Immunogenicity Strategies for Gene Therapies

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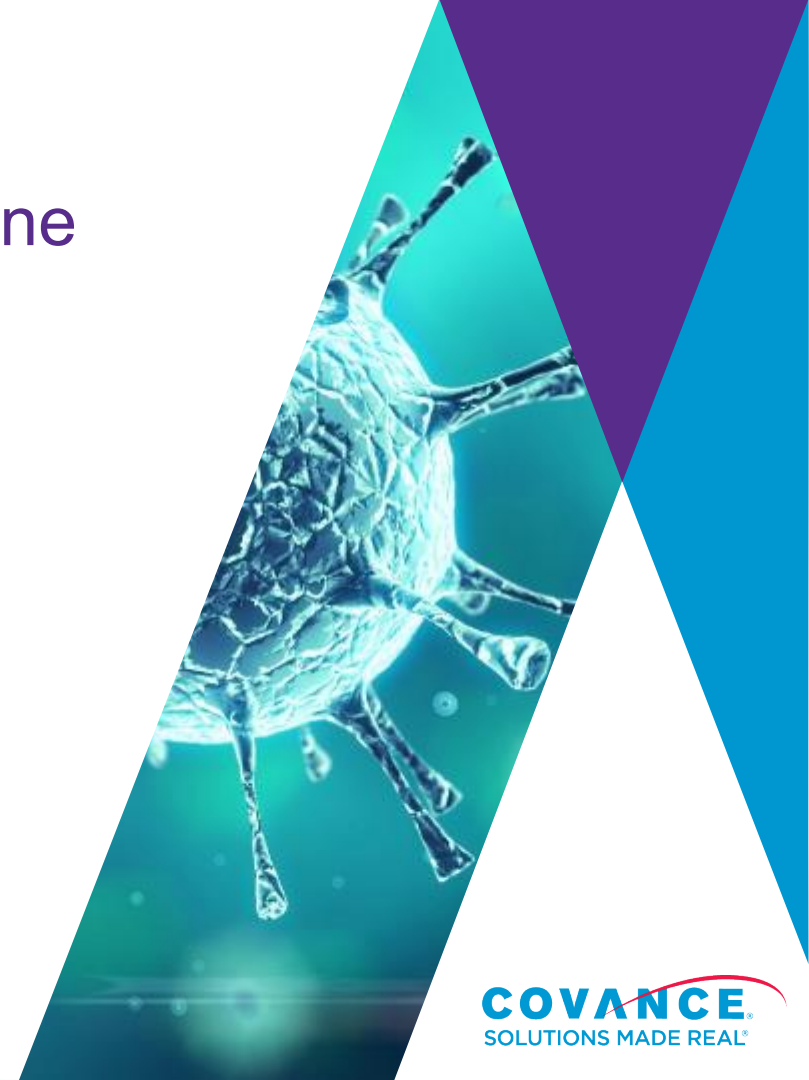

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Agenda

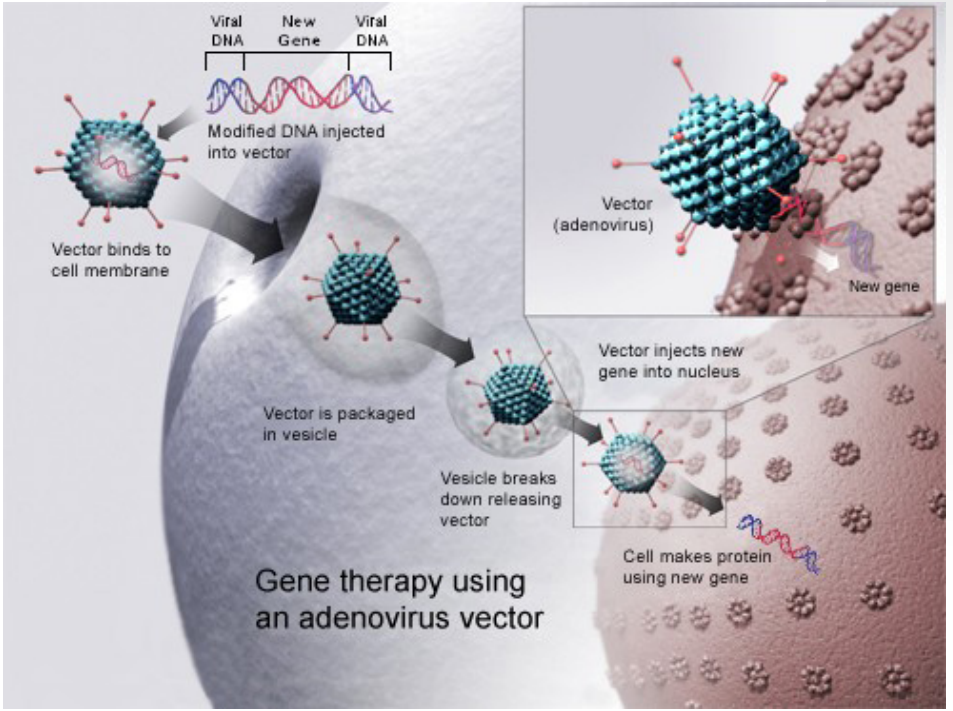
1. Gene therapy introduction
2. Anti-capsid assays
3. Anti-transgene antibody assays
4. Neutralizing antibody assays
5. Conclusions

Gene Therapy

- Gene therapy is a technique that delivers therapeutic nucleic acid polymers into patient's cells to modify gene expression at DNA or RNA level to treat or prevent disease
- First successful gene therapy study in humans was conducted in May 1989 using tumour-infiltrating lymphocytes modified by retroviral gene transduction as treatment for advance melanoma
- September 1990 a four-year-old received treatment for severe immune system deficiency. The defective gene of the patient's blood cells was replaced by the functional variant
- Unique ability to target 'undruggable' targets
- Gene therapy is now the third major drug platform in addition to traditional small-molecule and large-molecule therapeutics

Gene Therapy Medicinal Products (GTMPs)

EMA guidelines 2015 “Gene therapy medicinal products (GTMPs) generally consist of a vector or delivery formulation/system containing a genetic construct engineered to express a specific therapeutic sequence or protein responsible for the regulation, repair, addition or deletion of a genetic sequence”



https://en.wikipedia.org/wiki/Gene_therapy

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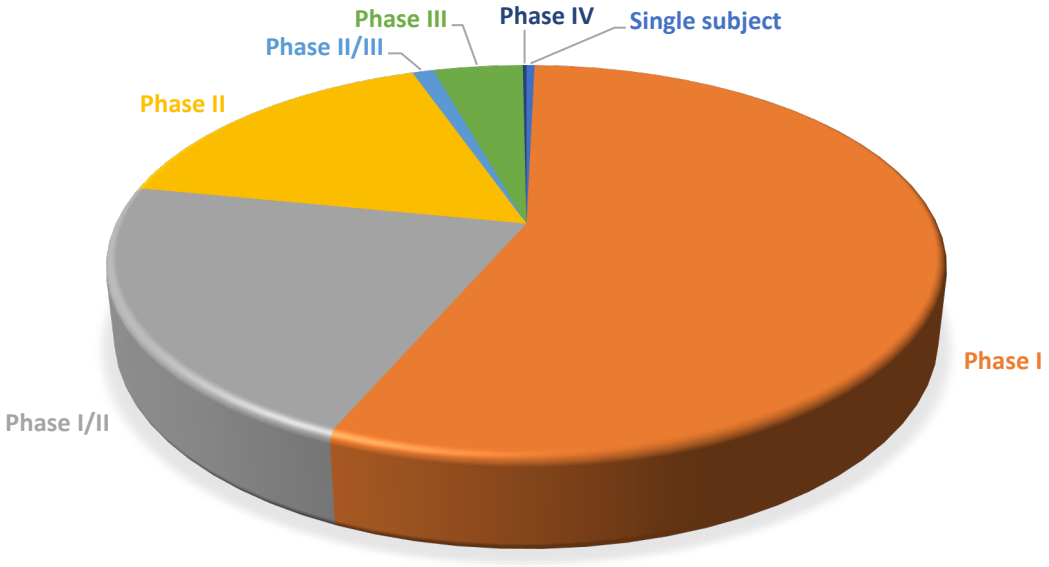
Gene Therapeutics In Current Clinical Trials

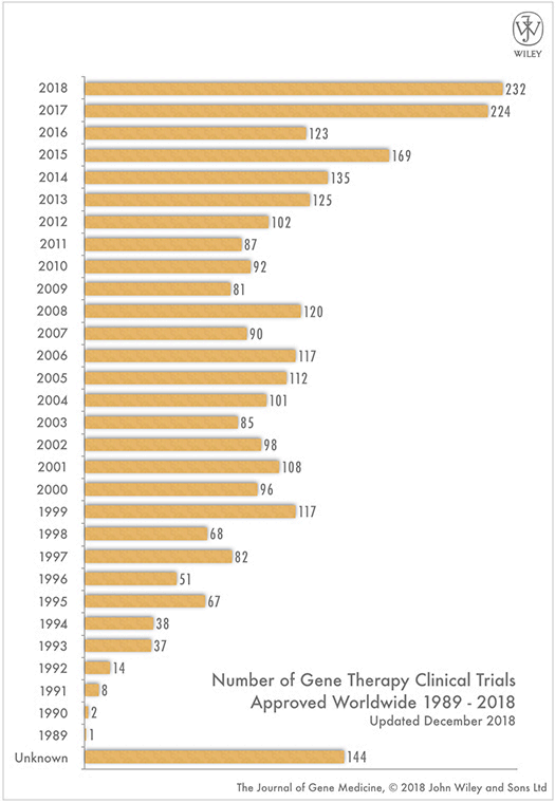
Company	Drug	Mechanism	Target	Status
Shenzhen SiBiono GeneTech	Gendicine	Oncolytic virus	Squamous cell carcinoma	SFDA approved
uniQure	Glybera	Gene therapy	Lipoprotein lipase deficiency	EMA approved
GlaxoSmithKline	Strimvelis	Gene therapy	Severe combined immunodeficiency due to adenosine deaminase deficiency	EMA approved
Biogen/Ionis	SPINRAZA	ASO	Spinal muscular atrophy	FDA approved
Sarepta Therapeutics	EXONDYS	ASO	Duchenne muscular dystrophy	FDA approved
Novartis	CTL019 (tisagenlecleucel)	Gene therapy	Relapsed or refractory pediatric and young adult patients with B-cell acute lymphoblastic leukemia	FDA review
Ionis	Volanesorsen	ASO	Familial chylomicronia syndrome	Phase III
Ionis	Inotersen (Ionis-TTRrx)	ASO	Familial amyloid polyneuropathy	Phase III
Alnylam Pharmaceuticals	Patisiran	siRNA	Hereditary ATTR amyloidosis	Phase III
Exicure	AST-005	SNA	Psoriasis	Phase II
eTheRNA immunotherapeutics	Unknown	mRNA	Melanoma	Phase II
CureVac	CV9104	mRNA	Prostate cancer	Phase II

ASO: Antisense oligonucleotides; SFDA: State FDA (Chinese FDA); SNA™: Spherical nucleic acid

Mark Ma et al - Bioanalytical Development, Alexion – “Challenges and opportunities in bioanalytical support for gene therapy” *Bioanalysis* (2017) **9**(18), 1423–1430

Gene Therapeutics Since 1989





The Journal of Gene Medicine. Gene Therapy Clinical Trials Worldwide Database. www.abedia.com/wiley/

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GTMPs

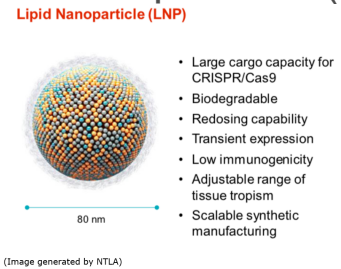
- **Transgene:** Segment of DNA containing a gene sequence that has been isolated from one organism and is introduced into a different organism.
- **Vector:**

Viral

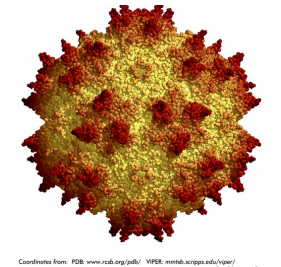
- Capsids are the protein cage derived from the protein shell of a virus.
- High transmission efficiencies – used in first generation GTMPs
- Possible mutations and post treatment recombination and high cost

Nonviral

- e.g. lipid nanoparticles (LNP) – are more simplistic and show less safety concerns



https://seekingalpha.com/article/4188960-intellia-therapeutics-left-behind-cas9-excitement



Virology.wisc.edu/virusworld

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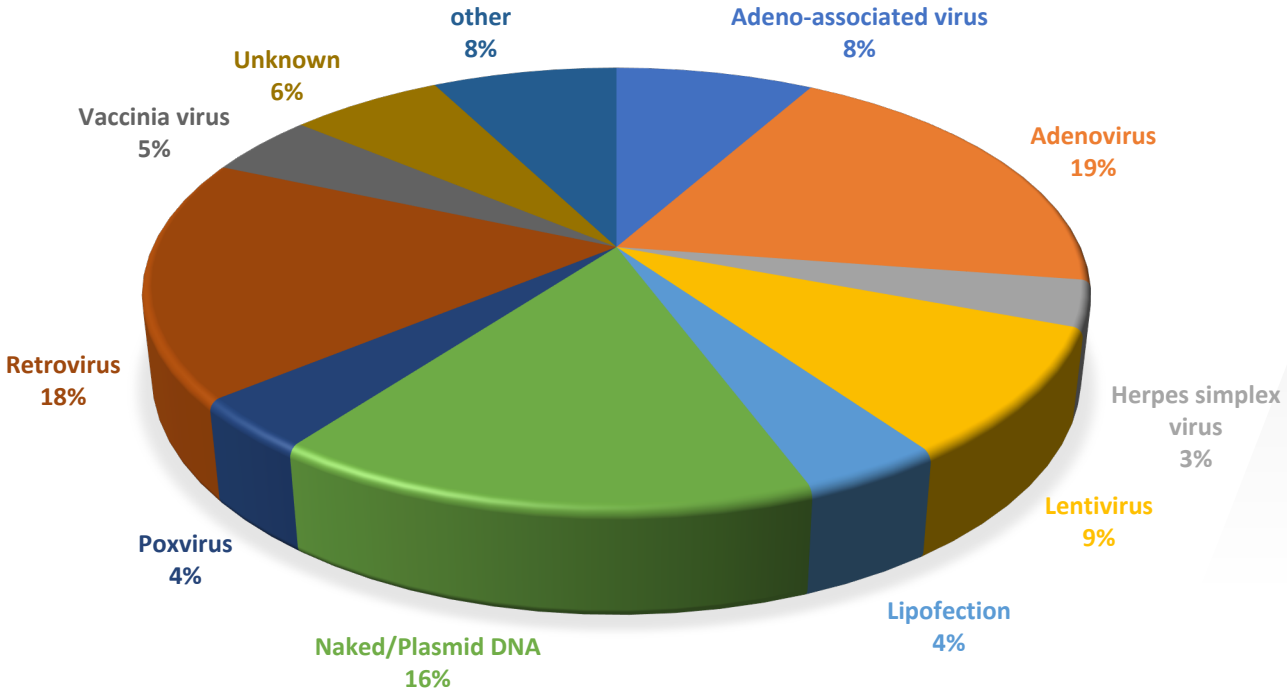
Vector Types



The Journal of Gene Medicine. Gene Therapy Clinical Trials Worldwide Database. www.abedia.com/wiley/

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Types Of Immunogenicity

- Immunogenicity against delivery vector – (Capsid) assay
  - Titre assay
- Immunogenicity against transgene products
  - ADA assays
  - Neutralizing antibodies (Nabs) assays

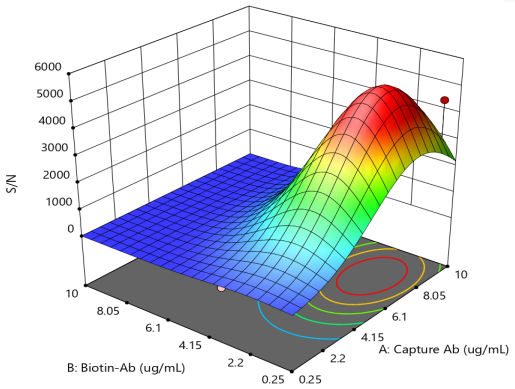
- There are many regulatory documents establishing the agreed requirements for PK assays however the requirements for the above assays are not as well established.
- Many bioanalytical labs follow the general method validation guidance for bioanalytical assays for ligand binding or chromatography assays. Is this the best approach?

Anti-Capsid Assay

- Repeat dose may generate anti-capsid antibodies and stop transgene effectiveness
- Expect pre-existing levels of antibodies against viruses
- If high levels of Abs are observed early, is this an issue? Is an assay really required?
- Membrane proteins are not soluble and difficult to use in immunoassays
  - Virus used as a capture reagent
- Sourcing a commercially available positive control
- CaptureSelect™ Biotin Anti-IgG-Fc (ms) conjugate as primary detection reagent

Anti-Capsid Assay – Development Parameters (Preclinical)

- Optimise Assay conditions (Design-Expert®)
- Titre assays are relatively easy to develop
- Titre of 3 levels of spiked material and 1 unspiked material. Inter assay variation established
- Cut point (3 times the SD of buffer blank) – spiked samples distinguish between unspiked samples
- Whole plate precision/Assay drift
- Freeze thaw stability



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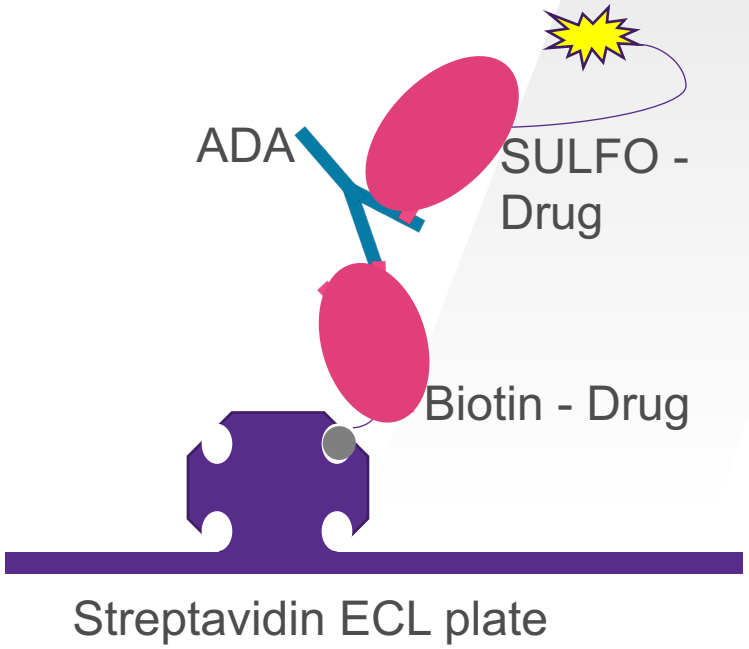
Preclinical Anti-Transgene Antibody - Homogenous Bridging Assay

- Drug is labelled and is used as both capture and detection.
- ChromaLink® Biotin Antibody Labelling Kit
  - Reacts with primary amines on antibody or protein
  - Buffer exchange to remove buffer components
  - Change in pH

- Positive control in correct species
- Require good quality protein
- Require a large amount of protein



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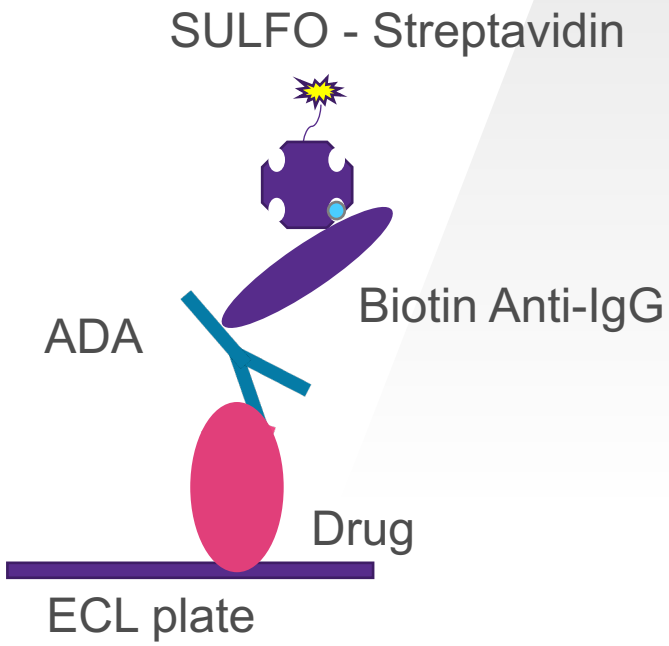
Preclinical Anti-Transgene Antibody - Stepwise Assay

- CaptureSelect™ Biotin Anti-IgG-Fc (ms) Conjugate that specifically binds to the Fc part of IgG from multiple species
  - Less sensitive
  - Higher background noise
  - Measure IgG only (not IgM) therefore problematic in clinical studies
- Biotin Anti species IgG+IgM solid-phase adsorbed to ensure minimal cross-reaction with human serum proteins (Jackson ImmunoResearch)



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Anti-Transgene Antibody Assay – Development Parameters

- Is a statistical (Shankar) cut point required? (18 plates of data)
- Prepare arbitrary cut point control – instrument responses above this control are positive, below are negative.
- Confirmatory assay? Is this really needed for preclinical studies?
  - Change the %false positive rate from 5% to 1%
- Assess assay variation and assay drift
- Drug Tolerance – using a positive control prepared at 500 ng/mL (recommended preclinical sensitivity level Mire-Sluis *et al*)

Nabs To Transgene *In Vitro* Assays - Titre Assays

- The immunogenicity associated with dosing of AAV based vectors are well understood – are Nabs assays in preclinical studies really necessary?
- The basis of the procedure is to measure the *in vitro* activity of an AAV vector that codify for luciferase
- The luciferase activity (expressed by the AAV-luc) can be measured with the luciferin-luciferase reaction
- The absence of Nabs allows luciferase expression while the presence of Nabs in the sample inhibits AAV-luc activity
- Samples are titrated and the titre around 50% neutralization is reported

Nabs To Transgene – Assay Parameters

- Assay sensitivity – FDA states sensitivity should not be reported as a titre level. However stock concentrations of positive controls are often not provided therefore a theoretical sensitivity level can not be calculated
- Drug tolerance - Low levels of virus expected in the toxicological samples and that the virus is usually non replicating - is drug tolerance required?
- Positive control titration and matrix interference
- Assay variation – titration of controls
- Inter analyst/inter analyser variation
- Linearity
- Freeze thaw stability

Conclusions

- Many assays required to obtain a complete picture of the GTMPs effectiveness and safety on the host
- Fit for purpose – Immunogenicity strategies are constantly evolving and a fit for purpose approach should be considered
- A strategy has to be agreed, established and implemented prior to supporting any preclinical and clinical studies between client and laboratory
- Constant review and discussion of all immunogenicity assays throughout the length of the project

Thank you

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