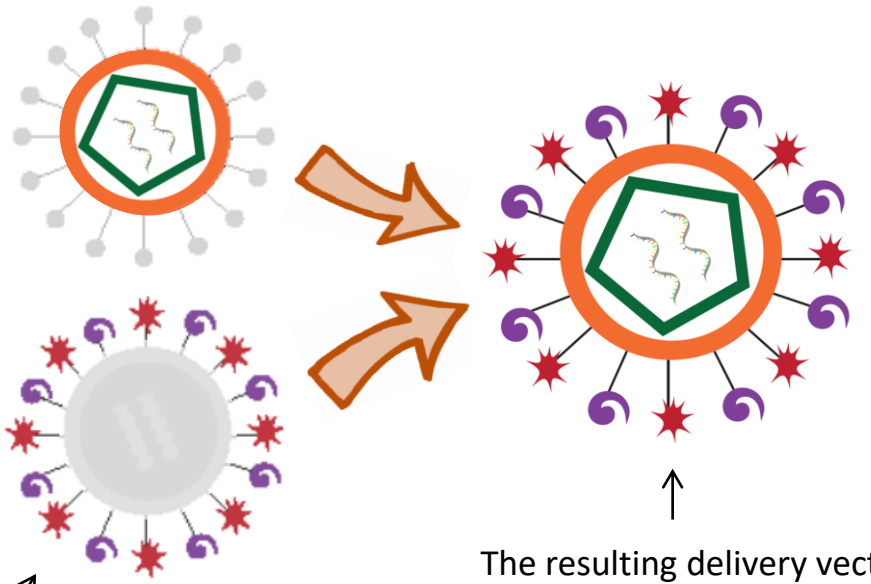
Lentivirus Gene Therapy Vector for Cystic Fibrosis

Simian Immunodeficiency Virus can last for a for a long time inside cells it enters

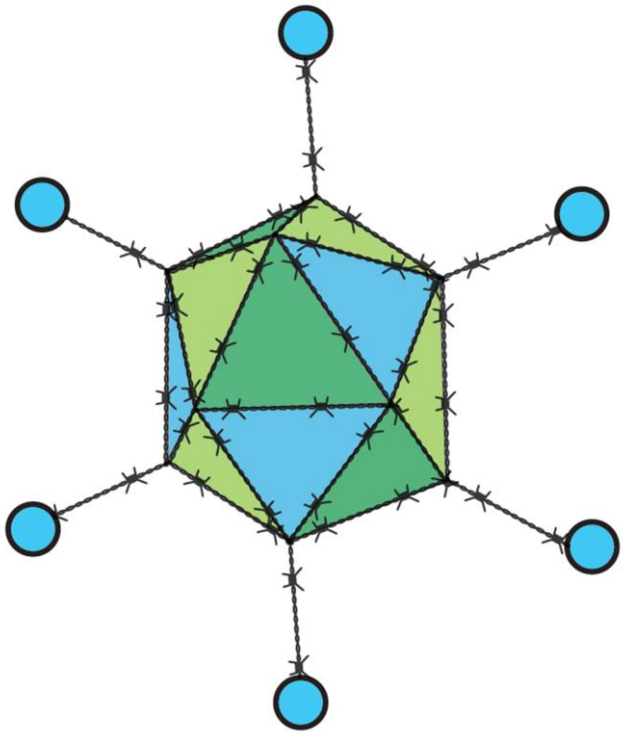


Sendai Virus has surface receptors that help it get into lung cells

The resulting delivery vector enters lung cells much more efficiently than previous vectors and a single dose can result in CFTR production for at least 2 years in a mouse

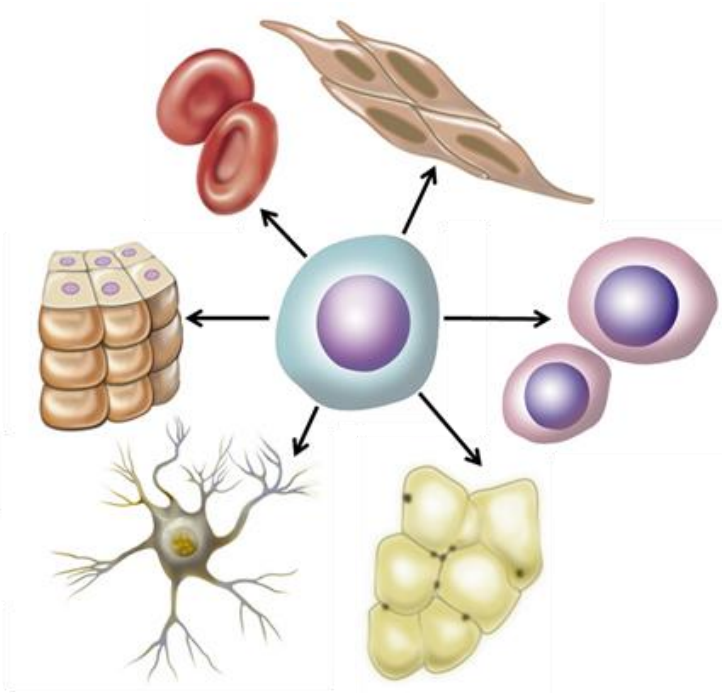
- The image shows how features from 2 viruses can be combined to create an ideal ‘vector’ or gene delivery vehicle for a particular task
- In cystic fibrosis lung disease, patients lack a healthy copy of a gene called CFTR
- This gene codes for a salt channel in lung cells without which lung mucus is thick and sticky and patients suffer repeated infections and a limited lifespan
- Gene therapy for Cystic Fibrosis aims to deliver healthy copies of the CFTR gene into lung cells so that they can make normal salt channels
- By using the best features of 2 different viruses we have designed a new modified virus which is very effective at delivering the gene into airway cells

Oncolytic Adenovirus



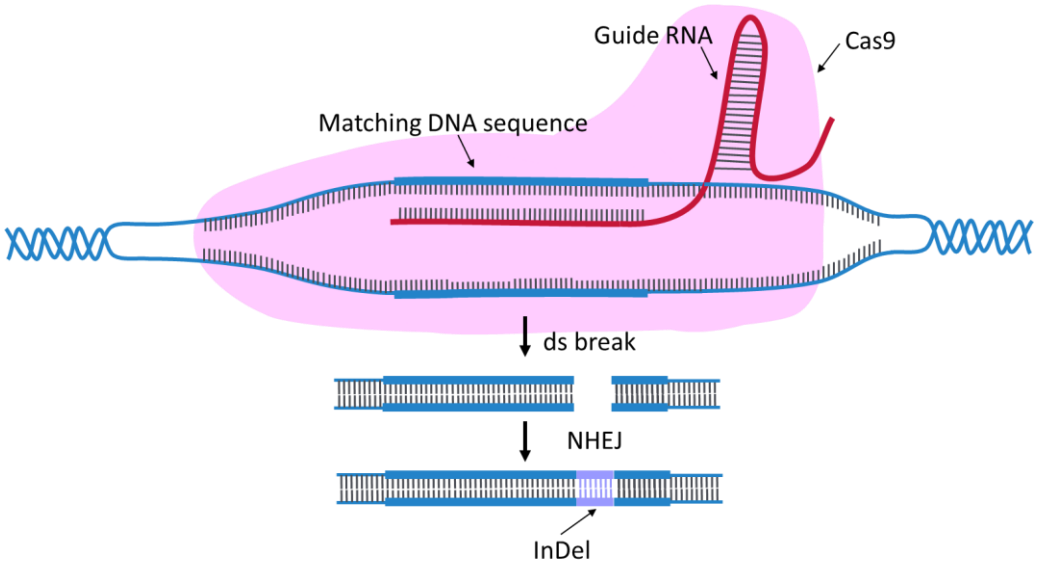
- The image depicts an adenovirus particle, which is icosahedral in nature.
- For cancer applications the virus is engineered so it can't infect healthy cells, but seeks out and infects cancer cells.
- Once inside cancer cells, the virus replicates, filling infected cells with daughter virions. Eventually the cell bursts (or "lyses") – as depicted by the barbed wire, spreading daughter virus to infect surrounding tumour cells, and repeating the process.
- Cell bursting, or "oncolysis" induced by the virus is immunogenic, helping the host immune system to recognise and destroy the tumour.
- This immunogenic, oncolytic activity can be enhanced through additional genetic engineering of the virus, which forces infected tumour cells to overexpress immunotherapies.

Stem Cell Self-renewal And Differentiation



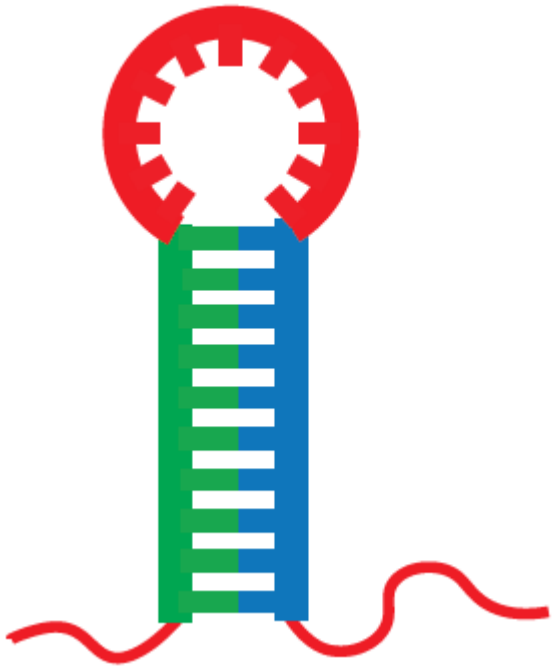
- Stem cells are undifferentiated cells. They don’t have a specific function in the organism yet, but they can give rise to differentiated/specialised cells with specific functions such as red blood cells, muscle cells, nerve cells or skin cells etc.
- Stem cells have a unique property: When they divide they can either produce a copy of themselves (i.e. a stem cell), this is also called self-renewal, or they produce a more differentiated cell (i.e. a cell on the way to becoming a skin cell for example).
- Stem cells act as a reservoir for more differentiated cells, because they can become more than one cell type and can therefore supply the organism with replenishment of certain cell types.
- Embryonic stem cells can differentiate into any specialised cell type in the organism, adult stem cells can only produce a specific subset of specialised cells for example red blood cells.

CRISPR



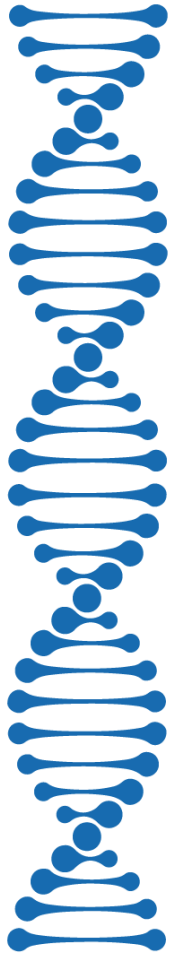
- CRISPR is a technology that allows for specific targeting of DNA sequences
- By coupling the CRISPR molecule with a cutting enzyme such a ‘Cas9’, genes can be edited accurately at very specific locations
- Compared to previous gene editing technologies, CRISPR is relatively simple to perform and can be easily customised to target any sequence
- CRISPR has enormous potential to cure diseases by silencing or making changes to disease causing genes
- Gene editing could be used to make heritable changes which are unrelated to health such as hair colour or height. This poses serious ethical dilemmas
- Recently, a group in China announced the birth of a CRISPR edited baby. The scientific community agreed that this research was not performed responsibly
- CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats. These short palindromic repeats were unusual DNA sequences found in the genomes of bacteria which were thought to be ‘memories’ left by previous viral invaders. Bacteria use these sequences to recognise dangerous viruses when encountered again and neutralise them

Short hairpin RNA



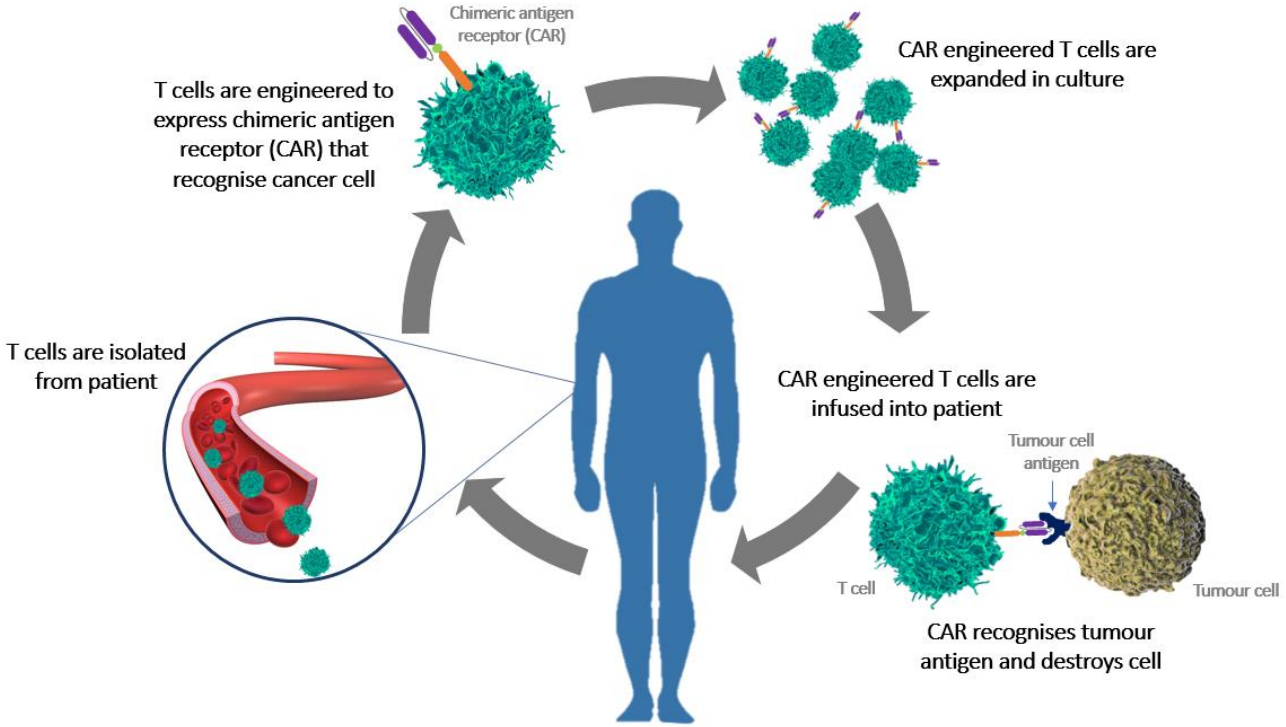
- Short-hairpin RNAs (or shRNAs) are artificial RNA molecules with a hairpin-like structure. They can be used to specifically silence gene expression in a process called RNA interference.
- A specific nucleotide sequence on the shRNA matches a sequence on the target gene. The shRNA specifically binds to the target gene's messenger RNA (mRNA) and deactivates it. Since mRNA's function is to carry a gene's coded instructions how its protein is assembled. The deactivation of mRNA by shRNA means that its protein is no longer produced.
- This technique can be used to treat genetic diseases that are caused by too much expression of a certain gene. A good example of this is a familial colon cancer known as familial adenomatous polyposis or FAP.

DNA



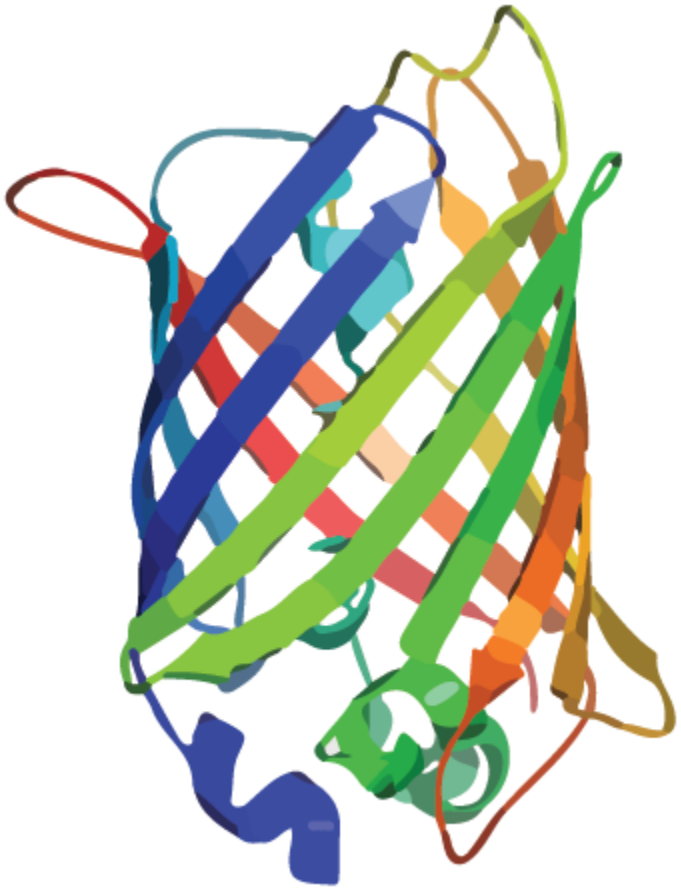
- DNA (Deoxyribonucleic acid) consists of 4 different ‘letters’ or bases: Adenine, Cytosine, Guanine and Thymine (A, C, G, T), and a sugar phosphate backbone. DNA is a double stranded molecule, two strings of DNA are held together by base-pairing of the bases. Adenine binds to Thymine, while Cytosine binds to Guanine. The pairing is called Watson-Crick base-pairing.
- DNA encodes genetic information. The DNA in each of our cells contains all the information needed to produce a whole organism. The information is encoded in the so called ‘genetic code’. The DNA in a human cell is like an instruction book to build a human body.
- The information is encoded in units called genes. A gene contains the information to build one specific protein.
- A combination of 3 bases encodes an amino acid. There are $4^3 = 64$ possible combinations for 20 amino acids. Several 3-base-combinations therefore produce the same amino acid. Amino acids fold to produce three-dimensional structures, proteins.

CAR-T Cell Therapy



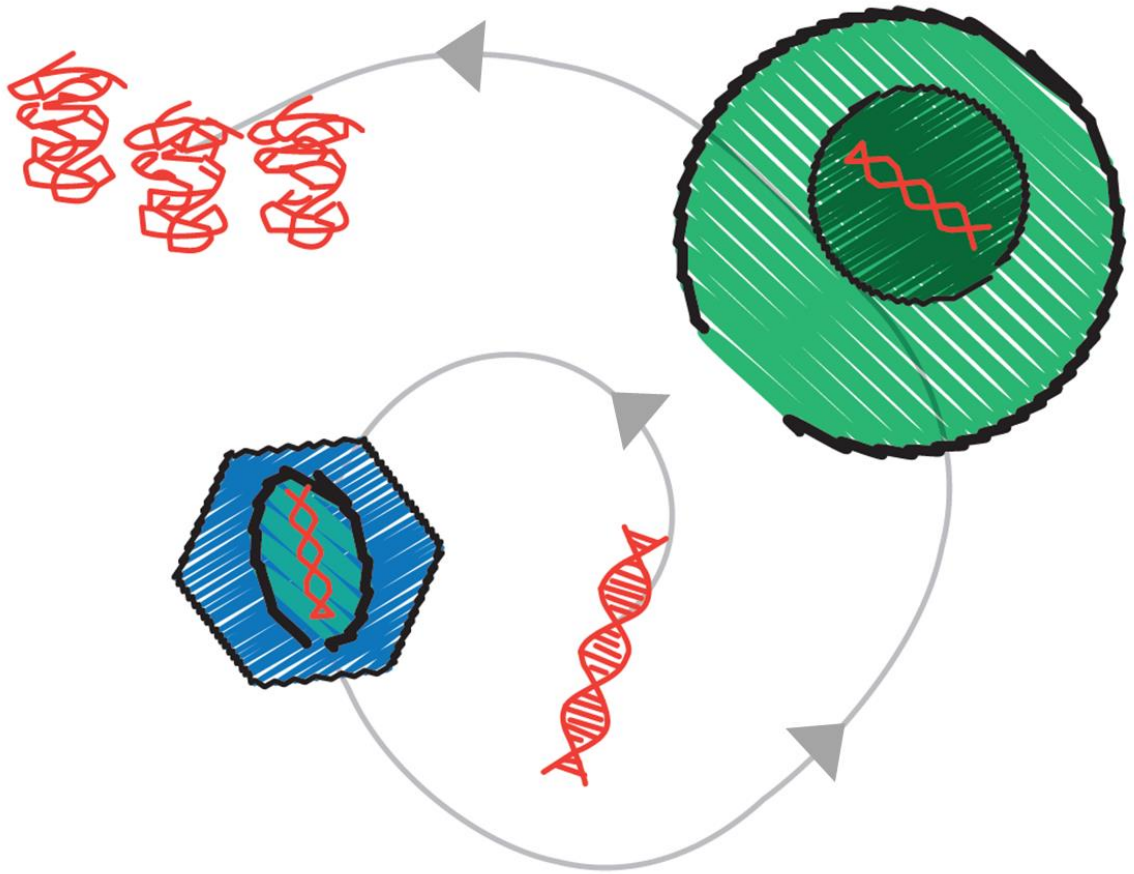
- CAR-T cell therapy is used to treat some types of cancer. This therapy involves the removal of immune system cells, known as T-cells, from the patients blood where they are reprogrammed and returned to the patient.
- A specific receptor known as CAR, or chimeric antigen receptor (shown in blue) is added to the T-cells' surface allowing the modified cells to target and kill the patients cancer cells.
- As the specific protein that CAR recognises and binds is only found on the cancer cells and not on healthy cells, only the cancer cells are killed.
- CAR-T cell therapy is used to treat some types of lymphoma and leukaemia.

Green Fluorescent Protein



- This is a protein structure of GFP (green fluorescent protein).
- GFP is a protein isolated from the jellyfish *Aequorea victoria*. It fluoresces green when excited with light of blue to ultraviolet range.
- It is a popular tool in molecular biology as a reporter system. This means that it will be introduced into a cell and can then be used to track the production, expression, location of another protein of interest.
- Scientists Roger Y. Tsien, Osamu Shimomura and Martin Chalfie were awarded the 2008 Nobel Prize in Chemistry for their discovery and development of the green fluorescent protein.

Gene Therapy



- Gene therapy is a type of technology in which DNA or RNA is delivered into cells
- The simplest example of this is in diseases caused by mutations in a single gene. Gene therapy can be used to deliver a healthy copy of the gene into cells to restore the function that was previously lost.
- For example, in Cystic Fibrosis patients have a faulty gene encoding a salt channel which regulates mucus in the lung. The result is frequent infections caused by this thick mucus. Gene therapy can deliver the gene encoding normal salt channels to solve this problem
- Gene therapy has other applications such as delivering DNA or RNA to silence mutated genes that cause harm
- For example in Huntington's Disease which is caused by a toxic accumulation of a mutated protein and may be silenced at a genetic level using gene therapy
- Various vehicles or 'vectors' can be used to deliver the DNA or RNA into cells. These include modified viruses and non-viral vectors such a lipid molecules and polymer nanoparticles