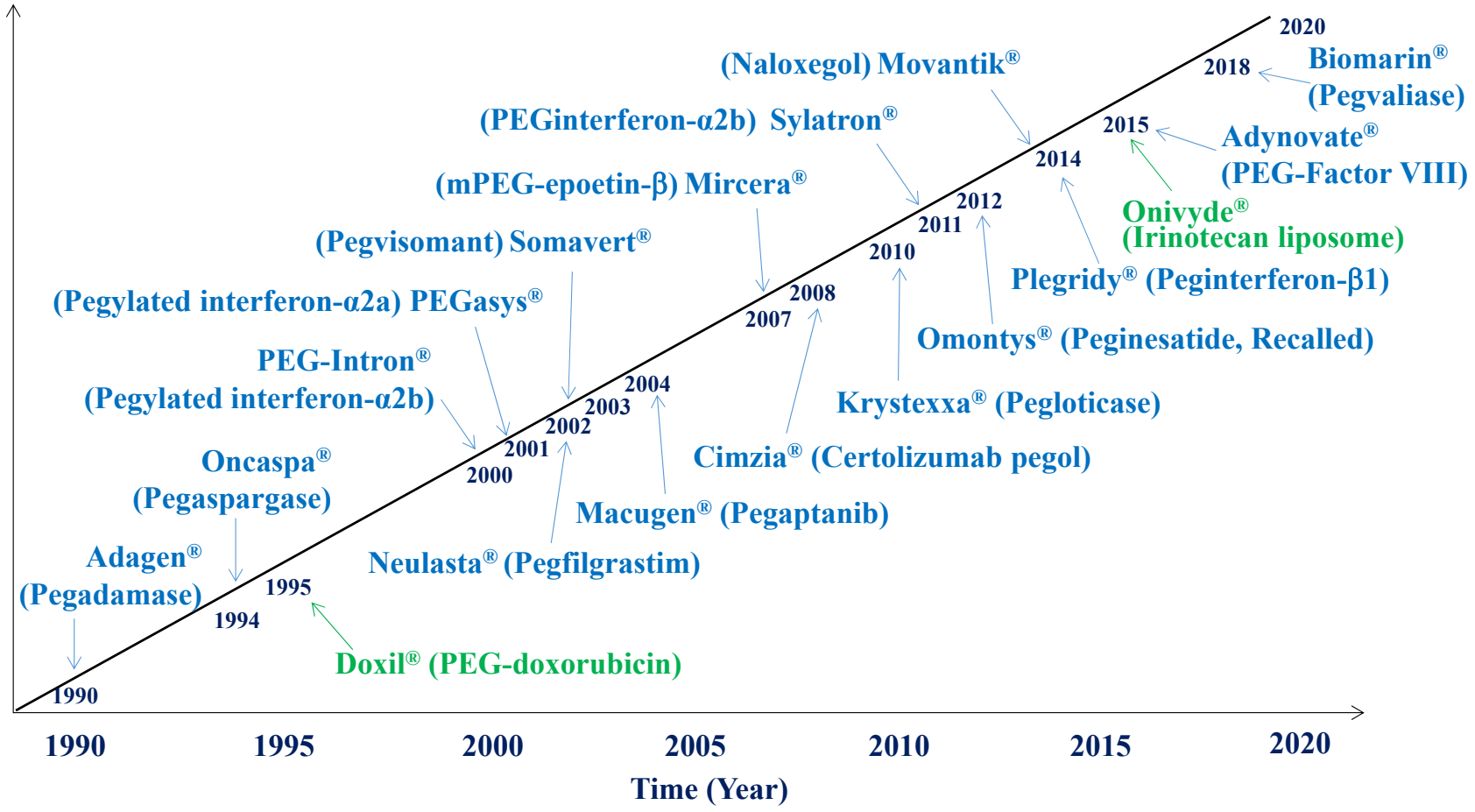


Figure 5. FcRn-mediated recycling of IgG and albumin.

Zhu 2025, Strategies to enhance protein delivery

PEGylated Protein Drugs



FDA-Approved PEGylated Protein Drugs

Table 1. FDA-Approved PEGylated Protein Drugs

generic name	brand name	approval year	description	half-life in humans	application
pegademase bovine	Adagen	1990	PEGylated enzyme adenosine deaminase	3–6 days	severe combined immunodeficiency disease
pegaspargase	Oncaspar	1994	PEGylated L-asparaginase	5.8 days	acute lymphoblastic leukemia
peginterferon alfa-2b	Pegintron	2001	PEGylated alfa-2b	40 h	chronic hepatitis C
peginterferon alfa-2a	Pegasys	2002	PEGylated alfa-2a	160 h	chronic hepatitis B and C
pegfilgrastim	Neulasta	2002	PEGylated G-CSF	15–80 h	stimulation of white cell production
pegvisomant	Somavert	2003	PEGylated growth hormone	74–172 h	acromegaly
pegaptanib	Macugen	2004	PEGylated aptamer	10 days	neovascular age-related macular degeneration
certolizumab pegol	Cimzia	2008	PEGylated TNF blocker	14 days	rheumatoid arthritis and Crohn's disease
methoxy polyethylene glycol-epoetin β	Mircera	2007	PEGylated erythropoietin	119 h	anemia associated with chronic kidney disease
pegloticase	Krystexxa	2010	PEGylated uricase	300 h	chronic gout
peginterferon alfa-2b	Sylatron	2011	PEGylated interferon alfa-2b	51 h	melanoma
peginterferon β-1a	Plegridy	2014	PEGylated interferon β-1a	78 h	multiple sclerosis
antihemophilic factor (recombinant), PEGylated	Adynovate	2015	PEGylated FVIII	13.4–14.7 h	hemophilia A
coagulation factor IX (recombinant), glycoPEGylated	Rebinyn	2017	PEGylated FIX	114.9 h	hemophilia B
pegvaliase-pqpz	Palynziq	2018	PEGylated phenylalanine ammonia lyase	47 h	phenylketonuria
ropeginterferon alfa-2b	Besremi	2021	PEGylated interferon	7 days	polycythemia vera
pegcetacoplan	Empaveli	2021	PEGylated pentadecapeptide	8 days	paroxysmal nocturnal hemoglobinuria
avacincaptad pegol	Izervay	2023	PEGylated ribonucleic acid aptamer	12 days	geographic atrophy
pegunigalsidase alfa-iwxj	Elfabrio	2023	PEGylated human GLA enzyme	96.5 h	Fabry disease
palopegteriparatide	Yorvipath	2024	PEGylated parathyroid hormone	60 h	hypoparathyroidism

Zhu 2025, Strategies to enhance protein delivery

Jivi (damoctocog alfa pegol) - 2018: A PEGylated factor VIII for Hemophilia A.

Esperoct (turoctocog alfa pegol) - 2019: A PEGylated antihemophilic factor for Hemophilia A.

Ziextenzo (pegfilgrastim-bmez) - 2019: A PEGylated G-CSF biosimilar.

Nyvepria (pegfilgrastim-apgf) - 2020: A PEGylated granulocyte colony-stimulating factor (G-CSF) biosimilar.

COVID-19 mRNA Vaccines (Comirnaty/Spikevax) - 2021/2022: These utilize PEGylated lipid nanoparticles (LNPs) for delivery.

Syfovre (pegcetacoplan) (2023): A PEGylated peptide molecule (complement C3 inhibitor) used to treat geographic atrophy secondary to age-related macular degeneration.

Aducanumab (Adelum) Withdrawal from the Market

Aducanumab, sold under the brand name Aduhelm, is a medication designed to treat Alzheimer's disease (AD). It is a monoclonal antibody that targets aggregated forms (plaque) of amyloid beta (Aβ) found in the brains of people with Alzheimer's disease to reduce its buildup. It was developed by Biogen and Eisai. Aducanumab is given via intravenous infusion. (Elimination half-life of 24.8 days)

Aducanumab was approved for medical use in the United States by the Food and Drug Administration (FDA) in June 2021, in a controversial decision that led to the resignation of three advisers to the FDA in the absence of evidence that the medication is effective. The FDA stated that it represents a first-of-its-kind treatment approved for Alzheimer's disease and that it is the first new treatment approved for Alzheimer's since 2003. Aducanumab's approval is controversial for numerous reasons including ambiguous clinical trial results regarding efficacy, the high cost of the medication and the very high rate of serious adverse events. The FDA considers it to be a first-in-class medication. https://en.wikipedia.org/wiki/Aducanumab

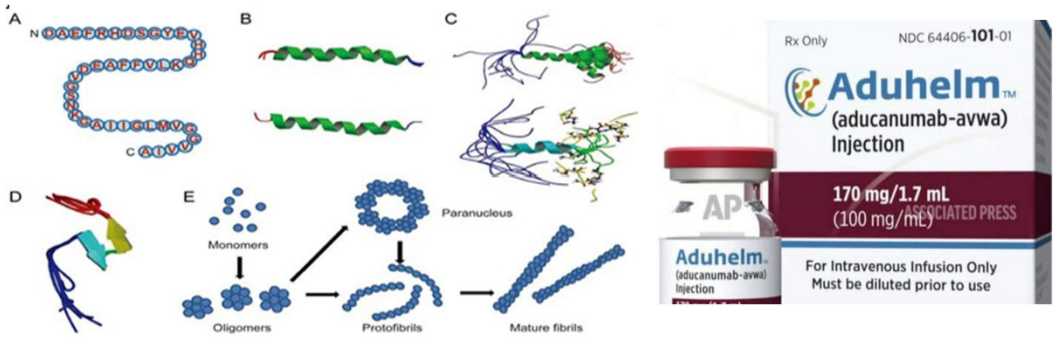


Figure 2. Structures of Aβ monomer, fibril, and oligomers. (A) The primary amino acid sequence. (B) The structure of Aβ peptide. (C) Solution structure of Aβ peptide (D) The collapsed coil structure formed by Aβ peptides. (E) Pathway of the conversion of Aβ monomers to higher order oligomers, protofibrils and fibrils.
(Kuang 2022, The progress of Aduhelm in the treatment for Alzheimer's disease (AD))

Aduhelm $56,000/year **Leqembi (Lecanemab) $26,500/year**

Biogen abandons Aduhelm efforts, focuses on Eisai-partnered Leqembi and pipeline drugs
By Eric Sagonowsky (January 31, 2024)

Biogen is ending its troubled Aduhelm journey, paying a $60 million one-time charge to end development and commercialization on the drug. More than two years after Aduhelm's controversial and ill-fated FDA accelerated approval, Biogen is discontinuing the Alzheimer's disease therapy. Wednesday, Biogen said it's pulling all efforts from the first-of-its-kind anti-amyloid beta therapy to focus on Leqembi, its Eisai-partnered newer medicine, and its pipeline candidates. The newer drug, Leqembi, won a full FDA approval early last year, making the partners' marketing efforts on the therapy much simpler than was the case with Aduhelm.

Biogen is taking a $60 million charge and is discontinuing all development and sales of Aduhelm, the company said. It's terminating the ENVISION clinical study, which sought to confirm the benefit of the medicine as required under its 2021 accelerated approval. The decision follows Biogen's move to launch a strategic review in early 2023 under new CEO Chris Viehbacher, the former Sanofi chief who joined the Massachusetts drugmaker in November 2022. During that review, Biogen weighed the ENVISION study commitments and the "likely advancements in the field" by the time Aduhelm gained a potential full FDA nod. Despite searching, Biogen wasn't able to find any external partners nor financing for the medicine, the company revealed.

Going forward, Biogen will work with its partner on Leqembi and will "accelerate development of potential new treatment modalities," including pipeline meds BIIB080 and BIIB113, the company said in a release. A "large portion" of resources freed by the Aduhelm halt will go toward Biogen's remaining Alzheimer's franchise, the company said. “When searching for new medicines, one breakthrough can be the foundation that triggers future medicines to be developed," Viehbacher said in a statement. "Aduhelm was that groundbreaking discovery that paved the way for a new class of drugs and reinvigorated investments in the field.”

While Biogen may tout Aduhelm as a groundbreaking drug, it wasn't received as such. The med's 2021 approval was shrouded in controversy, and the company had trouble convincing payers of its benefits. The Centers for Medicare & Medicaid Services, a key player in the launch, blocked straightforward access to the drug for patients on its healthcare plans.

In 2022, Aduhelm's sales weren't significant enough for Biogen to break out of its "other product revenue" category, which totaled $13 million for the year. The company last year started a layoff round about 1,000-people strong.
https://www.fiercepharma.com/pharma/biogen-abandons-aduhelm-efforts-focuses-eisai-partnered-leqembi-and-pipeline-meds?utm_medium=email&utm_source=nl&utm_campaign=LS-NL-FiercePharma&oly_enc_id=6566C0039234G4K

GLP-1 Agonist: Diabetes Drugs and Weight Loss

Are there any type 2 diabetes drugs that can help people lose weight and lower their blood sugar? Are there side effects? (M. Regina Castro, M.D.)
There's a class of type 2 diabetes drugs that not only improves blood sugar control but may also lead to weight loss. This class of drugs is commonly called glucagon-like peptide 1 (GLP-1) agonists. A second class of drugs that may lead to weight loss and improved blood sugar control is the sodium glucose cotransporter 2 (SGLT-2) inhibitors. These include canagliflozin (Invokana), ertugliflozin (Steglatro), dapagliflozin (Farxiga) and empagliflozin (Jardiance).
Weight loss can vary depending on which GLP-1 drug you use and your dose. Studies have found that all GLP-1 drugs can lead to weight loss of about 10.5 to 15.8 pounds (4.8 to 7.2 kilograms, or kg) when using liraglutide. Studies found people using semaglutide and making lifestyle changes lost about 33.7 pounds (15.3 kilograms) versus 5.7 pounds (2.6 kilograms) in those who didn't use the drug.

Zepbound
Tirzepatide: A dual GIP/GLP-1 receptor co-agonist.
FDA approved tirzepatide for weight loss (Eli Lilly).
(GIP: insulinotropic polypeptide)

Diabetes drugs in the GLP-1 agonists class are generally taken by a shot (injection) given daily or weekly and include:

- Dulaglutide (Trulicity) (weekly)
- Exenatide extended release (Bydureon bcise) (weekly)
- Exenatide (Byetta) (twice daily)
- Semaglutide (Ozempic for Type 2 diabetes) (Wegovy for weight loss) (weekly)
- Liraglutide (Victoza, Saxenda) (daily)
- Lixisenatide (Adlyxin) (daily)
- Semaglutide (Rybelsus) (taken by mouth once daily)

These drugs mimic the action of a hormone called GLP-1. When blood sugar levels start to rise after someone eats, these drugs stimulate the body to produce more insulin. The extra insulin helps lower blood sugar levels. Lower blood sugar levels are helpful for controlling type 2 diabetes. But it's not clear how the GLP-1 drugs lead to weight loss. Doctors do know that GLP-1s appear to help curb hunger. These drugs also slow the movement of food from the stomach into the small intestine. As a result, you may feel full faster and longer, so you eat less.

Along with helping to control blood sugar and boost weight loss, GLP-1s and SGLT-2 inhibitors seem to have other major benefits. Research has found that some drugs in these groups may lower the risk of heart disease, such as heart failure, stroke and kidney disease. People taking these drugs have seen their blood pressure and cholesterol levels improve. But it's not clear whether these benefits are from the drug or the weight loss.

The downside to GLP-1 drugs is that all but one has to be taken by a shot. And, like any drug, there is a risk of side effects, some serious. More common side effects often improve as you continue to take the drug for a while. Some of the more common side effects include: Nausea, vomiting, and diarrhea
Low blood sugar levels (hypoglycemia) are a more serious risk linked to the GLP-1 class of drugs. But the risk of low blood sugar levels often only goes up if you're also taking another drug known to lower blood sugar at the same time, such as sulfonylureas or insulin.

The GLP-1 class of drugs isn't recommended if you have a personal or family history of medullary thyroid cancer or multiple endocrine neoplasia. Lab studies have linked these drugs with thyroid tumors in rats. But until more long-term studies are done, the risk to humans isn't known. They're also not recommended if you've had pancreatitis. The drugs already discussed are indicated in people living with type 2 diabetes. There is also a drug that has a higher dose of liraglutide (Saxenda) that's approved for the treatment of obesity in people who don't have diabetes. If you have diabetes and wonder if one of these drugs may be helpful for you, talk to your diabetes doctor or health care provider.

https://www.mayoclinic.org/diseases-conditions/type-2-diabetes/expert-answers/byetta/faq-20057955
Haelle 2023, New obesity drugs are coming

What Happens If You Stop Taking Weight-Loss Drugs?

Wegovy and Ozempic are made of the same compound, known generically as semaglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist.
"These drugs are a synthetic version of natural gut hormones that are secreted after you eat to make you feel full," Dr. Coviello says. "The synthetic versions have a longer half-life, so they stay in your system longer. This has an amplified effect on your metabolism and appetite regulation."
However, it doesn't seem that the brain is automatically retrained to resist food or cravings once you stop taking the drug.

"You will still have the natural gut hormones in your system, but they won't last as long," Dr. Coviello says. "After stopping the drugs, people experience a return of cravings and hunger, and most people in clinical trials gain back 50 percent of weight lost in 12 to 18 months."
Because of the risk of rebound weight gain after stopping semaglutide, people prescribed Wegovy for obesity may need to take the drug for the rest of their lives. Lifestyle modifications on their own don't always prevent weight gain, research shows.

Some people opt to stop taking weight-loss medications because of their side effects, which can include nausea, diarrhea and constipation. Dr. Coviello says the side effects will cease once the medication is out of your system, which may take about six weeks.
Others may choose to discontinue the medication if they don't see results.
"Some people have more weight loss than is typical, some have less," Dr. Coviello says. "That reflects the variability of underlying reasons for weight excess. These gut hormones do work for most people to some extent, but in the future, we may have more nuanced tools to address what's causing obesity."

Andrea Coviello, MD, FACE (March 8, 2024)
https://healthtalk.unchealthcare.org/what-happens-if-you-stop-taking-weight-loss-drugs/#:~:text=%E2%80%9CYou%20will%20still%20have%20the,in%2012%20to%2018%20months.%E2%80%9D

Side effects of GLP-1 medications

GLP-1 medications can have effects on the gastrointestinal (GI) tract that include nausea, vomiting, stomach pain, bloating, diarrhea and constipation. For many people, these side effects are temporary and improve over time. However, in rare cases more serious side effects have also been reported. Examples include gastroparesis (a condition where the stomach takes longer to empty food than it should), gallbladder problems, bowel obstructions and pancreatitis.

Approximately 10 percent of patients have no treatment response to GLP-1 medications at all, according to Dr. Morton. "They get frustrated, blame themselves when the drugs don't work and just stop taking them," he said. "However, obesity is a difficult condition and not every treatment works for every patient."

Why you shouldn't suddenly stop taking GLP-1 medications

If you're taking a GLP-1 medication to treat diabetes, it can be dangerous to stop suddenly. "If you're diabetic, it can cause issues with your blood sugars," Dr. Morton said.

When it comes to treatment for weight loss, there may be a "rebound effect" when the medications are stopped. In other words, you may start to put back on the weight you've lost – and more. "The weight regain often occurs faster than the weight loss, and you may regain in four months what it took you a year to lose," he said. Patients may also experience a significant amount of gastrointestinal upset after a sudden halt.

Amy Brenner-Fricke (January 29, 2026)
https://www.ynhhs.org/articles/should-you-suddenly-stop-taking-glp1s

New Injectable Weight Loss Drugs Pose Ethical Issue

Arthur L. Caplan, PhD (The Division of Medical Ethics at New York University's Grossman School of Medicine in New York City. February 01, 2024)

There's never been anything like the revolution in the treatment of obesity that we are now living through. Historically, there's always been calorie counting and diets. Now, after a burst of interest in gastric bypass surgery, we have the amazing world of injectables. We all have heard about Ozempic, Mounjaro, and Wegovy. These are being used by millions of Americans at this point, some on prescription for conditions like diabetes and some to bring about weight loss in prediabetes, or in some instances — as is often seen on American television — weight control or weight loss by people who just want to look better. Celebrities getting behind these injectables has really powered an explosion of use.

There still are ethical issues out there for practitioners. For one thing, there are some forms of semaglutide, a key ingredient in some of these injectables, that are made by compounding pharmacies. They're not the name-brand prescription injectables made by large companies. They're brewed up, if you will, by a specialty pharmacy trying to mimic the ingredient. What we've seen in recent weeks is an explosion of overdoses. When a person uses one of these compounding pharmacies, usually in association with a spa or sometimes online sales of weight loss injectables, they're not always certain about how to dose themselves, how much to give, and what to take. They could misread the instructions. The more that it's up to them to determine the dose, the more there's risk for error. Reports show as much as 1500% increases in poisoning of people who took, instead of a 10th of a milliliter, 10 mL of these compounded versions of the injectable drugs.

Everybody needs to be alert, and not only for adverse events from the prescription injectables. It is important to track that, make sure that people aren't getting into trouble, and have contact with the FDA if you have a patient who reports some kind of adverse event they attribute to injectables. It's important to realize that there's this generic, cheaper path, but it's a more dangerous path. People need to know this if they're going to try that route. Doctors should be aware of it. People should be ready to call the poison control center number in their area to make sure that they know what to do if they overdose on this stuff. My own inclination is to try to discourage its use. I think it's still too dangerous to have people self-dosing with ingredients that really are not yet FDA approved in terms of knowing that they've been tested in clinical trials.

The other big issue, aside from **this Wild West world outside of prescribed injectables**, is what to say to people who are obese or trying to manage their weight. I think people need to know all their options. It's pretty easy to just say, "Let's put you on one of these injectables" and prescribe it. For one thing, they may not be able to get it; there's such huge demand that there are some shortages out there. **People may be better off trying to manage weight with diet, calorie counting, or lifestyle changes**. After all, you could stay on these drugs forever to maintain your weight, but it's not cheap. **We don't really know the long-term consequences of decades-long use of these drugs**.

I think people should hear their options and maybe try something less invasive to begin with. If that doesn't work, then move on to the injectables. It isn't so clear to me — given the cost, some of the unknowns of long-term use, and some of the dangers of people sneaking around and trying to get things cheaper on the side — that going straight to injectables is our best answer. I do think doctors should talk about weight with their patients, carefully, with the patient's consent. Make sure there's no stigma. Make sure we're not doing anything to raise anxiety as we talk about this condition. **After all, it is seen as a disease**.

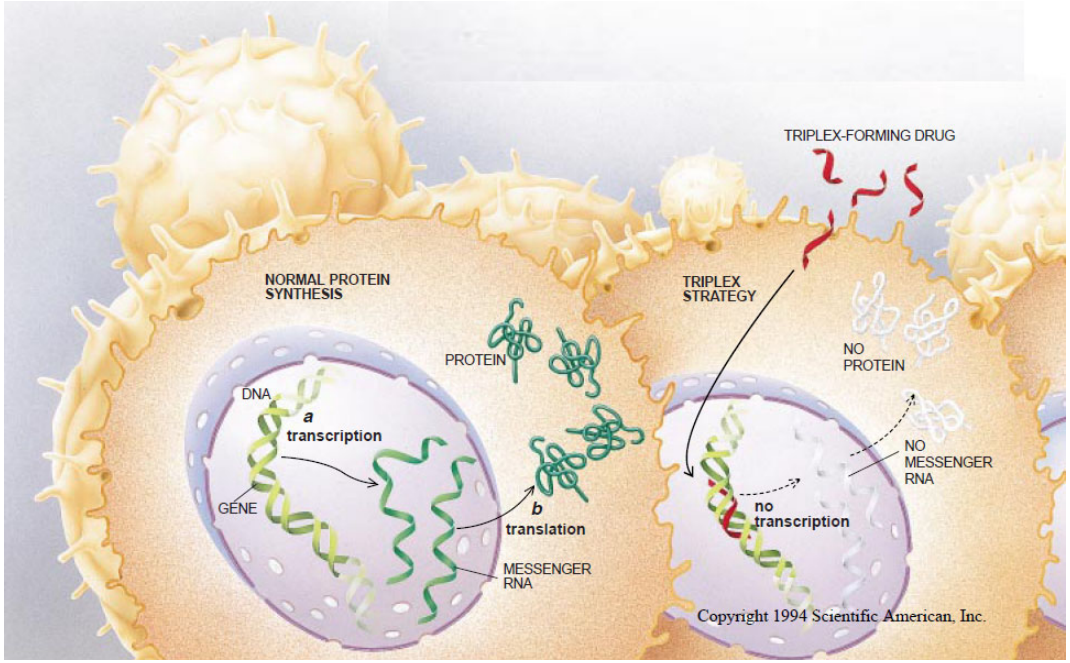
Then, maybe enter your way gradually into interventions, seeing if lifestyle change is possible. It's cheap and easier to implement: better diet, better exercise, or calorie counting. Some people succeed. When they don't, we should move on, but realize that we've got the equivalent of a black market. We need to encourage patients, if they use injectable weight loss drugs, to tell doctors so that they can be on alert about the dangers and risks of overdose.

https://www.medscape.com/viewarticle/999507?ecd=wnl_infocu10_broad_broad_persoexpansion-both_20240217_etid6317472&uac=70212FJ&impID=6317472

Gene Therapy

Gene Therapy: Genetic Modification

Synthetic strands of DNA are being developed as drugs. Called antisense and triplex agents, they can potentially attack viruses and cancers without harming healthy tissue. Jack S. Cohen and Michael E. Hogan



TWO INNOVATIVE STRATEGIES have been tested for inhibiting the production of disease-related proteins. For any protein to be synthesized (left), the gene that specifies its composition must be transcribed from DNA (a) into molecules of messenger RNA. Then the RNA must be translated (b) into copies of the protein. The triplex strategy (center) aims to stall production of an unwanted protein by selectively inhibiting transcription of its gene. The antisense strategy (right) aims to selectively impede translation.
Sci. Amer. December 1994.

For the last 3 decades, research on finding better, more effective gene delivery systems has been intense. Yet, there are still no easy way of delivering DNAs to the target cells in the body. This illustrates how difficult it is to execute conceptually simple, highly promising gene therapy. It is important to understand the magnitude of difficulties, and it will provide better ways to tackle the problem and find answers. Do not underestimate the problem at hand, and never overestimate your own capability.

Gene Therapy

What is gene therapy?

Gene therapy is a type of **treatment that uses genetic material to change the course of a disease**. It is a therapeutic approach under investigation for the treatment of multiple diseases.[1]

What is the goal of gene therapy?

The goal of gene therapy is to **treat diseases at the genetic level (the source)**. Gene therapy is a treatment method under study for a number of diseases, including inherited diseases and cancers.

There are 2 major types of gene therapy:

GENE ADDITION

The addition of genetic materials into the cell to enable the body to produce a functional protein that it could not adequately make before.[6,7,8]

GENE EDITING

The process of directly changing, or editing, a specific site in the genome. The techniques in this therapy include gene correction/insertion and gene inactivation/disruption.[6,9]

What are the potential risks of gene therapy?

As with any treatment, gene therapy carries risks. **Risk depends on the type of gene therapy, the type of vector (used to deliver the gene therapy), and the administration method**. Some risks can be serious.[11] The safety of gene therapy will continue to be assessed over time.

https://www.thegenehome.com/what-is-gene-therapy?msclkid=a14e8e985fe817be1c84ef66a597122b&utm_source=bing&utm_medium=cpc&utm_campaign=HV%20-%20Standard&utm_term=how%20does%20gene%20therapy%20work&utm_content=General

Overview of gene addition and gene editing

	Gene addition	Gene editing	
Mechanism (how it works)	Inserts functional copies of a gene into target cells using a vector to overcome the cells' use of a faulty gene[6,7]	Gene inactivation or disruption	Gene correction or insertion
		Creates targeted breaks in DNA without instructions to repair those breaks, with the aim of disrupting or inactivating the function of a gene[10]	Creates targeted breaks in DNA with instructions to repair those breaks, with the aim of correcting the function of a gene by inserting functioning genetic material[9]
Key components	Viral vectors containing functional genetic material[6,7]	A targeted editing nuclease, with or without genetic material to repair DNA breaks[9]	
Manufacturing	Therapeutic gene is engineered and packaged into vector for delivery to cells[6,7]	Nuclease and genetic material is engineered and delivered to cells[9]	

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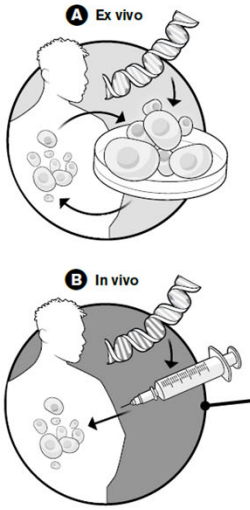
Gene Therapy

Editing the Book of Life

Since the concept of treating diseases by targeting their underlying genes arose half a century ago, gene therapy research has advanced dramatically. Recently the pace of progress has intensified. In the past five years the U.S. Food and Drug Administration has approved more than half a dozen gene therapy products aimed at several types of cancer and inherited conditions. These treatments work in various ways, such as delivering healthy genes to affected cells or reshaping the activity of existing genes. Some of the newest approaches, which have shown promise in early-stage clinical trials, aim to fix errors in the genome itself. And experts expect the pace of new product approvals will continue to pick up. —*E.L.*

Location

Ex vivo gene therapy involves removing blood, bone marrow or other tissues from a patient, isolating the cells of interest and correcting them in the lab before reinfusing them back into the body (A). In vivo approaches send therapeutic genes, gene modulators or gene-editing tools directly to cells in affected tissues within the patient's body (B).

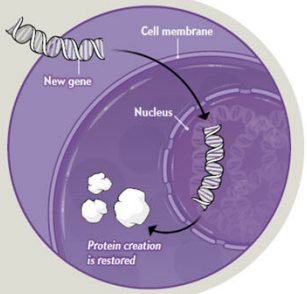


Technique

Gene therapies use various strategies to supply cells with healthy genes, influence gene activity or tweak the genome directly. Each of these methods has advantages and drawbacks, including treatment duration and potential side effects.

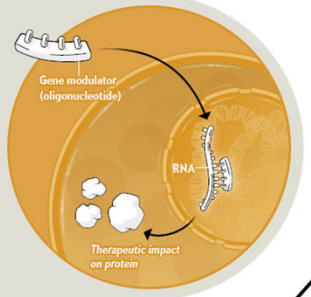
Introduce a New Gene

This approach, the first to be tested in humans, equips affected cells with a working copy of the gene that is missing or malfunctioning in the disease. Whereas this strategy can work for diseases traced to a single genetic glitch, many conditions involve multiple genetic and environmental factors.



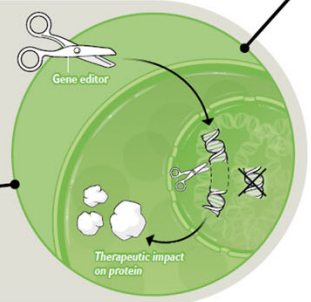
Modulate an Existing Gene's Activity

Other therapies send short sequences of nucleic acids, called oligonucleotides, into affected tissues where they can influence how cells build working proteins from underlying genetic code. Unlike gene replacement or correction, this approach is not permanent, and patients must receive regular infusions for continued benefits.



Edit Gene Directly

These approaches aim to fix errors in specific genes of affected cells. Newer methods use a gene-editing system called CRISPR-Cas9 to make precise changes in the genome.

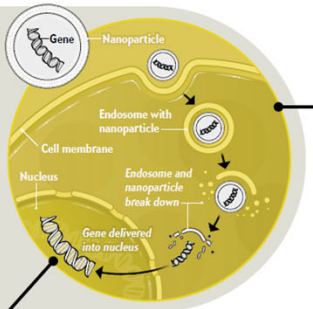


Cargo Delivery Method

Decades of research have honed several methods for carrying either genes, or tools to edit those genes, into target cells. Not only do they have to reach the cell, but they must also evade the immune responses that are often triggered when foreign substances enter the bloodstream.

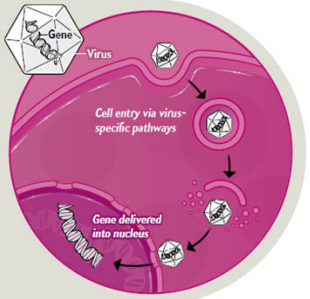
Nanoparticles

These gene therapies use nanoparticles to carry genes or gene-editing tools directly into cells of affected tissues. Nanoparticles can be chemically modified to avoid immune detection and to better target cells.



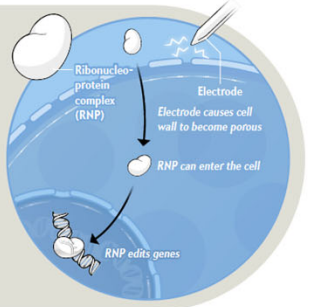
Virus

This approach delivers genes or gene-editing cargo with viruses that researchers have engineered to minimize chances of harmful immune responses and unintended effects on healthy cells.



Other

Clinical trials are testing newer approaches that send gene-editing machinery into cells as complexes of molecules that work together to target and make precise cuts within specific DNA sequences to delete or fix genes.



Examples

In a small study, people with an inherited disease called transthyretin amyloidosis that causes misfolded proteins responded well to an experimental in vivo gene-editing treatment, NTLA-2001 (Intellia Therapeutics/Regeneron), that uses nanoparticles to carry CRISPR-Cas9 into liver cells to inactivate the gene culprit.

A protein called SMN is necessary for motor neuron function, and people with spinal muscular atrophy have a mutation that decreases its production. Spinraza (Ionis Pharmaceuticals/Biogen)—an in vivo gene modulator—coaxes cells into making more SMN protein by boosting its production from a different, unmutated gene.

Leber congenital amaurosis and retinitis pigmentosa are forms of severe vision loss caused by genetic mutations. In certain cases, vision can be restored with Luxturna (Spark Therapeutics), which uses a virus to deliver the healthy gene into retinal cells.

Kymriah (Novartis) is the first approved gene therapy to equip a patient's own immune cells to fight cancer. The approach, known as chimeric antigen receptor (CAR) T cell therapy, involves isolating a patient's T cells and using a virus to equip them with receptors that enable them to recognize and kill certain kinds of tumor cells.

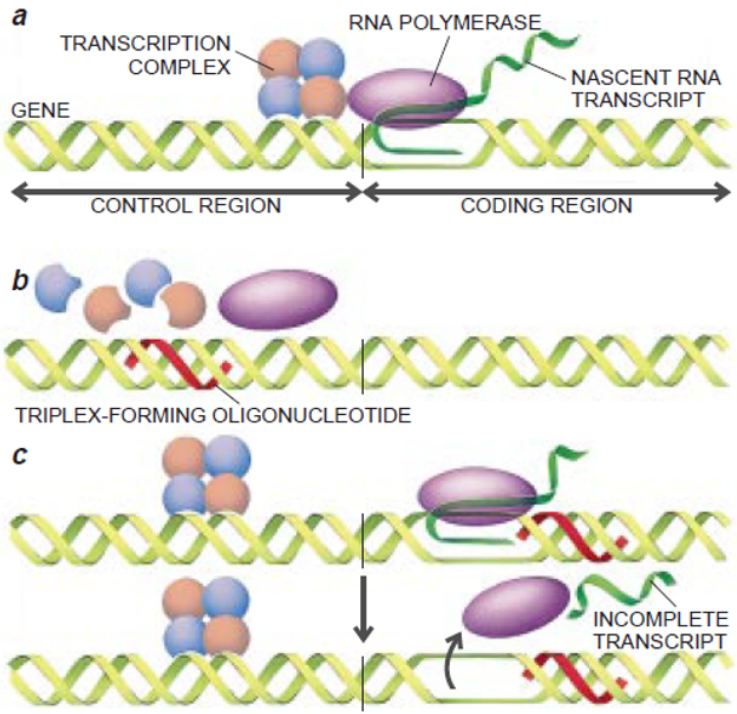
An experimental, ex vivo gene-editing treatment, CTX001 (CRISPR Therapeutics/Vertex Pharmaceuticals), boosted hemoglobin production in blood stem cells of trial participants with sickle cell disease or transfusion-dependent beta thalassemia.

Source: "The Once and Future Gene Therapy," by Karen Bulaklak and Charles Gersbach, in *Nature Communications*, Vol. 11; November 2020 (reference); Cynthia Dunbar National Institutes of Health (expert reviewer)

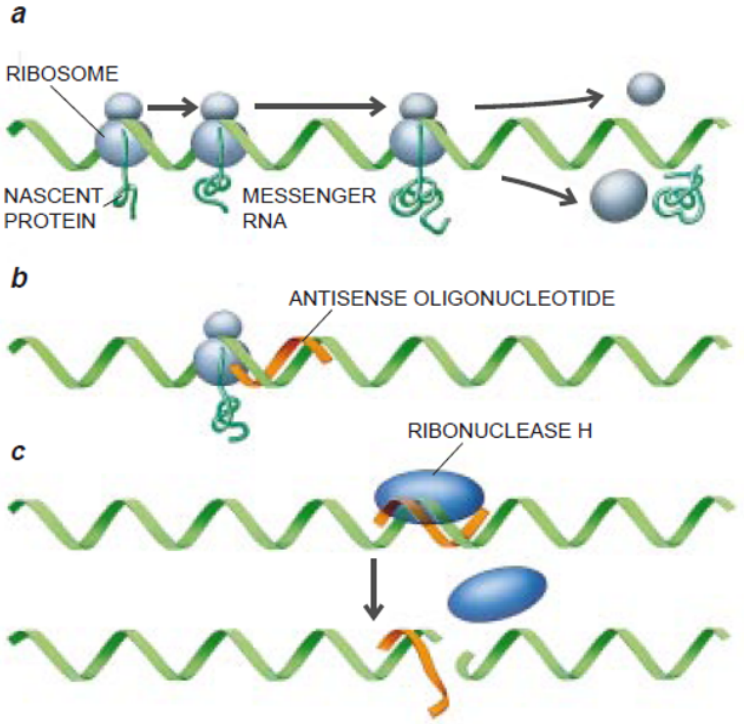
Graphic by Now Medical Studios

Scientific American
November 2021

Gene Transcription and Translation



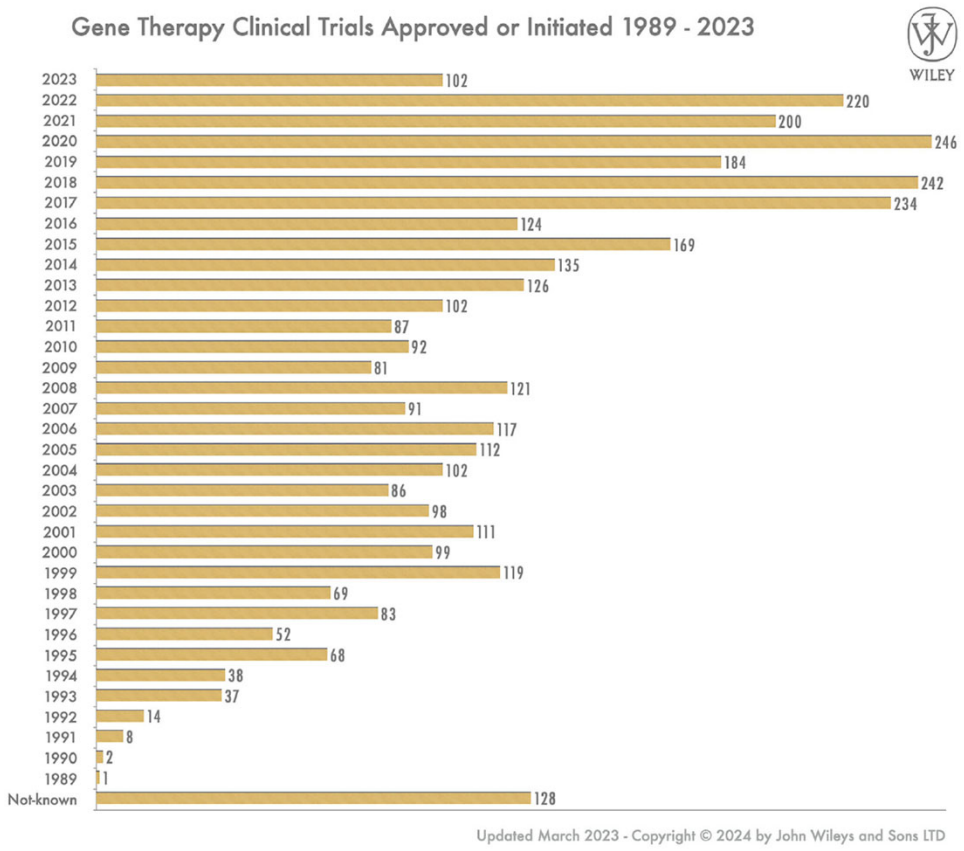
GENE TRANSCRIPTION OCCURS (*a*) after proteins attach to the control region of a gene, forming a transcription complex. This complex directs the enzyme RNA polymerase (*purple*) to copy the instructions in the coding region into messenger RNA (*dark green*). Most triplex-forming agents (*red*) are targeted to the control region, to prevent RNA polymerase from attaching to a gene (*b*). Drugs targeted to the coding region might also halt transcription midstream (*c*).

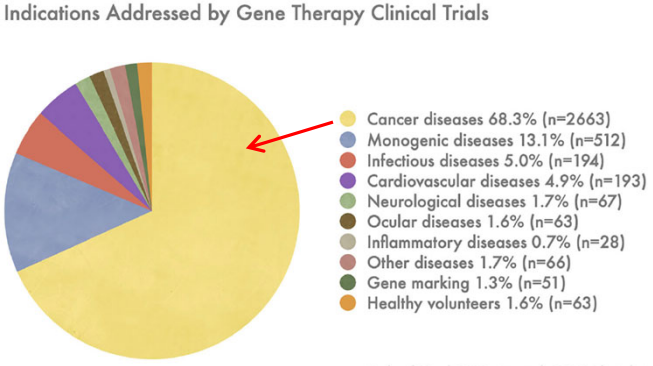


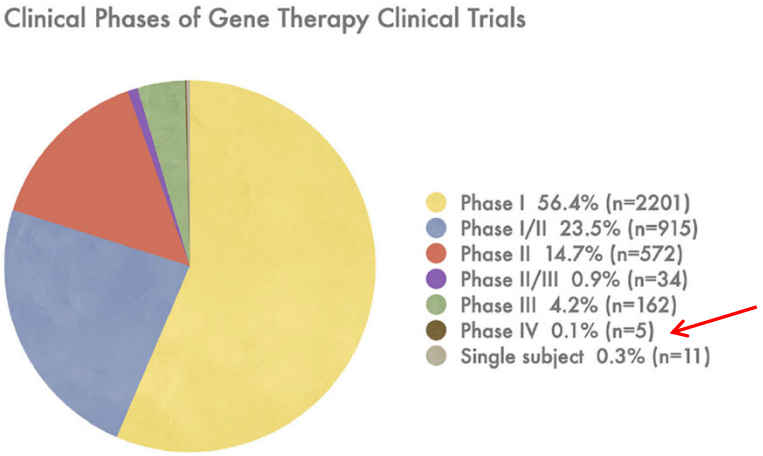
TRANSLATION IS ACCOMPLISHED (*a*) by structures called ribosomes, which travel along RNA transcripts, constructing proteins as they go. Binding of an antisense drug (*orange*) to messenger RNA can inhibit translation in at least two ways. It can prevent the ribosomes from beginning or completing their journey (*b*). It can also induce an enzyme, ribonuclease H, to cut the RNA at the site of drug binding (*c*). Cleaved RNA cannot be translated and is rapidly degraded in cells.

Gene Therapy Clinical Trials Worldwide to 2023

TABLE 1 Approved viral vector-based gene therapy products.
TABLE 2 Approved cloned T-cell receptor cell products.
TABLE 3 Approved non-viral gene therapy products.



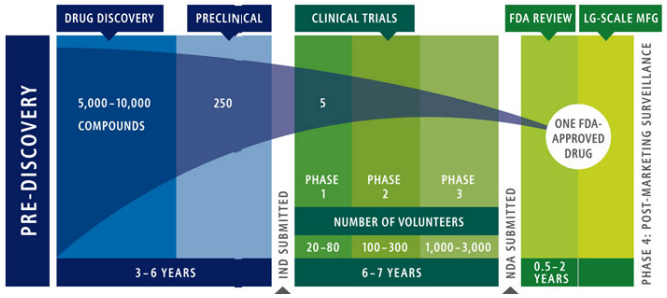




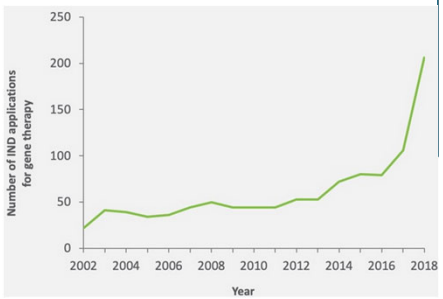
Ginn 2024, Gene therapy clinical trials worldwide to 2023 - an update

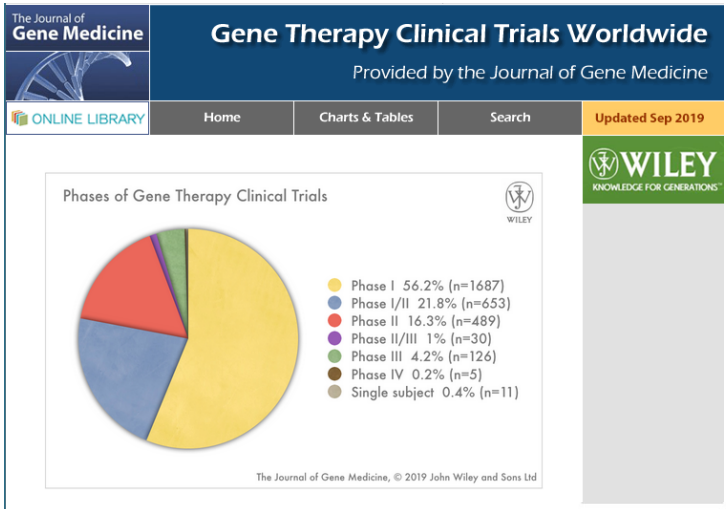
Gene Therapy Clinical Trials Worldwide

Because of enormous potential of DNA delivery or gene therapy, More than 3,000 clinical trials have done as of September 2019. The success rate is only 0.2%, i.e., 5 out of 3001. This is far below the average success rate of small molecular weight drugs, which is about 20% from Phase 1 to the final FDA approval (See below). The miniscule success rate of gene therapy indicates the difficulty of gene therapy, i.e., the lack of suitable DNA/gene delivery systems.



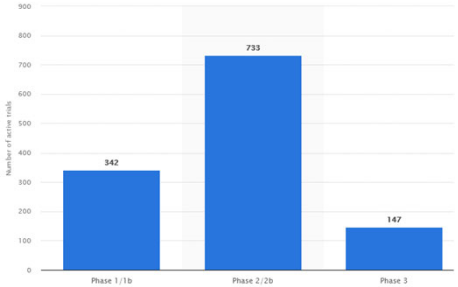
All New IND Applications for Gene Therapy Products by Year
*Data adapted with permission from Lorrie McNeill, Director, FDA Office of Communications. Data in graph are from Marks 2018, except 2018 data from Eisenman 2019.
FDA: U.S. Food and Drug Administration;
IND: investigational new drug.

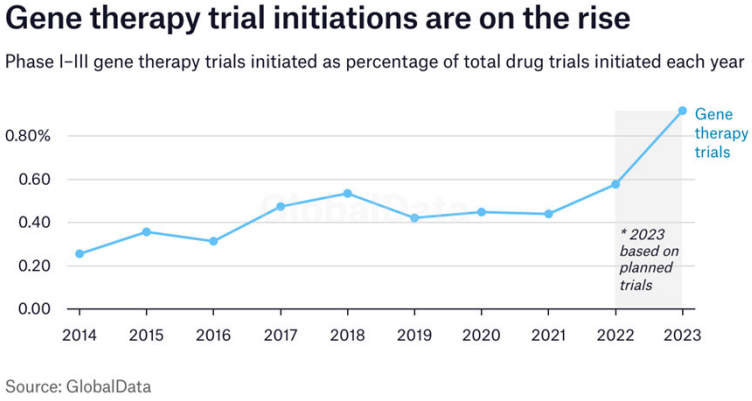




Phase	Gene Therapy Clinical Trials	
	Number	%
Phase I	1687	56.2
Phase I/II	653	21.8
Phase II	489	16.3
Phase II/III	30	1
Phase III	126	4.2
Phase IV	5	0.2
Single subject	11	0.4
Total	3001	

Number of active trials for cell and gene therapies in the global pipeline as of 2022, by trial phase

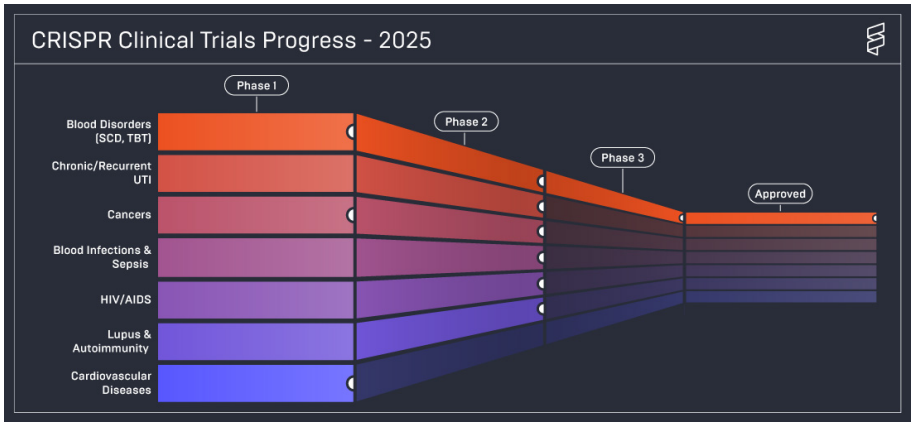




https://genetherapynetwork.com/current-therapeutics-research/gene-therapies-in-research-overview/
http://www.abedia.com/wiley/phases.php
http://www.wiley.com//legacy/wileychi/genmed/clinical/
https://www.statista.com/statistics/1249776/number-active-trials-cell-gene-therapies-by-trial-phase-worldwide/
https://www.clinicaltrialsarena.com/features/five-gene-therapy-trial-readouts-to-watch-in-the-first-half-of-2023/?cf-view

Clinical Trials

CRISPR Clinical Trials: A 2025 Update (Hope Henderson, July 9, 2025)



https://innovativegenomics.org/news/crispr-clinical-trials-2025/

2025 Q3: Key Takeaways

- Four new approvals across each of the gene, cell, and RNA categories took place.
- While regulatory progress continued, clinical development activity slowed, with 125 trials initiated across gene, cell, and RNA therapies. The global pipeline remains robust, with more than 3,200 trials currently underway worldwide.
- Dealmaking for advanced molecular therapy increased, with 99 transactions representing a 9% rise from the previous quarter.

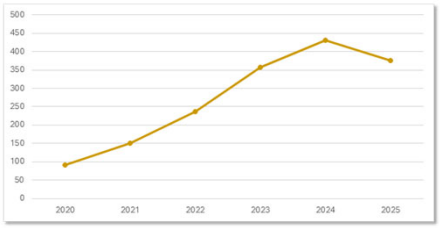
https://www.asgct.org/news-publications/landscape-report

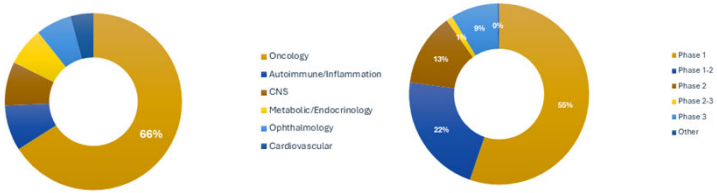
Clinical Trial Trends: Gene Therapy (Alexis Hobbins-White, October 2025)
Understanding the Gene Therapy Clinical Trial Landscape

Gene therapy is entering a period of measurable progress. Once viewed as highly experimental, it now underpins active programs in hematology, neurology, ophthalmology, and oncology. Since the first approvals for conditions such as spinal muscular atrophy and inherited retinal disease, sponsors have expanded development into broader, more complex populations. Per Citeline, as of late 2025, approximately 3,200 gene therapy trials are registered globally and in active stages (planned or ongoing). This number includes both industry and academic trials.

Most programs rely on viral vectors, primarily adeno-associated and lentiviral systems, although non-viral approaches are gaining attention as manufacturing and immunogenicity challenges persist. Across therapeutic areas, studies are assessing both in vivo and ex vivo delivery models, with increasing focus on dose durability and long-term monitoring. Oncolytic and gene-modified constructs are also reshaping cancer research, extending the reach of genetic medicine beyond rare disorders.

Regulatory frameworks continue to adapt to the unique requirements of these therapies. Agencies are refining guidance around vector characterization, potency assays, and follow-up duration. Sponsors are also adopting platform-based strategies that enable faster iteration and improved comparability across programs.

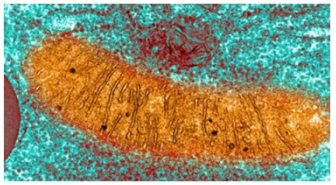


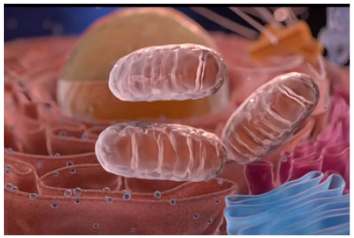


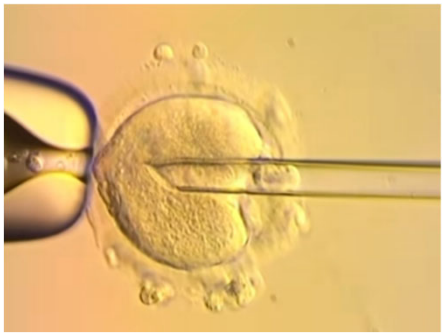
https://www.precisionformedicine.com/blog/clinical-trial-trends-gene-therapy

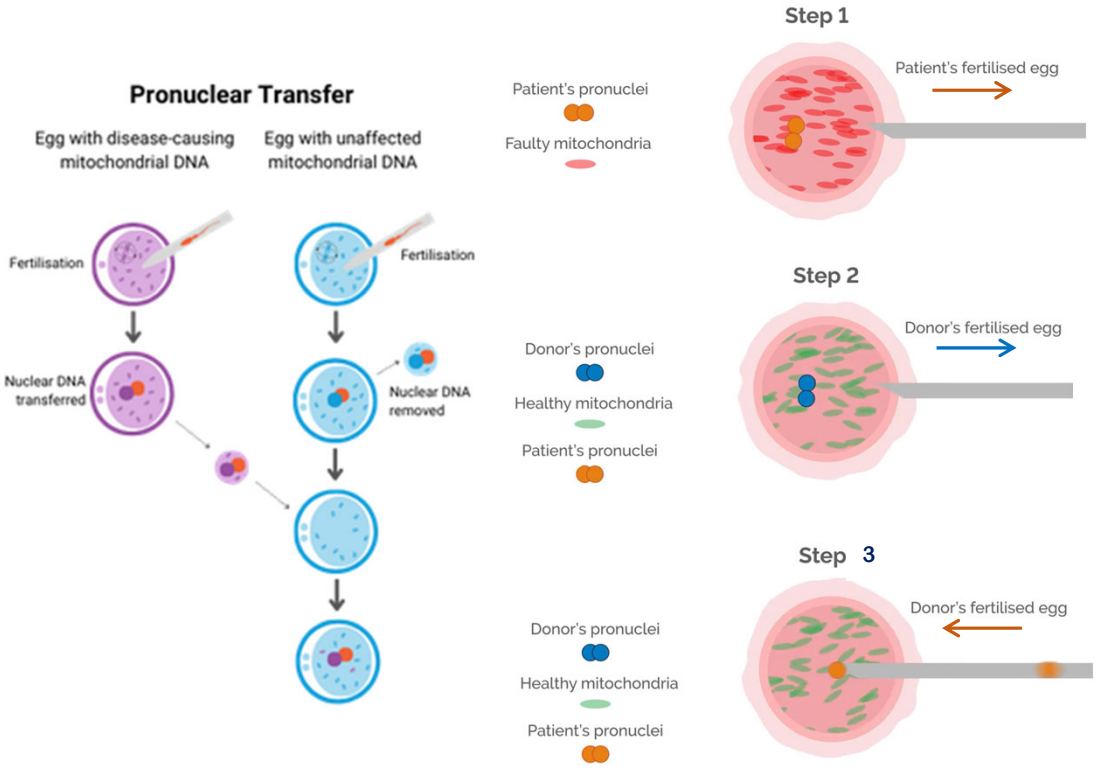
IVF Technique for Mitochondrial Diseases

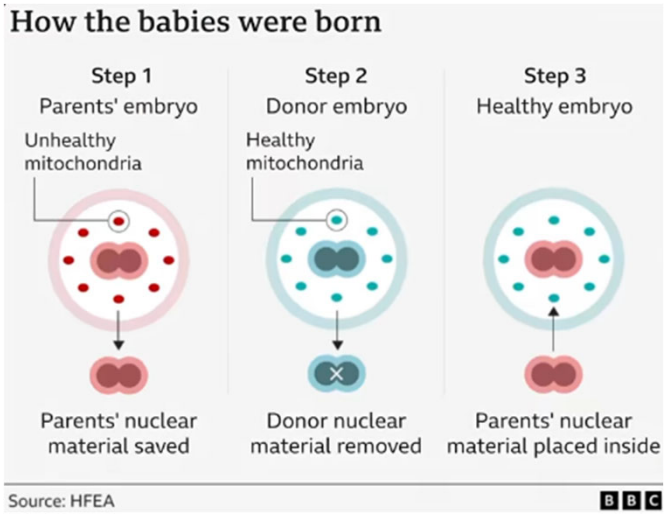
Eight babies born after Mitochondrial donation (July 16, 2025)
The UK's pioneering licensed IVF technique to reduce the risk of mitochondrial diseases carried out in Newcastle has seen eight babies born, published research shows.











https://www.ncl.ac.uk/press/articles/latest/2025/07/mitochondrialdonationtreatment/
McFarland 2025, Mitochondrial donation in a reproductive care pathway for mtDNA disease
https://www.tiktok.com/@tilscience/video/7530314174778379533?_r=1&_t=ZT-93jJlVY6lKf

RNA and DNA Structure Predictions

Critical Assessment of RNA and DNA Structure Predictions via Artificial Intelligence: The Imitation Game

Published as part of Journal of Chemical Information and Modeling special issue "Editing DNA and RNA through Computations".

Christina Bergonzo* and Alexander Grishaev*

Cite This: https://doi.org/10.1021/acs.jcim.5c00245

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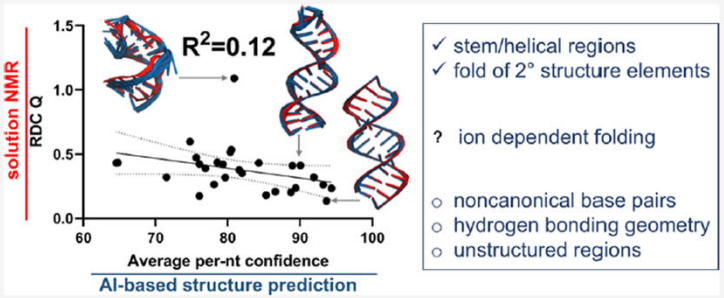
ABSTRACT: Computational predictions of biomolecular structure via artificial intelligence (AI) based approaches, as exemplified by AlphaFold software, have the potential to model of all life's biomolecules. We performed oligonucleotide structure prediction and gauged the accuracy of the AI-generated models via their agreement with experimental solution-state observables. We find parts of these models in good agreement with experimental data, and others falling short of the ground truth. The latter include internal or capping loops, noncanonical base pairings, and regions involving conformational flexibility, all essential for RNA folding, interactions, and function. We estimate root-mean-square (r.m.s.) errors in predicted nucleotide bond vector orientations ranging between 7° and 30°, with higher accuracies for simpler architectures of individual canonically paired helical stems. These mixed results highlight the necessity of experimental validation of AI-based oligonucleotide model predictions and their current tendency to mimic the training data set rather than reproduce the underlying reality.

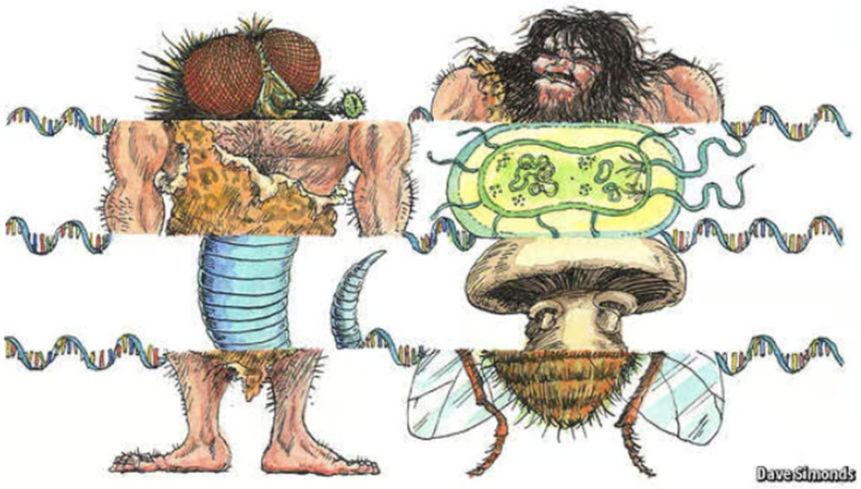


Bergonzo 2025, Critical assessment of RNA and dna structure predictions via artificial intelligence- The imitation game

Genetically Engineered People

Genetically Modified People: Horizontal Gene Transfer.

Human beings' ancestors have routinely stolen genes from other species.



OPPONENTS of genetically modified crops often complain that moving genes between species is unnatural. Leaving aside the fact that the whole of agriculture is unnatural, this is still an odd worry. It has been known for a while that some genes move from one species to another given the chance, in a process called horizontal gene transfer. Genes for antibiotic resistance, for example, swap freely between species of bacteria. Only recently, though, has it become clear just how widespread such natural transgenics is. What was once regarded as a peculiarity of lesser organisms has now been found to be true in human beings, too.

March 12, 2015
https://www.economist.com/science-and-technology/2015/03/12/genetically-modified-people

The human genome contains over 100 functional genes acquired through horizontal gene transfer (HGT) from bacteria, viruses, and other microorganisms, challenging the notion of strictly vertical, parent-to-offspring inheritance. These foreign genes, often acquired millions of years ago, play key roles in metabolism, immune response, and antioxidant generation.

You're not completely human, at least when it comes to the genetic material inside your cells. You—and everyone else—may harbor as many as 145 genes that have jumped from bacteria, other single-celled organisms, and viruses and made themselves at home in the human genome. That's the conclusion of a new study, which provides some of the broadest evidence yet that, throughout evolutionary history, genes from other branches of life have become part of animal cells.

"This means that the tree of life isn't the stereotypical tree with perfectly branching lineages," says biologist Alastair Crisp of the University of Cambridge in the United Kingdom, an author of the new paper. "In reality, it's more like one of those Amazonian strangler figs where the roots are all tangled and crossing back across each other."

Scientists knew that horizontal gene transfer—the movement of genetic information between organisms other than parent-to-offspring inheritance—is commonplace in bacteria and simple eukaryotes. The process lets the organisms quickly share an antibiotic-resistance set of genes to adapt to an antibiotic, for instance. But whether genes have been horizontally transferred into higher organisms—like primates—has been disputed. Like in bacteria, it's been proposed that animal cells could integrate foreign genetic material that's introduced as small fragments of DNA or carried into cells by viruses. But proving that a bit of DNA in the human genome originally came from another organism is tricky.

Crisp and his colleagues analyzed the genome sequences of 40 different animal species, ranging from fruit flies and roundworms to zebrafish, gorillas, and humans. For each gene in the genomes, the scientists searched existing databases to find close matches—both among other animals and among nonanimals, including plants, fungi, bacteria, and viruses. When an animal's gene more closely matched a gene from a nonanimal than any other animals, the researchers took a closer look, using computational methods to determine whether the initial database search had missed something.

In all, the researchers pinpointed hundreds of genes that appeared to have been transferred from bacteria, archaea, fungi, other microorganisms, and plants to animals, they report online today in Genome Biology. In the case of humans, they found 145 genes that seemed to have jumped from simpler organisms, including 17 that had been reported in the past as possible horizontal gene transfers.

"I think what this shows it that horizontal gene transfer is not just confined to microorganisms but has played a role in the evolution of many animals," Crisp says, "perhaps even all animals.

The paper doesn't give any hints as to how the genes—which now play established roles in metabolism, immune responses, and basic biochemistry—may have been transferred or the exact timeline of the jumps, he says. That will take more work.

The findings are critical to understanding evolution, says Hank Seifert, a molecular biologist at the Northwestern University Feinberg School of Medicine in Chicago, Illinois. "This is a very well-done paper. They used all the latest data they could find, all the genomes in the databases," he says. "It makes it clearer than ever that there has been a history, throughout evolution, of gene transfer between organisms."

But not all agree that the new evidence is indisputable. "I see little here that is particularly convincing evidence for horizontal gene transfer," says microbiologist Jonathan Eisen of the University of California, Davis. He doesn't rule out that horizontal gene transfer between bacteria and animals is possible, but says that there are other explanations for the identified genes being present in only some branches of the evolutionary tree—a gene that existed in a far-off ancestor could have simply been lost in many relatives other than two seemingly unrelated species, for instance. "It is up to [the researchers] to exclude other, more plausible alternatives, and I just do not think they have done that."

(https://www.science.org/content/article/humans-may-harbor-more-100-genes-other-organisms)

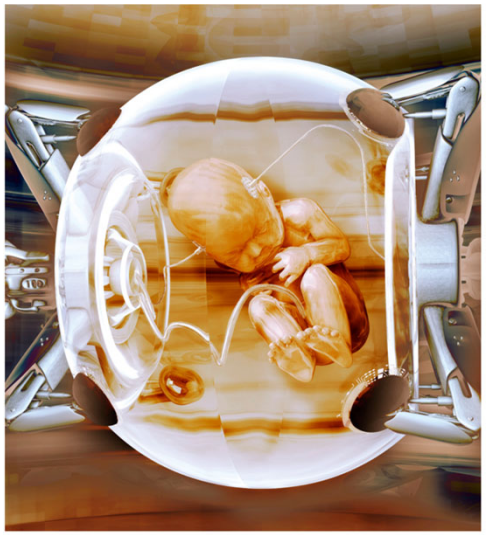
The End of Sex and the Future of Human Reproduction

Within twenty, maybe forty, years, most people in developed countries will stop having sex for the purpose of reproduction. Instead, prospective parents will be told as much as they wish to know about the genetic makeup of dozens of embryos, and they will pick one or two for implantation, gestation, and birth. And it will be safe, lawful, and free. In this work of prophetic scholarship, Henry T. Greely explains the revolutionary biological technologies that make this future a seeming inevitability and sets out the deep ethical and legal challenges humanity faces as a result.

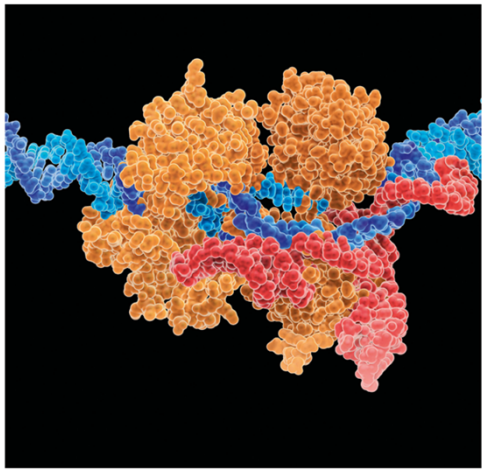
"Readers looking for a more in-depth analysis of human genome modifications and reproductive technologies and their legal and ethical implications should strongly consider picking up Greely's The End of Sex and the Future of Human Reproduction… [It has] the potential to empower readers to make informed decisions about the implementation of advancements in genetics technologies."
—Dov Greenbaum, Science

"[Greely] provides an extraordinarily sophisticated analysis of the practical, political, legal, and ethical implications of the new world of human reproduction. His book is a model of highly informed, rigorous, thought-provoking speculation about an immensely important topic."
—Glenn C. Altschuler, Psychology Today

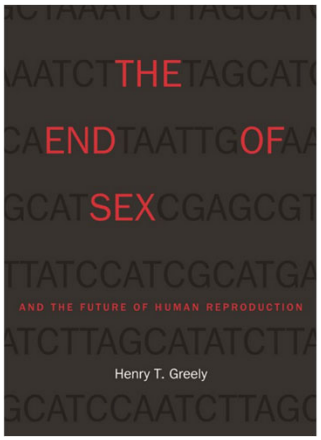
You've probably read about concerns over "designer babies," whose DNA is shaped by gene editing. Greely is focused on a different technology that has gotten much less attention: In a startling bit of biological alchemy, scientists have shown that in mice, they can turn ordinary cells into sperm and eggs. It's too soon to know if it could be done in people. But if it can, it could become a powerful infertility treatment, permitting genetic parenthood for people who can't make their own sperm or eggs. —Washington Post

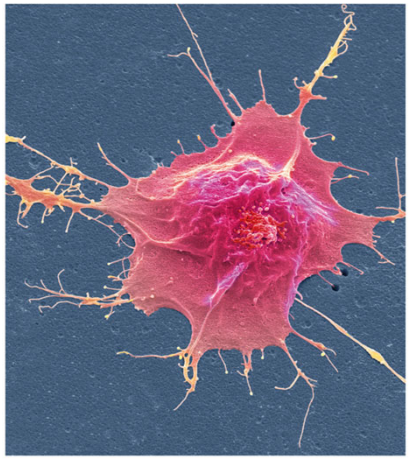


Artificial wombs could enable us to produce babies while eliminating the dangers associated with sex, pregnancy and childbirth



CRISPR technology can be used to edit genetic material, although if it's part of an embryo, those changes will be passed on to any offspring that embryo may go on to produce





Human induced pluripotent cells like this one, created from a skin cell, can be turned into eggs and sperm

Greely 2018, The end of sex and the future of human reproduction

The Ultimate Baby Bottle

Are artificial wombs in our future? Was Aldous Huxley right?

NOBUYA UNNO brings up the nightmarish novel *Brave New World* himself, marveling at Aldous Huxley's accurate prediction that the kids are likely to be anemic after they emerge from their artificial wombs. Actually, Unno's little ones are not quite kids yet. They're fetuses. Goat fetuses.

Raising the ticklish subject of Huxley's 67-year-old novel is pretty cheeky for a scientist who has devoted a decade to developing an artificial womb. But Unno, an obstetrician-gynecologist and researcher at the University of Tokyo, might simply be acknowledging the inevitable. The novel's clever and even now slightly shocking vision of human kids fostered in jars always lurks beneath any talk of artificial wombs.

It's hard to dismiss Huxley, even though the purposes of the artificial wombs being developed at several institutions around the world differ from those described in his book. They are not the government's way of breeding a citizenry specialized for particular chores, most of them menial. Quite the opposite. They are born of consumer demand for fertility treatments and better babies.

NOT YOUR AVERAGE SIBLING RIVALRY

Today's assisted-reproduction technologies, such as in vitro fertilization, have resulted in a boom of cases of a womb with a two—or a three or a four. Indeed, it is not so rare for five, six or even more fetuses to be jammed together in a berth that was really designed for just one. One consequence has been more babies born far too early. Their tiny lungs are not ready to breathe air, so we plunk them into incubators and hook them up to respirators. The result is what doctors delicately term iatrogenic injuries, meaning damage arising from medical intervention. To wit: brain damage, blindness, intestinal damage, delays in development, mental retardation and other lifelong handicaps. So the hunt is on for safer ways to help fetuses through the transition to becoming air-breathing creatures.

Hence the artificial womb. Unno and his colleagues at the University of Tokyo call their version the Extrauterine Fetal Incubation system, or EUFI. Although incubation is its middle name, EUFI is quite different from a conventional incubator. It attempts to simulate the fetal universe.

EUFI is a double-walled, vertical acrylic box filled with artificial amniotic fluid warmed to just under 40 degrees Celsius (104 degrees Fahrenheit), the normal temperature of a nanny goat's own. The furry fetus floats in the fluid and need not breathe air. The truly critical component of the artificial womb, however, is not the container itself but its substitute for the placenta.

A biological placenta adds oxygen and removes carbon dioxide from the fetus's circulating blood, just as its lungs will do once they are fully developed. Artificial-womb scientists must mimic that ability, building a detour into the fetus's circulation so that blood passes from umbilical artery to umbilical vein, exchanging gases as it goes. The Japanese design passes the blood through a membrane oxygenator made of hollow silicone fibers; the unit looks like a thick, clear plastic tube full of straws.

Unno and his co-workers have maintained a fetal goat in EUFI for more than three weeks. (Because goat gestation is about half as long as a human pregnancy, three weeks for a goat fetus is roughly comparable to six weeks for a human one.) But none of the kids the scientists have kept in EUFI for long periods have survived

TOM WAGNER, SABA

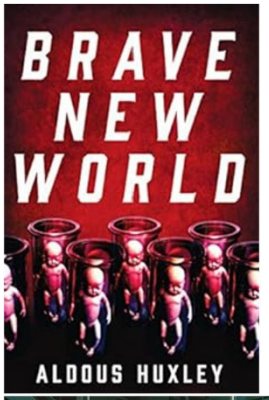
Goat-in-a-box? Using goat fetuses as guinea pigs, researchers at the University of Tokyo have developed the world's most advanced artificial uterus technology. They say their plastic box filled with synthetic amniotic fluid is almost ready to nurture a human fetus.

By Tabitha M. Powledge

Eventually a woman who wants a uterus could place her order, donate her cells and take delivery of her custom-made womb in just six weeks.

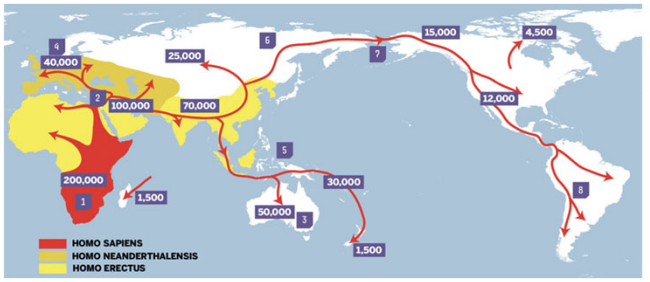
Your New Lifestyle. Scientific American. 1999, p. 96

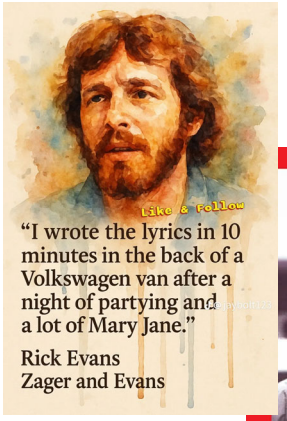
Brave New World (1932)



Year 2540

In The Year 2525





Zager & Evans in TIME Magazine in 1969.

https://www.forbes.com/sites/jimclash/2020/04/03/in-the-year-2525-if-man-is-still-alive/?sh=191cfebad9d2

CAR (Chimeric Antigen Receptor) T-Cell Therapy

Back to the Present!

Evolution of Controlled Drug Delivery Systems

1950 1960 1970 1980 1990 2000 2010 2020 2030

1952 Spansule®
Dissolution-control

1974 Ocusert®
Diffusion-control

1975 OROS®
Osmosis

1982 Delsym®
Ion exchange

1989 Lupron Depot®
PLGA Microparticle

Nanomedicine

1974 InFed®
Iron-Dextran Complex

1979 Transderm Scop®

1990 Norplant®
Implant

2000 Mylotarg™
Ab-Drug Conjugate

2000 Rapamune®
Nanocrystal

2019 Rebelsus®
Oral Peptide Tablet

1994 Taxol®
Paclitaxel in PEGylated Castor Oil

2005 Abraxane®
Paclitaxel-Albumin Complex

2018 Onpattro®
RNAi in PEGylated Lipid Nanoparticle

1990 Adagen®
PEGylated Protein

2014 Movantik
PEGylated naloxol

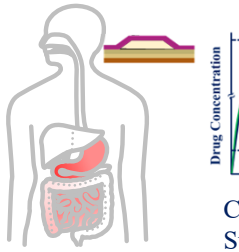
1995 Doxil®
PEGylated Liposome

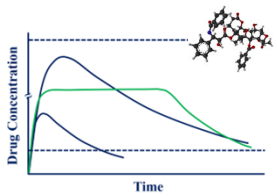
2017 Kymriah® CAR-T Gene Therapy

2021 Comirnaty®
PEGylated Lipid Nanoparticle

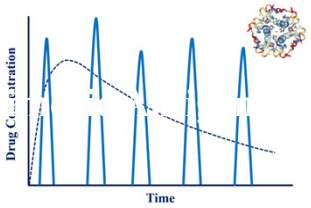
1964 Liposome (Bangosome)

Basic Drug Delivery Mechanisms





Controlled release ≈ Sustained Release



Modulated drug delivery

Drug release kinetics controls pharmacokinetic (PK) profile → **Body controls PK profile**

Small Molecules

Peptide & Protein Drugs

Targeting

Biological Barriers

Long-Term Treatment

CAR T-Cell Therapy

How CAR T-cell therapy works

Immune receptors and foreign antigens
The immune system recognizes foreign substances in the body by finding proteins called antigens on the surface of those cells. Immune cells called T cells have receptors that bind to foreign antigens and help trigger other parts of the immune system to destroy the foreign substance. The relationship between antigens and immune receptors is like a lock and key. Just as a lock can only be opened with the right key, each foreign antigen has a unique immune receptor that is able to bind to it. Cancer cells also express antigens, but if your immune cells don't have the right receptors, they can't bind to those antigens and help destroy the cancer cells.

Chimeric antigen receptors (CARs)
In CAR T-cell therapies, T cells are taken from the patient's blood and modified in the lab by adding **a gene for a receptor (a chimeric antigen receptor, or CAR)**, which helps the T cells bind to a specific cancer antigen. The CAR T cells are then given back to the patient. Since different cancers have different antigens, each CAR is made for **a specific cancer's antigen**. For example, in certain kinds of leukemia or lymphoma, the cancer cells have an antigen called CD19. CAR T-cell therapies for these cancers are designed to bind the CD19 antigen and will not work for cancers that lack it.

("Chimeric" means having the properties of a chimera, something that is made up of very disparate parts. In Greek mythology, the Chimera was a monster comprising parts of different animals, usually depicted as a combination of a lion, a goat, and a serpent.)

The immune system works by keeping track of all the substances normally found in your body. Any new substance the immune system doesn't recognize raises an alarm, causing the immune system to attack it.

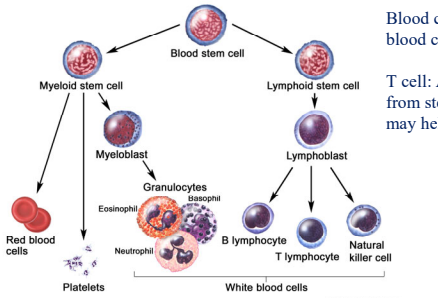
Chimeric antigen receptor (CAR) T-cell therapy is a way to get immune cells called T cells (a type of white blood cell) to fight cancer by changing them in the lab so they can find and destroy cancer cells. CAR T-cell therapy is also sometimes talked about as a type of cell-based gene therapy, because it involves altering the genes inside T cells to help them attack the cancer. This type of treatment can be very helpful in treating some types of cancer, even when other treatments are no longer working.

CAR T-cell therapy can take several weeks.

FDA-approved CAR T-cell therapies
Tisagenlecleucel, also known as tisa-cel (Kymriah)
Axicabtagene ciloleucel, also known as axi-cel (Yescarta)
Brexucabtagene autoleucel, also known as brexu-cel (Tecartus)
Lisocabtagene maraleucel, also known as liso-cel (Breyanzi)
Idecabtagene vicleucel, also known as ide-cel (Abecma)
Ciltacabtegene autoleucel, also known as cilta-cel (Carvykti)

https://www.cancer.org/cancer/managing-cancer/treatment-types/immunotherapy/car-t-cell1.html

CAR T-Cell Therapy



Blood cell development. A blood stem cell goes through several steps to become a red blood cell, platelet, or white blood cell.

T cell: A type of white blood cell. T cells are part of the immune system and develop from stem cells in the bone marrow. They help protect the body from infection and may help fight cancer. Also called T lymphocyte and thymocyte.

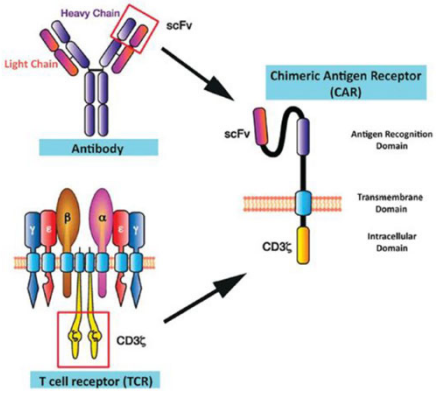
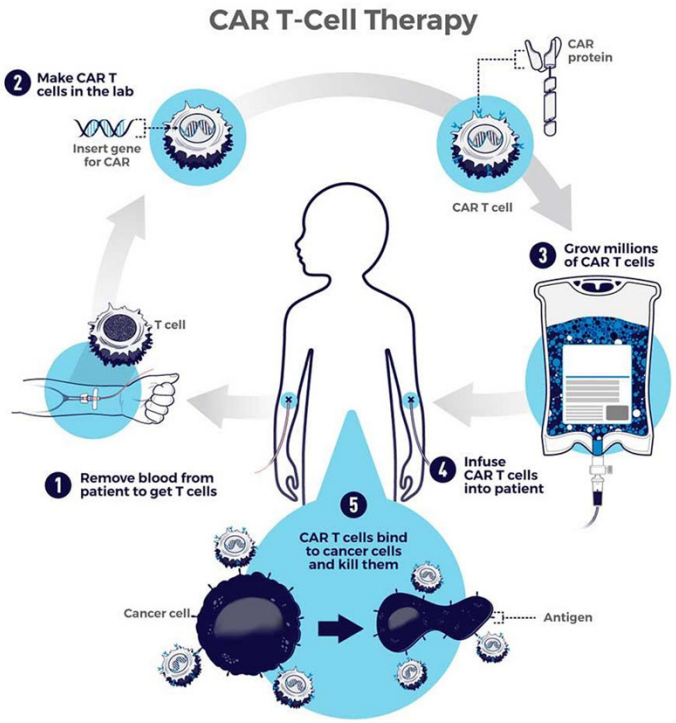


FIGURE 2: First generation CAR T cell design. A chimeric antigen receptor is composed of natural and artificial components. At its most basic form, the native T cell receptor signaling machinery (CD3ζ) is combined with a protein derived from antibodies called a single chain variable fragment (scFv). The single chain variable fragment can precisely recognize and bind to an antigen target such as CD19 or BCMA (B-cell maturation antigen).



CAR T-cell therapy is a type of treatment in which a patient's T cells are genetically engineered in the laboratory so they will bind to specific proteins (antigens) on cancer cells and kill them. (1) A patient's T cells are removed from their blood. Then, (2) the gene for a special receptor called a chimeric antigen receptor (CAR) is inserted into the T cells in the laboratory. The gene encodes the engineered CAR protein that is expressed on the surface of the patient's T cells, creating a CAR T cell. (3) Millions of CAR T cells are grown in the laboratory. (4) They are then given to the patient by intravenous infusion. (5) The CAR T cells bind to antigens on the cancer cells and kill them.

More than Just CAR T Cells: TILs and TCRs

CAR T cells have garnered the lion's share of attention when it comes to cellular therapies. But other types of cellular therapies have also shown promise in small clinical trials, including in patients with solid tumors.

One type, known as **tumor-infiltrating lymphocytes (TILs)**, uses immune cells that have penetrated the environment in and around the tumor. Researchers at NCI were the first to use TILs to successfully treat patients with advanced cancer—initially in melanoma and later in several other cancers, including cervical cancer. More recently, NCI researchers have developed a technique for identifying TILs that recognize cancer cells with mutations specific to that cancer and identifying people whose cancers are more likely to respond to TIL therapy.

The other type of cellular therapy involves engineering patients' T cells to express a specific **T-cell receptor (TCR)**. Unlike CARs, which use portions of synthetic antibodies that can recognize specific antigens only on the surface of cells, TCRs use naturally occurring receptors that can also recognize antigens that are inside tumor cells.

To date, TCR T cells have been tested in patients with a variety of solid tumors, showing promise in melanoma and sarcoma.

https://www.cancer.gov/publications/dictionaries/cancer-terms/def/t-cell#:~:text=A%20type%20of%20white%20blood,Enlarge
Haseltine 2023, How CAR T therapy reimagines cancer treatment and more
https://www.cancer.gov/about-cancer/treatment/research/car-t-cells

Cell Therapy using Tumor-Infiltrating Lymphocytes (TILs)

FDA approves Iovance's cell therapy for melanoma, the first treatment based on tumor-infiltrating lymphocytes (Ryan Cross. February 16, 2024)
An experimental approach to treating cancer more than 40 years in the making finally has a long-sought and repeatedly delayed green light from the FDA. On Friday, Iovance Biotherapeutics won accelerated approval for Amtagvi, a cell therapy for patients with advanced melanoma. It's **the first modern cell therapy for a solid tumor,** rather than blood cancer, and the first approved treatment based on **tumor-infiltrating lymphocytes (TILs)**. TIL therapies, pioneered by National Cancer Institute scientist Steve Rosenberg in the 1980s, are based on the observation that immune cells can penetrate and attack tumors, but often get stuck or lose steam before finishing the job due to cancer's relentless defenses. Iovance dissects those cells from a patient's tumor, nurtures them in its labs and reinfuses the rejuvenated cells into the patient where they hopefully target the tumor.

While the approach sounds simple, turning the idea into a bona fide medicine has been tricky. Unlike commercial CAR-T cell therapies for cancer, in which a patient's immune cells are genetically engineered to target a single protein on blood cells, TIL therapies target a different array of cancer antigens in each patient. Convincing the FDA that the bespoke product would be consistent across patients created an enormous headache for Iovance. After several years of back-and-forth with the agency on how best to assess the treatment's potency, Iovance hopes to leave those troubles behind and hit the ground running with the commercialization of the treatment. "Because of the delay, there is pent-up demand," interim CEO Frederick Vogt told Endpoints News in an interview. "We believe this will be the largest launch in cell therapy ever." Vogt said that Amtagvi will initially be available through 30 medical centers, with plans to expand to 50 by the end of May, and possibly more in the future.

Ahead of the approval, Vogt told Endpoints that the therapy's cost would be "in line with the CAR-T products," ranging from roughly $450,000 to $500,000. In a call with investors on Friday afternoon, he said Amtagvi would cost $515,000. Amtagvi is approved as a second- or third-line treatment option for patients who still have melanoma despite treatment with the commonly used checkpoint inhibitor immunotherapies. Patients also have to get chemo before receiving Amtagvi in order to clear space for the incoming cells. According to Iovance, the roughly 6,300 second-line patients in the US who don't carry BRAF V600 mutations, which are found in about half of melanomas, will be eligible to get Amtagvi after a checkpoint therapy. About 4,800 third-line patients with the mutations have to get BRAF inhibitor drugs before they're eligible for the cell therapy.

The therapy comes with a black box warning about risks of low blood count, infection, heart disorder, lung or kidney dysfunction, or lethal complications. Side effects also include chills, fatigue, fever, swelling and abnormally fast heart rate. **Amtagvi shrank tumors in about one-third of 150 patients, with an objective response rate of 31.4%**, in a Phase II clinical study. **Half of those responses lasted for at least a year**, and the mediation duration of the response was not yet reached after 21.5 months.

As a prerequisite of its accelerated approval, Iovance is currently conducting a large Phase III study of 670 people with melanoma to confirm the treatment's benefit. That study, which will test Amtagvi alone or with Merck's checkpoint inhibitor Keytruda as a frontline therapy for melanoma, is expected to wrap up between 2028 and 2030. Iovance is also testing Amtagvi in cervical cancer and is testing a similar TIL therapy in head and neck cancer and lung cancer. The company also has earlier-stage programs to supercharge the TILs and hopefully boost response rates to the therapy with gene editing. "We're not going to clip the pipeline and just focus on commercial," Vogt said. "TILs are coming back. I think you will see a renaissance." **(Expected to complete the Phase III study in March 2030).**

https://endpts.com/fda-approves-iovances-cell-therapy-for-melanoma-the-first-treatment-based-on-tumor-infiltrating-lymphocytes/

CAR T-Cell Therapy Successfully Used to Treat Brain Tumors

By Camille Mojica Rey, Ph.D. March 13, 2024

Researchers at City of Hope report the first-ever chimeric antigen receptor (CAR) T-cell treatment of recurrent brain tumors delivered directly to the tumor, bypassing the protective blood-brain barrier. The study marks the completion of the largest Phase I clinical trial to date for any solid tumor type.

"We're going after one of the most difficult to treat solid tumors," said Christine Brown, PhD, first author of the current study, which appears in Nature Medicine. "Our local delivery helped us give the most potent therapy," said Brown, who is The Heritage Provider Network Professor in Immunotherapy and deputy director of the T Cell Therapeutics Research Laboratories at City of Hope Beckman Research Institute and Medical Center in Duarte, California.

Brown described the study as foundational for the treatment of brain cancer with CAR-T cell therapies. "We learned how to deliver these cells, we learned about CAR-T cell manufacturing, and we learned the features of each patient that determined how well the treatment worked," she said. (City of Hope has its own GMP manufacturing facility.)

The current study evaluated CAR-T cells engineered to target the tumor-associated antigen interleukin-13 receptor alpha 2 (IL13Rα2). At the end of the six-year study, 29 of the 58 patients with recurrent high-grade glioma brain tumors, mostly glioblastoma, achieved stable disease after treatment with CAR-T cells for at least two months. There were two partial responses, one complete response and a second complete response after additional CAR-T cell therapy cycles were delivered under compassionate use.

Doses of the therapy were escalated as the trial progressed and all doses tested were well-tolerated. Researchers evaluated three methods of delivery: at the tumor site alone, at a brain ventricle alone, and at both sites. **The median overall survival for all patients was eight months.** The trial culminated in treating a patient cohort that used an optimized manufacturing process and injected CAR T cells at both delivery sites. For this final patient cohort, researchers were able to establish a maximal feasible dose and found that these patients had **the best median overall survival of 10.2 months**, which was higher than the expected survival rate of six months in patients with recurrent glioblastoma.

"These were heavily pretreated patients so we were not sure how they would do with CAR-T cell therapy," said Behnam Badie, MD, the Heritage Provider Network Professor in Gene Therapy, chief of neurosurgery at City of Hope and the study's senior author. "But some of them even did better than how they initially responded to standard of care treatments."

Badie, a clinician, credited the collaboration with Brown, who developed the CAR-T cell therapy, for the current results. "We knew from our animal models that local delivery could be ten times more effective than delivering the therapy systemically," he said. Badie also said **having a GMP facility readily available allowed for readily optimizing the CAR-T cell therapy.**

Delivery of the therapy evolved over time, Badie explained. The team learned initially that the treatment delayed local recurrence, but saw that tumors developed in other parts of the brain. "The more you treat the tumors, the more they become aggressive and become multifocal," he said. Implanting the CAR-T cell therapy at the two sites kept this from happening in some of the patients who remained stable.

In addition to optimizing the delivery method, the researchers determined what tumor characteristics impacted response to treatment. They found the best response rate was among those in which larger numbers of host CD4+ T-cells were present in tumor cavity and cerebrospinal fluid. "This suggests to us that there is important interaction between CAR-T cell therapy and the host immune system," Brown said. Future changes to CAR-T cell therapies could be designed to further optimize the treatment, Badie added. "We could deliver agents into the tumor microenvironment to engage the immune system in fighting this difficult to treat tumor," he said.

NOTE: Brown and Badie have financial interests in Mustang. Brown has previously been a paid consultant for Mustang.

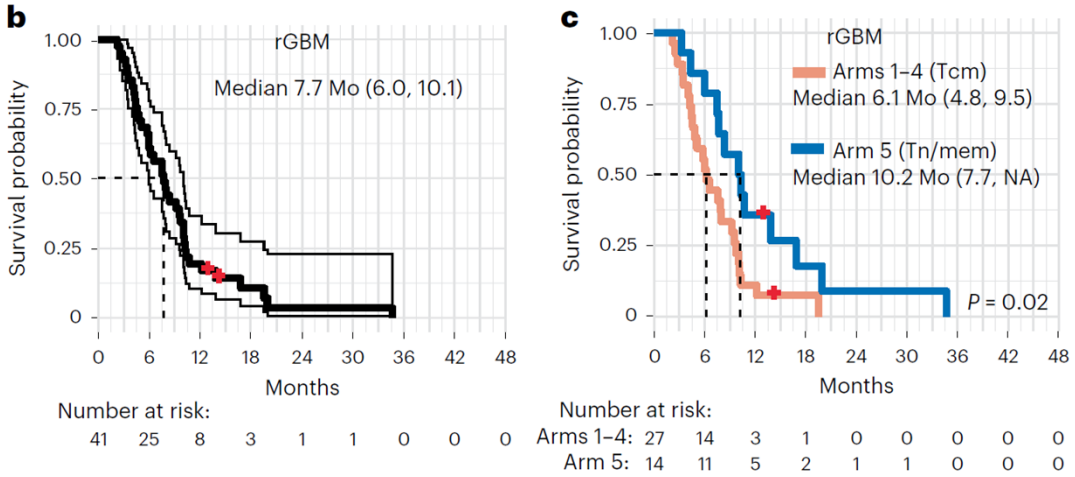
Brown 2024, Locoregional delivery of IL-13Rα2-targeting CAR-T cells in recurrent high-grade glioma: a phase 1 trial

Locoregional Delivery of IL-13Rα2-targeting CAR-T Cells

Brown 2024, Locoregional delivery of IL-13Rα2-targeting CAR-T cells in recurrent high-grade glioma: a phase 1 trial

Recurrent glioblastoma (rGBM).

The protocol was amended to include the Tn/mem manufacturing platform (arm 5) based on data suggesting superior activity against hematological malignancies for Tn/mem- versus Tcm-derived CAR-T cell products22–27, along with feasibility challenges with generating sufficient Tcm-derived CAR products for the highest dose schedule (DS3) in arm 4 (refs. 22–27). Arm 5, (Tn/mem manufacturing and dual ICT/ICV delivery), therefore, represents the foundation for ongoing and future clinical testing (NCT04003649, NCT04510051 and NCT04661384).



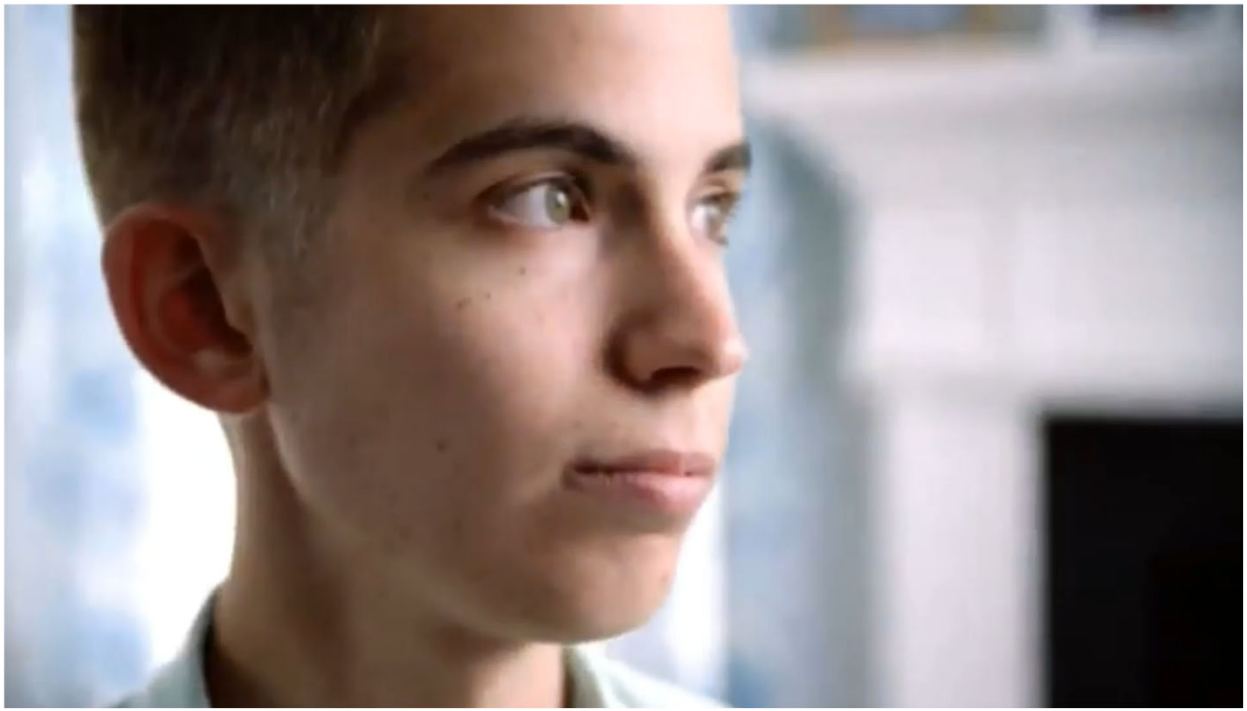
Chimeric antigen receptor T cell (CAR-T) therapy is an emerging strategy to improve treatment outcomes for recurrent high-grade glioma, a cancer that responds poorly to current therapies. Here we report a completed phase I trial evaluating IL-13Rα2-targeted CAR-T cells in 65 patients with recurrent high-grade glioma, the majority being recurrent glioblastoma (rGBM). Primary objectives were safety and feasibility, maximum tolerated dose/maximum feasible dose and a recommended phase 2 dose plan. Secondary objectives included overall survival, disease response, cytokine dynamics and tumor immune contexture biomarkers. This trial evolved to evaluate three routes of locoregional T cell administration (intratumoral (ICT), intraventricular (ICV) and dual ICT/ICV) and two manufacturing platforms, culminating in arm 5, which utilized dual ICT/ICV delivery and an optimized manufacturing process. Locoregional CAR-T cell administration was feasible and well tolerated, and as there were no dose-limiting toxicities across all arms, a maximum tolerated dose was not determined. Probable treatment-related grade 3+ toxicities were one grade 3 encephalopathy and one grade 3 ataxia. A clinical maximum feasible dose of 200×10^6 CAR-T cells per infusion cycle was achieved for arm 5; however, other arms either did not test or achieve this dose due to manufacturing feasibility. A recommended phase 2 dose will be refined in future studies based on data from this trial. Stable disease or better was achieved in 50% (29/58) of patients, with two partial responses, one complete response and a second complete response after additional CAR-T cycles off protocol. For rGBM, median overall survival for all patients was 7.7 months and for arm 5 was 10.2 months. Central nervous system increases in inflammatory cytokines, including IFNγ, CXCL9 and CXCL10, were associated with CAR-T cell administration and bioactivity. Pretreatment intratumoral CD3 T cell levels were positively associated with survival. These findings demonstrate that locoregional IL-13Rα2-targeted CAR-T therapy is safe with promising clinical activity in a subset of patients. ClinicalTrials.gov Identifier: NCT02208362.

The trial opened as a two-arm study treating patients ICT following either biopsy (arm 1) or resection (arm 2). Arms 3–5 were added by protocol amendments based on clinical observations (Methods). ICV delivery (arm 3) was added on the basis of clinical experience with UPN109, in which ICV administered IL-13Rα2-CAR-T cells mediated a CR against multifocal rGBM[6], along with preclinical data suggesting ICV was more effective against multifocal tumors[16,20]. Subsequently, the trial transitioned to dual delivery combining attributes of both ICV and ICT (arms 4 and 5)[16,20,21], and after noting that ICV delivery eliminated small, multifocal subpial lesions, whereas large intraparenchymal tumor progressed (Extended Data Fig. 1a). The protocol was amended to include the Tn/mem manufacturing platform (arm 5) based on data suggesting superior activity against hematological malignancies for Tn/mem- versus Tcm-derived CAR-T cell products[22–27], along with feasibility challenges with generating sufficient Tcm-derived CAR products for the highest dose schedule (DS3) in arm 4 (refs. 22–27). Arm 5, (Tn/mem manufacturing and dual ICT/ICV delivery), therefore, represents the foundation for ongoing and future clinical testing (NCT04003649, NCT04510051 and NCT04661384).

Fig. 2 | Clinical activity of locoregionally delivered IL-13Rα2-CAR-T cells. b, overall survival (OS) of evaluable rGBM patients from date of surgery. Thin lines denote 95% CIs; dashed line depicts median in months (Mo). Median survival with 95% CI in parentheses also indicated. c, Survival comparison of evaluable rGBM patients who were infused with either Tcm- or Tn/mem-derived cell products. Dashed lines depict medians in Mo. Median survival times with 95% CIs in parentheses also indicated; NA means infinity. P value for survival comparison was determined using the log-rank test. Red dots indicate censored participants (that is, lost to follow-up but had not passed away).

a, Swimmer plot of evaluable patients and their clinical outcomes. WHO grade is at time of treatment. Tumor IDH mutations are indicated by yes (Y) or no (N); ND, not determined; DS, dose schedule. Black lines indicate CAR-T cell cycles administered to route dictated by arm (arm 1: ICT-Biopsy; arm 2: ICT-Resection; arm 3: ICV; arms 4 and 5: dual ICT/ICV). White lines indicate additional CAR-T cell cycles administered ICV. Yellow lines indicate additional CAR-T cell cycles administered ICT. Bold UPN numbers, evaluable rGBM patients (n = 42, evaluable for survival n = 41); #, diffuse midline glioma, H3 K27-altered.

CAR T-Cell Therapy

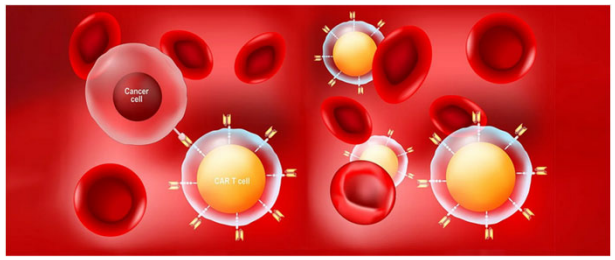


Researchers label early CAR-T therapy patient ‘cured’ after living a decade without cancer (By Angus Chen Feb. 2, 2022)

In 2010, Doug Olson became the second person in the world to receive CAR-T cell therapy, an experimental tactic to engineer his own immune cells to fight cancer. His doctors had tempered expectations for how well it would fight off Olson’s chronic lymphocytic leukemia, an incurable blood cancer — it was a last stab in the dark, one with no guarantees. But as the researchers tracked Olson and another patient, what they saw was remarkable: Year after year, the CAR-T cells persisted, actively watching for cancer cells. Olson has now been cancer-free for a decade,

The therapy works by isolating immune cells known as T cells from the patient’s body. Then, **researchers use a virus to genetically engineer a synthetic receptor** — known as a CAR, or chimeric antigen receptor — onto the T cell’s surface. This CAR can bind to a specific target, in this case a protein found on immune B cells called CD19, and it can activate the T cell to kill any cell bearing this target. Because chronic lymphocytic leukemia, the cancer that Olson and Ludwig had, are malignancies of the B cell, the engineered cells could recognize cancerous B cells and destroy them.

https://www.statnews.com/2022/02/02/cart-cancer-therapy-leukemia-treatment/



“The potential impact of CAR-T is tremendous,” National Cancer Institute pediatric hematologist Nirali Shah. This study “gives you a proof of concept about the safety of having long-term persistence and integration of the T cells into your body.” **Unfortunately, other patients who have received CAR T cell treatments have not been so lucky, especially those with solid tumors**. But Joseph Melenhorst, an immunologist at the University of Pennsylvania and the lead author on the new study, tells STAT that the team’s results could help scientists figure out why CAR T therapy works only for some and develop a next generation of treatments that can be more widely helpful.

https://www.the-scientist.com/news-opinion/ten-years-on-car-t-cell-recipient-is-still-cancer-free-69672?utm_campaign=TS_DAILY_NEWSLETTER_2022&utm_medium=email&_hsmi=202932173&_hsenc=p2ANqtz-_FLBusXKJ-aoVUftpDbZPdh3cwm5uEWjGGSdqs10oqb2PXzrdflybiLvXN0DRm_Z-CowPN1bUlbocQfhb1xW7TsUf1hQ&utm_content=202932173&utm_source=hs_email

CAR T-Cell Therapy

Cancer center leaders lay bare CAR-T makers' struggles—and an unexpected laggard

This year, the FDA moved two CAR-T therapies into earlier large B-cell lymphoma (LBCL) and cleared a second cell therapy for multiple myeloma. But despite five years of collective experience making and selling engineered human cell products, the biopharma industry is still struggling to ensure smooth and timely access.

Cell therapy leaders at three top U.S. cancer hospitals—Memorial Sloan Kettering Cancer Center, Moffitt Cancer Center and City of Hope—are not satisfied with CAR-T availability and their manufacturers' operations. During separate interviews at the recent American Society of Hematology annual meeting, the experts said **manufacturing constraints** were their top sticking point, especially for the myeloma CAR-Ts from Bristol Myers Squibb and Johnson & Johnson. But the problems go beyond well-documented manufacturing bottlenecks. And, in the case of J&J and Legend Biotech's Carvykti, having witnessed other drugmakers' struggles didn't guarantee immediate success.

Thanks to limited manufacturing slots, doctors at Sloan Kettering can only treat **about two to three myeloma patients with commercial CAR-Ts out of the 10** they would like to in a month, Jae Park, M.D., the center's acting chief of cellular therapy service, told Fierce Pharma.
"That's a very frustrating part for the patients and for clinicians, too," Park said. "That has to improve." To Sloan Kettering's Park, BMS and J&J/Legend are "equally suboptimal" on the operational side of CAR-T treatment. But City of Hope's Budde and Moffitt's Locke have a clearer preference. "The obvious winner is Abecma by far, and not because the efficacy is better. It's not. But because the company that's making it knows what they're doing," Locke said of BMS' track record in CAR-T. But BMS' early struggles with Abecma were well publicized. The company launched the therapy nationwide last year and immediately hit a manufacturing bottleneck—both because of **a shortage of viral vectors** used to deliver the cell therapy and **limited production slots**.

Liu 2022, Cancer center leaders lay bare CAR-T makers' struggles—and an unexpected laggard
https://www.fiercepharma.com/pharma/johnson-johnson-bristol-myers-kite-pharma-car-t-cell-therapy-struggle-sloan-kettering

CAR-T hype faces infrastructure reality check
By Angus Liu. Jan 15, 2024

Since the FDA approved the first CAR-T therapy back in August 2017, **high prices, small patient pools, and limited manufacturing capacity** have at times hindered these cell-based treatments. As biopharma companies clear those hurdles, a larger, more systemic problem now threatens the drug class.

Six CAR-T therapies targeting either CD19 or BCMA have reached the U.S. market to treat various blood cancers. Impressive efficacy data, wide reimbursement acceptance, earlier-line approvals and steady production expansions have fueled blockbuster revenue predictions. But drug developers and Wall Street may have underestimated **the bottlenecks from the healthcare infrastructure needed to deliver a cell therapy**, Leerink Partners analyst Daina Graybosch, Ph.D., warns.

In recent interviews, experts said manufacturing constraints were their top sticking point as the cell therapy field continues to evolve. (Gerard Julien/AFP/Getty Images)

A "revolutionary paradigm shift" in cell therapy delivery and patient care is necessary to remove the limitations ahead for CAR-T therapies. Hospitals, manufacturers and others are working to resolve bottlenecks, but it will take time. (z_wei/iStock/Getty Images Plus)

Intrathecal Bivalent CAR T Cells Targeting EGFR and IL13Rα2 in rGBM

Bagley 2024, Intrathecal bivalent CAR T cells targeting EGFR and IL13Rα2 in recurrent glioblastoma- phase 1 trial interim results.
KP: Basically minimal response even after bivalent CAR T Cells treatment.

Recurrent glioblastoma (rGBM) remains a major unmet medical need, with a median overall survival of less than 1 year. Here we report the first six patients with rGBM treated in a phase 1 trial of intrathecally delivered bivalent chimeric antigen receptor (CAR) T cells targeting epidermal growth factor receptor (EGFR) and interleukin-13 receptor alpha 2 (IL13Rα2). The study's primary endpoints were safety and determination of the maximum tolerated dose. Secondary endpoints reported in this interim analysis include the frequency of manufacturing failures and objective radiographic response (ORR) according to modified Response Assessment in Neuro-Oncology criteria. All six patients had progressive, multifocal disease at the time of treatment. In both dose level 1 (1×10^7 cells; $n = 3$) and dose level 2 (2.5×10^7 cells; $n = 3$), administration of CART-EGFR-IL13Rα2 cells was associated with early-onset neurotoxicity, most consistent with immune effector cell-associated neurotoxicity syndrome (ICANS), and managed with high-dose dexamethasone and anakinra (anti-IL1R). One patient in dose level 2 experienced a dose-limiting toxicity (grade 3 anorexia, generalized muscle weakness and fatigue). Reductions in enhancement and tumor size at early magnetic resonance imaging timepoints were observed in all six patients; however, none met criteria for ORR. In exploratory endpoint analyses, substantial CAR T cell abundance and cytokine release in the cerebrospinal fluid were detected in all six patients. Taken together, these first-in-human data demonstrate the preliminary safety and bioactivity of CART-EGFR-IL13Rα2 cells in rGBM. An encouraging early efficacy signal was also detected and requires confirmation with additional patients and longer follow-up time. ClinicalTrials.gov identifier: NCT05168423.

Although the sample size treated thus far is small, and the follow-up duration is relatively brief, CART-EGFR-IL13Rα2 cells mediated reductions in enhancement and tumor size at early post-treatment timepoints in all six patients with multifocal, treatment-refractory rGBM. Although none met criteria for an objective response according to mRANO criteria (that is, ≥50% decrease in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks), tumor shrinkage of at least 30% was observed in three of six patients, and stable disease was maintained on scans performed at least 2 months after CAR T cell therapy in three of the four patients who had at least 2 months of follow-up time, arguing against a 'pseudo-response' phenomenon. Although the effects on tumor enhancement and size were observed more quickly (that is, within 24–48 h) after CAR T cell injection than what is expected with other immunotherapies, such as immune checkpoint inhibitors, such rapid tumor cell killing is (1) consistent with intrathecally delivered CAR T cells coming into contact with tumor cells soon upon CSF entry and (2) occurring on a timeline that corresponds to the onset of neurotoxicity and peak CAR T cell and pro-inflammatory cytokine levels in the CSF. It is also notable that two patients experienced apparent pseudoprogression, with marked increases in enhancing tumor burden at the day +28 and day +14 timepoints, respectively, followed by substantial tumor regressions on short-term follow-up MRI scans in the absence of intervening anti-neoplastic therapy. Similar radiographic patterns of pseudoprogression were described in patients with non-Hodgkin's B cell lymphoma treated with anti-CD19 CAR T cells[27] and in children with H3K27M-mutated diffuse midline gliomas after intrathecal administration of GD2-targeted CAR T cells[28]. More complete characterization of this phenomenon will require treatment of additional patients along with serial tissue sampling when feasible.

FDA Investigating Serious Risk of T-cell Malignancy

FDA Investigating Serious Risk of T-cell Malignancy Following BCMA-Directed or CD19-Directed Autologous Chimeric Antigen Receptor (CAR) T cell Immunotherapies (November 28, 2023)

The Food and Drug Administration (FDA) has received reports of T-cell malignancies, including chimeric antigen receptor CAR-positive lymphoma, in patients who received treatment with BCMA- or CD19-directed autologous CAR T cell immunotherapies. Reports were received from clinical trials and/or postmarketing adverse event (AE) data sources. FDA has determined that the risk of T-cell malignancies is applicable to all currently approved BCMA-directed and CD19-directed genetically modified autologous CAR T cell immunotherapies. T-cell malignancies have occurred in patients treated with several products in the class. Currently approved products in this class (listed alphabetically by trade name) include the following:

Abecma (idecabtagene vicleucel)
Breyanzi (lisocabtagene maraleucel)
Carvykti (ciltacabtagene autoleucel)
Kymriah (tisagenlecleucel)
Tecartus (brexucabtagene autoleucel)
Yescarta (axicabtagene ciloleucel)

Although the overall benefits of these products continue to outweigh their potential risks for their approved uses, FDA is investigating **the identified risk of T cell malignancy with serious outcomes, including hospitalization and death, and is evaluating the need for regulatory action**. As with all gene therapy products with integrating vectors (lentiviral or retroviral vectors), the potential risk of developing secondary malignancies is labeled as a class warning in the U.S. prescribing information (USPIs) for approved BCMA-directed and CD19-directed genetically modified autologous T cell immunotherapies. The initial approvals of these products included postmarketing requirements (PMRs) under Section 505(o) of the Federal Food, Drug, and Cosmetic Act (FDCA) to conduct 15-year long term follow-up observational safety studies to assess the long-term safety and the risk of secondary malignancies occurring after treatment.

Patients and clinical trial participants receiving treatment with these products should be monitored life-long for new malignancies. In the event that a new malignancy occurs following treatment with these products, contact the manufacturer to report the event and obtain instructions on collection of patient samples for testing for the presence of the Chimeric Antigen Receptor (CAR) transgene.
To report suspected adverse events including T cell malignancies, contact the FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.
Healthcare providers, clinical investigators, patients, and caregivers who have questions may contact FDA's Center for Biologics Evaluation and Research (CBER) at ocod@fda.hhs.gov.

https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/fda-investigating-serious-risk-t-cell-malignancy-following-bcma-directed-or-cd19-directed-autologous

Issues with CAR T-Cell Immunotherapies

T-cell Malignancy From CAR-T Cell Immunotherapies Gets FDA Investigation (December 1, 2023)

After receiving reports of T-cell malignancies in patients who received treatment with BCMA- or CD19-directed autologous CAR-T cell immunotherapies, the agency has announced an investigation into the issue and is evaluating the need for regulatory action.

According to the agency, reports from clinical trials and postmarketing adverse events, the risk — which includes chimeric antigen receptor CAR-positive lymphoma — is applicable to all currently approved B-cell maturation antigen (BCMA) directed and CD19-directed genetically modified autologous CAR-T cell immunotherapies.

Although the agency said **the overall benefits of these products continue to outweigh their risks**, they noted that all gene therapy products with **integrating vectors (lentiviral or retroviral vectors) pose the potential risk of developing secondary malignancies**.

https://www.fdanews.com/articles/212925-t-cell-malignancy-from-car-t-cell-immunotherapies-gets-fda-investigation?utm_source=DRW&utm_medium=email&mkt_tok=ODM4LUxVWi00MjcAAAGP3OaMxTKCMyIPK1GFtXmvhSfnTmdeWttna_cLQGn6oKnRX13forPlZB8GIo6RJqJ-ESlaBciGGwDHnE3xxRcUZiL4n0b6fF_nJxS2SJYq2Wk

FDA puts up CAR-T roadblock, slapping holds on 3 CARsgen cell therapies after inspecting facility
(By Nick Paul Taylor. Dec 13, 2023)

The FDA has put three CAR-T cell therapy candidates from CARsgen Therapeutics on clinical hold after paying a visit to its manufacturing facility, setting back the development of an asset that caught the eye of Moderna and a key enabler of the Chinese biotech's global expansion plan. **(In November 2024, the FDA lifted the holds, allowing trials to resume.)** CARsgen started (PDF) clinical manufacturing at the North Carolina facility targeted by the FDA early last year and released its first batch 15 months ago. Things appeared to be progressing according to plan, with the biotech telling investors that the site was "under full operation" and "overall manufacturing operational efficiency improved" in a corporate presentation (PDF) released at the start of this month. But the FDA reset the narrative this week by putting three CAR-Ts on hold until the findings of its inspection of the facility are resolved. CARsgen said it will "conduct a comprehensive review and improvement on the current good manufacturing practice" but its notice (PDF) lacks details of what the inspectors found.

It is also unclear how long it will take the biotech to resolve the manufacturing findings and get the hold lifted. Investors see the hold as a significant setback and sent CARsgen's share price down 30% to 6.57 Hong Kong dollars after the news broke.

The reaction reflects the potential for the setback to harm CARsgen's chances of carving out a nook in the highly competitive spaces targeted by its CAR-Ts. The three cell therapies affected by the hold target BCMA, Claudin18.2 and GPRC5D, receptors that are priorities for a flock of leading drug developers.

CARsgen moved its BCMA candidate into a phase 1b/2 multiple myeloma clinical trial at sites in the U.S. and Canada in 2019. ClinicalTrials.gov lists the primary completion date as the end of next year but that estimate was provided before the FDA hold. CARsgen has already given a head start to the approved BCMA CAR-Ts, Johnson & Johnson's Carvykti and Bristol Myers Squibb's Abecma.

The Claudin18.2 candidate, CT041, is also in phase 1b/2 and is the subject of a clinical collaboration with Moderna. CARsgen sees CT041 as a potential first-in-class CAR-T but, while it gained an early lead over other cell therapies, it is surrounded by companies that are applying different modalities to the target. The third CAR-T, CT071, targets GPRC5D, a receptor that AstraZeneca, BMS, J&J and Roche are pursuing.

CARsgen opened the facility in North Carolina to go toe-to-toe with such companies outside its native China, identifying the site as a way to treat 700 patients a year and support clinical trials and early launch activities in North America and Europe.

https://www.fiercebiotech.com/biotech/fda-puts-car-t-roadblock-slapping-holds-3-carsgen-cell-therapies-after-inspecting-facility

Fully-Automated CAR-T Bioprocessing Platform

Fully-Automated CAR-T Bioprocessing Platform Aids Scalability (By Gail Dutton. February 5, 2025)

Manufacturing platforms for **cell and gene therapies (CGT)** are becoming more automated and integrated but, despite recent gains, there's still a lot of room for improvement. The challenge is that the current manufacturing process isn't sufficiently scalable to service a market that, with a predicted 26% compound annual growth rate through 2028, may exceed $23 billion in four years' time. The challenges include processes that typically require human intervention, timelines that are measured in weeks rather than days, and a paper trail that is not fully-digitized, Jon Ellis, co-founder of Trenchant BioSystems, tells GEN.

CGT-in-a-box

To address those issues, Trenchant is developing a fully-automated cell therapy manufacturing platform for CAR-T cells. It recently partnered with Autolomous, integrating its digital solution into Trenchant's manufacturing platform, for faster manufacturing and lower production costs.

Currently at the prototype stage and awaiting its final name, Ellis says the platform can:

- Reduce CGT costs by 80%
- Cut manufacturing time to 2.5 days
- Increase cell processing efficiency by eightfold
- Increase overall CGT manufacturing efficiency from 8% to 65%
- Reduce the required manufacturing footprint and labor requirements significantly

"It is an end-to-end manufacturing platform," Ellis stresses, that was designed from the ground up specifically to manufacture CAR-T cells. Trenchant's team used what he calls a "smart engineering" approach to automate proprietary cell-selection technology to isolate T cells or NK cells from other blood cells and to improve viral transduction. And, because cells aren't lost during the early phases of manufacturing, cell expansion isn't necessary, he points out. "Those pieces, combined, mean we get a more efficient manufacturing process."

This fully automated, CAR-T-in-a-box manufacturing approach also means the data from each step is digitized, making reports easier and faster to develop. "You don't need a bunch of people sorting through paper," he points out. The goal, he says, is to "completely change the paradigm of manufacturing by speeding it up and making it more affordable."

Transformational potential

Trenchant is about to announce a collaboration with a leading academic research center to test the platform, generating data that Ellis hopes will validate the company's internal results. "From there, we'll go into productization," which he says may take about two years.

Ellis says he expects to partner with clinical centers rather than developing Trenchant manufacturing centers. If the platform performs as expected, it could transform CAR-T therapies into first-line treatments for patients.

"CAR-T therapies will likely deliver better clinical outcomes (than competing therapies), and also a much better journey for the patients," he suggests. But for them to do that for the broad population who may benefit, they must be made at scale and at an affordable price point. "That's the bit we're trying to resolve at Trenchant."

https://www.genengnews.com/topics/bioprocessing/fully-automated-car-t-bioprocessing-platform-aids-scalability/?_hsmi=345933804

Keeping CAR-T Cells Fresh

Keeping CAR-T Cells Fresh (By Mike May, PhD. February 5, 2025)

Despite the powerful cancer-fighting capabilities of treatments based on T cells containing an engineered chimeric antigen receptor (CAR) to fight a patient's cancer, one key challenge is getting these treatments to more patients. To accomplish that, these CAR-T cells must remain alive and active from the time they're manufactured until they are injected in a patient.

In the abstract for an article in press, Alina Kunitskaya, PhD, then working on her doctoral degree in biomedical engineering at the University of British Columbia (UBC) in Vancouver, and James M. Piret, ScD, professor of chemical and biological engineering at UBC, reported: "During concentrated cell washing, the cells are frequently exposed to transiently reduced oxygen, temperature, pH, and nutrient levels."

The question is: How do variable conditions impact CAR-T treatments? To find out, Kunitskaya and Piret exposed concentrated T cells—about 250 million cells per milliliter—to temperatures from 4 to 37 °C. The highest temperature damaged the T cells the most and the impact on the cells increased over time. As Kunitskaya and Piret wrote: "The concentrated cell conditions at 37 °C resulted in by far the greatest losses in viable cell numbers with, on average, only 58% and 41% of the cells recovered after three and six h, respectively."

When Kunitskaya and Piret applied lower temperatures, the quality of the cells lasted longer. "At 4°C the transient conditions were less extreme, and the cells well maintained for six h," the scientists noted.

So, the conditions provide fairly narrow windows for keeping CAR-T cells fresh. Kunitskaya and Piret emphasized, "when developing processes and devices for T-cell therapy manufacturing that involve concentrated cells, the results of this study indicate that more practically feasible room temperatures can be used for up to three hours to obtain high viable cell recoveries, whereas lower temperatures such as 4 °C should be used if there is a need for more prolonged concentrated T-cell conditions."

Consequently, temperature plays a crucial role in the efficacy of CAR-T cells. Here, time and temperature are dangerous enemies of these cancer therapies.

https://www.genengnews.com/topics/bioprocessing/keeping-car-t-cells-fresh/?_hsmi=345933804

Moving the Needle of CAR-T Beyond Oncology

Chimeric antigen receptor (CAR) T cell therapy has emerged as a pivotal treatment modality for advanced hematological malignancies. However, clinical evidence suggests that CAR T cell therapy has a low response rate, poor efficacy for solid tumor, and a high complication rate. Recent research highlighted the crucial role of epigenetics in tumor immunity, particularly in modulating the fate and function of T cells. The epigenetic landscapes among T cell subpopulations show substantial differences, which in turn have a profound impact on the effector function and persistence of T cells. Epigenetic reprogramming holds promise for enhancing the persistence of CAR T cells, augmenting T cell infiltration, and ameliorating the immunosuppressive microenvironment while impeding immune evasion. In addition, biomarkers derived from the epigenetics serve as indicators to predict patient prognosis. In recent years, a growing number of clinical trials have been initiated to explore the combination of epigenetic drugs with CAR T cell therapy, highlighting the therapeutic promise of this synergistic approach in improving efficacy and overcome therapeutic resistance. However, the non-specificity of epigenetic drugs, side effects of epigenetic gene editing, poor efficacy in solid tumors, and instability of epigenetic biomarkers for predicting prognosis remain areas for further exploration. In this review, we explored the characterization of epigenetic modification landscapes across CAR T cell subpopulations, discussed how epigenetic reprogramming addresses challenges associated with CAR T cell therapy, and provided insights into the limitations of combining epigenetic strategies with CAR T cell therapy.

Epigenetics means changes in gene activity that are heritable without changing the DNA sequence. Epigenetic modifications include DNA methylation, histone modification, chromatin accessibility, non-coding RNA, and RNA modification (Dawson and Kouzarides, 2012). Emerging evidence shows that epigenetics plays an important role in controlling how immune cells work (Henning et al., 2018) and regulating the immunosuppressive tumor microenvironment (TME)(Niu et al., 2024). Therefore, targeting epigenetic modifications and subsequent transcriptional changes, a process known as epigenetic reprogramming, has great potential to improve the therapeutic performance of CAR T cells(Akbari et al., 2021). Epigenetic reprogramming covers all stages of CAR T cell therapy: before treatment, during CAR T cell manufacturing, and after CAR T cell infusion.

Epigenetic editing enhances CAR T therapy by modifying gene expression without altering the DNA sequence, improving T-cell persistence, reducing exhaustion, and overcoming tumor immunosuppression. Using tools like CRISPRoff, researchers can safely silence multiple genes simultaneously to create more effective, "off-the-shelf" CAR T cells, particularly for treating solid tumors.

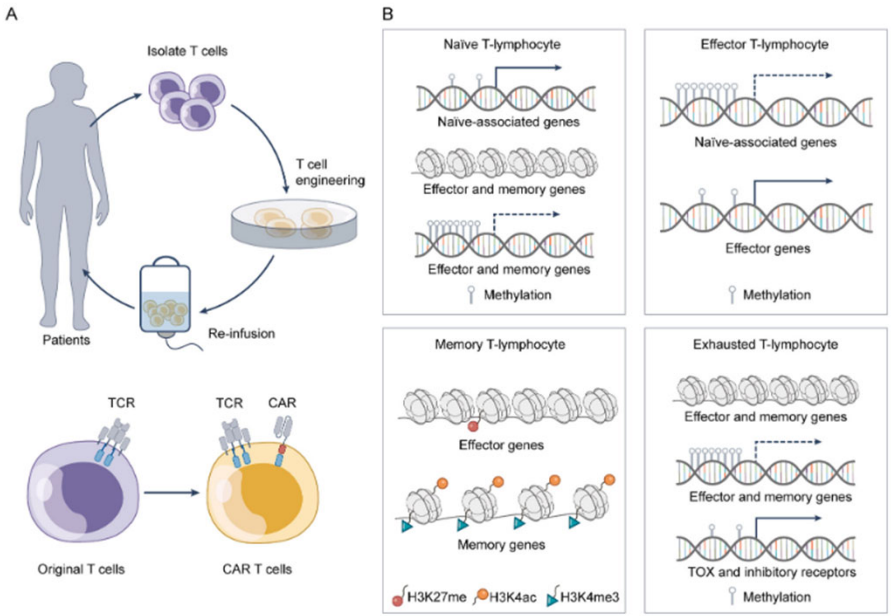


Fig. 1. Production process of CAR T cells and epigenetic landscape of different CAR T cell subpopulations. A. CAR T cell production process. T cells are first isolated from the patient, then engineered in vitro to express CAR targeting specific antigen, and finally expanded and infused back into the patient. B. Epigenetic landscape of different CAR T cell subpopulations. Naïve T-lymphocytes have considerable demethylation of naïve-associated genes. The effector and memory genes of naïve T cells are highly methylated and have closed chromatin. Effector T-lymphocytes are characterized by
hypermethylation of naïve-associated genes and demethylation of effector genes. The motifs encoding effector genes in memory T-lymphocytes have decreased chromatin accessibility owing to a decrease in methylation of histone 3 lysine 27 (H3K27me), whereas the histones of the memory genes undergo H3K4ac and H3K4me3, with increased chromatin accessibility. High methylation and closed chromatin of effector and memory genes, as well as substantial demethylation of TOX and inhibitory receptors, were observed in exhausted T-lymphocytes

Zheng 2025, Epigenetic reprogramming holds promise in enhancing anti-tumor efficacy of CAR T cell therapy

CRISPR

CRISPR

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

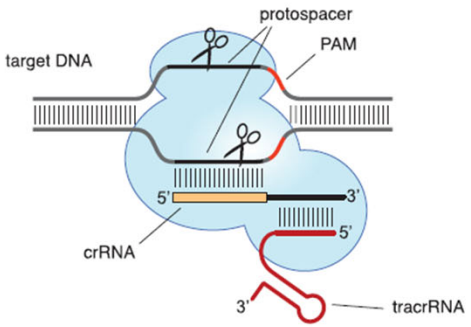
Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]†
Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded (ds) breaks in target DNA. At sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand. The dual-tracrRNA:crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage. Our study reveals a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlights the potential to exploit the system for RNA-programmable genome editing.

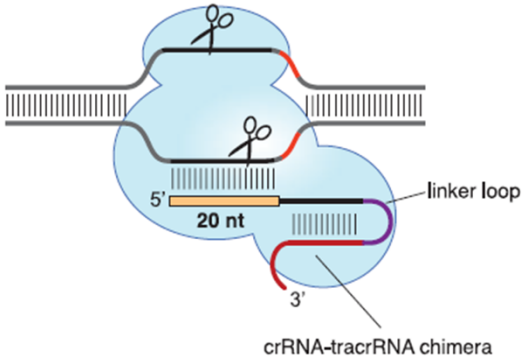
Emmanuelle Charpentier & Jennifer Doudna

Jinek 2012, A Programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science 337: 816-821, 2012

Cas9 programmed by crRNA:tracrRNA duplex



Cas9 programmed by single chimeric RNA



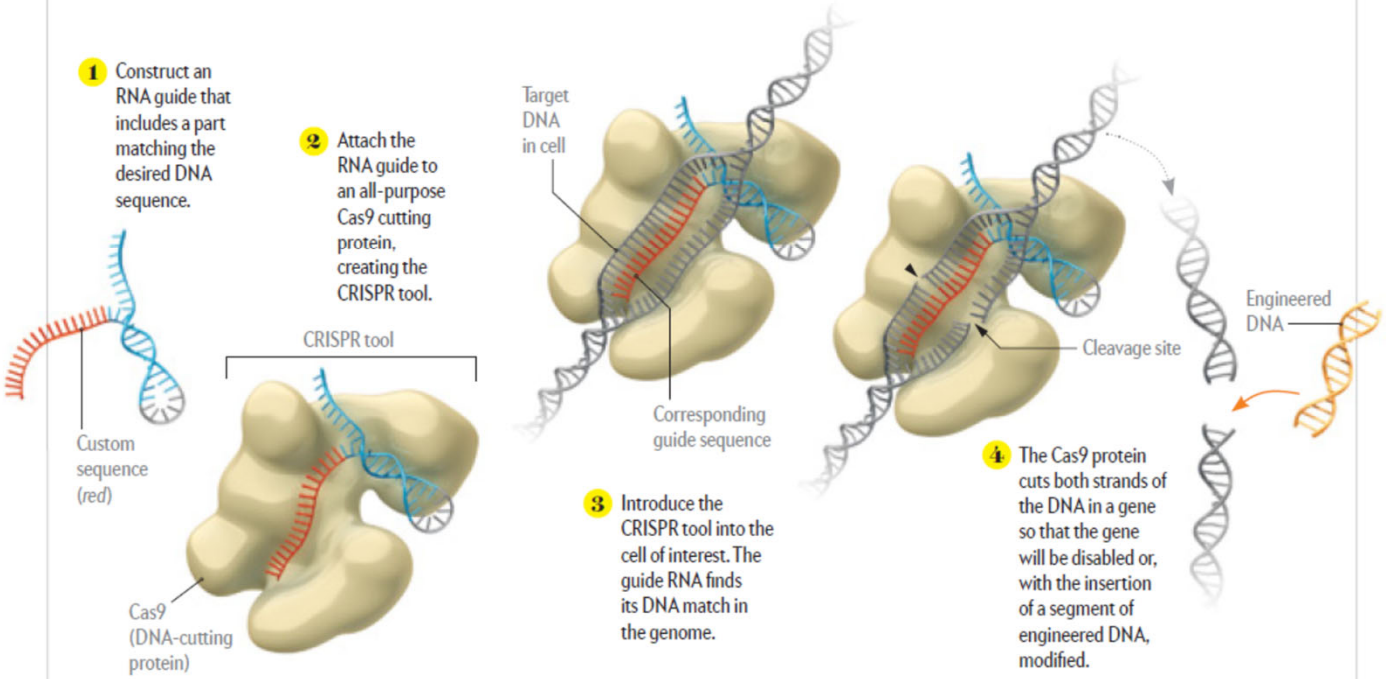
Conclusions. We identify a DNA interference mechanism involving a dual-RNA structure that directs a Cas9 endonuclease to introduce site-specific double-stranded breaks in target DNA. The tracrRNA:crRNA-guided Cas9 protein makes use of distinct endonuclease domains (HNH and RuvC-like domains) to cleave the two strands in the target DNA. Target recognition by Cas9 requires both a seed sequence in the crRNA and a GG dinucleotide-containing PAM sequence adjacent to the crRNA-binding region in the DNA target. We further show that the Cas9 endonuclease can be programmed with guide RNA engineered as a single transcript to target and cleave any dsDNA sequence of interest. The system is efficient, versatile, and programmable by changing the DNA target-binding sequence in the guide chimeric RNA. Zinc-finger nucleases and transcription-activator–like effector nucleases have attracted considerable interest as artificial enzymes engineered to manipulate genomes (*35*–*38*). We propose an alternative methodology based on RNA-programmed Cas9 that could offer considerable potential for gene-targeting and genome-editing applications.

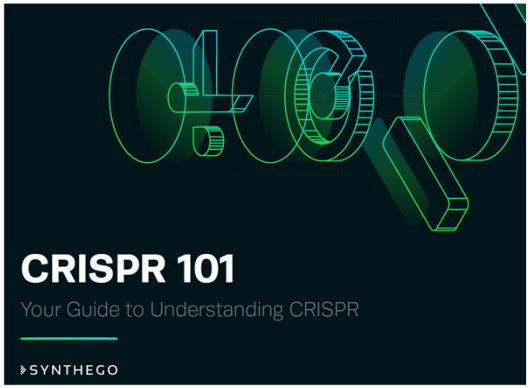
How CRISPR Works

BASICS

How CRISPR Works

Bacteria use a weapon called CRISPR to julienne invading viruses. Scientists can hijack this process to chop up sequences of DNA they would like to modify instead. Unlike previous genome-editing methods, the CRISPR system uses a single, all-purpose enzyme, called Cas9, to do the slicing. All the researcher has to do is create an RNA "guide" to steer it there; RNA is vastly easier to synthesize than enzymes.



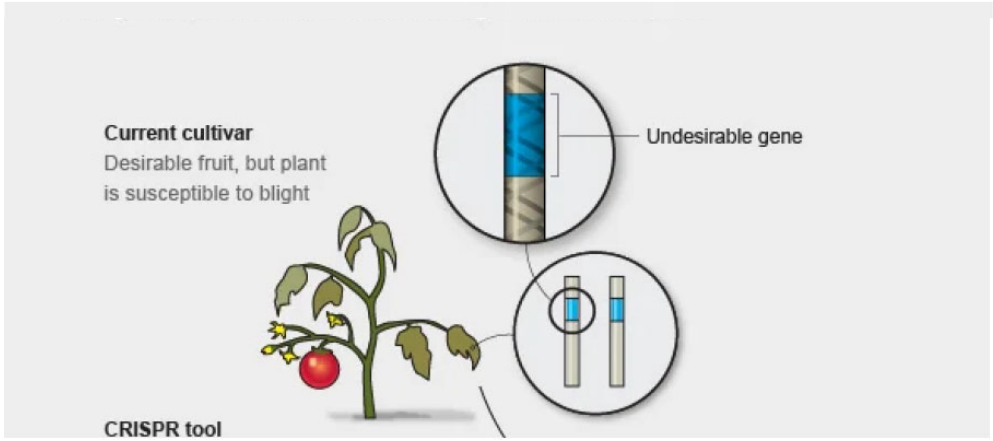


Synthego 2021, CRISPR 101

A Visual Guide to Genetic Modification

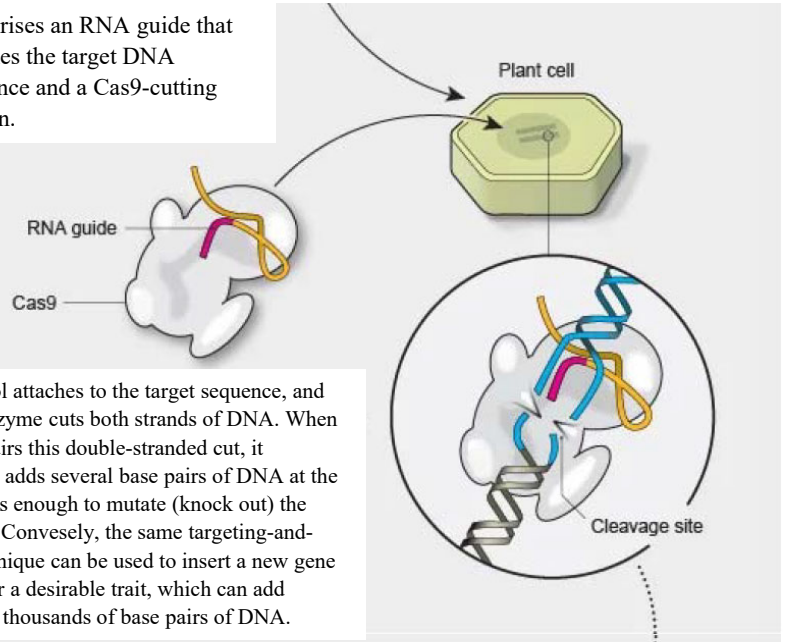
Second-Generation Gene Editing

With precision gene-editing technologies (zinc fingers, TALENs and CRISPR), biologists can target a specific gene and either deactivate it (depicted below) or replace it. A replacement gene can come from an unrelated species (transgenic) or from a related variety (cisgenic). Although CRISPR can be targeted to a specific location, its accompanying Cas9 enzyme occasionally makes unprogrammed, "off-target" cuts; limited data indicate that off-target cuts are rare in plants.



Montanez 2016, A visual guide to genetic modification

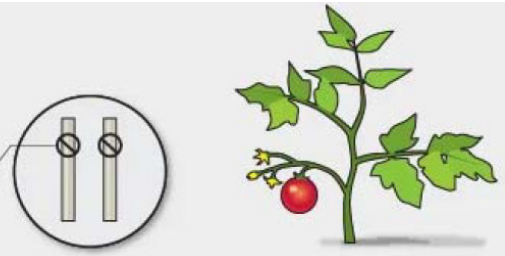
Comprises an RNA guide that matches the target DNA sequence and a Cas9-cutting protein.



CRISPR tool attaches to the target sequence, and the Cas9 enzyme cuts both strands of DNA. When the cell repairs this double-stranded cut, it accidentally adds several base pairs of DNA at the site, which is enough to mutate (knock out) the entire gene. Convesely, the same targeting-and-cutting technique can be used to insert a new gene encoding for a desirable trait, which can add hundreds or thousands of base pairs of DNA.

Cells containing the modified DNA divide, then regenerate into plantlets

Engineered plant
Blight-resistant plant with desirable fruit

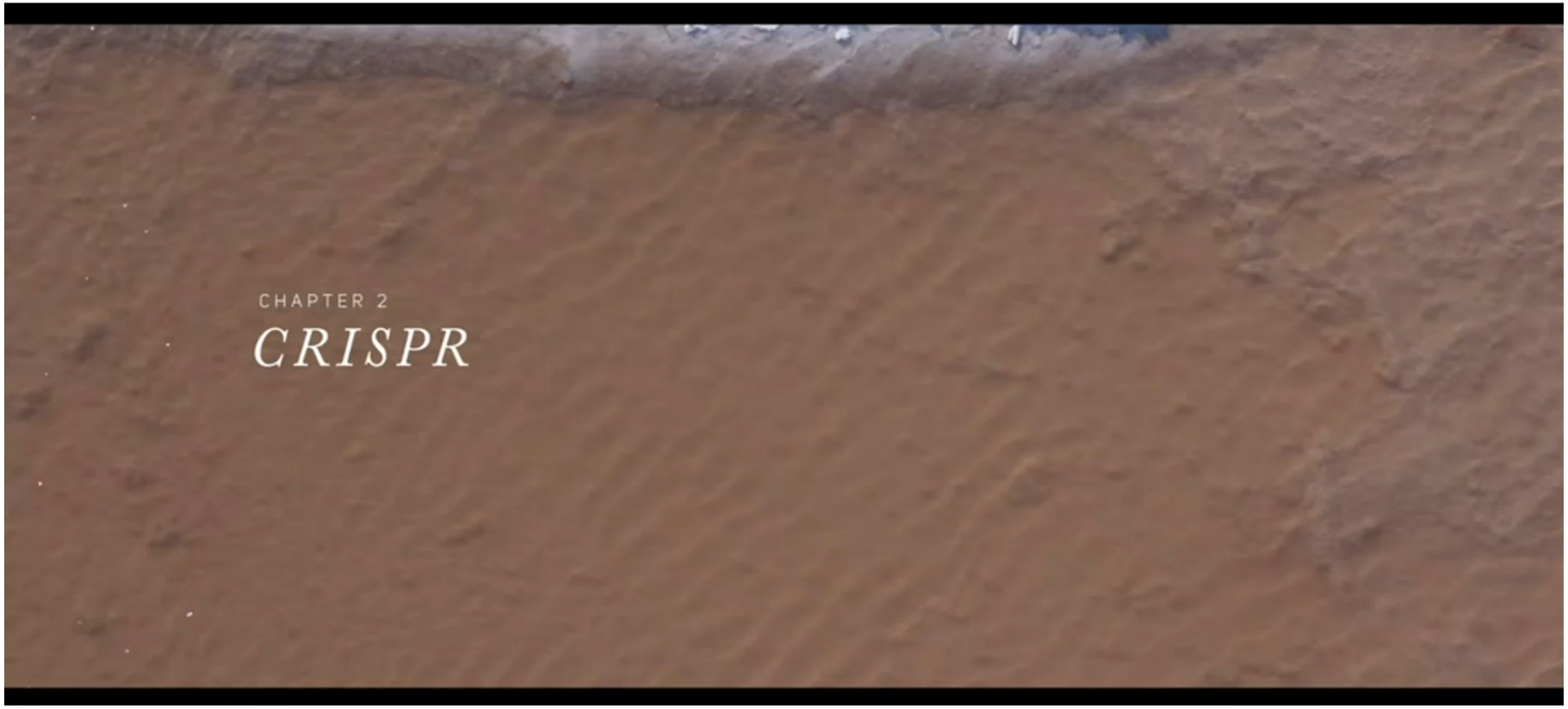


Disabled undesirable gene

Graphic by Jen Christiansen

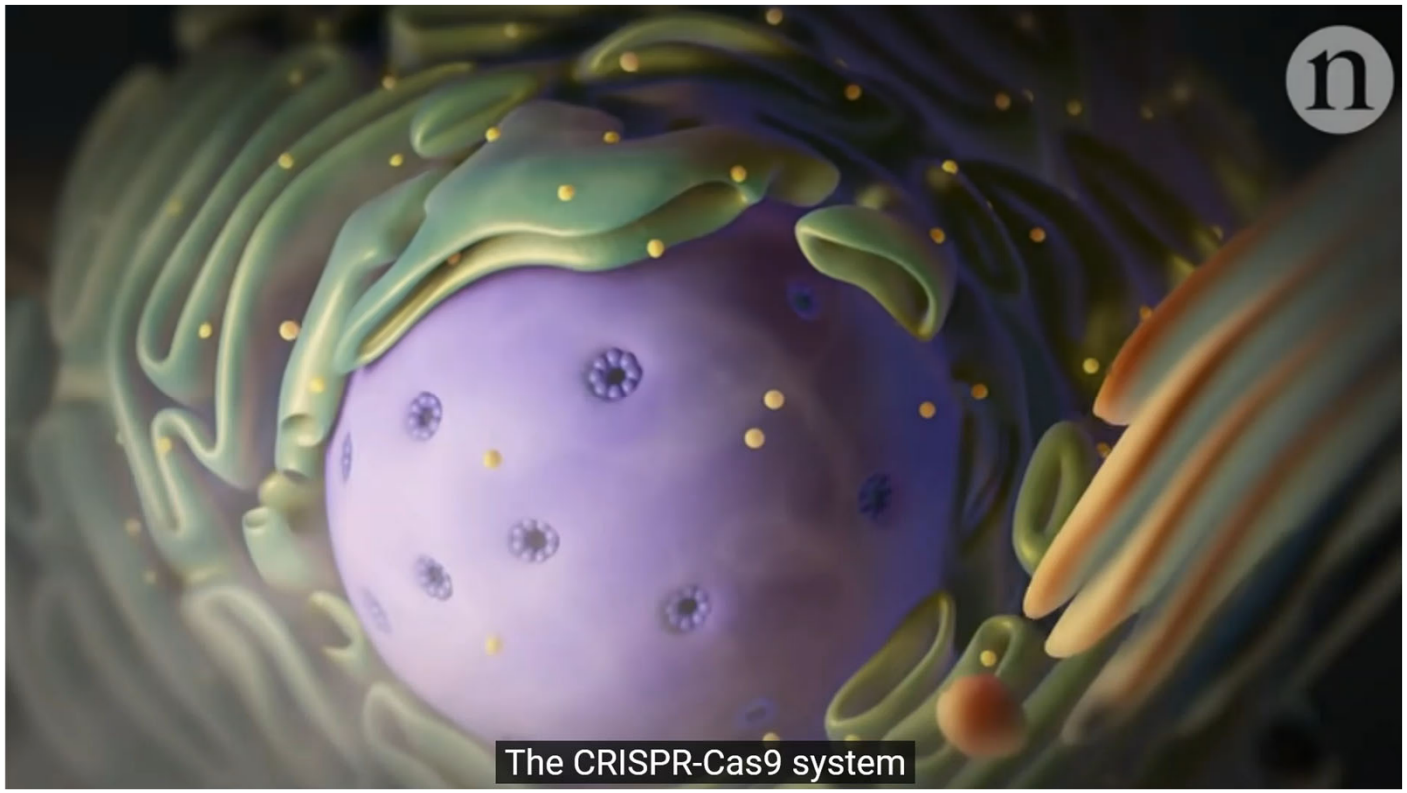
What is CRISPR?

(Human Nature. Netflix)



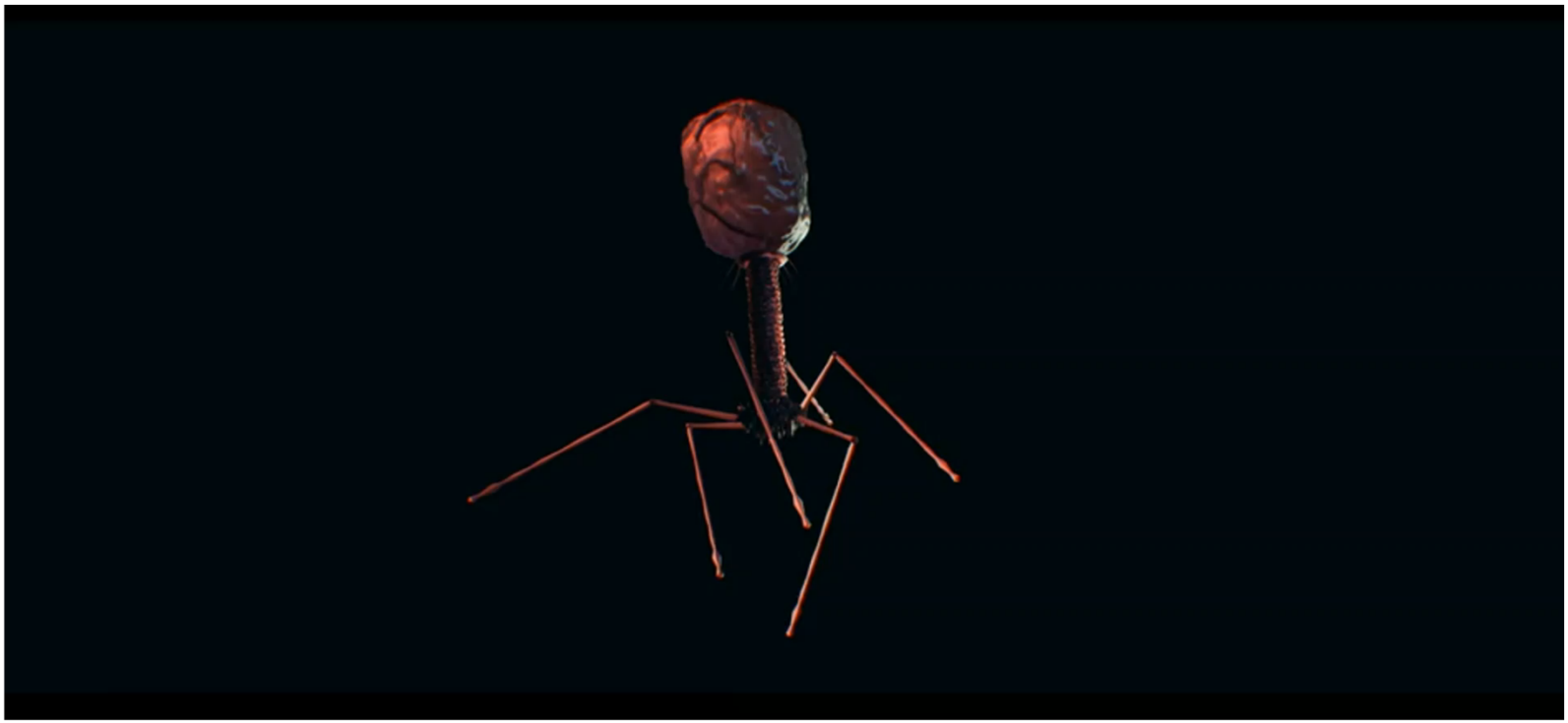
1993: Francisco Mojica discovered Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
2007: CRISPR's function is related to prokaryotic immunity
2012: The CRISPR-Cas9 bacterial immune system could be repurposed as a gene editing tool
2020: Nobel Prize in Chemistry to Dr. Jennifer Doudna and Dr. Emmanuel Charpentier

CRISPR: Gene Editing



CRISPR: Gene editing and beyond
https://www.youtube.com/watch?v=4YKFw2KZA5o

How CRISPR was found?



Human Nature by Netflix

CRISPR's Potential

Human Nature by Netflix

Programmable CRISPR-Responsive Smart Materials

Stimuli-responsive materials activated by biological signals play an increasingly important role in biotechnology applications. We exploit the programmability of CRISPR-associated nucleases to actuate hydrogels containing DNA as a structural element or as an anchor for pendant groups. After activation by guide RNA-defined inputs, Cas12a cleaves DNA in the gels, thereby converting biological information into changes in material properties.

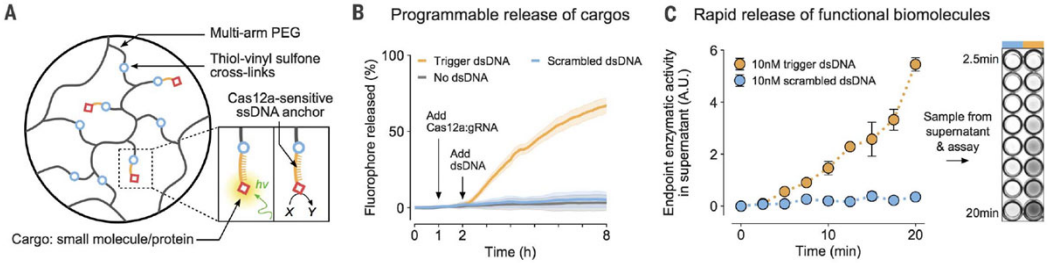
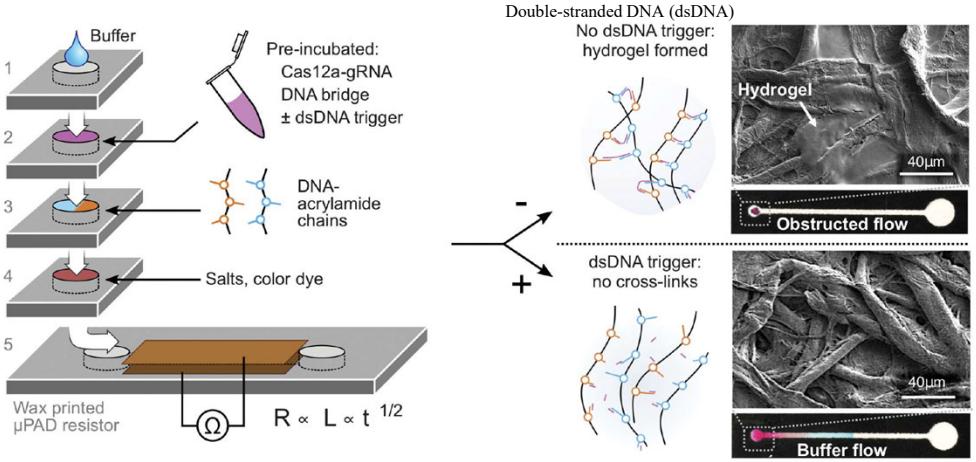


Fig. 1. Cas12a-mediated release of small molecules and enzymes from PEG hydrogels. (A) ssDNA acts as a cleavable linker for attaching payloads to an inert PEG matrix. $h\nu$, light energy. (B) Release of a tethered fluorophore by Cas12a is initiated only upon introduction of a specific dsDNA trigger and not a scrambled dsDNA control sequence. (C) Functional enzymes can be anchored into the hydrogel and released by Cas12a in sufficient quantities for visual detection in an HRP activity assay within minutes. A.U., arbitrary units.



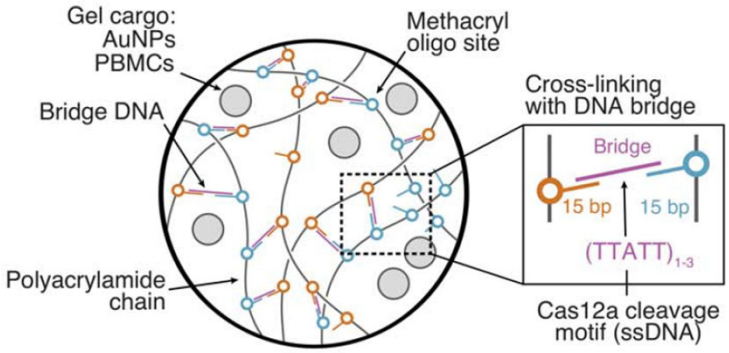
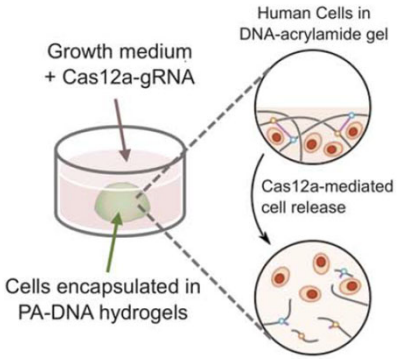


Fig. 2. Programmable release of NPs and live cells from PA-DNA hydrogels. (A) ssDNA bridges lock DNA-functionalized PA chains into a 3D network.



Sequence-specific degradation of PA-DNA gels leads to the release of encapsulated nonadherent PBMCs.

Fig. 4. Cas12a digestion of hydrogel precursors modulates permeability of a paper-based microfluidic device (mPAD) with dual visual and electronic readouts for diagnostic applications. (A) Schematic of the stackable mPAD design modified for operation with CRISPR gels and electrical readout. Layers 1 to 4 contain hydrophilic regions that form a continuous channel on folding and feed into a lateral flow channel in layer 5. The channel in layer 5 was covered with conductive tape to measure conductivity as a function of buffer wicking. In the presence of target trigger, Cas12a cleavages the DNA linker, preventing hydrogel cross-linking in the channel and enabling flow. The inset shows SEM images of channels with (top) and without (bottom) cross-linked hydrogel.

English 2019, Programmable CRISPR-responsive smart materials

CRISPR for Plant Protection

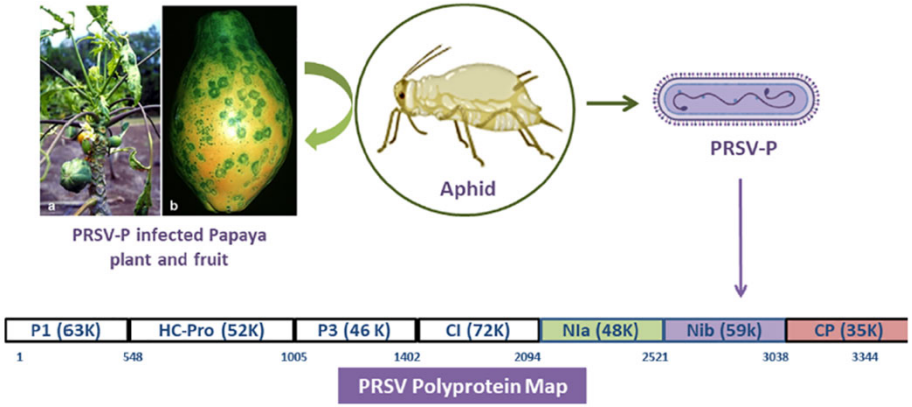


Figure 3. Schematic representation of CRISPR-Cas9 system-mediated immunization to PRSV NIa/Nib gene silencing in papaya.

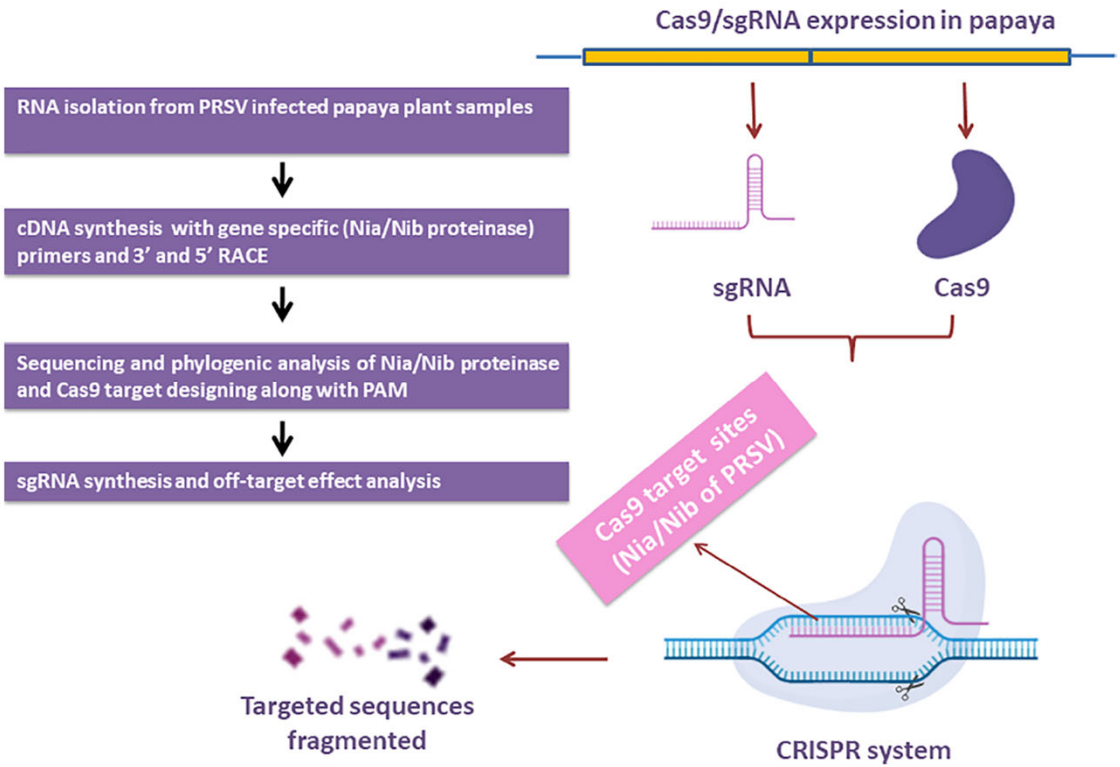
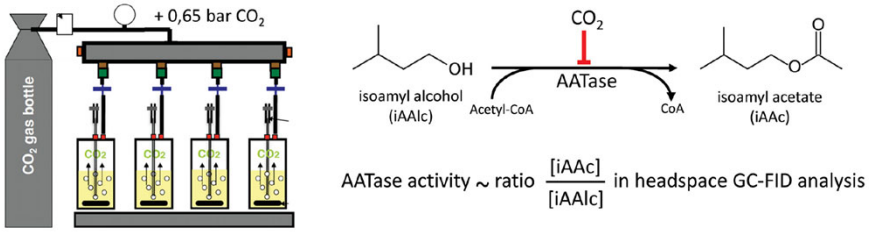


Figure 4. Papaya ringspot virus infection symptoms and transformation and the PRSV polyprotein gene map.

Mujtaba 2021, Nanocarrier-mediated delivery of miRNA, RNAi, and CRISPR-Cas for plant protection

CRISPR/Cas9 and Beer

ABSTRACT The introduction in modern breweries of tall cylindroconical fermentors, replacing the traditional open fermentation vats, unexpectedly revealed strong inhibition of flavor production by the high CO_2 pressure in the fermentors. We have screened our collection of *Saccharomyces cerevisiae* strains for strains displaying elevated tolerance to inhibition of flavor production by +0.65 bar CO_2, using a laboratory scale CO_2 pressurized fermentation system. We focused on the production of isoamyl acetate, a highly desirable flavor compound conferring fruity banana flavor in beer and other alcoholic beverages, from its precursor isoamyl alcohol (IAAc/Alc ratio). We selected the most tolerant *Saccharomyces cerevisiae* strain, saké yeast Kyokai no. 1, isolated a stable haploid segregant seg63 with the same high IAAc/Alc ratio under CO_2 pressure, crossed seg63 with the unrelated inferior strain ER7A and phenotyped 185 haploid segregants, of which 28 displaying a high IAAc/Alc ratio were pooled. Mapping of Quantitative Trait Loci (QTLs) by whole-genome sequence analysis based on SNP variant frequency revealed two QTLs. In the major QTL, reciprocal hemizygosity analysis identified *MDS3* as the causative mutant gene, a putative member of the TOR signaling pathway. The $MDS3^{Seg.63}$ allele was dominant and contained a single causative point mutation, T2171C, resulting in the F274S substitution. Introduction of $MDS3^{Seg.63}$ in an industrial tetraploid lager yeast with CRISPR/Cas9 enhanced isoamyl acetate production by 145% under CO_2 pressure. This work shows the strong potential of polygenic analysis and targeted genetic modification for creation of cisgenic industrial brewer's yeast strains with specifically improved traits.



Souffriau 2022, Polygenic analysis of tolerance to carbon dioxide inhibition of isoamyl acetate "banana" flavor production in yeast reveals MDS3 as major causative gene

Scientists Just Figured Out a Way to Make Beer Taste Even Better (08 October 2022. By David Nield)

Today's tall cylindrical fermentation tanks that have replaced the shorter vats of breweries in the past have tended to negatively impact the taste of the resulting beer – but now scientists have stepped in to improve the taste of our booze. These tall tanks can produce more beer for less money – they're easier to fill, empty and clean – but their widespread adoption also means excess pressure from the carbon dioxide produced during fermentation, and that affects flavor. The researchers began by identifying strains of the *Saccharomyces cerevisiae* yeast that were particularly CO_2-resistant, focusing on the production of isoamyl acetate that gives beer its fruity, banana-like flavor. After finding a particularly robust strain, the team then used a whole-genome sequence analysis to figure out what made it so adept at being able to keep its fruity flavor even under the pressure of modern fermentation tanks. "To our surprise, we identified a single mutation in the MDS3 gene, which codes for a regulator apparently involved in production of isoamyl acetate, the source of the banana-like flavor that was responsible for most of the pressure tolerance in this specific yeast strain," says molecular biologist Johan Thevelein, from Katholieke Universiteit Leuven in Belgium.

With this discovery, the researchers were then able to use the CRISPR/Cas9 gene editing technique to engineer the same mutation in other yeast strains. After editing, these strains could better withstand CO_2 pressure and better retain their flavor. Further down the line, many yeast strains could be modified in the same way, leading to beers with a fuller flavor when they're poured. So far, it doesn't appear that other traits of the yeast strain are affected by the genetic edits. "The mutation is the first insight into understanding the mechanism by which high carbon dioxide pressure may compromise beer flavor production," says Thevelein.

Before now, it hasn't been clear exactly how high CO_2 pressure has been having an impact on beer flavor at the molecular level, even though the end results in terms of the drop in fruitiness have been easy to taste. In the future, the researchers want to run experiments with even higher CO_2 pressures to see if different genes are identified. A number of other genes showed promise in this study too, though MDS3 was the dominant one.

The same gene identification technology has also previously been used to highlight other important traits in yeast, including the production of glycerol (a sugary alcohol that adds to the taste), and tolerance towards increased temperatures. The authors are up front about the fact the work was supported by a brewing company, which hopes to make use of the technology in a patent. While other brands of brew might miss out on the technology, the study does demonstrate the potential benefits in applying CRISPR to tweaking yeast's talents for making an exceptional drop. "This work shows the strong potential of polygenic analysis and targeted genetic modification for creation of cisgenic industrial brewer's yeast strains with specifically improved traits," write the researchers in their published paper. The research has been published in *Applied and Environmental Microbiology*.

https://www.sciencealert.com/scientists-just-figured-out-a-way-to-make-beer-taste-even-better

7 Medical Breakthroughs That Gave Us Hope In 2023

7 medical breakthroughs that gave us hope in 2023 **(**By Sanjay Mishra. December 6, 2023)

COVID-19 has continued to claim lives in 2023, killing more than 50 thousand patients in the United States alone and bringing the global death toll to almost seven million people. The pandemic has also created an epidemic of survivors who continue to suffer from long COVID. But it wasn't all bad news in 2023. With more people becoming immune against the virus, the World Health Organization decided, on May 5, that COVID-19 no longer constitutes a public health emergency of international concern. Updated boosters of existing vaccines helped reduce the number of cases, hospitalizations, and deaths, and a new COVID vaccine from Novavax was approved this year.
Aside from COVID-19 vaccines, there were many other interesting and groundbreaking discoveries made this year, some of which are especially notable for their potential impact on health and medicine.

1. The world's first CRISPR-based gene therapy becomes available
The world's first CRISPR-based gene therapy was approved by the drug regulators in the United Kingdom. It treats sickle cell disease and beta thalassemia, genetic disorders that affect the red blood cells. Hemoglobin, found in red blood cells, carries oxygen around the body. The errors in hemoglobin genes create fragile red blood cells that cause a shortage of oxygen in the body, a condition known as anemia. Patients with sickle cell disease also suffer from infections and severe pain when sickled cells form clots and impede blood flow, while patients with beta thalassemia must receive blood transfusion every three to four weeks. The newly approved gene therapy, named CASGEVY, corrects faulty hemoglobin genes in a patient's bone marrow stem cells so they can produce functioning hemoglobin. A patient's stem cells are harvested from their bone marrow, edited in a laboratory, and then infused back into the patient. A single treatment can potentially cure some patients for life. Two inventors who fine-tuned CRISPR (short for "clustered regularly interspaced short palindromic repeats") to work as a precise gene-editing tool, Emmanuelle Charpentier and Jennifer Doudna, were awarded the Nobel Prize in Chemistry just three years ago in 2020. This is just the first of dozens of potential treatments in development to treat other genetic diseases, cancer, or even infertility.

2. The first drug that slows down Alzheimer's disease gets approved
The U.S. Food and Drug Administration approved the first drug for Alzheimer's that targets one underlying cause of the disease. While the drug, Leqembi, isn't a cure or improve symptoms in late-stage disease, after 18 months of treatment it slows declines in memory and thinking by about 30 percent if the medicine is given in the early stage of disease. Leqembi is a monoclonal antibody that works by targeting amyloid plaques in the brain that are a defining feature of Alzheimer's disease. When abnormal levels of a naturally occurring protein, called beta amyloid, clump together to form sticky plaques in brain, they trigger inflammation and damage neuronal connections. Accumulation of amyloid plaques leads to loss of memory and thinking causing Alzheimer's disease. Clinical trials indicate that Leqembi removes amyloid plaques from the brain, which slows the progression of the disease.

3. Researchers produce healthy mice pups from two fathers; no female required
Yes, you read that right. Researchers from Japan presented evidence at a scientific conference that it is possible to produce healthy, fertile mice without an egg from a female mouse. First, eggs were made from the stem cells derived from the skin cells of a male mouse. These eggs were fertilized with sperm of another male and then the fertilized egg was transferred into a female mouse where it grew and matured. Although just seven out of more than 600 implanted embryos developed into baby mice, the pups grew normally and were fertile as adults. It is not yet known if the mouse pups will develop exactly like those born through conventional breeding. These findings have not yet been published in a peer reviewed journal and similar preliminary steps have so far failed in humans.

4. Scientists map all the connections in an insect brain
Scientists have produced the first complete brain-wiring diagram of an insect brain. This may not sound impressive but the brain, even that of a fruit fly, contains vast networks of interconnected neurons called the connectome. Until now, only the brains of a roundworm, a sea squirt, and a marine worm have been completely mapped; each of which contains just a couple of hundred connections. But a complete map of the connectome of a fruit fly larva reveals it contains more than 3,000 neurons and more than half a million connections between them. Developing this map took an international team of scientists more than five years. Although a fruit fly brain is much simpler than that of humans, the techniques developed will help map more complex brains in the future. The neural circuits In the fruit fly brain look similar to neural networks used in machine learning. Understanding the similarities and complexities of the fly brain connectome can help to decipher how the human brain works and how neurological diseases develop. It can also lead to the development of new machine learning methods and more efficient artificial intelligence systems.

5. Pigment-producing cells get "stuck" causing gray hairs
Scientists show that when pigment-producing cells, called melanocytes, get stuck in an immature state, they fail to develop their blonde, brown, red, or black, hair color. This arrested state leads to graying hairs. New hair grows from follicles, found in the skin, where melanocytes also reside. The scientists at New York University observed single melanocyte stem cells migrate up and down the individual hair folicle of mice over two years. To their surprise, they found that melanocyte stem cells can switch back and forth from gray immature stem cells to mature colored cells as they traverse up and down during the life cycle of the hair. But as hair ages, the melanocyte stem cells get sluggish after multiple cycles and become trapped near the base of the hair as immature melanocytes. With no pigment being produced, the hair turns gray.

6. Bacteria shown to help cancer cells spread more aggressively
Scientists have found that some bacteria that are frequently found in many gastrointestinal tract tumors directly help cancer cells evade the body's immune response. Not only do these bacteria cooperate with tumor cells to promote cancer progression, they also help them spread more rapidly by breaking down anticancer drugs and causing the treatment to fail. This research suggests that some anticancer drugs are effective because they also kill the tumor dwelling bacteria. Understanding how the tumor's microenvironment affects its survival and progression can open new doors of treating cancer.

7. AI identifies people at the highest risk of pancreatic cancer
A new artificial intelligence (AI) tool can predict pancreatic cancer up to three years before actual diagnosis, by identifying specific patterns of conditions that occurred in patients' health records. Pancreatic cancer is rare but it is the third largest cause of cancer-related deaths. It is so deadly because it is generally detected in the late stages when the disease has already spread to other areas of body. Symptoms of early stage pancreatic cancer are easily misdiagnosed, but many patients could live longer if the cancer was detected early. That led scientists to train an AI algorithm on the medical records of 6.2 million people from Denmark spanning 41 years to detect the patterns hidden in the records of 24,000 patients who later developed pancreatic cancer. In the medical records, each disease is recorded with a code. The AI model analyzed the combinations of these disease codes and the timing of their occurrence. By comparing specific sequences of conditions that preceded a diagnosis of pancreatic cancer, the AI model learned to identify those at greatest risk for the disease. The scientists then tested the AI tool by analyzing the records of nearly 3 million U.S. veterans spanning 21 years. The computer algorithm correctly identified almost 4,000 individuals, up to three years before they were actually diagnosed with pancreatic cancer. The study shows that AI models can be as accurate as genetic testing in predicting the risk of pancreatic cancer. Because pancreatic cancer is so rare, genetic screening is currently recommended only for high risk individuals, or with those with a family history of the disease.

FDA OKs First Two Gene-Editing Therapies for Sickle Cell Disease

On December 08, 2023, the US Food and Drug Administration (FDA) approved two gene-editing treatments for patients aged 12 years or older with severe sickle cell disease. These "milestone treatments" mark the first cell-based gene therapies for this debilitating and potentially life-threatening blood disorder that affects about 100,000 people in the US.
One therapy - exagamglogene autotemcel or exa-cel (Casgevy) from Vertex Pharmaceuticals and Crispr Therapeutics — is the first to use the gene-editing tool CRISPR. The other - lovotibeglogene autotemcel or lovo-cel (Lyfgenia) from bluebird bio — uses a different gene-editing tool called a lentiviral vector. "The approval of the first gene therapies for [sickle cell disease] represents a tremendous step forward for the [sickle cell] community, which has been historically overlooked and underfunded," said Robert A. Brodsky, of Johns Hopkins University School of Medicine, in a statement from the American Society of Hematology, following the approval. "We are excited to advance the field, especially for individuals whose lives have been severely disrupted by the disease, by approving two cell-based gene therapies today," added Nicole Verdun, MD, of the FDA's Center for Biologics Evaluation and Research, in an agency press release. Sickle cell disease involves a mutation in hemoglobin, a protein in red blood cells that provides oxygen to tissues. The mutation leads red blood cells to develop a crescent or sickle shape, which can restrict blood flow and cause severe pain and organ damage, known as vaso-occlusive events or crises. Treatment options prior to these approvals primarily included red blood transfusions and hydroxyurea alongside pain management. The only potential curative option has been allogeneic hematopoietic stem cell transplantation, but that comes with significant risks and most patients don't have an appropriate donor.

Exa-cel

Exa-cel uses CRISPR gene-editing technology. Before the infusion, patients undergo myeloablative conditioning, which removes cells from the bone marrow. These cells are genetically modified to produce fetal hemoglobin. Patients then receive an infusion of the edited cells, which can help restore normal hemoglobin production. The FDA approval was based on data from the pivotal CLIMB SCD-121 trial. In an October advisory committee meeting, the FDA highlighted trial data demonstrating that 29 of 31 patients reached the trial's primary endpoint: freedom from severe vaso-occlusive crises over a 12-month period. In addition, 28 of these patients remained free of vaso-occlusive crises for almost 2 years. The committee noted that one of the 31 patients died about 9 months after receiving an exa-cel infusion. The cell-based gene therapy also increased both fetal and total hemoglobin, with total hemoglobin levels increasing to > 11 g/dL by month 3 and remaining at that level afterward. No patients experienced graft failure or rejection. The most common side effects included low platelets and white blood cell counts, mouth sores, nausea, musculoskeletal pain, vomiting, and febrile neutropenia. Exa-cel could "provide a one-time functional cure" for patients with severe sickle cell disease, according to Franco Locatelli, MD, of Sapienza University of Rome, who presented initial findings last year. While the current approval is for patients with infusion-dependent sickle cell disease, exa-cel is also being evaluated in patients with another blood disorder, beta-thalassemia.

Lovo-cel

Lovo-cel, a cell-based gene therapy, uses a different technology — a lentiviral vector, or gene delivery vehicle — that can also genetically modify a patient's blood stem cells. Like exa-cel, lovo-cel is a one-time, single-dose infusion that contains the patient's modified cells. Before the infusion, patients undergo myeloablative conditioning. The patient's stem cells are then genetically modified to allow them to produce the most common form of hemoglobin, HbA This approval was based on data from a single-arm, 24-month study in patients aged 12-50 years who had sickle cell disease and a history of vaso-occlusive events. Overall, 88% of patients (28 of 32) achieved complete resolution of vaso-occlusive events 6-18 months after the infusion. The most common side effects included stomatitis; febrile neutropenia; and low platelet, white blood cell, and red blood cell counts. The FDA noted that hematologic cancer has occurred in patients treated with lovo-cel, and the label includes a black-box warning about the risk. Brodsky noted, however, that "while these new gene therapies are potentially life-changing for individuals living with [sickle cell disease], they must be accessible to be effective." **Access is a potential concern. Exa-cel and lovo-cel could cost about $2 million.**

Victoria Stern. https://www.medscape.com/viewarticle/fda-oks-first-two-gene-editing-therapies-sickle-cell-disease-2023a1000uqp?ecd=WNL_trdalrt_pos1_231208_etid6139049&uac=70212FJ&impID=6139049
See also, https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapies-treat-patients-sickle-cell-disease

CRISPR Gene Therapy

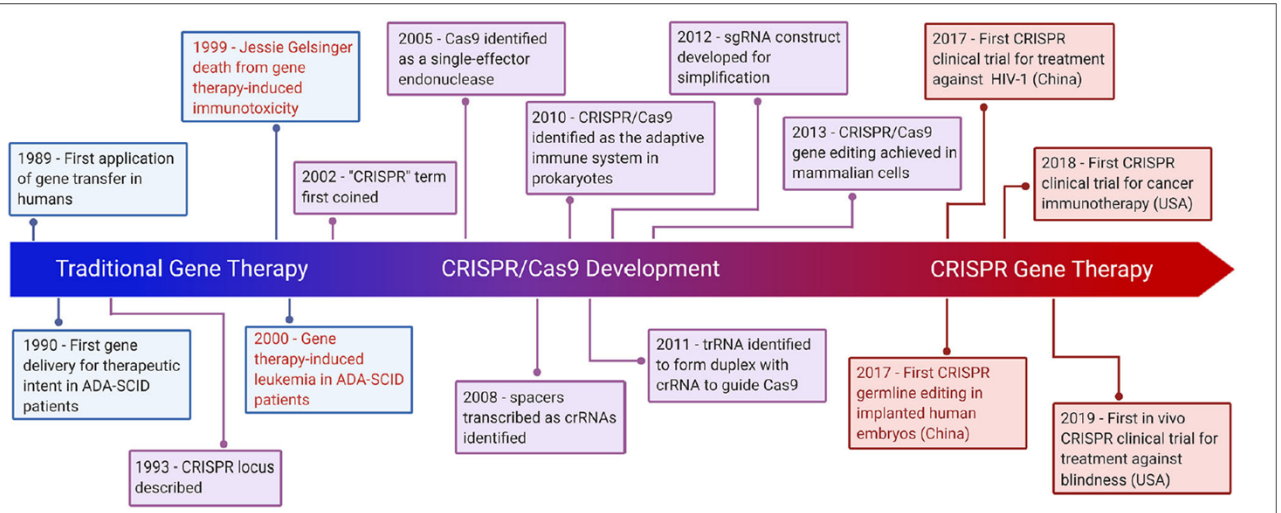


FIGURE 1 | Hallmarks of CRISPR Gene Therapy. Timeline highlighting major events of traditional gene therapy, CRISPR development, and CRISPR gene therapy. The text in red denotes gene therapy events which have raised significant ethical concerns.

LIMITATIONS AND ADVANCEMENTS OF CRISPR/Cas9

1. Off-Target Effects (OTEs)
A major concern for implementing CRISPR/Cas9 for gene therapy is the relatively high frequency of OTEs, which have been observed at a frequency of ≥50%.

2. Protospacer Adjacent Motif Requirement
Cas9 from the bacteria Streptococcus pyogenes (SpCas9) is one of the most extensively used Cas9s. However, SpCas9 is relatively large and difficult to package into AAV vectors, the most common delivery vehicle for gene therapy.

3. DNA-Damage Toxicity
CRISPR-induced DSBs often trigger apoptosis rather than the intended gene edit.

4. Immunotoxicity
In addition to technical limitations, CRISPR/Cas9, like traditional gene therapy, still raises concerns for immunogenic toxicity.

5. Precision Gene Editing With CRISPR
Precise-genome editing is essential for prospects of CRISPR gene therapy. Its low efficiency renders its utility for precise gene editing for clinical intervention highly limiting,

6. Delivery of CRISPR Gene Therapy
The delivery modality of CRISPR tools greatly influences its safety and therapeutic efficacy.

Uddin 2020, CRISPR gene therapy- Applications, limitations, and implications for the future

Delivery of CRISPR Gene Therapy

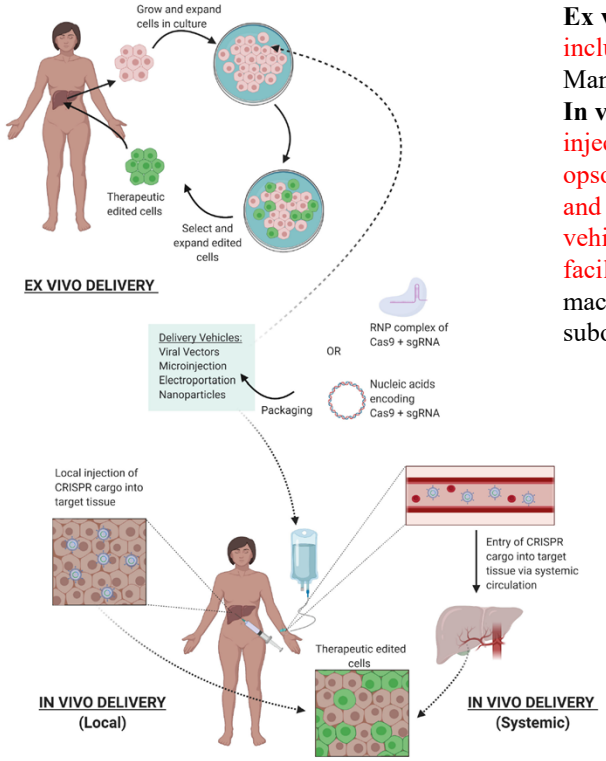


FIGURE 4 | Delivery of CRISPR Therapy. Nucleic acids encoding CRISPR/Cas9 or its RNP complex can be packaged into delivery vehicles. Once packaged, edits can be facilitated either ex vivo or in vivo. Ex vivo editing involves extraction of target cells from the patient, cell culture, and expansion in vitro, delivery of the CRISPR components to yield the desired edits, selection, and expansion of edited cells, and finally reintroduction of therapeutic edited cells into the patient. In vivo editing can be systemically delivered via intravenous infusions to the patient, where the CRISPR cargo travels through the bloodstream via arteries leading to the target tissue, or locally delivered with injections directly to target tissue. Once delivered, the edits are facilitated in vivo to provide therapeutic benefit.

Uddin 2020, CRISPR gene therapy- Applications, limitations, and implications for the future

Ex vivo delivery: Greater safety, technical feasibility, and tighter quality control of the edited cells. However, challenges to this method include survival and retention of in vivo function of cells outside the patient after genetic manipulation and extensive culture in vitro. Many tissue types are not suited for this method, severely limiting its therapeutic utility for other genetic diseases.

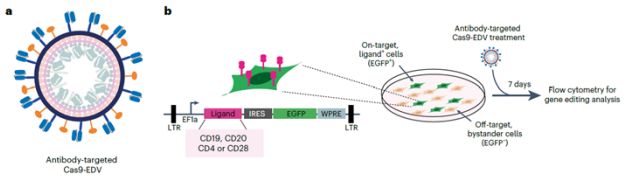
In vivo delivery: **CRISPR components can be delivered in vivo systemically through intravenous injections** or can be locally injected to specific tissues. However, challenges of in vivo delivery include degradation by circulating proteases or nucleases, opsonization by opsonins, or clearance by the mononuclear phagocyte system (MPS). Furthermore, the cargo must reach the target tissue and bypass the vascular endothelium, which are often tightly connected by cell-cell junctions, preventing accessibility to larger delivery vehicles (>1 nm diameter). Additionally, once the cargo has reached the target cells, they must be internalized, which is generally facilitated through endocytosis where they can be transported and degraded by lysosomal enzymes. In addition, localization of the editing machinery near the point of injection can result in uneven distribution of the edited cell repertoire within the tissue, which may result in suboptimal therapeutic outcomes. (Uddin 2020, CRISPR gene therapy- Applications, limitations, and implications for the future)

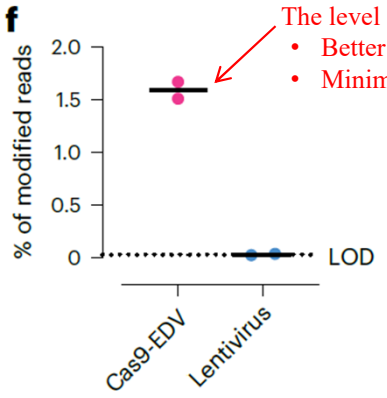
FDA OKs First Two CRISPR Gene-Editing Therapies for Sickle Cell Disease: Exa-cel & Lovo-cel ($2 million cost)
https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapies-treat-patients-sickle-cell-disease

Jennifer R. Hamilton[1,2,9], Evelyn Chen[1,2,9], Barbara S. Perez[1,2], Cindy R. Sandoval Espinoza[1,2], Min Hyung Kang[1,2], Marena Trinidad[1,2], Wayne Ngo[3,4] & Jennifer A. Doudna[1,2,3,4,5,6,7,8]

Viruses and virally derived particles have the intrinsic capacity to deliver molecules to cells, but the difficulty of readily altering cell-type selectivity has hindered their use for therapeutic delivery. Here, we show that cell surface marker recognition by antibody fragments displayed on membrane-derived particles encapsulating CRISPR–Cas9 protein and guide RNA can deliver genome editing tools to specific cells. Compared to conventional vectors like adeno-associated virus that rely on evolved capsid tropisms to deliver virally encoded cargo, these Cas9-packaging enveloped delivery vehicles (Cas9-EDVs) leverage predictable antibody–antigen interactions to transiently deliver genome editing machinery selectively to cells of interest. Antibody-targeted Cas9-EDVs preferentially confer genome editing in cognate target cells over bystander cells in mixed populations, both ex vivo and in vivo. By using multiplexed targeting molecules to direct delivery to human T cells, Cas9-EDVs enable the generation of genome-edited chimeric antigen receptor T cells in humanized mice, establishing a programmable delivery modality with the potential for widespread therapeutic utility.

Hamilton 2024, In vivo human T cell engineering with enveloped delivery veh





The level of editing by Cas9: **1.5%**
- Better delivery
- Minimized off-target effects

Fig. 4. CAR-expressing human T cells are detectable in the spleens of PBMC-humanized mice 10 days post administration of 1.5×10^9 Cas9-EDV (n = 2 animals) or lentivirus (n = 3 animals) but not in mice administered PBS (n = 3).

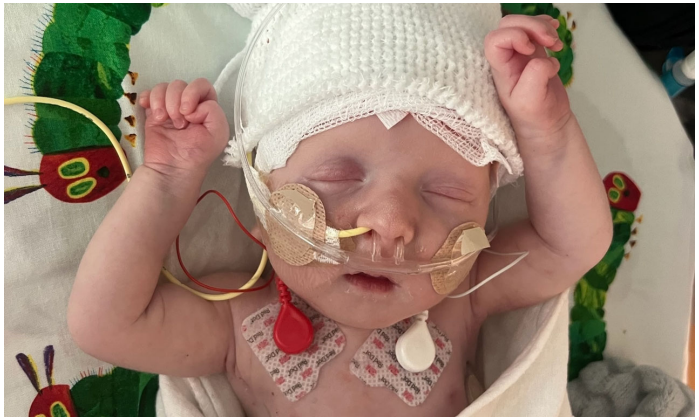
Personalized CRISPR Gene Editing Therapy

World's First Patient Treated with Personalized CRISPR Gene Editing Therapy at Children's Hospital of Philadelphia. May 15, 2025.

"Years and years of progress in gene editing and collaboration between researchers and clinicians made this moment possible, and while KJ is just one patient, we hope he is the first of many to benefit from a methodology that can be scaled to fit an individual patient's needs," said Rebecca Ahrens-Nicklas, MD, PhD, director of the Gene Therapy for Inherited Metabolic Disorders Frontier Program (GTIMD) at Children's Hospital of Philadelphia and an assistant professor of Pediatrics in the Perelman School of Medicine at the University of Pennsylvania.

CRISPR (clustered regularly interspaced short palindromic repeats)-based gene editing can precisely correct disease-causing variants in the human genome. Gene editing tools are incredibly complex and nuanced, and up to this point, researchers have built them to target more common diseases that affect tens or hundreds of thousands of patients, such as the two diseases for which there currently are U.S. Food and Drug Administration-approved therapies, sickle cell disease and beta thalassemia. However, relatively few diseases benefit from a "one-size-fits-all" gene editing approach since so many disease-causing variants exist. Even as the field advances, many patients with rare genetic diseases – collectively impacting millions of patients worldwide – have been left behind.

https://www.chop.edu/news/worlds-first-patient-treated-personalized-crispr-gene-editing-therapy-childrens-hospital



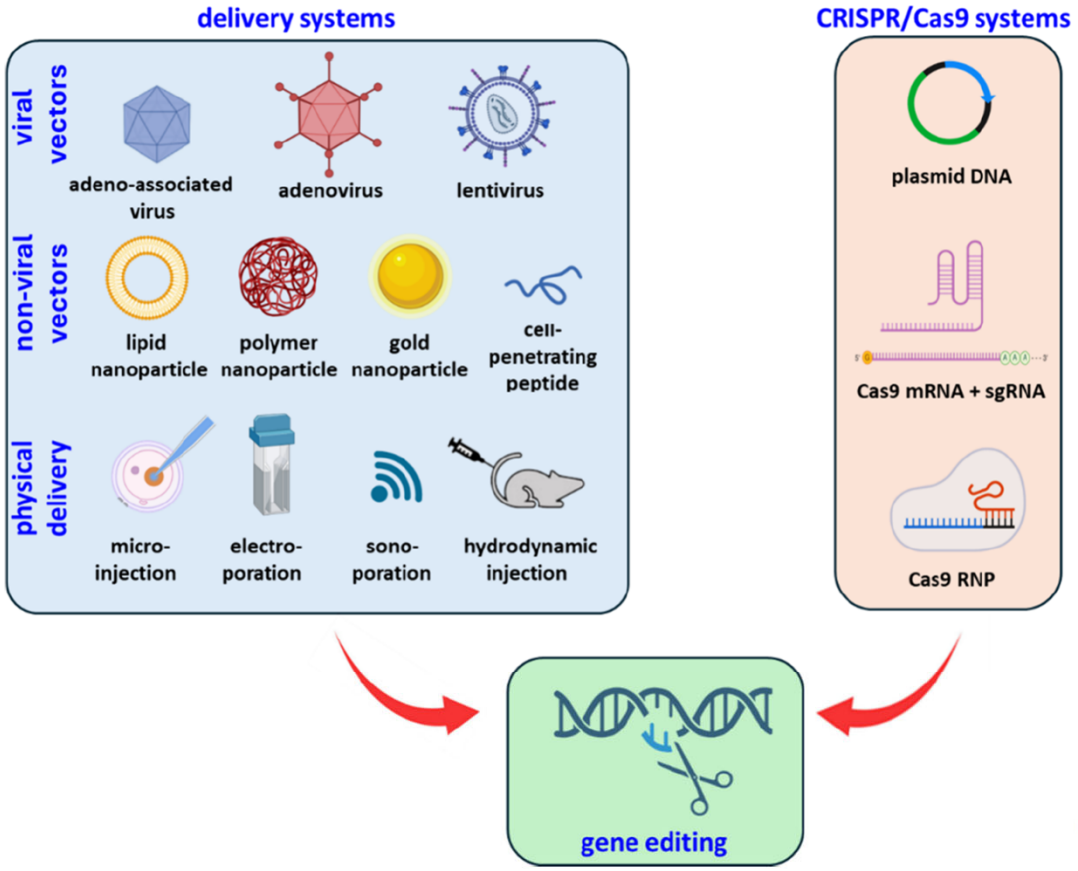
KJ was only days old when he was diagnosed with a rare metabolic disorder and transferred to Children's Hospital of Philadelphia where doctors were actively researching new cell and gene therapies.

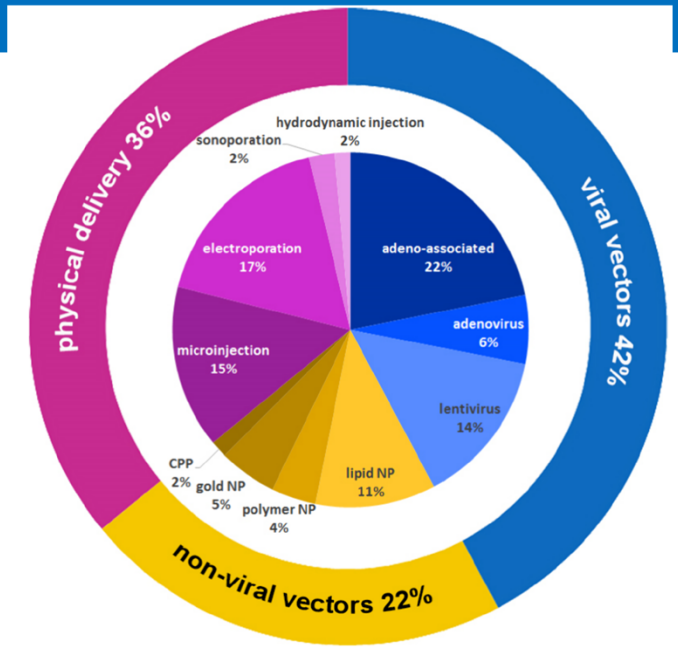
Patient-Specific In Vivo Gene Editing to Treat a Rare Genetic Disease

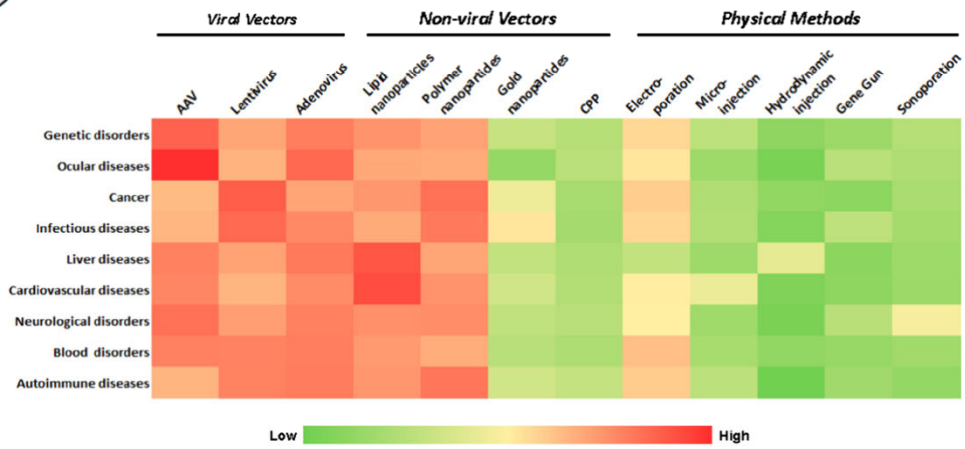
Base editors can correct disease-causing genetic variants. After a neonate had received a diagnosis of severe carbamoyl-phosphate synthetase 1 deficiency, a disease with an estimated 50% mortality in early infancy, we immediately began to develop a customized lipid nanoparticle–delivered base-editing therapy. After regulatory approval had been obtained for the therapy, the patient received two infusions at approximately 7 and 8 months of age. In the 7 weeks after the initial infusion, the patient was able to receive an increased amount of dietary protein and a reduced dose of a nitrogen-scavenger medication to half the starting dose, without unacceptable adverse events and despite viral illnesses. No serious adverse events occurred. Longer follow-up is warranted to assess safety and efficacy. (Funded by the National Institutes of Health and others.)

Kiran Musunuru et al. N, Engl, J, Med, 392(22):2235-2243, 2025.

CRISPR: Delivery Systems







Schematic representation of the various CRISPR/CAS9 delivery systems.

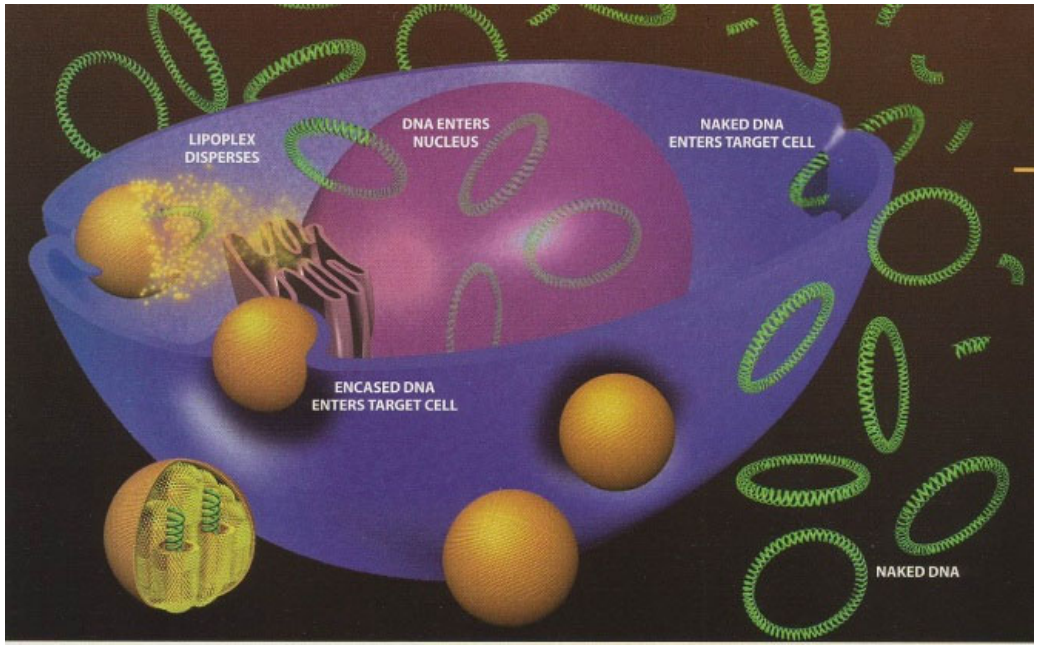
Iyer 2025, CRISPR technology- Transforming the future of medicine and diagnostics

Delivery of DNA and RNA

DNA Delivery

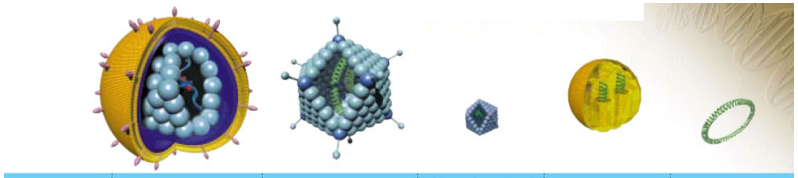
Instead of making bioactive proteins, DNA itself can be administered to have desired pharmacological effects. This is frequently called 'gene delivery' or 'gene therapy'. But delivery of naked DNAs is not easy, because of their large size and highly charged (and thus, highly hydrophilic) nature. The lack of proper delivery system is the major hurdle.

Usually, viral vectors are used for their effectiveness in gene delivery, but they are also dangerous with their own drawbacks. Thus, non-viral vectors, such as lipoplex and triplex, have been developed. They are safer, but the effectiveness is lower.



Gene Delivery Vectors

Using DNAs and genes as an active pharmaceutical ingredient (API) has a practical problem of delivering it to the target cells. DNAs and genes are very large molecules and are charged. This makes delivery through the cell membrane very difficult, as cell membranes are made of bilayers of lipid molecules. This is why various delivery systems (called vectors) are used to transport DNAs and genes through the cell membrane.



	Retroviruses	Adenoviruses	Adeno-Associated Viruses	Liposomes	"Naked" DNA
Some Potential Advantages	Integrate genes into host chromosomes, offering chance for long-term stability	Most do not cause serious disease; large capacity for foreign genes	Integrate genes into host chromosomes; cause no known human diseases	Have no viral genes, so do not cause disease	Same as for lipo-somes; expected to be useful for vaccination
Some Drawbacks of Existing Vectors	Genes integrate ran-domly, so might disrupt host genes; many infect only dividing cells	Genes may function tran-siently, owing to lack of integration or to attack by the immune system	Small capacity for foreign genes	Less efficient than viruses at transfer-ring genes to cells	Inefficient at gene transfer; unstable in most tissues of the body

Friedmann 1997, Overcoming the obstacles. Sci. Am. June 1997, p. 96.

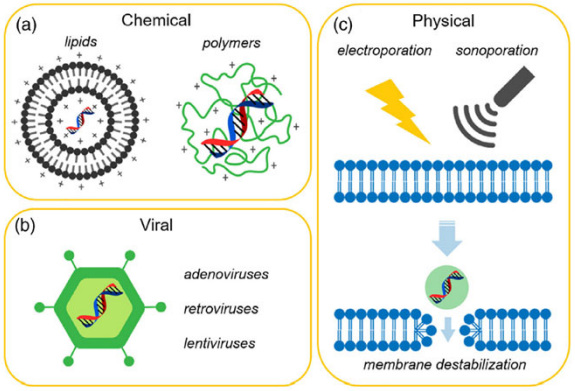


FIGURE 1. Summary of gene delivery approaches (viral, physical, and chemical): (a) chemical systems involve cationic lipids or polymers which complex negatively charged nucleic acids; (b) biological systems utilize deactivated viral vectors; and (c) physical methods, such as electroporation and sonoporation, create temporary pores in the cell membrane using electronic pulses or ultrasound.

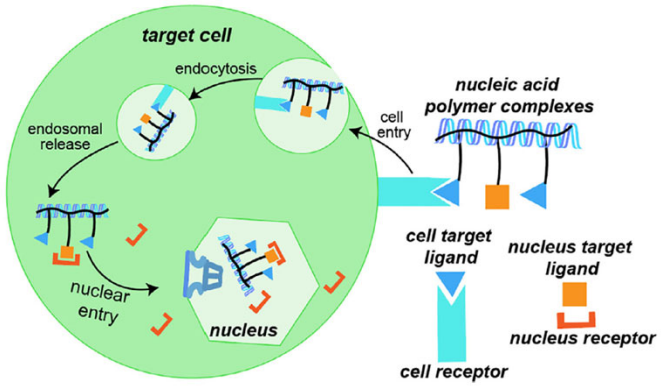


FIGURE 2. Schematic diagram of gene delivery using polyplexes, with steps including cell entry, lysosomal escape, and nuclear entry.

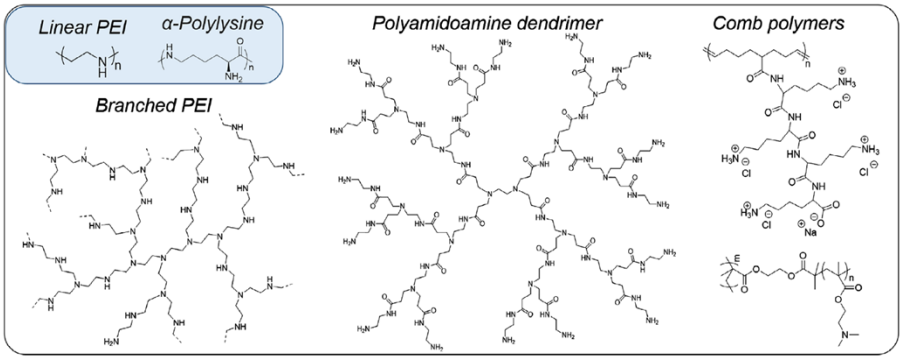


FIGURE 3. Branched PEI, poly(amidoamine) dendrimers, and comb polymers represent examples of polymer vectors with tunable nucleic acid binding and targeting capacity. Upper-left: chemical structures of linear polyethyleneimine (PEI) and poly-l lysine, two widely used polymers in gene delivery research.

Salameh 2019, Polymer-mediated gene therapy

Non-viral Vectors: Protection from Degradation

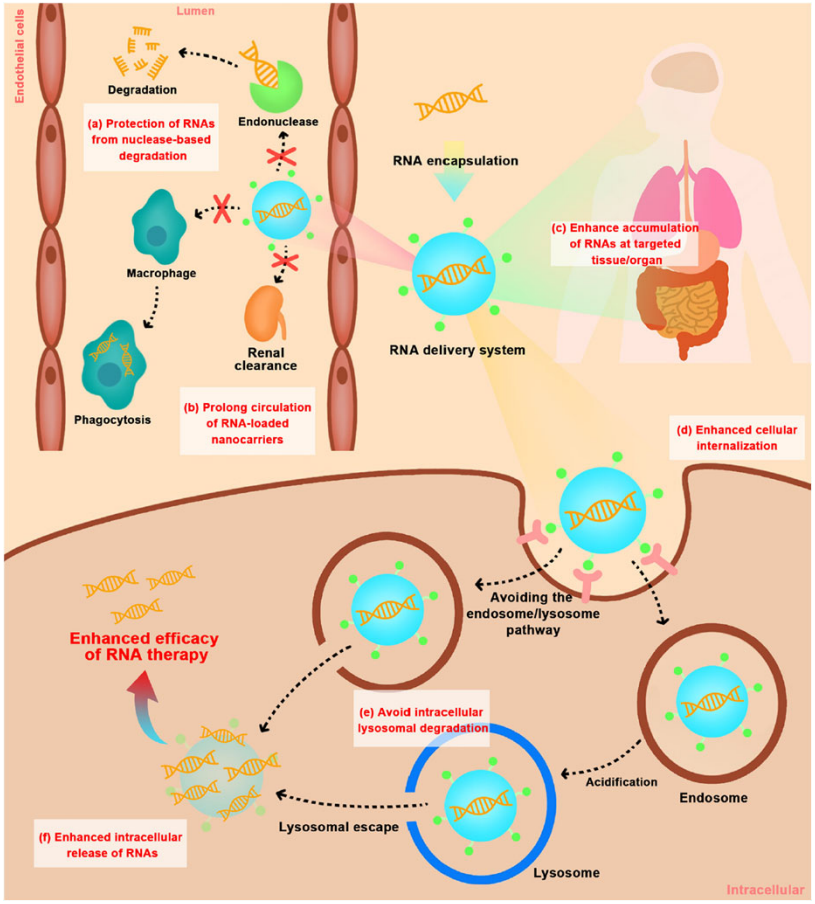


Fig. 2. Extracellular and intracellular barriers for in vivo delivery of RNAs using non-viral vectors. (a) protection of RNAs from nuclease-based degradation; (b) prolong circulation of RNA-loaded nanocarriers by avoiding phagocytosis by mononuclear phagocytic system and rapid kidney clearance; (c) enhance tissue/organ-selective accumulation of RNAs; (d) enhance cellular internalization; (e) avoid intracellular lysosomal degradation; (f) enhance intracellular release of RNAs.

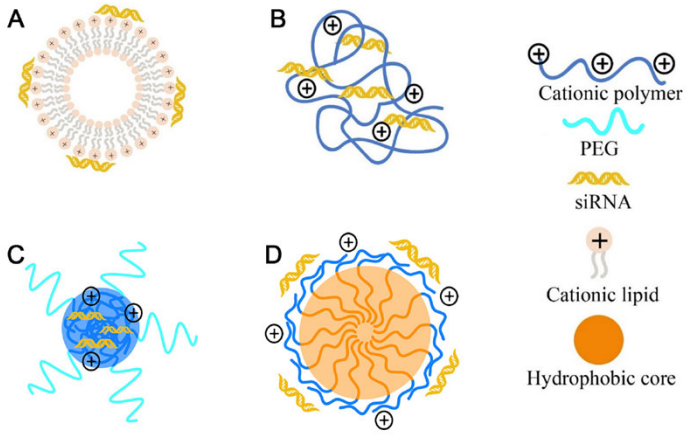


Fig. 3. Protection of RNAs from nuclease degradation by electrostatic adsorption. The common cationic nanovectors for RNAs delivery including (A) cationic lipids-based lipoplexes, (B) cationic polymers-based polyplexes, (C) PEG-based cationic block copolymer formed NPs, and (D) cationic amphipathic-based block copolymer formed NPs.

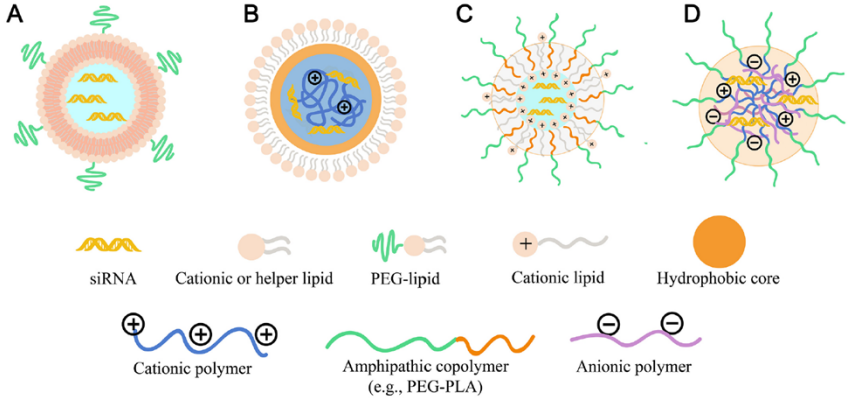
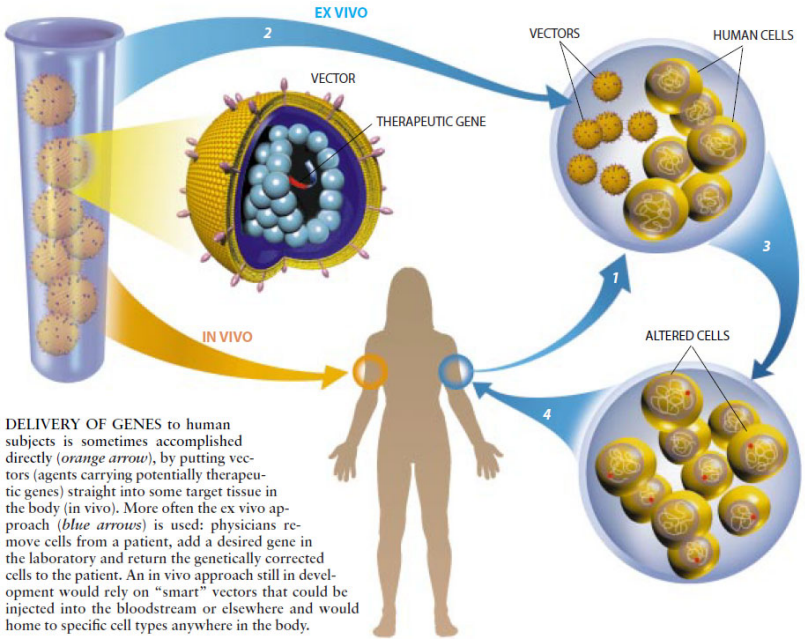


Fig. 5. Protection of RNAs from nuclease degradation by electrostatic interaction-based layer-by-layer encapsulation and core-shell encapsulation. Illustration of (A) Stable Nucleic-Acid Lipid Particle (SNALP) nanostructure; (B) lipid-polymer hybrid nanostructure (reverse micelle inner core); (C) polymer-lipid hybrid nanostructure (named as “CLAN”); and (D) Polyionic complex (PIC) nanostructure.

Yan 2022, Non-viral vectors for RNA delivery

Overcoming the Obstacles

Viruses are the most effective vesicles for delivery of DNA and genes. At the same time, they are very dangerous.



DELIVERY OF GENES to human subjects is sometimes accomplished directly (*orange arrow*), by putting vectors (agents carrying potentially therapeutic genes) straight into some target tissue in the body (in vivo). More often the ex vivo approach (*blue arrows*) is used: physicians remove cells from a patient, add a desired gene in the laboratory and return the genetically corrected cells to the patient. An in vivo approach still in development would rely on "smart" vectors that could be injected into the bloodstream or elsewhere and would home to specific cell types anywhere in the body.

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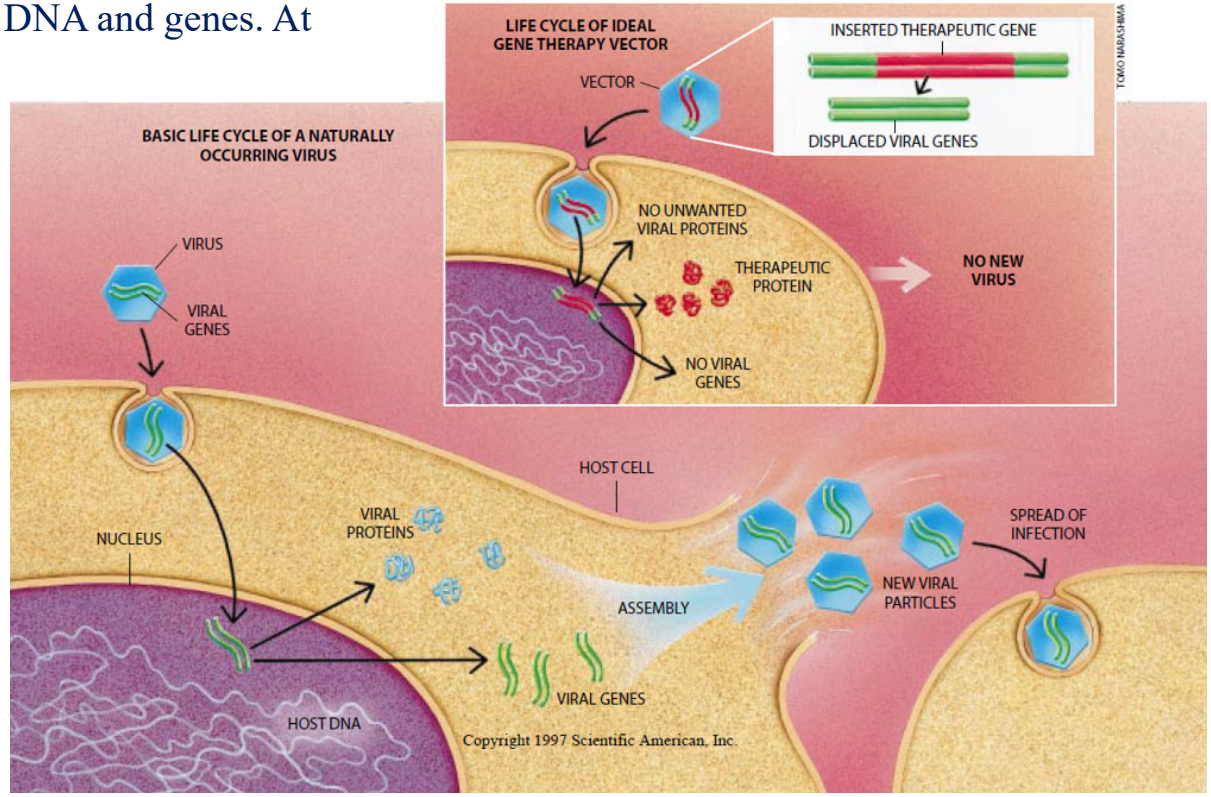


NATURALLY OCCURRING virus (bottom panel) releases its genetic material into cells. Whether or not the genes become integrated into the DNA of the infected cell, they soon direct the synthesis of new viral particles that can injure the cell and infect others. To convert a wild-type virus into a safe gene therapy vector, scientists replace viral genes with ones specifying therapeutic proteins (top panel), while ideally leaving only the viral elements needed for gene expression. Such vectors should enter cells and give rise to helpful proteins but should not multiply.

Friedmann 1997, Overcoming the obstacles

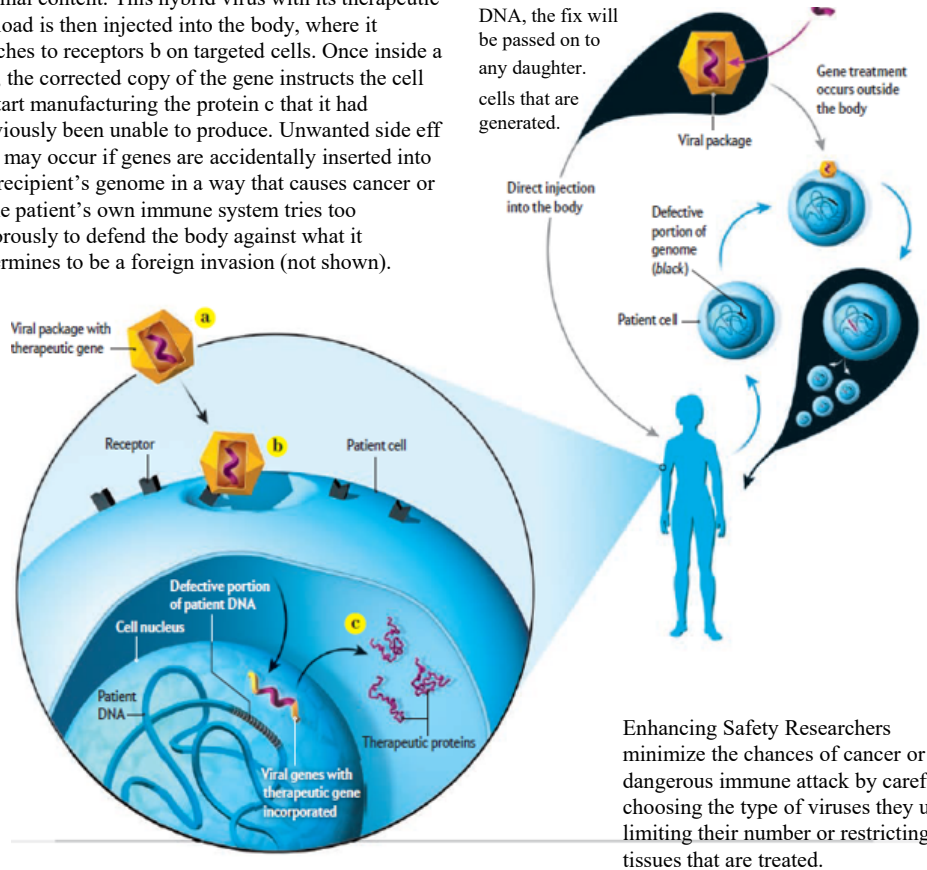
Overcoming the Obstacles

How to Fix a Defective Gene

Gene therapy attempts to undo the damage caused by broken or defective genes. The most common approach (below) packages a copy of a working gene into a virus a that has been stripped of most of its original content. This hybrid virus with its therapeutic payload is then injected into the body, where it attaches to receptors b on targeted cells. Once inside a cell, the corrected copy of the gene instructs the cell to start manufacturing the protein c that it had previously been unable to produce. Unwanted side eff ects may occur if genes are accidentally inserted into the recipient's genome in a way that causes cancer or if the patient's own immune system tries too vigorously to defend the body against what it determines to be a foreign invasion (not shown).

Two Delivery Choices

In addition to injecting viruses into patients directly, investigators may remove cells from the body, insert the therapeutic-gene-bearing viruses into those cells (below right) and reinject the altered cells. Because the corrected genetic information is incorporated into the cells' DNA, the fix will be passed on to any daughter. cells that are generated.



Enhancing Safety Researchers minimize the chances of cancer or a dangerous immune attack by carefully choosing the type of viruses they use, limiting their number or restricting the tissues that are treated.

Lewiw 2014, Gene therapy's second act. (Sci. Am. March 2014, p. 52.)

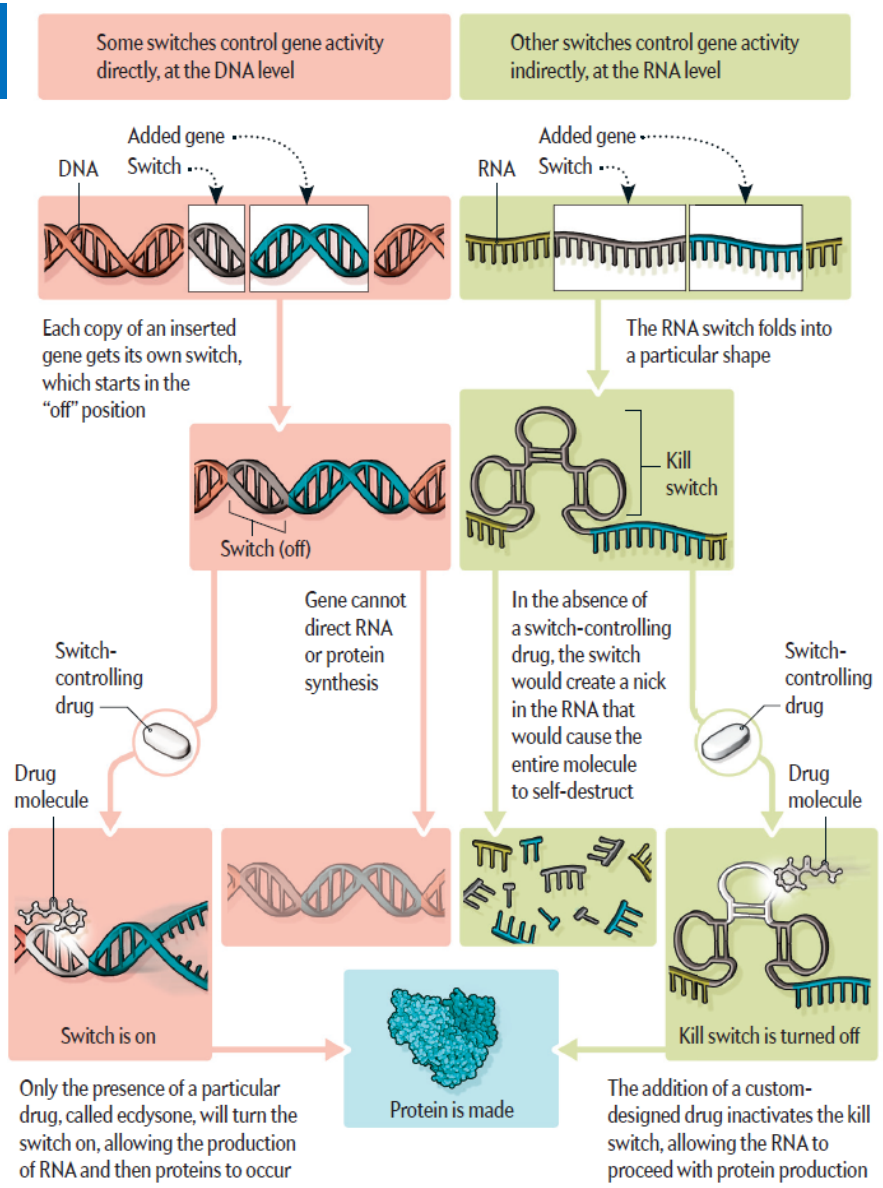
Rethinking the Technology Given the propensity of adenoviruses to provoke lethal immune reactions and of retroviruses to trigger cancer, investigators began paying more attention to other viruses to see if they offered better results. They soon focused on two more widely suitable entrants. The first new delivery system, adeno-associated virus (AAV), does not make people sick (although most of us have been infected by it at one time or another). Because it is so common, it is unlikely to cause extreme immune reactions. This virus has another feature that should also help minimize side effects: it is available in several varieties, or serotypes, that favor specific types of cells or tissues. For example, AAV2 works well in the eye, whereas AAV8 prefers the liver, and AAV9 slips into heart and brain tissue. Researchers can choose the best AAV for a specific body part, decreasing the number of individual viruses that need to be injected and thus minimizing the chances of an overwhelming immune response or other unwanted reaction. Plus, AAV depos chunky genes," he says. "There's no toxicity and no adverse immune reaction." Stripped-down lentiviruses are now being used in a number of clinical trials, including treatments for adrenoleukodystrophy - the disease featured in the 1992 movie Lorenzo's Oil. To date, a few of the boys who have received this treatment have become healthy enough to return to school. Although clinical trials using AAV and HIV are on the rise, researchers have also redirected or modified the older viral delivery systems so that they can be used in limited circumstances. For example, non-HIV retroviruses are now genetically edited so that they inactivate themselves before they can trigger leukemia. Even adenovirus, which caused Gelsinger's death, is still in clinical trails as a gene therapy vector. Investigators restrict its use to parts of the body where it is unlikely to cause an immune response. One promising application is to treat "dry mouth" in patients undergoing radiation for head and neck cancer, which damages the salivary glands, located just under the surface of the inside of the cheek.

An On-Off Switch for Genes

In gene therapy, it is usually the case that delivered genes may not work long enough, requiring repeated treatment. At the same time, it is important to prepare for a possible scenario that the delivered genes are too active.

Two Strategies for Controlling Gene Activity with Pills

A major challenge to the development of successful gene therapies is making sure that the newly inserted genes are not too active - which can cause cancer, among other things. All genes, which are made up of DNA, instruct cells to make another molecule, called RNA, which in turn often directs the manufacture of proteins. Researchers are studying various approaches (two of which are pictured right) for creating biological switches that can shift a gene's operation (and thus the production of proteins) into gear—or shut it down altogether.



Kozubek 2016, An On-off switch for genes

DNA Drugs

DNA drugs are simply in concept, but very difficult in practice. One of the difficulties facing the applications of DNA drugs is that unexpected results are observed during clinical trials.

DNA Drugs Come of Age

After years of false starts, a new generation of vaccines and medicines for HIV, influenza and other stubborn illnesses is now in clinical trials BY MATTHEW P. MORROW AND DAVID B. WEINER

IN A HEAD-TO-HEAD COMPETITION held 10 years ago, scientists at the National Institutes of Health tested two promising new types of vaccine to see which might offer the strongest protection against one of the deadliest viruses on earth, the human immunodeficiency virus (HIV) that causes AIDS. One vaccine consisted of DNA rings called plasmids, each carrying a gene for one of five HIV proteins. Its goal was to get the recipient's own cells to make the viral proteins in the hope they would provoke protective reactions by immune cells. Instead of plasmids, the second vaccine used another virus called an adenovirus as a carrier for a single HIV gene encoding a viral protein. The rationale for this combination was to employ a "safe" virus to catch the attention of immune cells while getting them to direct their responses against the HIV protein.

One of us (Weiner) had already been working on DNA vaccines for eight years and was hoping for a major demonstration of the plasmids' ability to induce immunity against a dreaded pathogen. Instead the test results dealt a major blow to believers in this first generation of DNA vaccines. The DNA recipients displayed only weak immune responses to the five HIV proteins or no response at all, whereas recipients of the adenovirus-based vaccine had robust reactions. To academic and pharmaceutical company researchers, adenoviruses clearly looked like the stronger candidates to take forward in developing HIV vaccines.

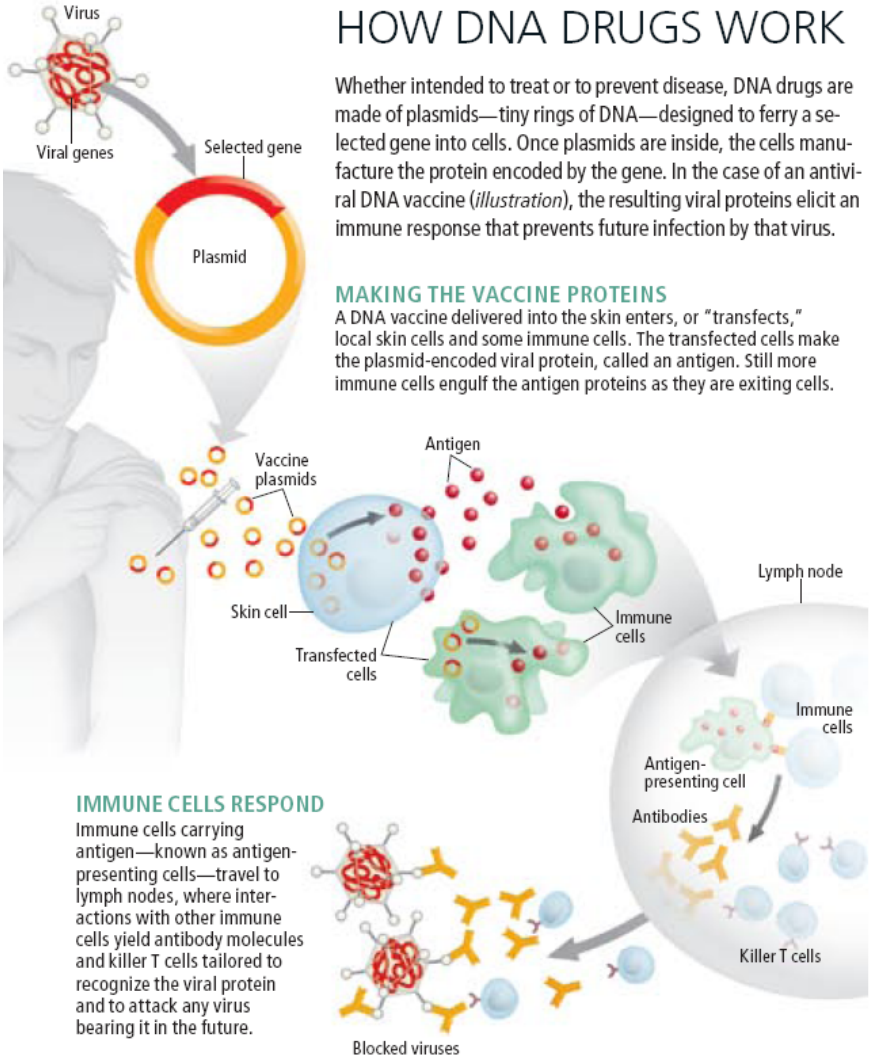
To DNA vaccine investigators, the results were not entirely surprising, because poor responses had been seen in some previous trials. Still, the failures were disappointing because we had good reasons for expecting the plasmid vaccine to be both safe and powerful. Convinced that the original concept was still strong, scientists went back to the drawing board to find ways to boost the effectiveness of the technology. Now these efforts are beginning to pay off. A new generation of plasmid-based vaccines is proving in human and animal trials that it can produce the desired responses while retaining the safety and other benefits that make DNA so appealing. The same DNA-based technology is also now expanding to other forms of immune therapy and the direct delivery of medicines. In their mature form, such DNA-based vaccines and treatments are poised to become a success story by addressing several conditions that now lack effective treatments.

KEY CONCEPTS

- Vaccines and therapies containing DNA rings called plasmids have long held promise for treating and preventing disease, but the plasmids made a weak showing in early tests.
- Improvements to the plasmids and new methods for delivering them have dramatically enhanced their potency.
- DNA vaccines and therapies now used in animals or in late-stage human trials demonstrate that plasmids are reaching their potential.

—*The Editors*

DNA Drugs



Unfortunately, in the early DNA vaccine tests the problem of weak immune responses was a significant pitfall. The main reasons for those failures seemed to be that vaccine plasmids were not getting into enough cells and, where they did penetrate, the cells were not producing enough of the encoded proteins. As a result, the immune system was not being sufficiently stimulated.

The rival technology would ultimately face a bigger problem, however. In 2007 pharmaceutical company Merck initiated a large trial of an HIV vaccine that used an adenovirus called AdHu5 to deliver HIV viral genes. In light of the potent immune responses seen in previous experiments with adenoviruses, great hope and excitement surrounded the beginning of this test, known as the STEP trial. In all, about 3,000 HIV-negative individuals received the vaccine or a placebo shot.

As the trial progressed, though, a disturbing difference between the two groups began to emerge: people who got the vaccine were no better protected than those who received the placebo, and eventually they appeared to be *more* vulnerable to being infected by HIV. An early

The result that volunteers who received vaccine plasmids are more prone to infection than the control group.

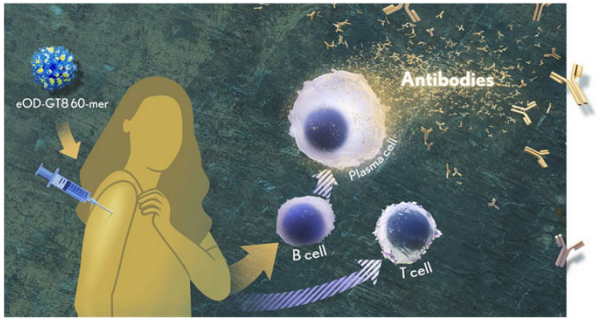
IAVI and Moderna launch trial of HIV vaccine antigens delivered through mRNA technology
(January 27, 2022)

Phase 1 trial aims to build on response seen in proof-of-concept trial

https://investors.modernatx.com/news/news-details/2022/IAVI-and-Moderna-Launch-Trial-of-HIV-Vaccine-Antigens-Delivered-Through-mRNA-Technology/default.aspx

Encouraging first-in-human results for a promising HIV vaccine
Lawrence Tabak, DDS, PhD, Acting Director, National Institutes of Health. June 08, 2023

https://www.hiv.gov/blog/encouraging-first-in-human-results-for-a-promising-hiv-vaccine/



Researchers used a customized nanoparticle (top left) to learn more about guiding the immune system to mount a desired robust response, the type needed for an effective HIV vaccine. Credit: Donny Bliss, NIH

Last of the HIV Vaccine Trials Fails, Scientists Regroup

The PREP vaccine trial testing two experimental HIV prevention regimens in Africa has been stopped after investigators announced that there is "little to no chance" the trial will show the vaccines are effective. This phase 2b trial was the last attempt in this generation of vaccine development, which has used non-neutralizing antibodies, experts say, and scientists will have to begin anew. "We cannot and will not lose hope that the world will have an effective HIV vaccine that is accessible by all who need it, anywhere," International AIDS Society (IAS) Executive Director Birgit Poniatowski said in a statement.

The African-led, European-supported HIV prevention study has been running since 2018 at four sites in Uganda, Tanzania, and South Africa and has involved 1513 participants aged 18-40 years. The vaccine trial was testing two experimental combination regimens designed to protect against HIV and a new form of pre-exposure prophylaxis. The PREP trial will continue. Almost 21 million of the 39 million people in the world with AIDS live in the test-site region of eastern and southern Africa, according to the IAS.

The news isn't a huge surprise, as the last two HIV studies testing non-neutralizing antibodies showed a lack of efficacy, Larry Corey, MD, virologist and principal investigator of HIV Vaccine Trials Network, headquartered in Seattle, told Medscape Medical News. But the results of this trial may help answer a question. "There is a small group of people who have really high immune responses to a part of the virus we call the V1V2 loop," he explained. "But the frequency of people with that immune response is only **8%-10% of the population**. If this trial, using a different vector, shows the same thing, then we have to spend a lot more time making an immunogen that gets 100% of the people to the levels of immunity that might be correlated with the V1V2 loop, and we'd walk out of this with a really good insight as to what to do next."

So far, he said, **scientists working to develop a vaccine haven't determined which part of the virus to target**. "The clinical trial was very well done, and that's the success," Corey said. But "are we going to learn from it?" The lack of an HIV vaccine continues to frustrate patients and scientists, especially because vaccines for other diseases, such as COVID-19, have been developed at record speed. But **COVID-19 presented a completely different challenge for vaccine development**, he explains. "We're doing **something much harder with HIV than we did with COVID**," he said. **"COVID vaccines prevent you from getting sick; you still get infected. With HIV, you have to prevent someone from getting infected in the first place. And that requires way more antibody and a way better immune response."**

Although there are other prevention tools for HIV, a vaccine is the only way to meet global elimination goals, he noted, pointing out that people who are at the highest risk for HIV "have an unchanged incidence over the past 15 years of 4% a year." "We are nowhere close to ending the HIV epidemic," Corey said. "We're not going to meet the 2030 goal. And one of the reasons we're not going to meet the 2030 goal is that between 40% and 50% of the cases of HIV globally are in people who don't self-identify as high risk." That's the limitation with PREP, which has been available for nearly two decades. It counts on the acceptance, intake, and adherence of people at a high risk for HIV. "That has been abysmal with oral PREP," he said.

There are long-acting antiretrovirals or long-acting monoclonal antibodies. But from a cost and effort standpoint, those would also be focused on people who consider themselves high risk, he pointed out. "In some countries, you could make a case that everybody's high risk." The only way we've really ever controlled a disease on a population base is with a vaccine, he said. "Just because it's hard, doesn't mean you give up. All the data say you've got to have it."

Marcia Frellick. December 18, 2023. https://www.medscape.com/viewarticle/last-hiv-vaccine-trials-fails-scientists-regroup-2023a1000vot

PrEPVacc is an African-led, European-supported HIV prevention study running in East and Southern Africa from 2018 to 2024. For the first time, it is combining evaluation of experimental HIV vaccines and pre-exposure prophylaxis (PrEP) at the same time. In December 2023, the study announced that it had stopped further vaccinations as there is little or no chance of the trial demonstrating vaccine efficacy in preventing HIV acquisition. The oral PrEP component of the study is continuing to completion.
https://www.prepvacc.org/#msdynttrid=2BDC8VtoJxA3Yk_OsOi9yg-afJJJ-qgnuTtt-GyClv4

PrEP is a proven intervention that has been shown to prevent HIV, where an Anti-Retroviral pill is taken prior to being exposed to HIV. PrEPVacc will test whether a new form of oral PrEP, TAF/FTC (Descovy) taken daily, is equivalent or more effective than TDF/FTC (Truvada) taken daily, in the context of the current PrEP availability situation at the study sites. The PrEP results of PrEPVacc will be valuable for informing future implementation and uptake strategies by local stakeholders and champions in settings where PrEP uptake is low even when accessible, but is likely to be more widely available in the future.
https://www.prepvacc.org/prep

Moderna's mRNA Cancer Vaccine

A cancer vaccine based on the messenger RNA (mRNA) technology, provided alongside the checkpoint inhibitor pembrolizumab (Keytruda), has shown encouraging results in an open label phase 2b clinical trial. The trial found that the combination regimen reduced the risk of cancer recurrence or death among melanoma patients by 44% compared with pembrolizumab alone, according to the vaccine's manufacturer Moderna.

Here are four things to know about the mRNA-4157/V940 cancer vaccine and what the company has in store for upcoming clinical trials.

1. The mRNA vaccine is personalized

Moderna's mRNA vaccine is personalized for each patient. The vaccine is designed to prime the immune system in a way that allows a patient to generate a tailored antitumor response specific to their tumor mutations.

To identify a patient's specific mutations, researchers sequence DNA from the patient's normal tissue as well as DNA from the tumor. Results are compared to identify a set of mutations unique to the patient's cancer. Researchers then develop a single synthetic mRNA coding for up to 34 neoantigens, designed based on the tumor's specific mutational signature. The aim is for mRNA-4157/V940 to help the patient's immune system identify and attack the tumor cells only.

2. Development, distribution happens quickly

The process of personalizing the vaccine happens over several weeks, according to Moderna's Head of Development for Oncology Kyle Holen.

By itself, "the RNA sequencing takes only 2 hours to develop, which is just mind-boggling that it can happen so quickly," Holen said. "It's important to do this quickly because patients with cancer don't have much time to wait."

After acquiring samples from patients, sequencing, running the algorithm to identify specific mutations, manufacturing the RNA, and delivering the vaccine to patients take about 6 weeks in total.

3. Adverse events higher in the experimental arm

Serious treatment-related events occurred in 14.4% of patients who received the combination of mRNA-4157/V940 and pembrolizumab vs 10% receiving pembrolizumab monotherapy. The adverse events observed were consistent with those seen in phase 1 of the trial, and Merck/Moderna did not report any new categories of treatment-related adverse events in the phase 2b trial.

4. Moderna's plans to expand beyond melanoma

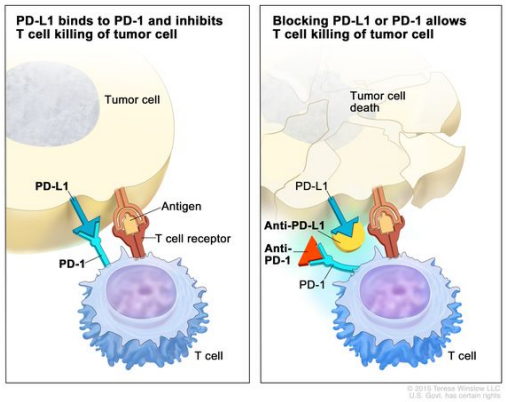
Moderna is still developing a phase 3 clinical trial for mRNA-4157/V940, which the company hopes to launch sometime in 2023, Holen said during a press conference. Moderna also plans to expand its personalized mRNA vaccine approach beyond melanoma to other tumor types but has not begun that expansion yet.

Patricia McKnight, December 16, 2022

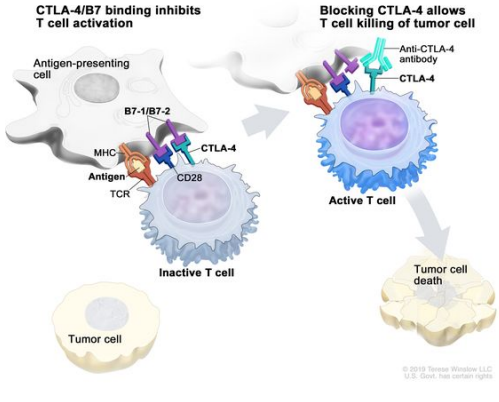
https://www.medscape.com/viewarticle/985744?src=WNL_trdalrt_pos1_221223&uac=70212FJ&impID=5024329

Immune Checkpoint Inhibitor

A type of drug that blocks proteins called checkpoints that are made by some types of immune system cells, such as T cells, and some cancer cells. These checkpoints help keep immune responses from being too strong and sometimes can keep T cells from killing cancer cells. When these checkpoints are blocked, T cells can kill cancer cells better. Examples of checkpoint proteins found on T cells or cancer cells include PD-1/PD-L1 and CTLA-4/B7-1/B7-2. Some immune checkpoint inhibitors are used to treat cancer.



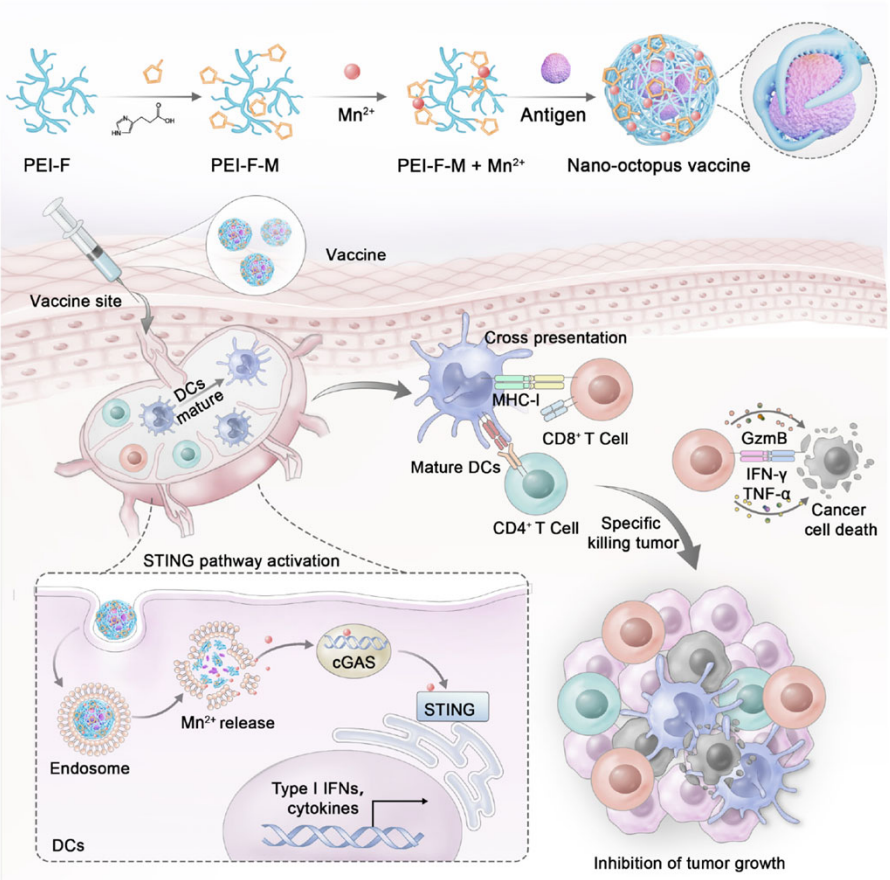
Immune checkpoint inhibitor. Checkpoint proteins, such as PD-L1 on tumor cells and PD-1 on T cells, help keep immune responses in check. The binding of PD-L1 to PD-1 keeps T cells from killing tumor cells in the body (left panel). Blocking the binding of PD-L1 to PD-1 with an immune checkpoint inhibitor (anti-PD-L1 or anti-PD-1) allows the T cells to kill tumor cells (right panel).



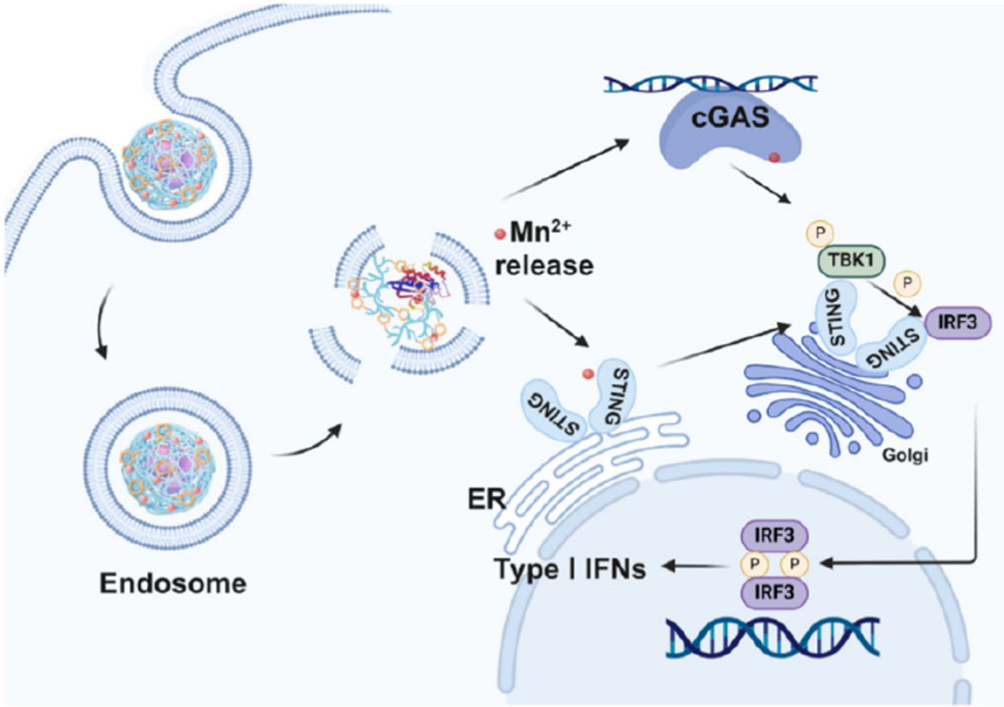
Immune checkpoint inhibitor. Checkpoint proteins, such as B7-1/B7-2 on antigen-presenting cells (APC) and CTLA-4 on T cells, help keep the body's immune responses in check. When the T-cell receptor (TCR) binds to antigen and major histocompatibility complex (MHC) proteins on the APC and CD28 binds to B7-1/B7-2 on the APC, the T cell can be activated. However, the binding of B7-1/B7-2 to CTLA-4 keeps the T cells in the inactive state so they are not able to kill tumor cells in the body (left panel). Blocking the binding of B7-1/B7-2 to CTLA-4 with an immune checkpoint inhibitor (anti-CTLA-4 antibody) allows the T cells to be active and to kill tumor cells (right panel).

https://www.cancer.gov/publications/dictionaries/cancer-terms/def/immune-checkpoint-inhibitor

Polymeric Nanovaccine



Scheme 1. Schematic illustration of octopus inspired vaccine design to overcome multiple barriers in antigen delivery. The nano-octopus vaccine captures antigens through PEI "tentacles" and imidazole-Mn2+ "suction cups", enabling high antigen loading and efficient cytosolic delivery. It facilitates dendritic cell (DC) maturation, activates the STING pathway, and enhances antigen cross-presentation, ultimately eliciting strong CD8+ T cell responses for tumor suppression.

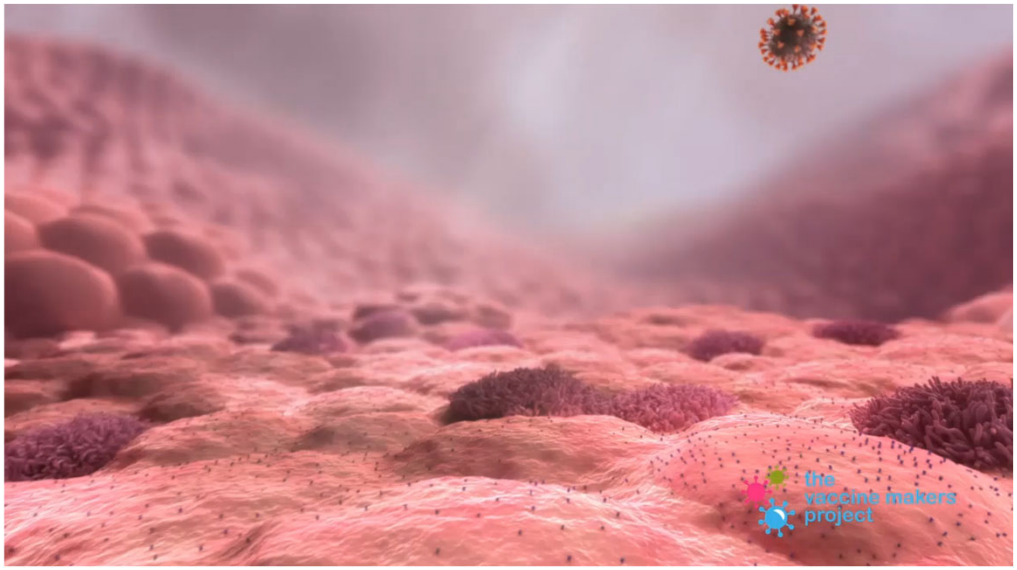


Proposed mechanism of STING (Stimulator of Interferon Genes) activation by the nano-octopus vaccine.

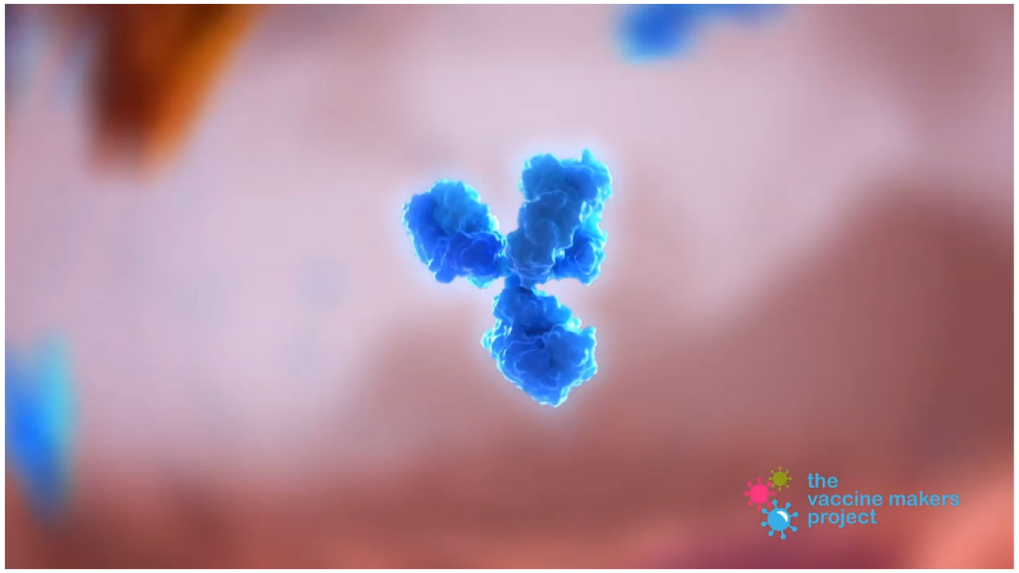
Wang 2026, Octopus-inspired polymeric nanovaccine enables high antigen loading and robust T cell activation for cancer immunotherapy

DNA & RNA Drugs

COVID-19 Viral Vector Vaccine



COVID-19 mRNA Vaccine



https://vaccinemakers.org/resources/videos-animations

Moderna's mRNA Flu Vaccine Rejected

FDA refusal to review Moderna’s mRNA flu shot adds to vaccine scrutiny under Trump

The Food and Drug Administration's refusal to review Moderna's mRNA flu vaccine is raising questions about how the Trump administration intends to treat other new products built around the technology.

Illustration: Maura Losch/Axios

https://www.axios.com/2026/02/12/moderna-vaccine-rejection-trump-mrna

Moderna rejection adds to vaccine cloud under Trump (Peter Sullivan • FEB 12, 2026)

The Food and Drug Administration's refusal to review Moderna's mRNA flu vaccine is raising questions about how the Trump administration intends to treat other new products built around the technology.

Why it matters: Some medical experts warn that the U.S. will miss out on promising new vaccines if it does not embrace the technology, which powered the COVID-19 shots and could be adapted to target other diseases, including cancer.

Driving the news: The FDA's decision, which the company disclosed on Tuesday, comes after Health and Human Services last year canceled nearly $500 million in grants for developing new mRNA vaccines.

• Health Secretary Robert F. Kennedy Jr. argued at the time that mRNA vaccines are not effective against infections like COVID-19 and flu.

• Some conservative-led states have targeted mRNA vaccines amid ongoing suspicion of public health officials and the tools they used to fight the COVID-19 pandemic.

State of play: The FDA said that it rejected Moderna's application because the company refused to fallow guidance on which current flu vaccine its new product should be compared with in its study, with FDA saying a higher dose vaccine should have been used.

• Moderna countered that while the higher dose was recommended, the FDA had previously been open to using the standard dose vaccine as a control, and the agency had not objected before the study began.

What they're saying: "It seems that the federal government, at this point, has decided unjustifiably against mRNA vaccines," said Amesh Adalja, a senior scholar at the Johns Hopl<ins Center for Health Security.

• He added that position "disincentivizes companies to use mRNA vaccine technology, which is really instrumental when it comes to infectious disease emergency preparedness and pandemic preparedness, because of the rapidity and the adaptability of that platform."

• Drug development experts raised concern that companies won't invest in long and expensive clinical trials if they think the FDA could move the goalposts at the end of the process.

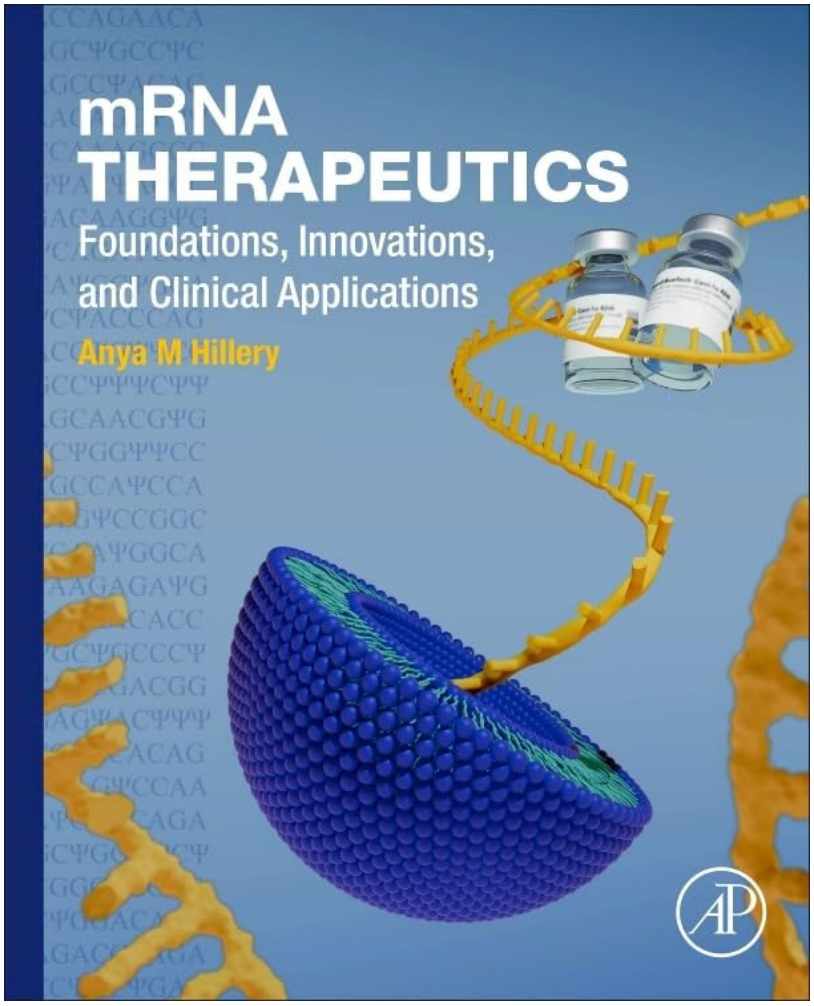
The other side: A senior FDA official told reporters the decision was based on the design of Moderna's study and not any overarching objections to mRNA vaccines.

• Kennedy was "not directly or indirectly involved" in the FDA's decision on the Moderna shot, the senior FDA official said.

• "I would like to see more research with mRNA technology," the official added. "But I don't think the government should pay for it. These companies made over $50 billion on the mRNA COVID shot. They can fund their own research."

The intrigue: The decision on the Moderna shot drew swift condemnation from Democrats in Congress. • "You could spend billions of dollars developing a drug, and then the FDA's going to not even consider it because of the quacks," said Rep. Frank Pallone (D-N.J.), the top Democrat on the House Energy and Commerce Committee.

mRNA Therapeutics



mRNA Therapeutics: Foundations, Innovations and Clinical Applications aims to provide a comprehensive text that covers all aspects of mRNA therapeutics, from the foundational science that underpins this disruptive new drug class, through the scientific and technological breakthroughs crucial for therapeutic success, to the current clinical applications and the innovative advances driving future directions.

The book begins with foundational knowledge covering mRNA biology, the immune system, and vaccines. The second section addresses the major challenges associated with mRNA as a therapeutic modality, and the molecular engineering innovations and delivery technologies that have allowed these hurdles to be largely overcome.

The third section describes the current and future clinical applications of mRNA therapeutics that are transforming, or are poised to transform, medicine and health. This includes the use of mRNA vaccines for COVID-19 and other infectious diseases, as well as mRNA's role in revolutionizing cancer immunotherapy, covering immunostimulants, cancer vaccines including personalized neoantigen vaccines, and CAR T cell technologies. Additional chapters describe the use of mRNA therapeutics for protein replacement therapy and gene-editing, as well as newer mRNA constructs, including self-amplifying mRNA. The final section addresses the safety and regulatory considerations of mRNA therapeutics, along with broader cultural issues including vaccine hesitancy, global vaccine inequality, and pandemic preparedness.

Currently, mRNA texts either provide personal accounts from key players involved in COVID-19 vaccine development, with limited scientific depth, or focus on highly specialized, more esoteric applications of mRNA in advanced molecular biology. This book aims to bridge this gap by providing a scientifically rigorous and wide-ranging exploration of mRNA's role in therapeutics.

This pioneering textbook serves as a vital addition to the academic canon, providing an essential tool for the current and next generation of students, scientists, researchers and professionals in a wide variety of related disciplines including molecular biology, biomedical engineering, pharmaceutical science, oncology, and the health sciences.

https://shop.elsevier.com/books/mrna-therapeutics/hillery/978-0-443-28934-7

Anya M. Hillery 2024, mRNA Therapeutics. Foundations, Innovations, and Clinical Applications

mRNA Drugs

Unlock the sustained therapeutic efficacy of mRNA

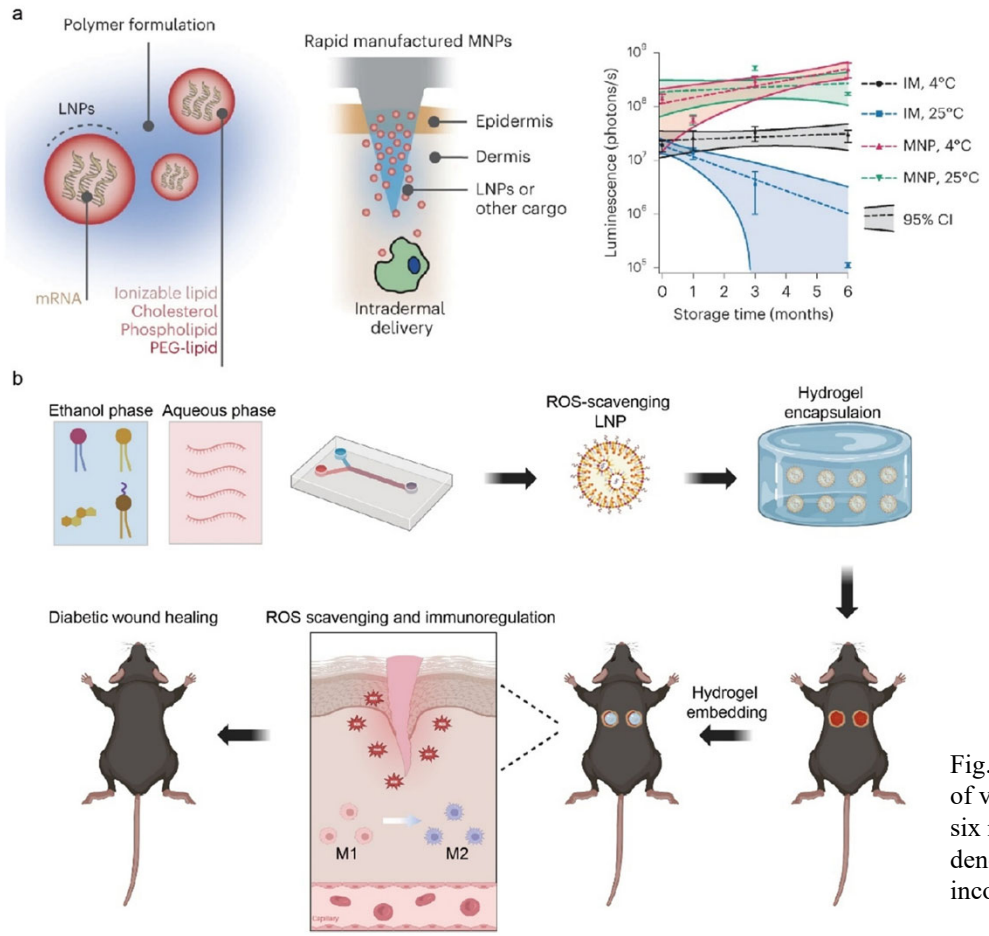


Fig. 2. (a) LNP-mRNA encapsulated in a dissolvable microneedle for intradermal delivery of vaccine. The microneedles maintain their capacity for high protein expression even after six months of storage at room temperature. MNPs refer to microneedle patches, while IM denotes intramuscular injection with conventional LNP-mRNA. (b) LNP-mRNA incorporated into a hydrogel for diabetic wound healing therapy.

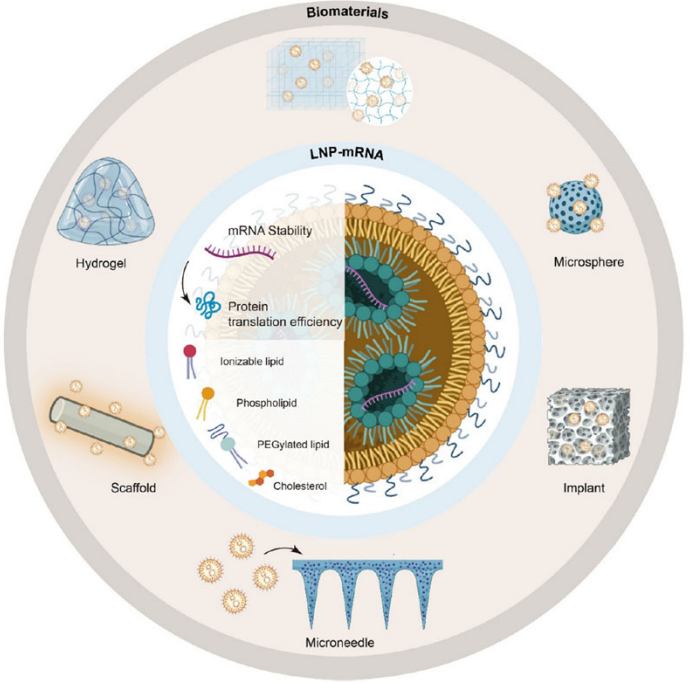


Fig. 4. Strategies for combining LNPs with macroscopic biomaterials to achieve sustained mRNA delivery.

Cao 2025, Unlock the sustained therapeutic efficacy of mRNA

Delivery of mRNA

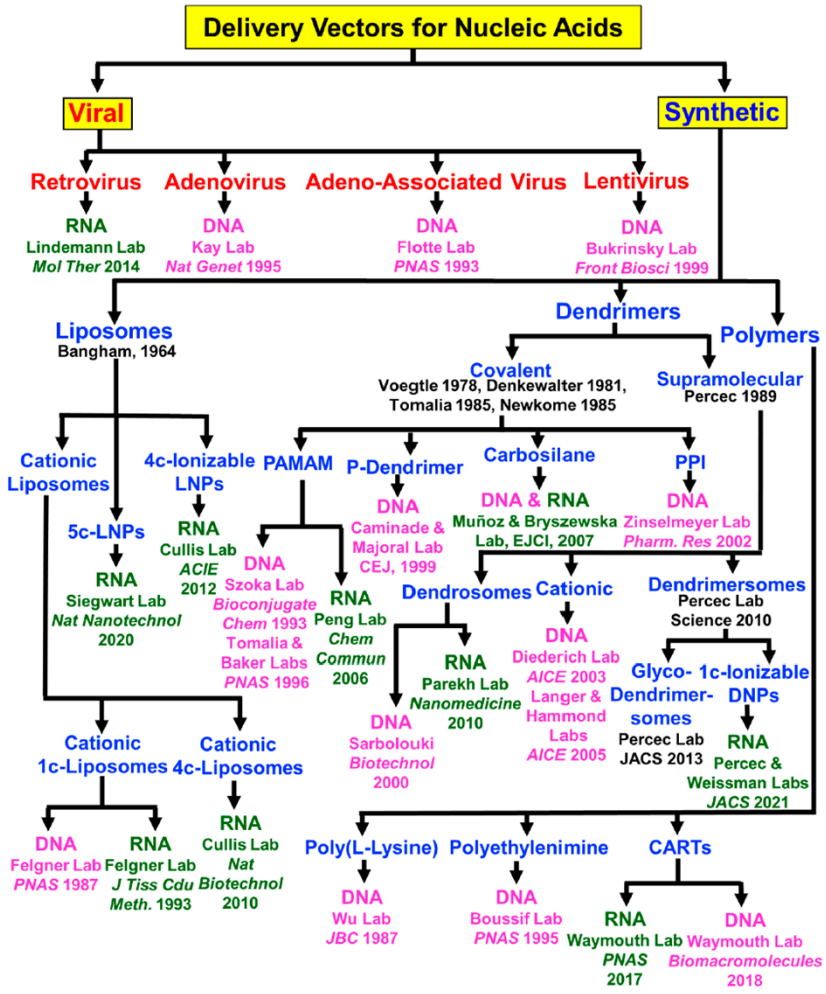


Figure 16. Summary of the viral and nonviral vectors for the delivery of nucleic acids and the evolution of methodology development.

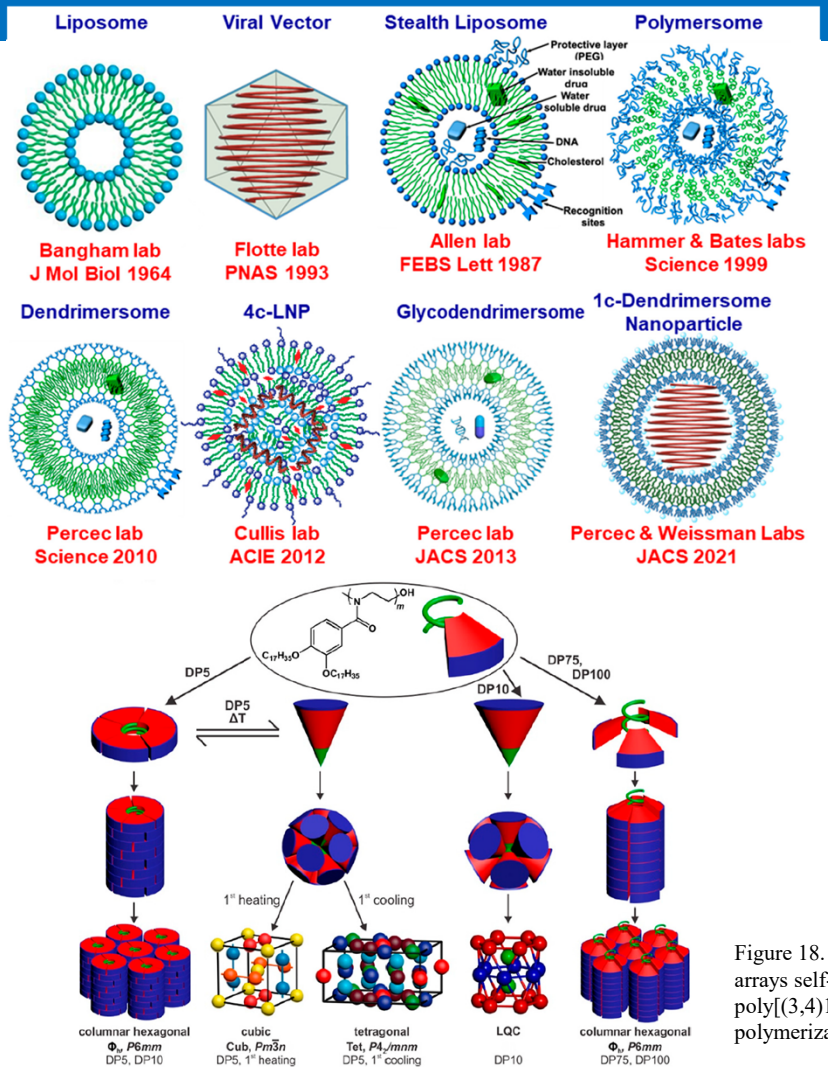


Figure 17. A brief summary of the evolution, development, and discovery of ionizable LNPs and DNPs.

Figure 18. Summary of periodic and quasiperiodic arrays self-organized from assemblies of poly[(3,4)17G1-Oxz] at different degrees of polymerization (DP) and temperature.

Lu 2023, Screening libraries to discover molecular design principles for the targeted delivery of mRNA

Self-Amplifying mRNA Vaccine

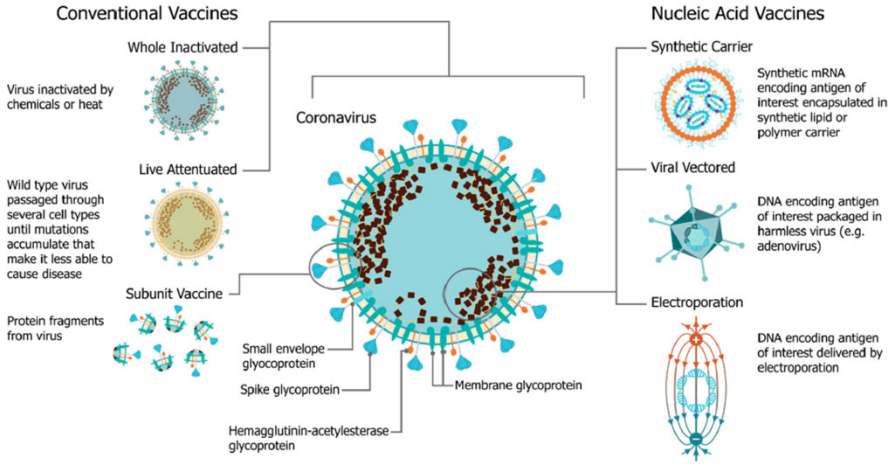


Figure 1. A comparison of vaccine platforms including vaccines derived from the virus itself and are formulated as a part or whole modified version of the virus (left) and nucleic acid vaccines, such as self-amplifying RNA vaccines (right). Nucleic acid vaccines are derived from knowledge of the viral genome, where glycoproteins are encoded into nucleic acids and delivered with either a synthetic carrier such as a lipid nanoparticle or an inert viral delivery system such as adenoviruses. The encoded antigen sequences are then expressed by the host cells.

4. Delivery Systems

The main challenge for saRNA vaccines is achieving sufficient delivery of saRNA to the target cells or tissue. saRNA constructs are relatively large (9000 to 15,000 nucleotide (nt)), anionic molecules, which precludes efficient cellular uptake of unformulated saRNA. Despite the use of "naked" saRNA in some studies, three predominant delivery platforms have emerged: Polymeric nanoparticles, lipid nanoparticles, and nanoemulsions. These delivery strategies share a central dogma wherein the anionic saRNA is condensed by a cationic (or ionizable cationic) carrier to a nanoparticle of ~100 nm in size, that protects the saRNA from degradation and encourages uptake into target cells (Figure 6).

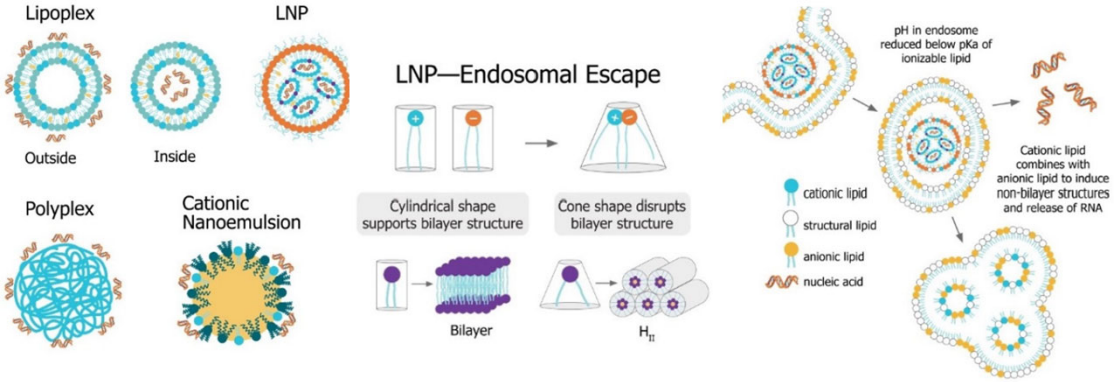


Figure 6. Non-viral saRNA delivery systems. Lipid-, polymer-, and emulsion-based delivery systems all use cationic groups to mediate condensation of the anionic RNA as well as delivery across the cell membrane. LNP systems, which have been found to be the most potent vaccine formulatinos, utilize a pH-sensitive ionizable cationic lipids and are taken up in cells through receptor-mediated endocytosis. In the endosome, the lower pH environment ionizes the cationic lipids, which then interacts electrostatically with anionic lipids in the endosomal membrane. These ion pairs cause a phase transition into a porous hexagonal phase (Hn) that disrupts the endosome and facilitates release of the RNA into the cytoplasm.

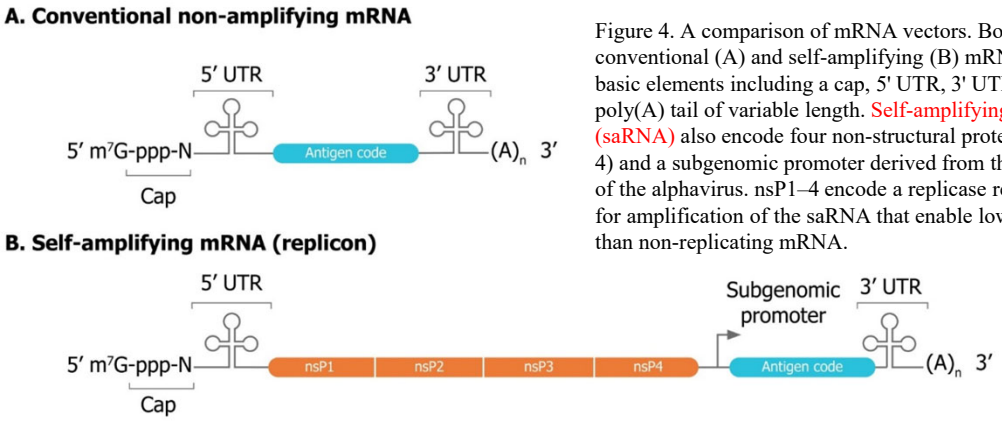
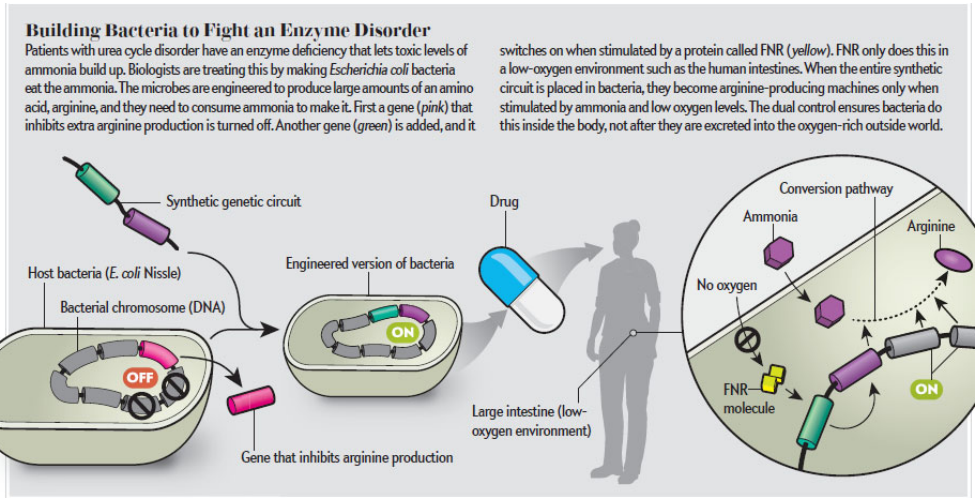


Figure 4. A comparison of mRNA vectors. Both conventional (A) and self-amplifying (B) mRNAs share basic elements including a cap, 5' UTR, 3' UTR, and poly(A) tail of variable length. Self-amplifying RNA (saRNA) also encode four non-structural proteins (nsP1–4) and a subgenomic promoter derived from the genome of the alphavirus. nsP1–4 encode a replicase responsible for amplification of the saRNA that enable lower doses than non-replicating mRNA.

Blakney 2021, An update on self-amplifying mRNA vaccine development

Transformers: Microbes for Patient-Saving Drugs

Billions of tiny, toxin-gobbling contraptions can be used to cure a crippling disease. The devices are not made from the usual machine parts of metal, wire or plastic. They are rebuilt organisms: bacteria, reconstructed from the inside out to perform an intricate feat of medical care. The circuit is designed to first fabricate a cancer drug inside the bacterium. It then directs the microbe to slip into the interior of a tumor, carried by the bloodstream, and self-destruct. When the microbe bursts apart, it releases its payload of drugs.



Unnatural Responsibilities Synthetic biology offers unusual rewards and risks.
By Kevin M. Esvelt

To fight an evolving pathogen, use an evolving cure. There are problems, though, in bending nature to our own ends. Adopting an organism to work for us means it is using energy that could otherwise be spent replicating, so it will not reproduce as well as competitors. Evolution will constantly select for faster-reproducing mutants that no longer do what we want. Biology's greatest strength is its capacity to replicate and evolve, but that also presents the greatest challenge. One way around this is to incorporate limits on the ability to change, particularly for those few cases where our changes might be able to spread in the wild. For example, one approach is to employ unnatural amino acid tethers: they make essential proteins within cells wholly dependent on chemicals that do not exist in nature. If the amino acids are withheld, the proteins will not function, and the bacteria cannot grow out of control. We are also better at building within the scope of evolutionary limits: microbes are now programmed to release a burst of complex molecules and then die, mostly avoiding evolutionary selection against production.

Engineered viruses that target bacteria will kill invading pathogens, multiply until the invaders are gone and then stop, leaving the patient untouched. We must also be careful to make sure benefits always outweigh the risks of reworking organisms. Mistakes are inevitable. Thus, the projects have to be worth it, especially the earliest examples that must justify the technology to the world.

Building cells that can selectively destroy cancer or cure diabetes is something everyone can get behind. The greatest biological risk to civilization stems from pandemics of infectious disease. Until now, these were inevitable, but we might soon use biotechnology to stop them. Ordinarily, a person's body confronts an invading pandemic pathogen by evolving its own defenses, creating a whole series of antibodies in the hope that one will effectively neutralize the invader. It is a process of trial and error that takes time; this is why you are typically sick for three to four days before getting well. Sometimes that is just too long, and people die. A better strategy is to give the human body a head start: Take the genes for several known protective antibodies, put them into the harmless shell of a virus and inject that virus into people. The virus enters their cells, which then start to churn out already optimized protective antibodies against the invader, ending the threat. c

Waldholz 2017, Transformers. Sci. Am. April 2017, p. 46.

Pancreatic Islet Cell Replacement Therapy

Vertex Pauses Islet Cell Study After Patient Deaths (Miriam E. Tucker, January 10, 2024)

Vertex Pharmaceuticals, Inc. has paused a study of its investigational allogeneic stem cell–derived, fully differentiated pancreatic islet cell replacement therapy (VX-880) following two patient deaths. Neither death is related to VX-880, the company said in a January 8, 2024, investor statement, noting that "Vertex has placed the study on a protocol-specified pause, pending review of the totality of the data by the independent data monitoring committee and global regulators." No further information about the deaths was provided. In response to an inquiry from Medscape Medical News, a Vertex spokesperson said, "We plan to share the full data set at an upcoming medical meeting." In the phase 1/2 study, 14 patients with type 1 diabetes and impaired hypoglycemia awareness or recurrent hypoglycemia received portal vein infusions of VX-880 along with standard immunosuppression. As of the last data cut, all 14 patients demonstrated islet cell engraftment and production of endogenous insulin. After more than 90 days of follow-up, 13 of the patients have achieved A1c levels < 7% without using exogenous insulin. The safety profile of VX-880 to date is consistent with immunosuppression, the perioperative period, and past medical history, Vertex says.

One of the two patients who died was 66-year-old Brian E. Shelton, the first person to receive VX-880 after living 40 years with type 1 diabetes. Vertex first reported his results in October 2021. At 90 days after a single half-dose of VX-880, his C-peptide level rose from undetectable to 280 pmol/L fasting, his A1c dropped from 8.6% to 7.2%, and his daily insulin requirements dropped from 34 to just 2.9 units per day. In contrast to the five severe hypoglycemic episodes Shelton had experienced in the year prior to the transplant, he had only some mild episodes soon after the procedure but none thereafter. In November 2021, Shelton's story appeared in The New York Times. Vertex provided subsequent study updates at the 2022 American Diabetes Association (ADA) annual Scientific Sessions, the 2023 ADA Scientific Sessions, and the 2023 European Association for the Study of Diabetes meeting. By fall 2023, three patients, including Shelton, had achieved insulin independence by day 180 post-transplant. According to Shelton's obituary, "Brian was the first human with Type I Diabetes to receive lab grown stem cells to replicate the natural action of insulin-producing cells, and to be independent of insulin injections until his death. The clinical trial was performed at Massachusetts General Hospital in Boston in July of 2021. He was extremely proud of this accomplishment, and what it means for the future of diabetes research and the health of diabetics worldwide." In a statement on Facebook, the type 1 diabetes advocacy group JDRF said it mourns the loss. "Brian was a type 1 diabetes trailblazer whose participation in this clinical trial showed that cures for type 1 diabetes are possible…. Our thoughts are with Brian's family and friends."

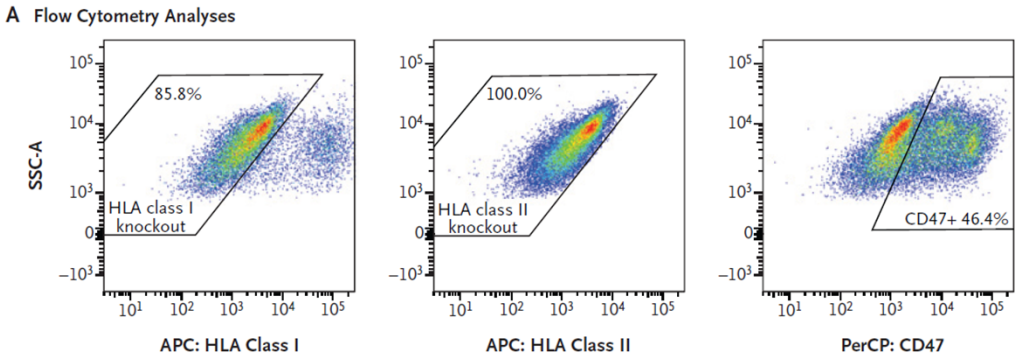
Vertex is continuing with a phase 1/2 clinical trial of a different product, VX-264, which encapsulates the same VX-880 cells in a device designed to eliminate the need for immunosuppression.

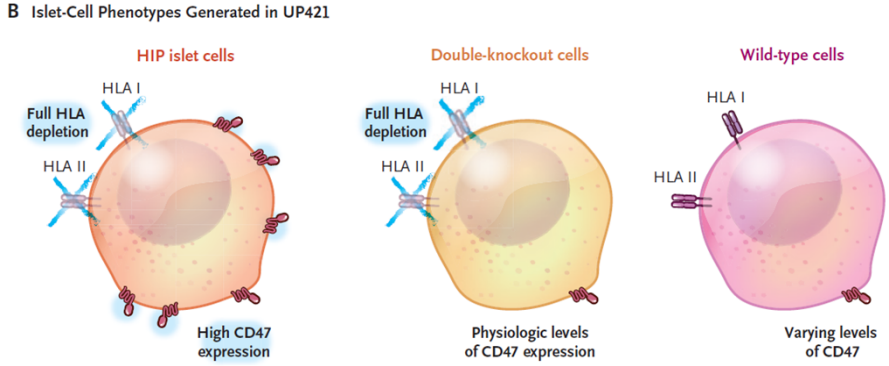
Miriam E. Tucker is a freelance journalist based in the Washington, DC, area. She is a regular contributor to Medscape, with other work appearing in the Washington Post, NPR's Shots blog, and Diabetes Forecast magazine. She is on X (formerly known as Twitter) @MiriamETucker.

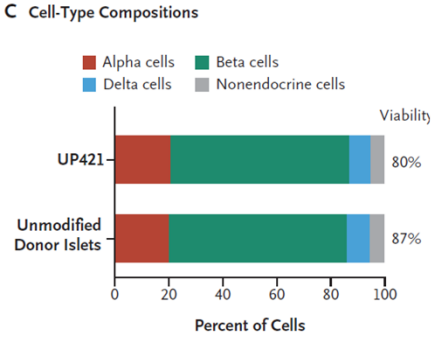
https://www.medscape.com/viewarticle/vertex-pauses-islet-cell-study-after-patient-deaths-2024a10000oe?ecd=mkm_ret_240128_mscpmrk_endo_top_etid6267024&uac=70212FJ&impID=6267024

Allogeneic Beta Cells with No Immunosuppression

The need to suppress a patient's immune system after the transplantation of allogeneic cells is associated with wide-ranging side effects. We report the outcomes of transplantation of genetically modified allogeneic donor islet cells into a man with long-standing type 1 diabetes. We used clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 12b (Cas12b) editing and lentiviral transduction to genetically edit the cells to avoid rejection; the cells were then transplanted into the participant's forearm muscle. He did not receive any immunosuppressive drugs and, at 12 weeks after transplantation, showed no immune response against the gene-edited cells. C-peptide measurements showed stable and glucose-responsive insulin secretion. A total of four adverse events occurred, none of which were serious or related to the study drug.







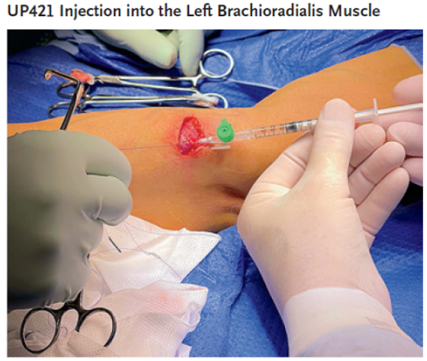


Figure 1. Characterization and Transplantation of the Engineered Allogeneic Islet-Cell Product UP421.
Panel A shows the results of the flow cytometry analyses of the final gene-edited islet-cell product (UP421) for the surface expression of HLA class I, HLA class II, and CD47, with the percentages for HLA class I and II depletion and CD47 overexpression. Panel B shows the three islet-cell phenotypes that were generated in the UP421 product. The hypoimmune platform (HIP) islet cells showed both full HLA depletion and CD47 overexpression, whereas HLA class I– and class II–depleted double-knockout islet cells showed physiologic CD47 expression. Wild-type islet cells retained physiologic HLA expression and showed varying levels of CD47 expression. Panel C shows the cell-type compositions of the unmodified donor islets and the final UP421 cell product. Gene editing did not change the cell makeup of the islets. Panel D shows 1 of the 17 injections into the left brachioradialis muscle in the participant. APC denotes allophycocyanin, PerCP peridinin–chlorophyll–protein, and SSC-A side scatter area.

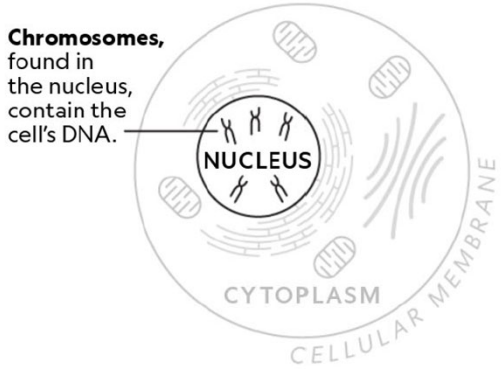
Carlsson 2025, Survival of transplanted allogeneic beta cells with no immunosuppression

Reversing the Aging Process

How Our Cells Age

Over the past three decades, biomedical researchers have identified a number of mechanisms, or “hallmarks,” of aging to explain the cellular and molecular processes that damage our cells and cause our bodies to age. Grouped here into three categories, nine of these hallmarks are at the core of cutting-edge efforts to slow aging—the leading risk factor for many major diseases including cancer.

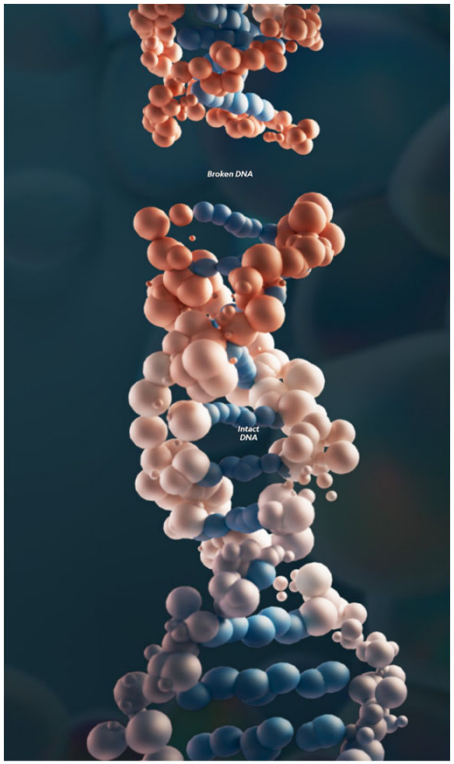
by Jason Treat, Eve Conant, and Kelsey Nowakowksi
Illustrations and animation by Markos Kay
Published December 28, 2022



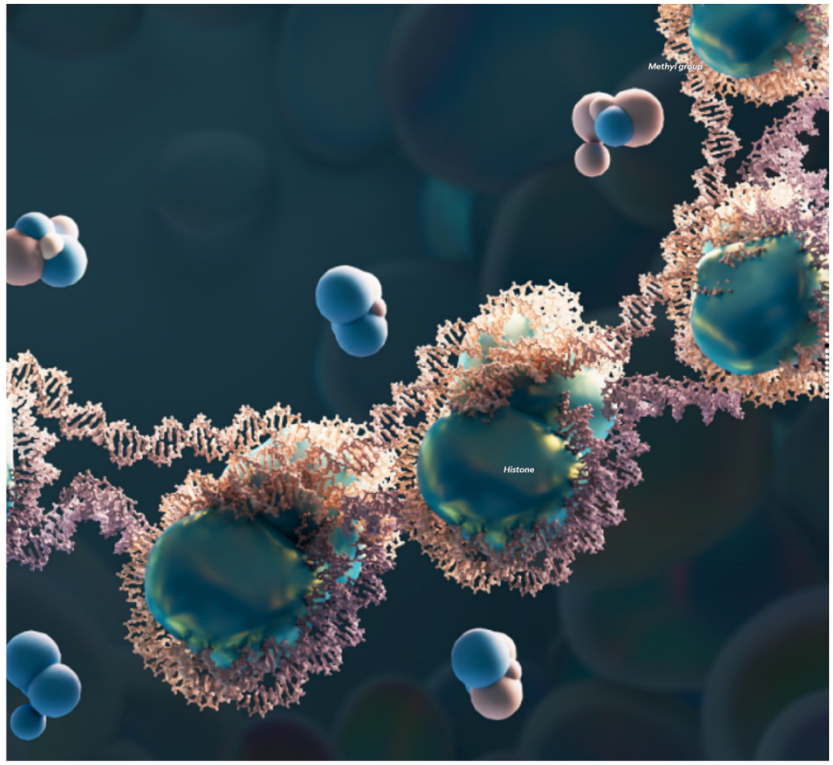
Inside the nucleus
The nucleus is the heart of the cell. Because it contains DNA, the blueprint for all cellular activity, any damage inside the nucleus is serious and can be transmitted to the entire cell, causing a torrent of negative effects.

https://www.nationalgeographic.com/magazine/graphics/aging-hallmarks-damage-cells-disease-feature

How Our Cells Age



Unrepaired DNA
Myriad hazards, such as pollution, are a constant threat to DNA. Our genomes encode processes that address assaults, but the repair isn't always successful and flaws can accumulate, leading to cancer and other diseases.



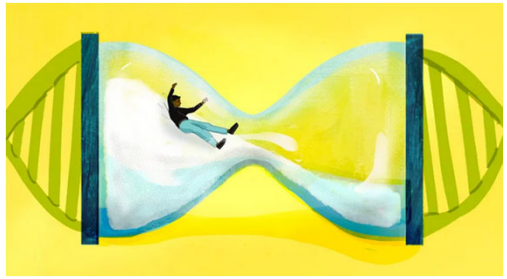
Defects in DNA regulation
DNA strands are wound around spools of proteins called histones. Genes are turned on and off depending on where methyl groups attach to DNA and histones. When those attachments malfunction, precise coordination of gene activity can be compromised.

https://www.nationalgeographic.com/magazine/graphics/aging-hallmarks-damage-cells-disease-feature

Methylation

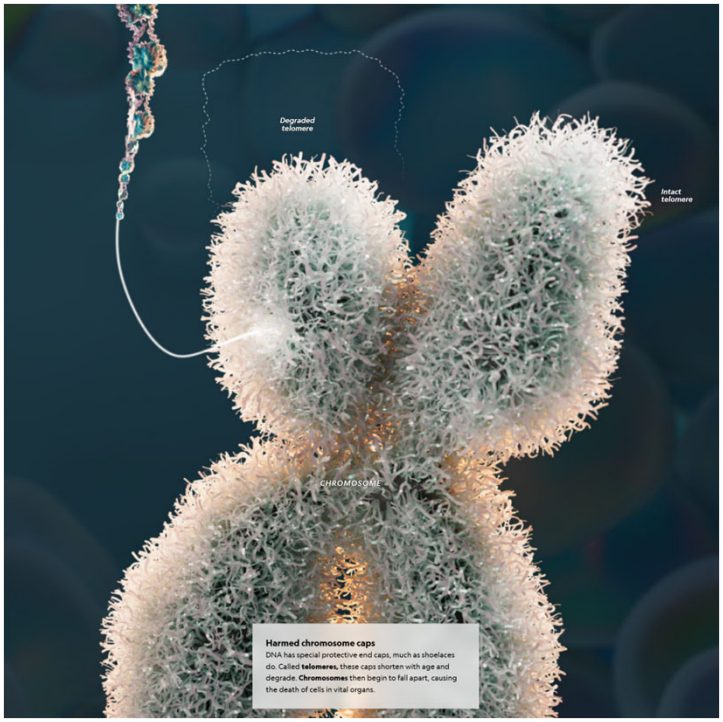
Methylation is a chemical modification of DNA and other molecules that may be retained as cells divide to make more cells. When found in DNA, methylation can alter gene expression. In this process, chemical tags called methyl groups attach to a particular location within DNA where they turn a gene on or off, thereby regulating the production of proteins that the gene encodes.

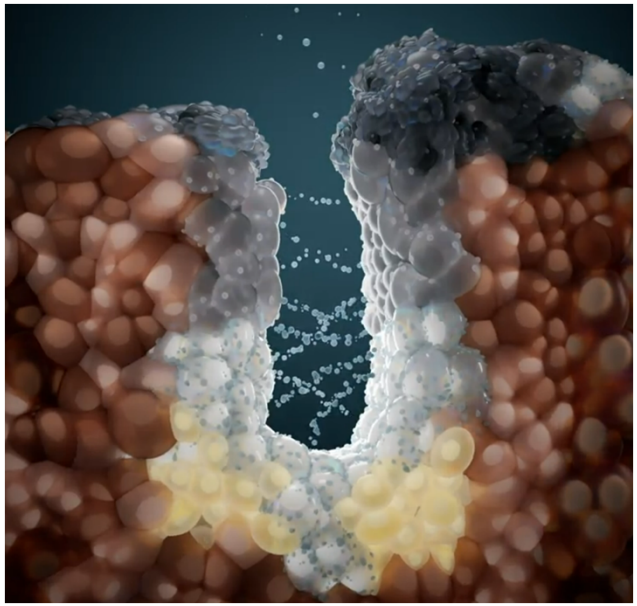
https://www.genome.gov/genetics-glossary/Methylation

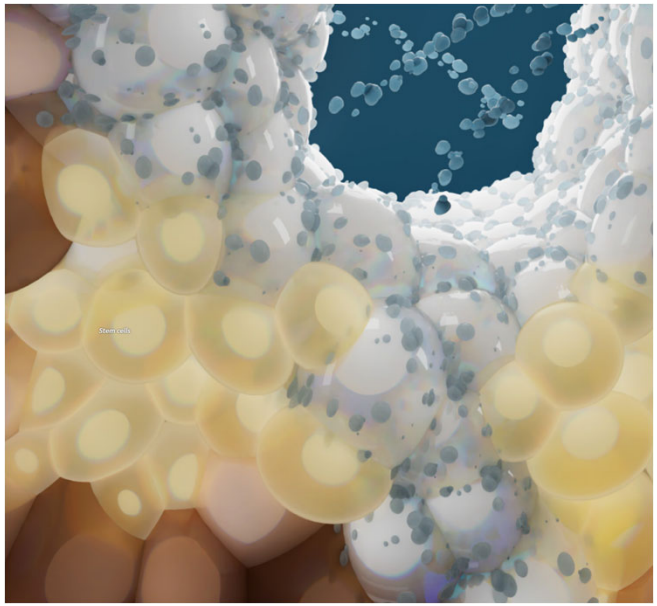


Aubrey 2024, You can order a test to find out your biological age

How Our Cells Age







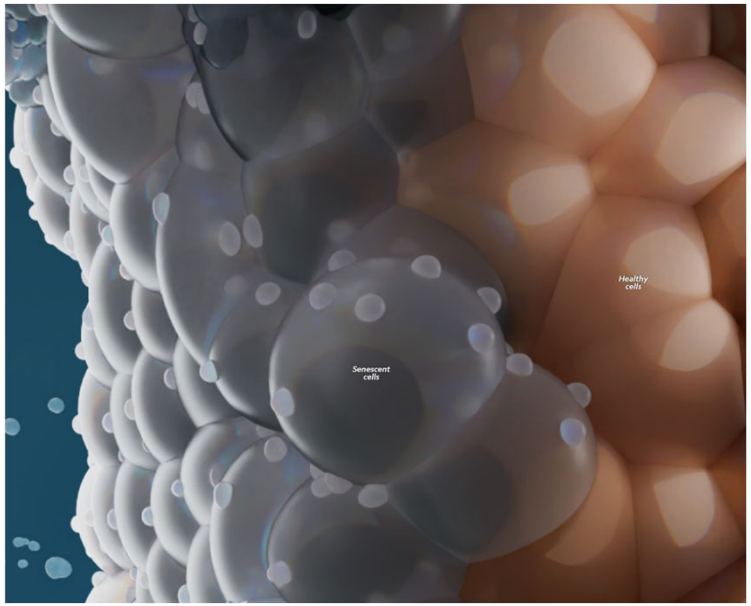
Harmed chromosome caps
DNA has special protective end caps, much as shoelaces do. Called telomeres, these caps shorten with age and degrade. Chromosomes then begin to fall apart, causing the death of cells in vital organs.

Cellular interactions
Cells need to be able to communicate with one another for our body's organs to function in an optimal way. When DNA or cells become damaged, as shown here in the intestinal wall, cells can't receive the proper signals.

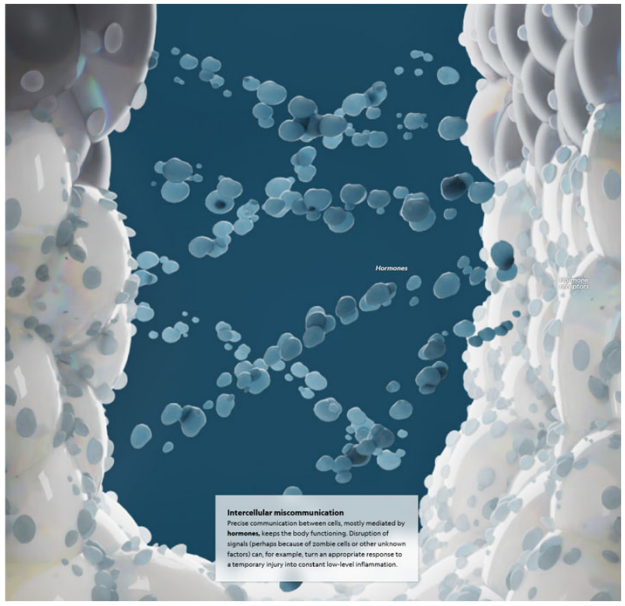
Loss of stem cell function
Our body's ability to repair tissues and organs depends on healthy stem cells—the main source of new cells. But stem cells replicate only on demand, an ability that declines with age.

https://www.nationalgeographic.com/magazine/graphics/aging-hallmarks-damage-cells-disease-feature

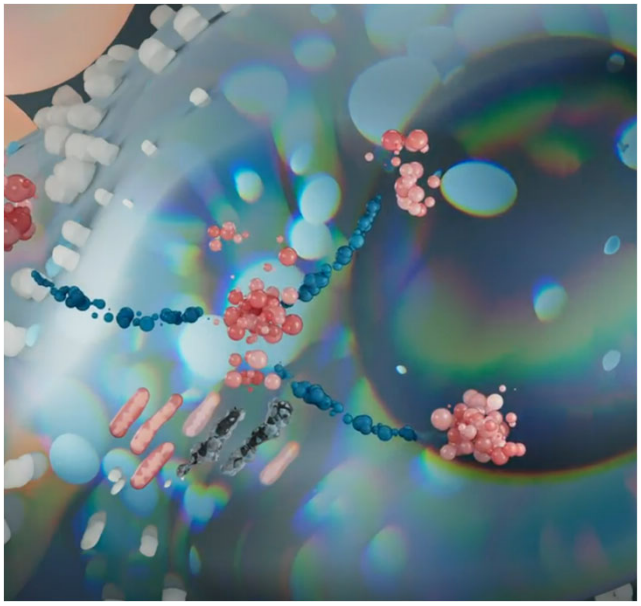
How Our Cells Age



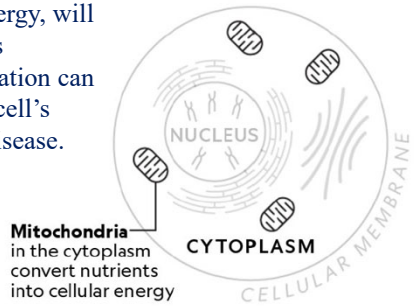
Formation of zombie cells
Defective cells can enter a permanent nondividing state called senescence. Sometimes called zombie cells, they can play important roles at times, such as in wound healing. But they accumulate with age and never die. These rogues also secrete molecules that harm neighboring cells.



Intercellular miscommunication
Precise communication between cells, mostly mediated by hormones, keeps the body functioning. Disruption of signals (perhaps because of zombie cells or other unknown factors) can, for example, turn an appropriate response to a temporary injury into constant low-level inflammation.

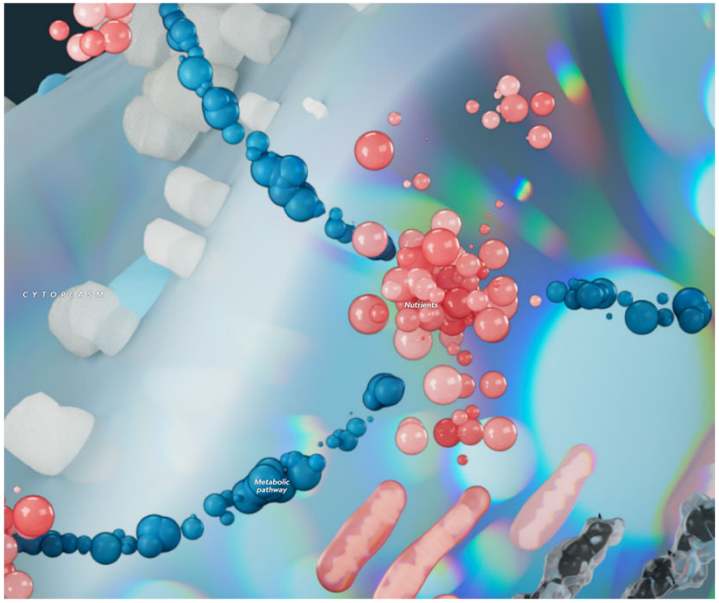


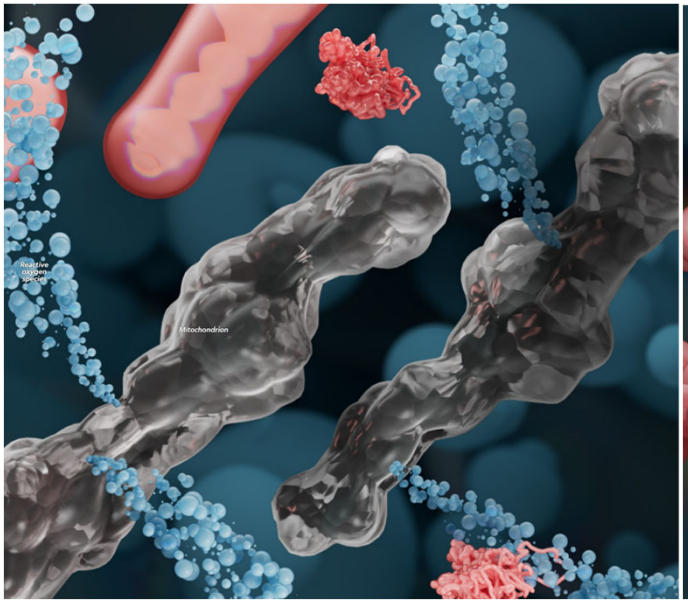
Inside the cell
Cells are like factories with many critical, interacting parts. Damage to any of them, including the mitochondria that turn food into energy, will compromise the cell's function. This degradation can eventually affect the cell's nucleus and lead to disease.

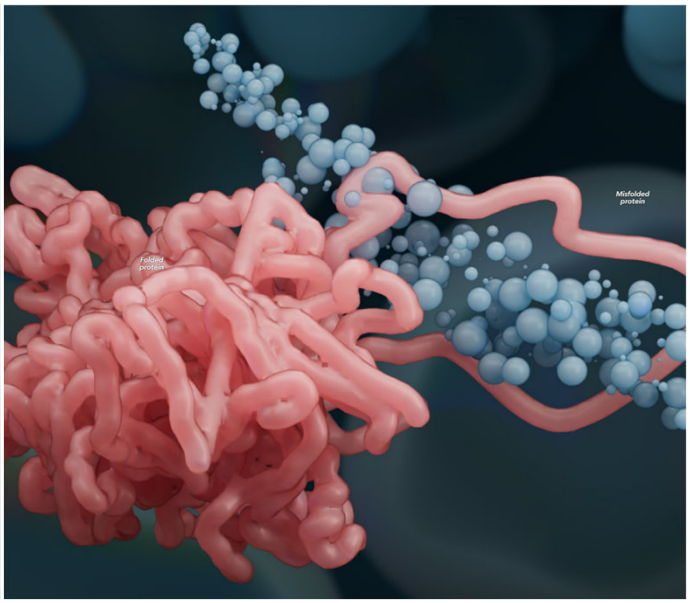


https://www.nationalgeographic.com/magazine/graphics/aging-hallmarks-damage-cells-disease-feature

How Our Cells Age







Deregulated response to nutrients
When we eat, we supply our cells with nutrients that they need to keep us healthy. But excessive nutrients can exceed the capacity of cells to store and metabolize them, resulting in toxic reactions.

Mitochondrial dysfunction
Mitochondria produce more than 90 percent of a cell's energy and almost all of its free radicals, also called reactive oxygen species. In low amounts these unstable molecules can be useful for signaling stress and triggering maintenance and repair, but too many can be toxic.

Compromised proteins
To regulate chemical reactions and provide cell structure, proteins must fold in precise, origami-like shapes. When they're injured, they misfold and become sticky, clumping together and gumming up the cellular machinery in ways that can lead to diseases such as Alzheimer's and Parkinson's.

https://www.nationalgeographic.com/magazine/graphics/aging-hallmarks-damage-cells-disease-feature

Sources: Steven Austad, University of Alabama at Birmingham; Manuel Serrano, Institute for Research in Biomedicine, Barcelona

Drink the Water of Life using the Right Cup