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Recent Advances in the Development of Gene Delivery Technologies

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1

Factors required for successful CGT transgene delivery

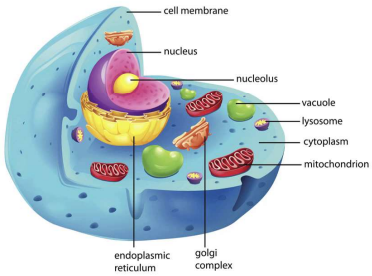
Cell structure and defences to beat: Uptake

When trafficked towards the cell there are interactions with multiple organelles depending on whether it is a DNA or RNA vector

2

Factors required for successful CGT transgene delivery

Cell structure and defences to beat: Uptake



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Factors required for successful CGT transgene delivery

Uptake of vector by appropriate cells

↓

Vector entry into nucleus (DNA vectors) or cytoplasm (RNA vectors)

↓

Evade host immune defence

Factors required for successful CGT transgene delivery

Evade host immune defence

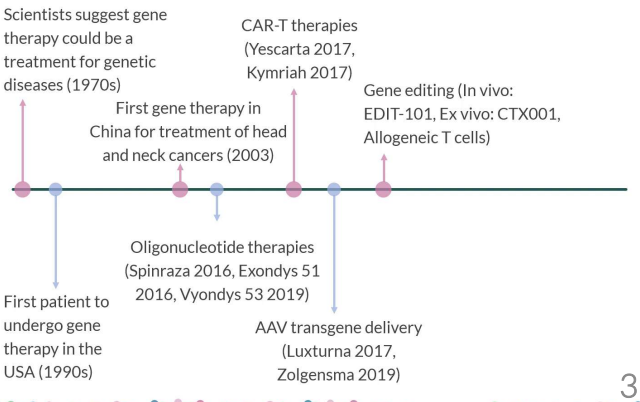
Innate Response

- Release of pro-inflammatory cytokines, recruitment of neutrophils, macrophages, dendritic cells or other leukocytes
- Activation and maturation of the antigen presenting cells

Adaptive Priming

Adaptive Response

Major milestones in Gene therapies



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Major milestones in Gene therapies

Base and prime editing and advancements in regulatory approval landscape

Personalized therapies

Universal donor cell therapies (Allogeneic cell products (engineered iPSCs)

Genome annotation and functional genomics

Common disease

Epigenome editing

Schematic of *ex vivo* and *in vivo* strategies

In vivo GENE THERAPY

1) The therapeutic gene is cloned into the genome of a recombinant virus

3) The cells that are transduced by the recombinant virus produce the therapeutic protein

2) The recombinant virus carrying therapeutic gene is infused into the patient.

Ex vivo GENE THERAPY

1) Cells from the patient are extracted via a biopsy and expanded in culture

2) Cells are transduced using a recombinant virus carrying the therapeutic gene

3) Transduced cells that produce the therapeutic protein are infused back into the patient

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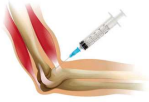
Where/How do you want to apply CGT?

1 *In vivo*

Viral or non viral | In situ | Loco regional | Systemic

Tourniquet

Extract injected under the skin



Injection into the joint

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Where/How do you want to apply CGT?

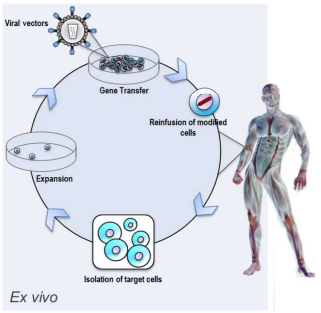
1 ***In vivo***

Viral or non viral | In situ | Loco regional | Systemic

- All current gene therapy approaches have been directed at somatic cells, germline engineering remains controversial
- One of the key challenges to *in vivo* approaches is maximising the on target tissue transduction

Where/How do you want to apply CGT?

2 ***Ex vivo***



Ex vivo

Autologous or Allogeneic

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Where/How do you want to apply CGT?

3 ***In utero?***

- During foetal development a window of opportunity exists for the therapeutic insertion of genes
- An insertion of genes into this receptive environment could provide opportunities to correct genetic deficiencies and prevent permanent or semi-permanent diseases

- However, there are important ethical and regulatory concerns and considerations are still to be applied

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What is an ATMP?

Advanced therapy medicinal products (ATMPs) are medicines for human use that are based on genes, tissues or cells. They offer ground breaking new opportunities for the treatment of disease and injury

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What is an ATMP?

ATMPs can be classified into three main types:

- Gene therapy medicines: these contain genes that lead to a therapeutic, prophylactic or diagnostic effect. They work by inserting 'recombinant' genes into the body, usually to treat a variety of diseases, including genetic disorders, cancer or long-term diseases. A recombinant gene is a stretch of DNA that is created in the laboratory, bringing together DNA from different sources
- Somatic-cell therapy medicines: these contain cells or tissues that have been manipulated to change their biological characteristics or cells or tissues not intended to be used for the same essential functions of the body. They can be used to cure, diagnose or prevent diseases

What is an ATMP?

- Tissue-engineering medicines: these contain cells or tissues that have been modified so they can be used to repair, regenerate or replace human tissue

In addition, some ATMPs may contain one or more medical devices as an integrate part of the medicine which are referred to as combined ATMPs. An example of this is cells embedded in a biodegradable matrix or scaffold

For detailed definitions of the different groups of advanced therapy medicinal products, refer to Regulation (EC) No 1394/2007 and Directive 2001/83/EC

Find full link in links tab

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Why do we need CGT and ATMPs?

- Over 6000 genetic diseases, with many affecting very few patients (rare), whilst others are far more common (cancer, diabetes, atherosclerosis etc.)

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Why do we need CGT and ATMPs?

A rare disease is defined by the European Union as one that affects less than 5 in 10,000 of the general population. There are between 6,000 and 8,000 known rare diseases and around five new rare diseases are described in medical literature each week

7% of the population, will be affected by a rare disease at some point in their lives. This equates to approximately 3.5 million people in the UK and 30 million people across Europe

Why do we need CGT and ATMPs?

- Over 6000 genetic diseases, with many affecting very few patients (rare), whilst others are far more common (cancer, diabetes, atherosclerosis etc.)
- Many acquired diseases can be potentially amenable by cell/gene therapies: unmet medical need
- Standard treatments often deal with symptoms rather than the cause of disease (focus on genetics!)
- Current medical treatments are unavailable or often inadequate
- This field is emerging as a promising approach to personalised medicine and a lot of large pharma companies are investing into this area

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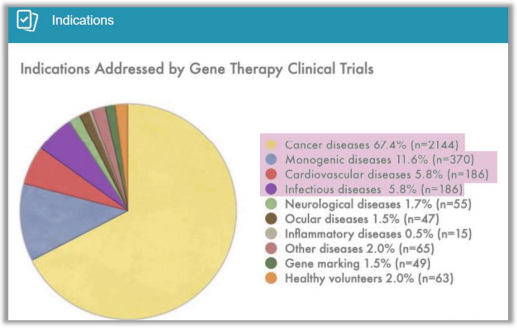
What are the indications addressed by and vectors used in CGT clinical trials?



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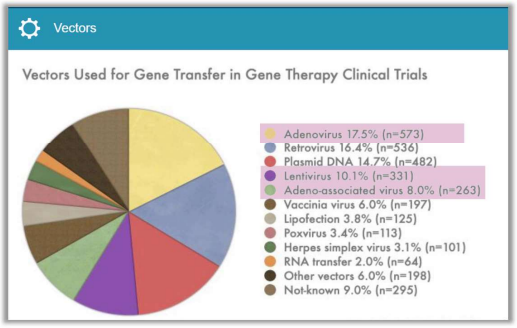
What are the indications addressed by and vectors used in CGT clinical trials?



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What are the indications addressed by and vectors used in CGT clinical trials?

Gene therapy has made important medical advances in less than the last two decades. Within this short time span it has moved from a conceptual stage to technology development and laboratory research to clinical translational trials for a variety of diseases

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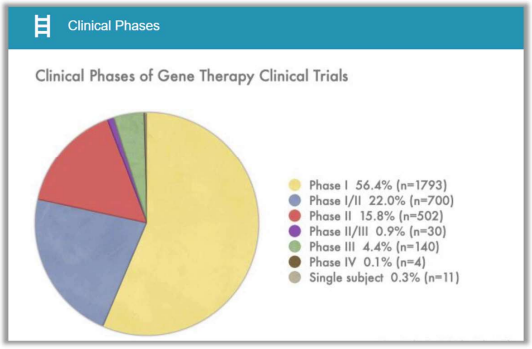
Clinical phases and gene types transferred in CGT clinical trials



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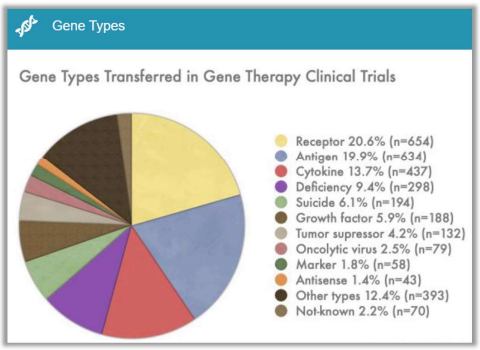
Clinical phases and gene types transferred in CGT clinical trials



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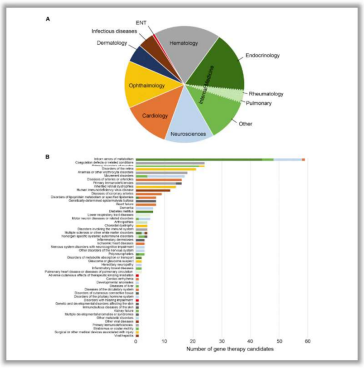
Landscape of early clinical CGT (non-oncology)



Rittié L, *et al. Mol Ther* 27(10),1706-1717 (2019)

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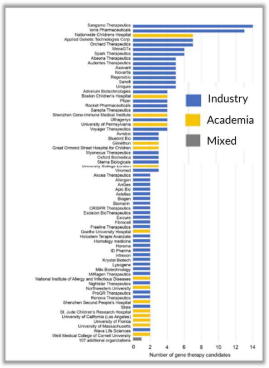
Landscape of early clinical CGT (non-oncology)



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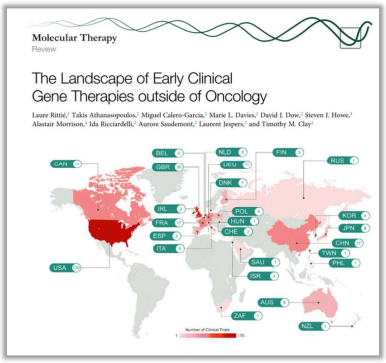

Rittié L, *et al. Mol Ther* 27(10),1706-1717 (2019)

Vector Development: using the right vector for the right target/modality

An ideal gene therapy vector would -

- Target the right cells, cell type-specific
- Show appropriate expression levels (stable or transient; physiological levels or over expression; regulated or constitutive)
- Be available and stable in a highly concentrated active form
- Have low immunogenicity
- Achieve site-specific integration (not random), be tissue specific/immuno-modulated
- Be safe-minimally toxic or non-toxic (to the cells and to the patient!)

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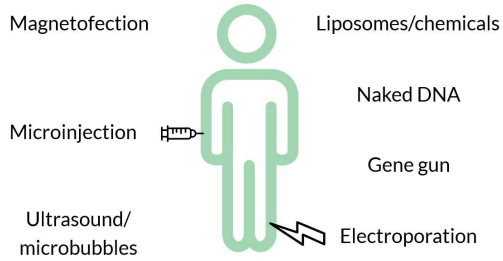
CGT gene delivery approaches: non viral



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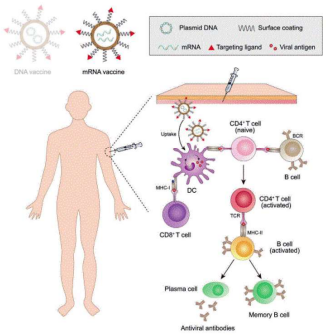
CGT gene delivery approaches: non viral



CGT gene delivery approaches: current toolbox of viruses

1	Viruses are active gene transfer vehicles
2	Viruses have evolved to deliver genetic information to cells
3	They have methods of avoiding immune systems
4	Viral structural proteins offer multiple functions
5	They exploit cellular mechanisms (receptors, endosomal processing, nuclear transport)
6	**BUT** they are normally associated with causing disease and need to be modified

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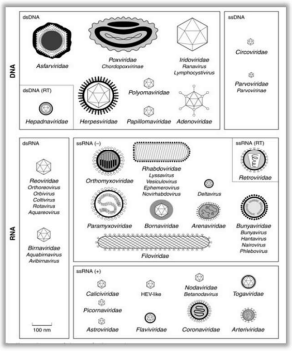
CGT gene delivery approaches: current toolbox of viruses

- Different types of viruses can be used as gene delivery "vectors", chosen for their different characteristics
- Viral vectors derived from families of retroviruses, adenoviruses, AAV and herpes simplex viruses are employed in more than 7% of clinical gene therapy trials



CGT gene delivery approaches: current toolbox of viruses

- The most recent example is the adenovirus based vaccine for COVID-19
- Adenovirus causes common illness including symptoms such as minor fevers and coughs
- Through this research scientists have learned early how to disable genes that can cause the illness whilst keeping their ability to get into cells to treat or prevent disease
- The engineered virus in the vaccine cannot replicate or make copies in the human body to cause illness

CGT platforms & delivery technologies

	Gamma-retrovirus	Lentivirus	Adenovirus	AAV	Non-viral
Nucleic acid	RNA	RNA	DNA	DNA	RNA and DNA
Packaging capacity	~9kb	~10kb	~30kb	4.6kb	Unlimited
Integration into host genome	Yes	Yes	No	Rarely	Rarely
Duration of transgene expression	Long	Long	Transient	Long in post-mitotic tissues	Transient
Transduction of post-mitotic cells	-	+	+++	++	++
Pre-existing host immunity	No	No	Yes	Yes	No
Immunogenicity	++	++	+++	+	-
Safety concerns	Insertional mutagenesis	Insertional mutagenesis	Inflammatory response	Low risk of insertional mutagenesis	-

Adapted from Nathwani *et al., Br J Haematol* (2004)

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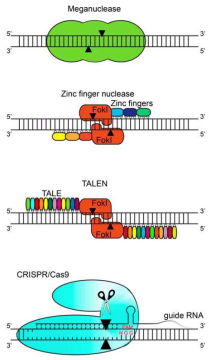
Gene editing technologies for the treatment of human diseases

Different technologies include meganucleases, zinc finger nucleases, TALEN and CRISPR/Cas9



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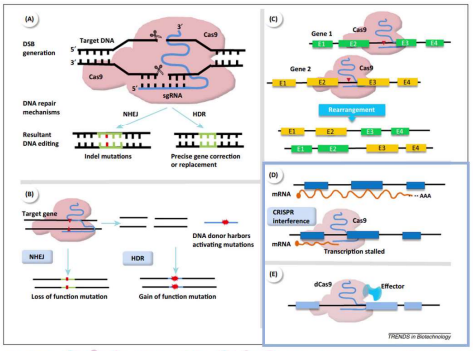
Gene editing technologies for the treatment of human diseases

CRISPR/Cas9



CGT PD&S: vector production at large scale

- Production of vectors by transient transfection has unlimited potential
- For example variable plasmid preparation, variable transfection efficacies, variable quality of packaging cells and variable vector outputs
- This led to vector manufacturing on a fully disposable and scalable platform, this in theory delivers the product on a commercial scale

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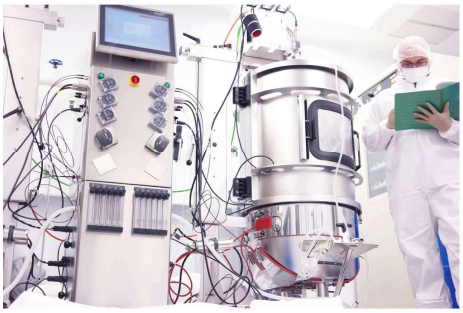
CGT PD&S: vector production at large scale

Research → Early Phase → Commercial

Small bottles and flasks	Cell factory	Wave bag bioreactor	Single-use stirred tank bioreactor

CGT PD&S: vector production at large scale

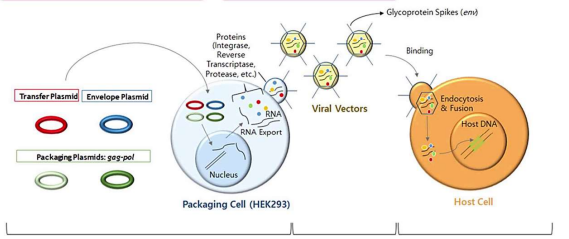


Bioreactor

CGT PD&S: potential variability and control of transient production

Potential for variable plasmid preparation	Potential for variable transfection efficiency
Potential for variable quality of packaging cell	Potential for variable viral vector output

pDNA Manufacturin: impact on LV CoG



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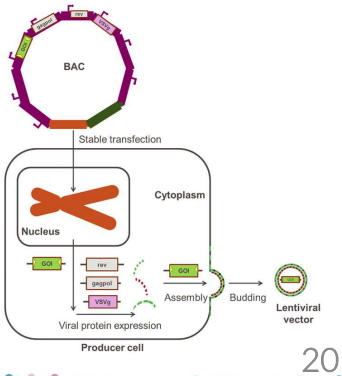
CGT PD&S: potential variability and control of transient production

- × There are low available known markers for engraftment or efficacy of the cell product
- ✓ In future studies the aim is to characterise the product by identifying markers for engraftment and efficacy and analyse specific markers of RNA, DNA and protein from individual studies

CGT PD&S: Innovation in lentiviral vector production

- Single transfection
- Selection
- Cloning and screen
- Bacterial artificial chromosome encoding all vector component
- Integration into HEK293T cells



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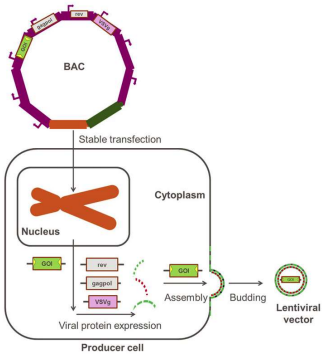
CGT PD&S: Innovation in lentiviral vector production

- ✓ Serum free
- ✓ Suspension
- ✓ Stable
- ✓ Scalable



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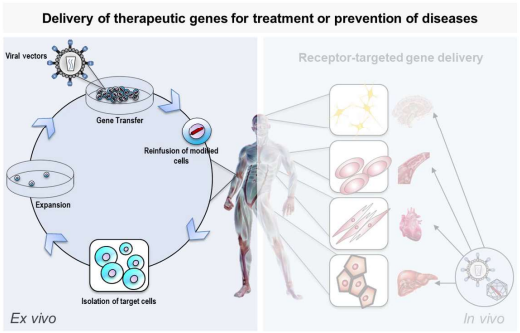
The use of viral vectors in gene therapy

Understanding different gene therapy approaches



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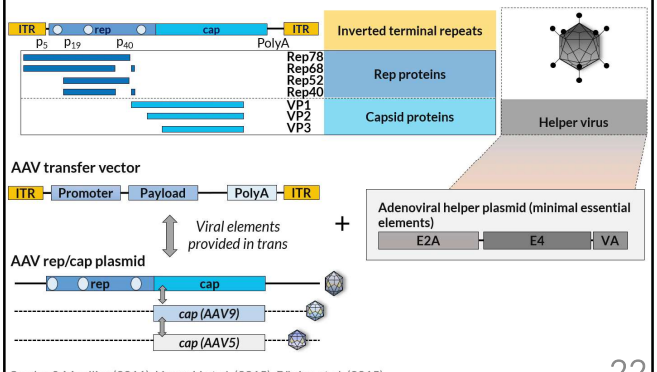
The use of viral vectors in gene therapy

Understanding different gene therapy approaches

- Adeno-associated virus is a replication defective single stranded DNA virus that normally requires a helper adenovirus for their replication
- × Relatively small transient capacity
- × Vectors establish latency normally in their wild type format by preferential integration into human chromosome 19, however, genetically modified AAV vectors lose this property

AAV technology: from virus to vector



Snyder & Moullier (2011), Lisowski et al. (2015), Büning et al. (2015)

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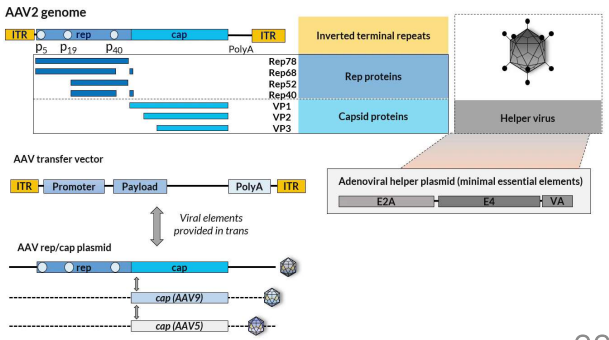
AAV technology: from virus to vector

AAV vector production for pre-clinical research projects



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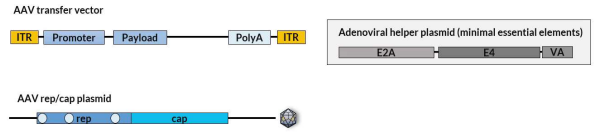

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AAV technology: from virus to vector

AAV vector production for pre-clinical research projects

Snyder & Moullier (2011), Lisowski et al. (2015), Büning et al. (2015)

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AAV technology: from virus to vector

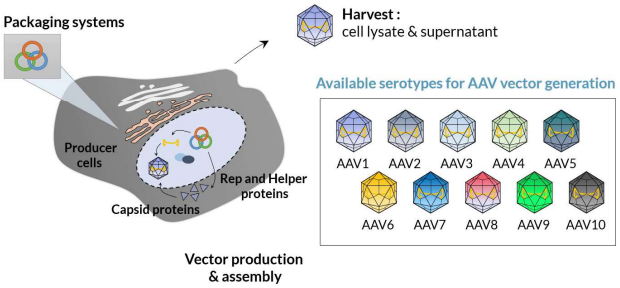
AAV vector production for pre-clinical research projects

Packaging systems

Snyder & Moullier (2011), Lisowski et al. (2015), Büning et al. (2015)

Setting up the system

AAV vector production for pre-clinical research projects



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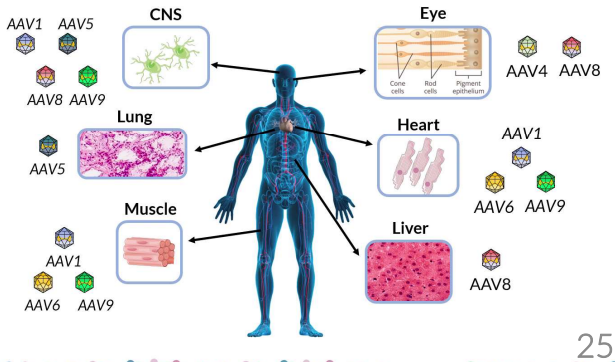
Serotype specificity for disease targets

AAV serotype determines tissue specificity/preference



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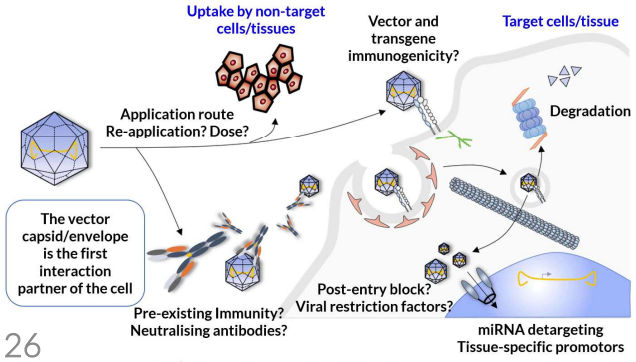
Challenges of vector-cell interactions

Advantages of vector capsid/envelope engineering



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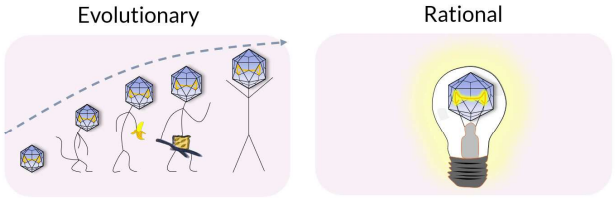
AAV Engineering

Different strategies, same goal



- Evolutionary pressure
- Library screening
- High through-put

- Ablation of natural tropism
- Defined targeting receptor
- Need of suitable binding domain

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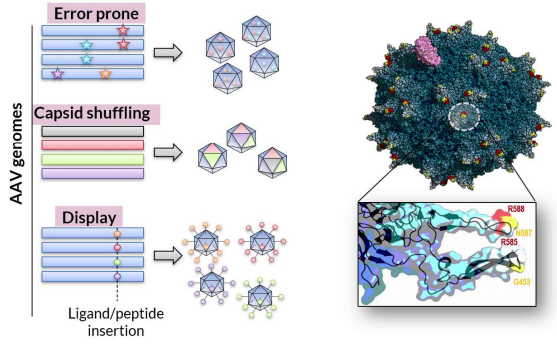
Engineering strategies for AAV vectors

An overview of commonly used strategies



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Surface engineering lentiviral vectors

Lymphocyte are the prime target in gene therapy

Modification of the lentiviral vectors glycoproteins, that are recognising lymphocyte surface markers as entry receptors, might increase the efficacy of the system

Vector	Envelope proteins	Degree of retargeting	Targeted receptors: Lymphocytes	Targeted receptors: Other cells
VSV-LV	VSV G	Preferential retargeting	CD30[80]	EGFR[80]
tVSV-LV	Truncated VSV G, non-viral membrane anchor	Preferential retargeting	None	Unknown human APC epitope[81]
VSV-MLV-LV	VSV G, MLV Env	Preferential retargeting	CD3[82] IL7R[83]	CD117[104] CD110[104]
SINV-LV	SINV E1, 2	Full retargeting Off-target: 1 - 10% (estimated from Fig. 5 in[84])	CD19[85] CD4[86] CD3[87] BCR/Ig[105]	DC-SIGN[107] CD34[106] CD117[104] P-glycoprotein[83] Mucin 4[110]
MV-LV	MV H, F	Full retargeting Off-target: 1 - 5% (estimated from Fig. 4, 5 in[88])	CD20[89] CD19[90] CD30[91] CD8[92] CD4[93]	CD133[111,112] CD105[111] IL3R[113] Her2/neu[78]
MV-D-LV	MV H, F non-viral membrane anchor	Full retargeting Off-target: < 1% (estimated from Fig. 6 in[88])	None	Her2/neu[89]
TPMV-LV	TPMV H, F	Full retargeting Off-target: 1 - 5% (estimated from Fig. 3 in[94])	CD20[95]	None
NiV-LV	NiV G, F	Full retargeting Off-target: < 1% (estimated from Fig. 3,7 in[96])	CD20[95] CD80,86[97]	EpCAM[95] Her2/neu[95] GlyA[95] CD117[98]

targeting domain · VSV G · SINV E1, E2/mut · MV Hmut, F · non-viral membrane anchor · truncated VSV G · MLV Env · TPMV Hmut, F · NiV Gmut, F

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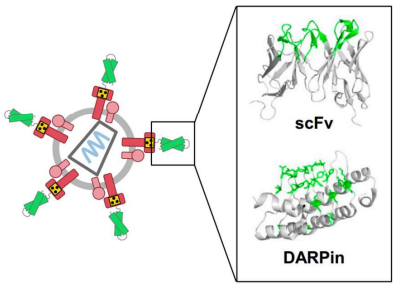
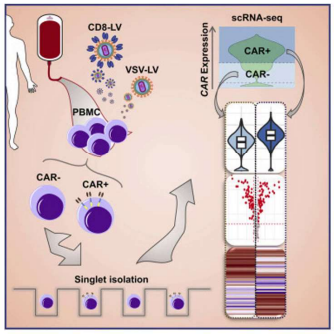
Surface engineering lentiviral vectors



Surface engineering lentiviral vectors

CD8 targeting lentivector

In vivo reprogramming of CAR T cells



Dr. Takis Athanasopoulos – GSK, UK

Avoiding immune responses in gene therapy

1. Hide vector from immune system
2. Hide immune system from vector

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Avoiding immune responses in gene therapy

1. Hide vector from immune system

Decrease vector dose
- Capsid modification
- Transgene modification
- Serotype switch
- Hydrodynamic injection

Delivery to immune privileged site
- Liver
- Eye
- Brain

Prevent APC expression
- Tissue-specific promoter
- miRNA targeting approaches

Avoiding immune responses in gene therapy

2. Hide immune system from vector

Immune suppression
- Block cell division/proliferation
- Deplete specific cell type with antibody

Immune modulation
- Block co-stimulation
- Induce Treg cells
- Adoptively transfer Treg cells

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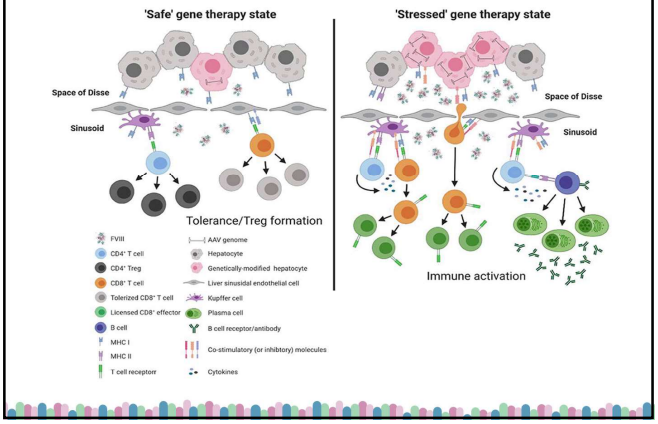
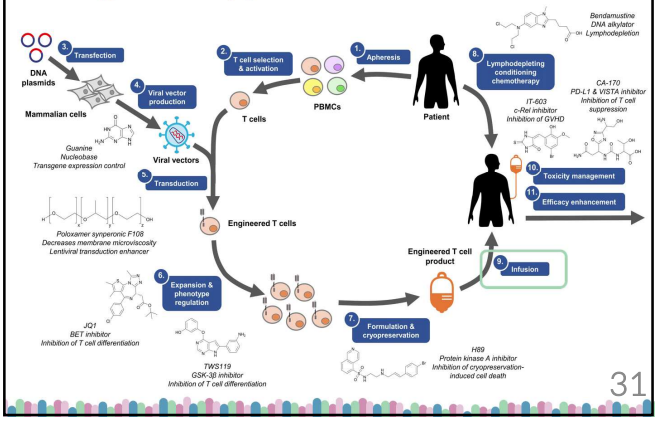
Avoiding immune responses in gene therapy



The role of small molecules in cell and gene therapy



GSK's CGT strategy

Identify areas of high unmet medical need, coupled with compelling genetic evidence for disease causation

Invest in treatment paradigms that maximize safety and delivery of CGT drug product

Develop manufacturing and supply chain solutions that deliver reproducible, high-quality therapies at a commercially viable cost

Engage regulators proactively and create a strong innovation culture

Continue to build patient-focused engagement approaches

Establish product pricing that will provide value and be accepted by prescribers and payers

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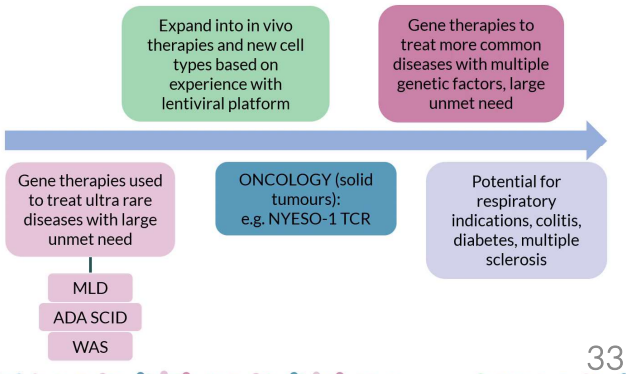
Dr. Takis Athanasopoulos – GSK, UK

GSK's CGT strategy: break through innovation therapies



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GSK's CGT strategy: break through innovation therapies

- Pharmaceutical companies have made large investments over decades in people, technology and infrastructure to discover, develop, test and market molecules and biopharmaceuticals
- There are differences in how cell and gene therapy treatment is manufactured and commercialised which requires knowledge and expertise in the area

GSK's CGT strategy: break through innovation therapies

- Over the last two decades academic institutes and small biotech companies have worked towards leading discovery tools and pre-clinical and clinical translation capabilities in CGT
- There are increased opportunities for partnerships between companies
  - In 2010, GSK collaborated with Fondazione Telethon and Ospedale San Raffaele
- It has been recognised that the use of genetically modified living cells as medicine will pose new challenges including from regulatory, manufacturing and supply chain standpoints

Dr. Takis Athanasopoulos – GSK, UK