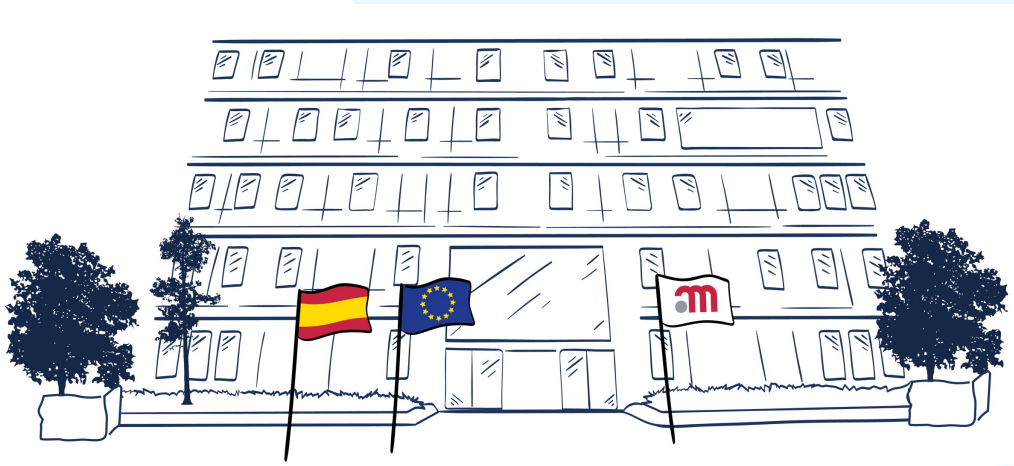
Quality attributes of rAAV-based Gene Therapy Medicinal Products



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AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

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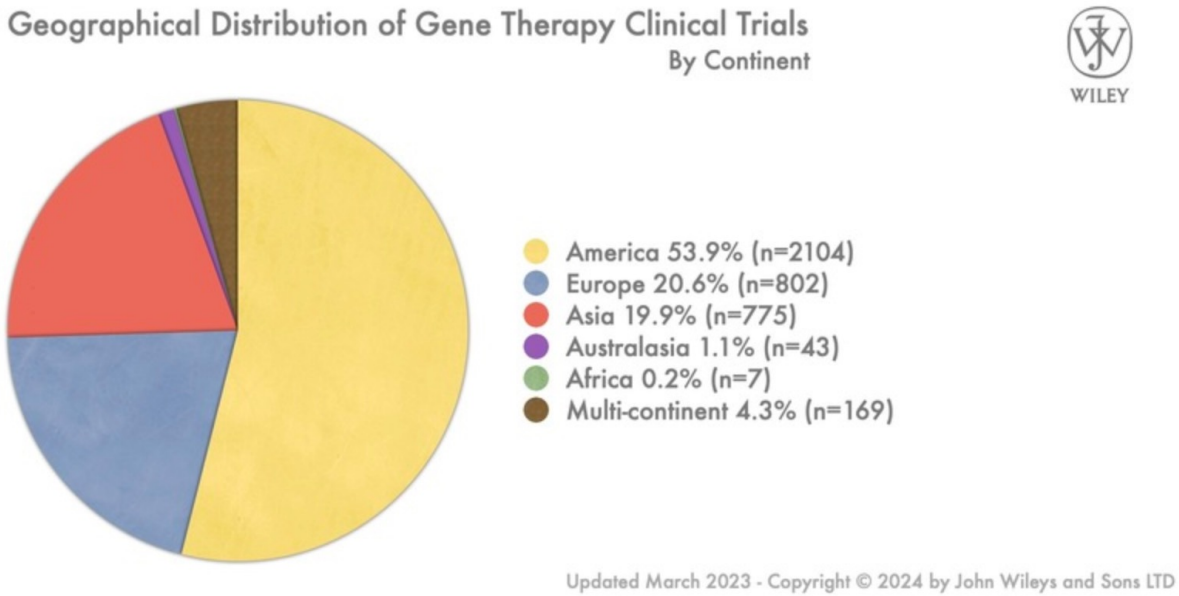
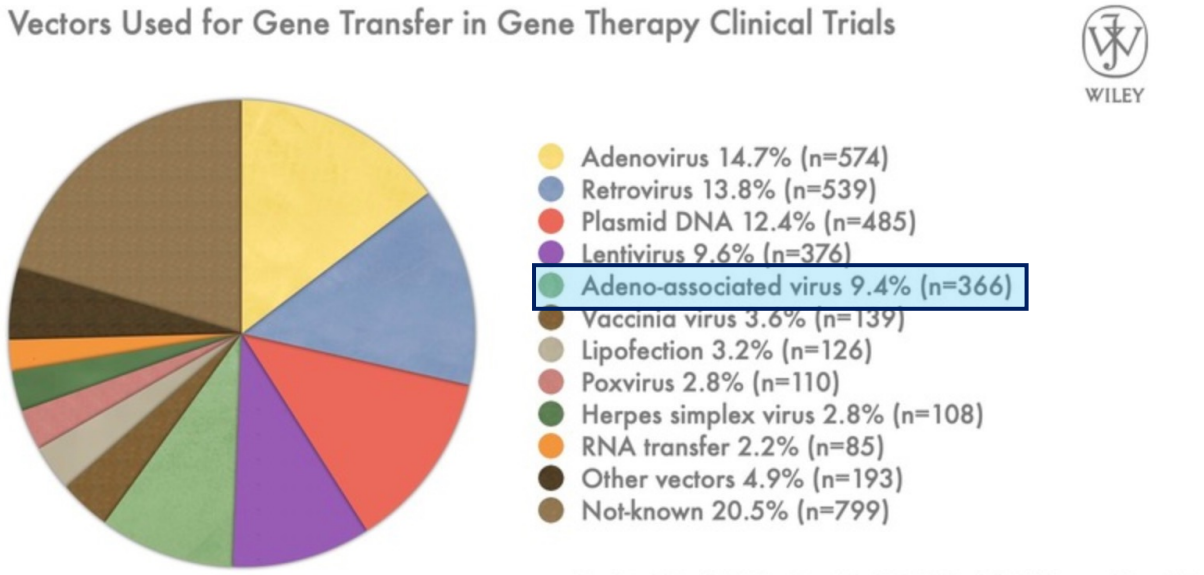
1.

Gene Therapy Context

Gene therapies in clinical trials worldwide and Vectors used

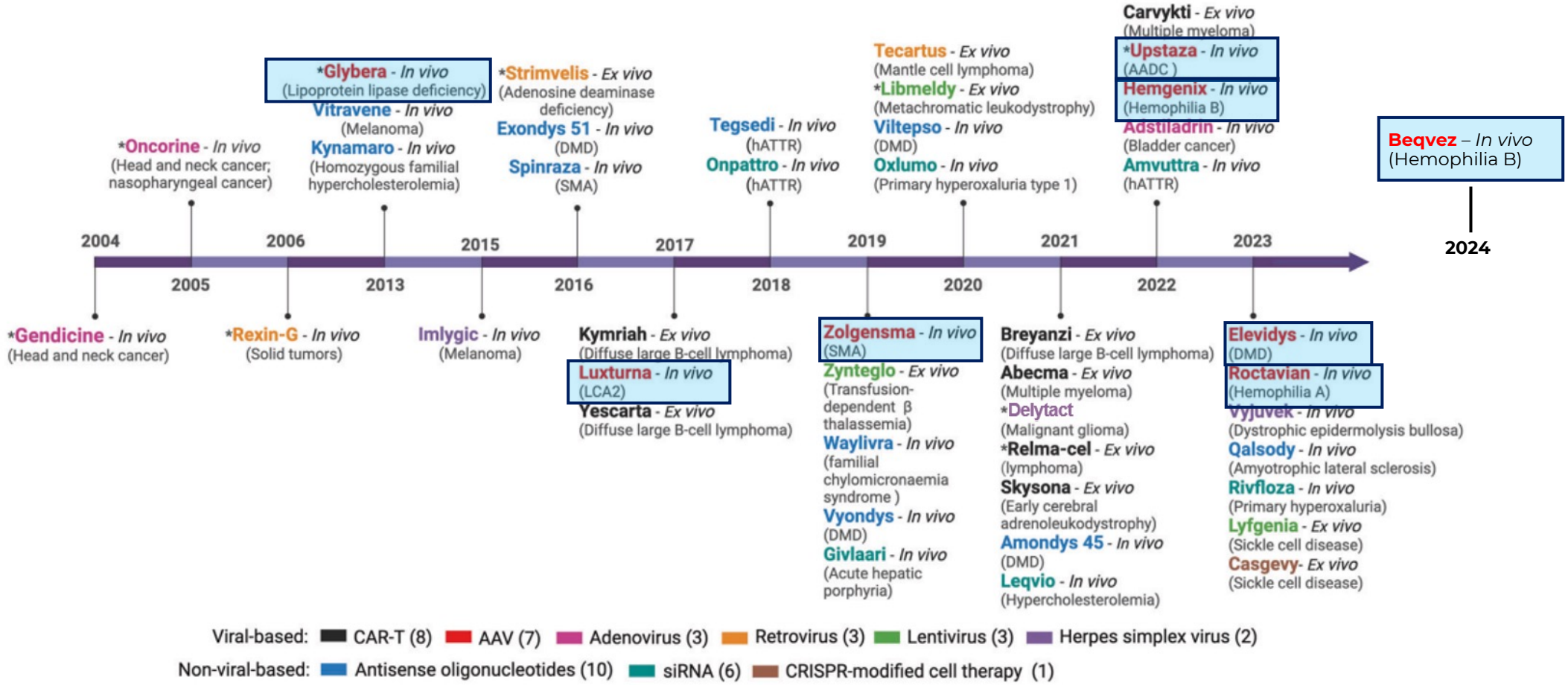




https://a873679.fmphost.com/fmi/webd/GTCT

Elevated number of Gene therapy Clinical trials (3900) → **9,4 % AAVs vectors**

Approved gene therapy products and delivery platforms

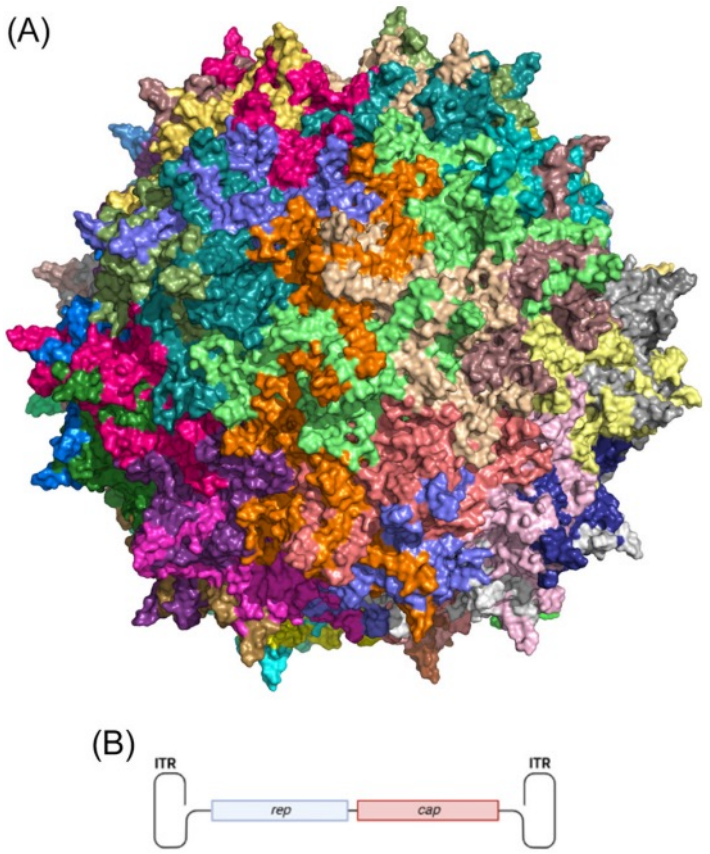


Wang JH et. al. Adeno-associated virus as a delivery vector for gene therapy of human diseases. Signal Transduct Target Ther. 2024 Apr 3;9(1):78

2.

Introduction to AAVs

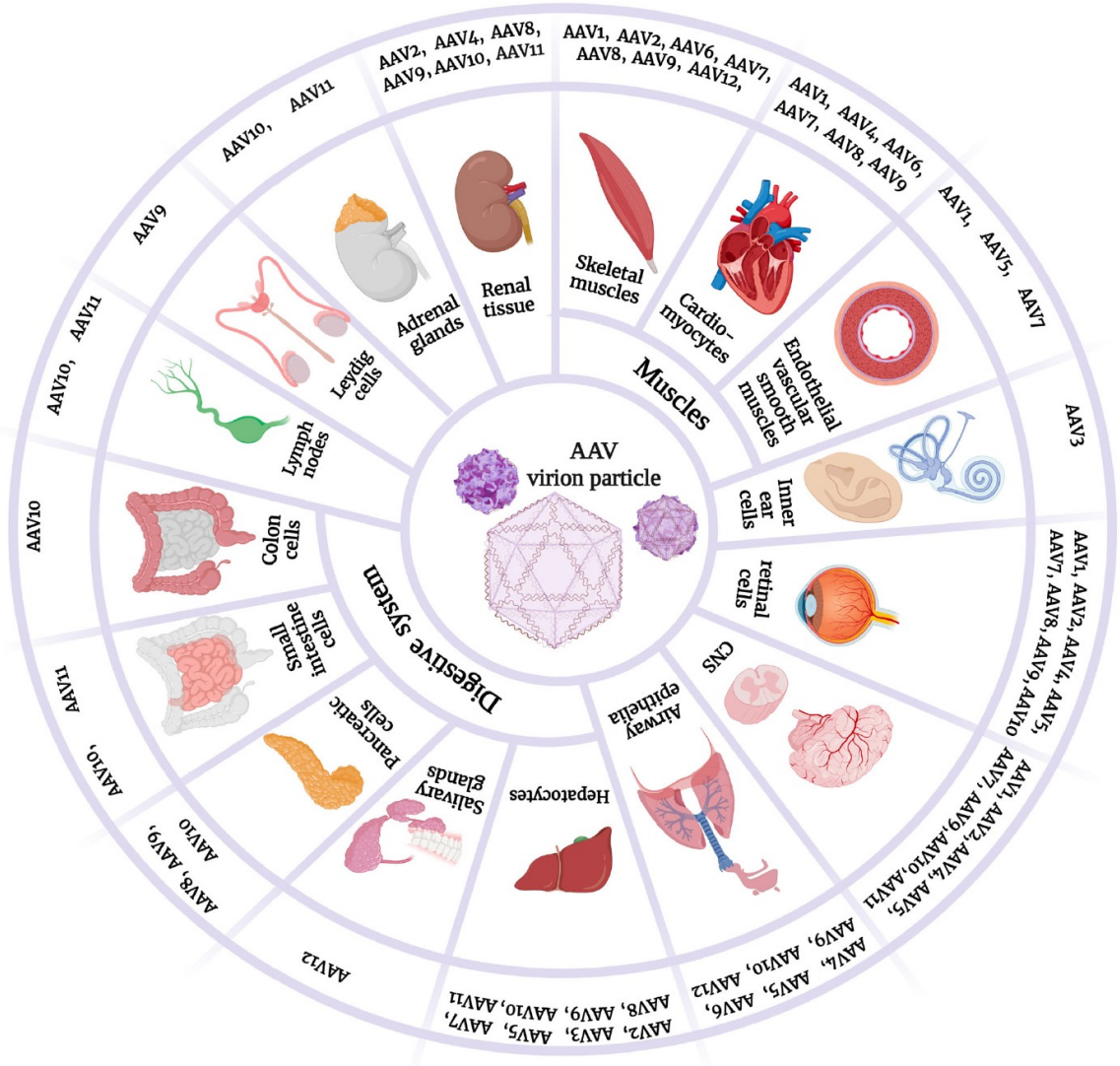
AAVs characteristics and features



- AAVs are single stranded non-enveloped non-pathogenic DNA viruses belonging to the family Parvoviridae (genus *Dependoparvovirus)*
- The capsid is approximately 25 nm in size and icosahedral
- Encloses a 4.7-kb ssDNA genome. Two ORFs express genes required for replication (**rep**) and capsid structure (**cap**) and are flanked by two inverted terminal repeat (**ITR**). An alternate reading frame overlapping the cap gene encodes the assembly-activating protein (**AAP**), promoting capsid formation and stability
- Replication-defective and dependent on the presence of a helper virus
- Transduced and expressed in both dividing and non-dividing cells.
- The AAV vector DNA forms episomal concatemers in the host cell nucleus:
    - Episomal stability enables long-term transgene expression, especially in non-dividing cells
    - Episomal persistence provides lower risk of DNA integration

Z. Jiang and P.A. Dalbi. Challenges in scaling up AAV-based gene therapy manufacturing. Trends in Biotechnology, October 2023, Vol. 41, No. 10

AAV Serotypes and tropism



Different tropism depending on the AAV serotype

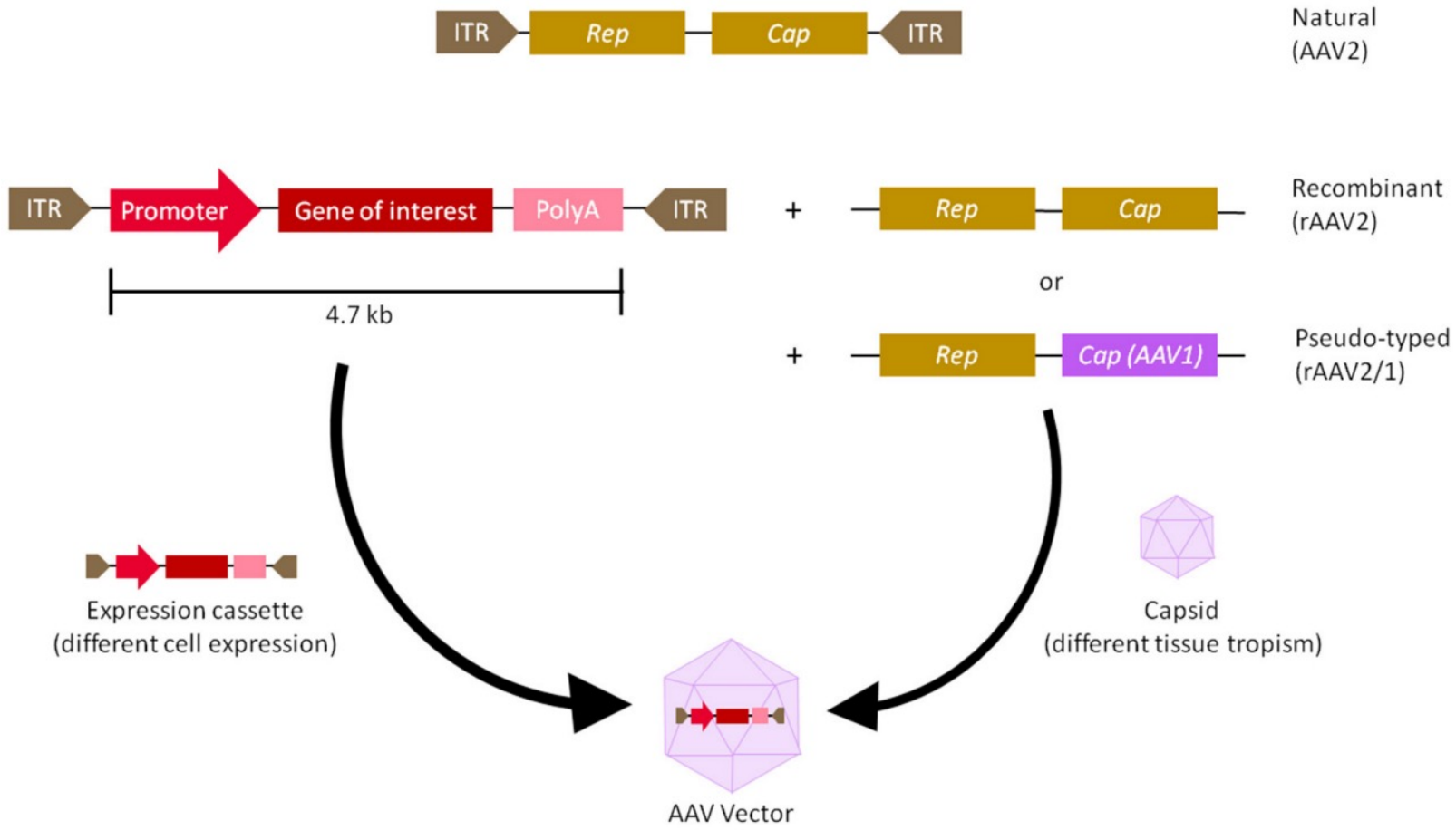
Different rAAV serotypes used depending on the illness treated

S. S. Issa, et all. Various AAV Serotypes and Their Applications in Gene Therapy - An Overview. Cells, 2023, 12, 785

3.

rAAV vectors

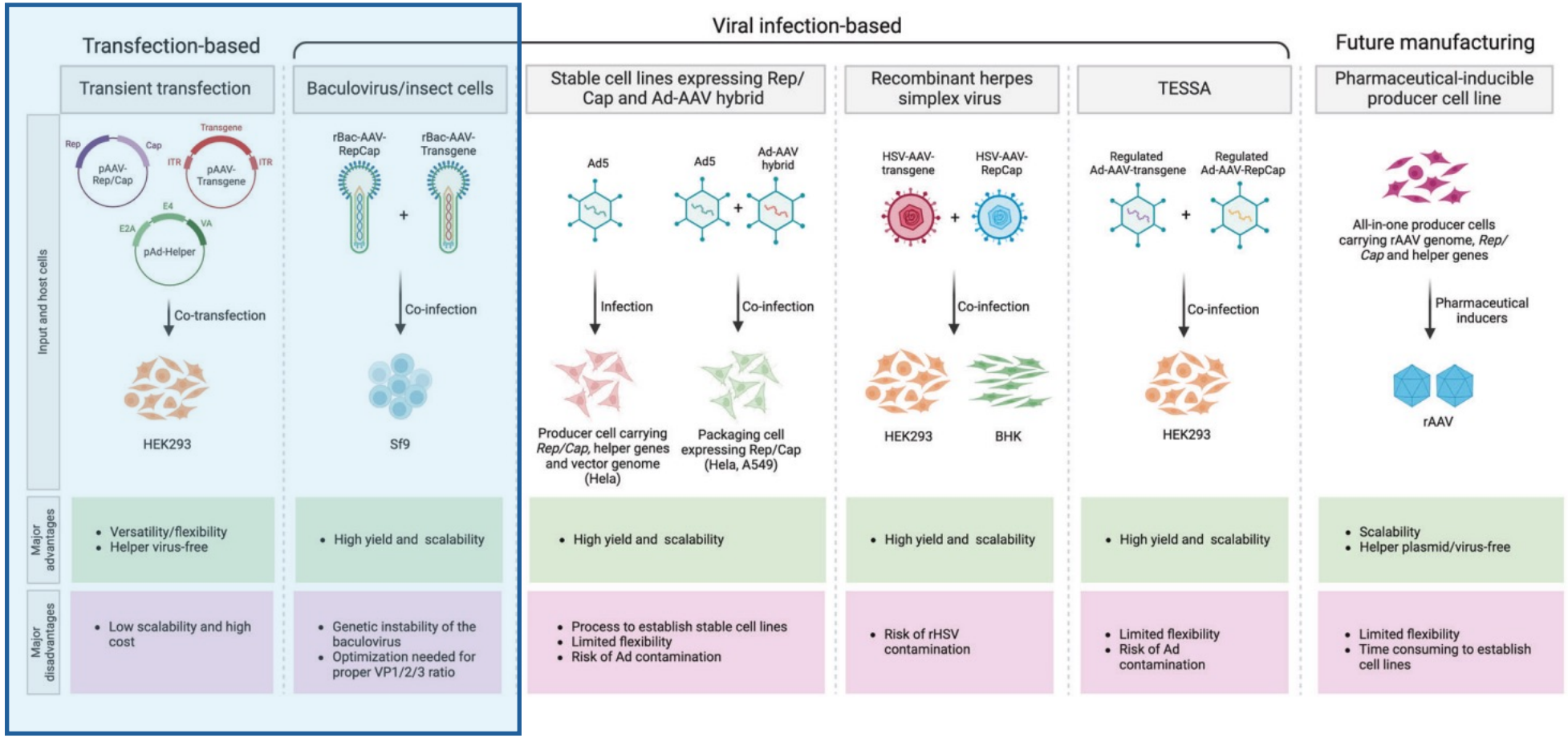
rAAV vector design – Expression cassette and capsid



H. K. E. Au et all. Front Med. 2022, 8:809118.

Current approaches for rAAV manufacture

Commercial products



JH Wang et all. Adeno-associated virus as a delivery vector for gene therapy of human diseases. Signal Transduction Target Therapy. 2024 Apr 3;9(1):78

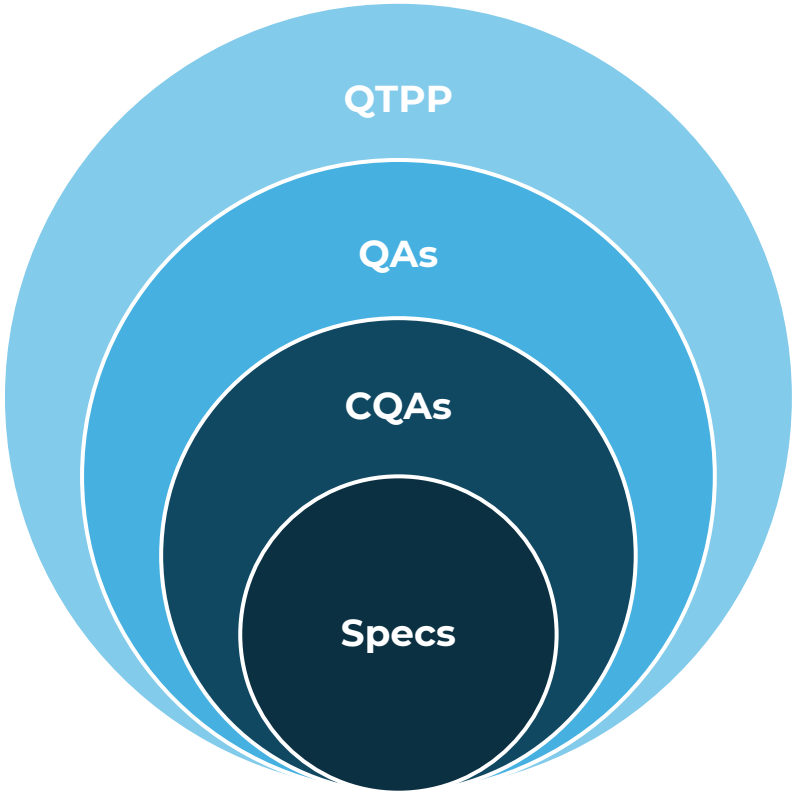
4.

rAAV Characterization

Critical quality attributes, characterization and release tests

CQAs

- are influencing quality, safety and efficacy of a product or are otherwise required by regulations (default CQAs)
- are associated with DP, DS, intermediates, excipients
- should address identity, quantity, potency, purity and safety of these substances
- should be within an appropriate limit, range, or distribution



QTPP: Defines overall quality of the product (Q, NC, C parameters)

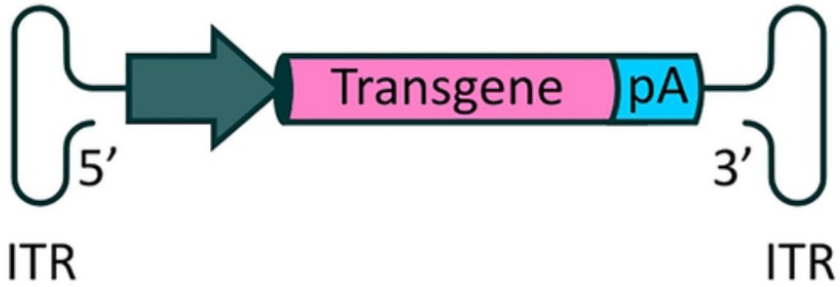
QAs: Attributes defining the quality of a product

CQAs: Quality attributes relevant for desired product quality. Impacting safety and efficacy

Specifications: CQAs that ensure safety and efficacy of the product

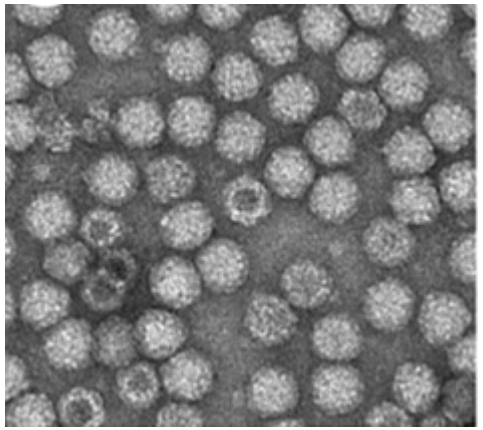
Drug Substance characterization

1. **Genome characterization**:
   a) **Vector genome identity**: Sequencing of the expression cassette (gene and regulatory sequences).
      Methods: ddPCR, qPCR, NGS, etc
   b) **Genome integrity** in respect to specific mutations, sequence rearrangements
   c) **Characterization of ITR-adjacent DNA sequences**: risk of pre-dominant packaging
   d) **Vector genomes only partially present**



Drug Substance characterization

2. **Structure characterization**:
   a) **Capsid protein identity**: protein composition, protein identity confirmation, integrity of capsid proteins, protein sequence confirmation . Methods: SDS-PAGE, Western-Blot, ELISA, LC-MS, etc
   b) **Capsid morphology**:
      - **Size and shape**: Methods: TEM, dynamic light scattering (DLS), SEC-MALS, etc
      - **Capsid hydrodynamic size**: Methods: DLS, etc
      - **Content and distribution of viral capsid proteins**: Methods: capillary gel electrophoresis (CGE), RP-HPLC, etc
        - **Stoichiometry**: VP1 : VP2 : VP3 (1 : 1 : 10 or similar). Methods: CE-SDS, CGE, RP-HPLC, etc
   c) **Capsid protein Post-translational modifications** . Methods: Peptide mapping, LMS, MS, etc



Andrei Hutanu et all. Stronger together: Analytical techniques for recombinant adeno associated virus Electrophoresis 2022, 43, 1107–1117

Drug Substance characterization

3. **Quantity/Content**:
   a) **Vector particle concentration**: overall number of particles independently of the content and functionality (vp/mL). Methods: ELISA, SEC-HPLC, AUC, etc
   b) **Infectious titer**: number of vector particles carrying an intact genome and able to transduce cells (ip/mL). Method: TCID50 endpoint assay in presence of rep/cap genes and helper components to allow replication in an initially transduced cell.
      Highly variable, complex, laborious assay
   c) **Vector genome titer**: vector genome carrying particles (gc or vg/mL). Method: qPCR against standard.
   Residual plasmid DNA might impair correct read-out
      Dosing is on the vg titer rather than on the infectious titer

- **Ratio of total particles to genome copy particles (vp/vg)**. Methods: Calculated values or determination by AUC
- **Ratio of genome copy particles to infectious units (vg/ip)**

Drug Substance characterization

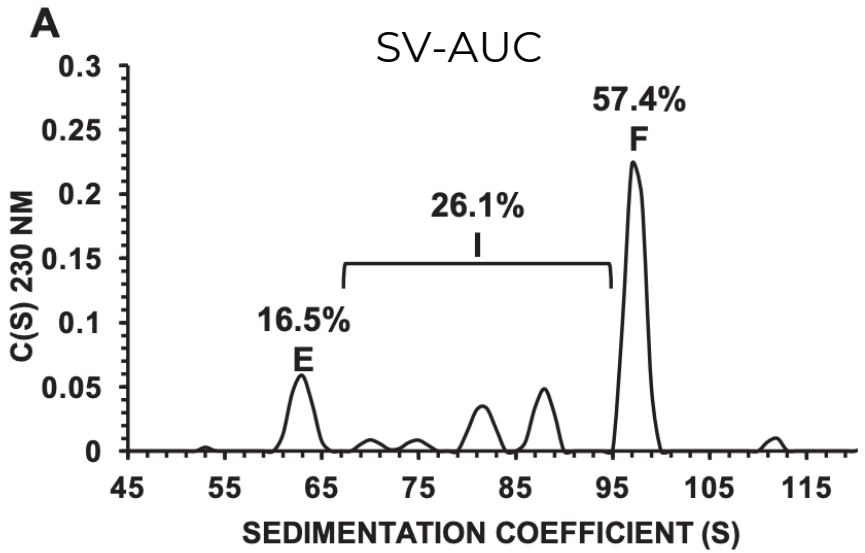
4. **Biophysical Characterization:** :

   a) **Evaluation of particles subpopulations regarding their content**: % full, empty and incomplete vector particles. Affect immunogenicity, efficacy and stability. Methods: SV-AUC, cryo-TEM, etc

      - Incomplete vector particles: DNA encapsidated from plasmids, cellular DNA. Specific DNA quantification (qPCR) (antibiotic resistance-, rep and cap- and helper genes (e.g. E1A, E1B of HEK293).

   b) **Evaluation of particles subpopulations regarding their size**: % monomers and aggregates. Methods: SEC-MALS, AUC, DLS , SEC-FS, cryo-TEM, etc

      - Aggregates:
        - Depends on titer, formulation buffer, storage, stress conditions
        - Some serotypes are less susceptible to aggregation



A. McColl-Carboni et al. Analytical characterization of full, intermediate, and empty AAV capsids. Gene Therapy (2024) 31:285 – 294

Drug Substance characterization

5. **Characterization of functionality**:
   a) **Infectivity**: ration genome copy titer / Infectious titer
   b) **Expression level of the therapeutic gene product** after transduction of reference cells or a cell line (ideally indicative for the target cell type)
      - Transduction efficiency *in vitro* may differ dependent on virus serotype, e.g. AAV9 reveals low infectivity *in vitro*
      - Different promoter activity in test system and patient
   c) **Biological activity**: functional assay that reflects biological activity of the therapeutic protein and correlates with the clinical efficacy.
      - Shows consistency of the product and indicates sub-potent batches.
      - Development of an *in vitro* biological activity assay is preferred over a *in vivo* assay (3R principle)
      - Ideally before the pivotal clinical Phase

TABLE 1. RELATIVE *IN VITRO* INFECTIVITY OF AAV1, AAV8, AND AAV9

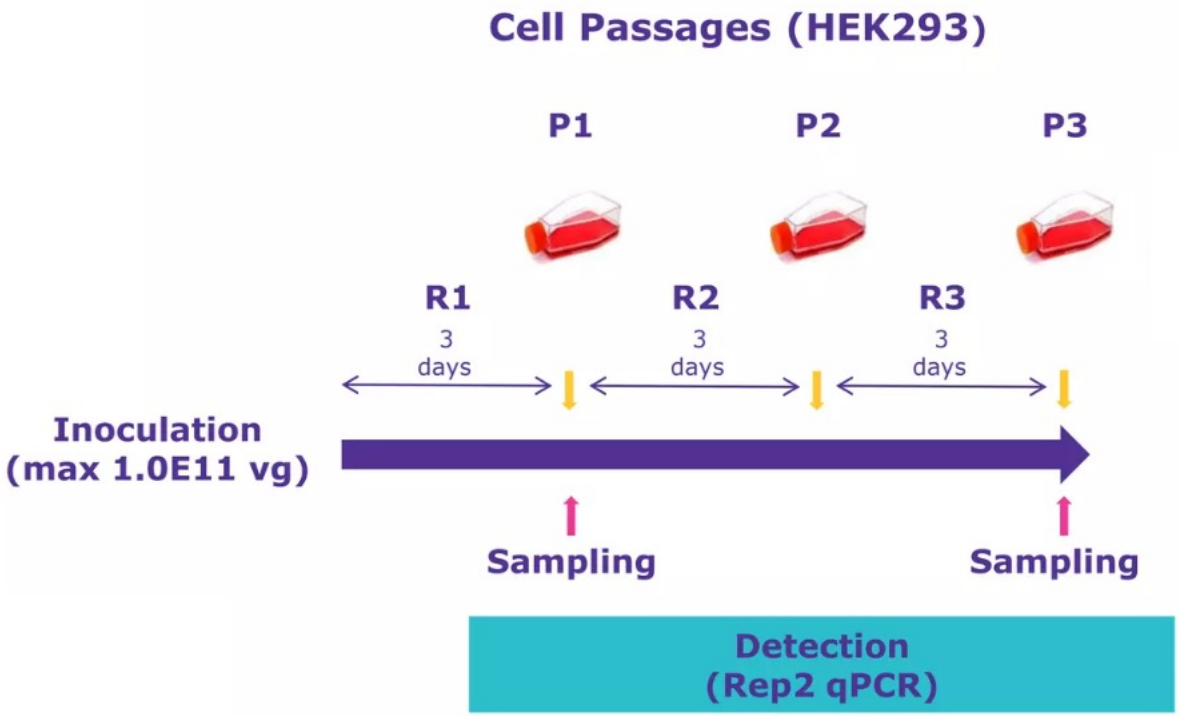
	Physical titer (GC/ml)	*Infectious titer (IU/ml)*	*P/I ratio*
AAV1			
Lysate	7.38×10^{12}	2.00×10^{11}	36.90
Medium	9.65×10^{12}	1.58×10^{11}	46.75
AAV8			
Lysate	3.92×10^{12}	8.29×10^{9}	472.62
Medium	7.38×10^{12}	1.77×10^{10}	416.69
AAV9			
Lysate	1.13×10^{13}	1.67×10^{10}	678.00
Medium	1.12×10^{12}	7.44×10^{8}	1506.27

Vandenberghe et al., Efficient Serotype-Dependent Release of Functional Vector into the Culture Medium During Adeno-Associated Virus Manufacturing. Human Gene Therapy (2010) 21:1251–1257

Drug Substance characterization

6. Test for replication competent AAVs (rcAAV):

- During manufacture, in presence of all helper sequences, virus components and vector genome: risk of **recombination and formation of Wild Type AAV (rcAAV)**
- Product related impurity
- Safety-relevant parameter
- Method is a Cell-based assay:
  - Usually at the DS level (higher vector concentration)
  - Complex assay. Requires several rounds of amplification and detection by PCR
  - Acceptance criterion: non-detected. The LOD should be indicated : ≤10 IU in $1x10^{9}$ to $1x10^{11}$ vg



Drug Substance characterization

7. **Characterization of impurities**:

   a) **Product related impurities**: affect immunogenicity, efficacy and stability

      - Capsid protein impurities: protein composition. Methods: SDS-PAGE, Western-Blot, ELISA, LC-MS, etc
      - % empty and incomplete vector particles. Methods: AUC, cryo-TEM, SEC-MALS, ratio vp / gc, etc
      - Non-infectious particles: ratio gc / ip
      - % aggregates. Methods: AUC, SEC-MALS, DLS, SEC, etc

   b) **Process related impurities**:

      - ***From raw materials***:
        - Residual Triton X-100 or other detergents (used for cell lysis). Methods: RP-HPLC, etc
        - Residual benzonase (used to digest non-packaged DNA). Methods: ELISA, etc
        - Residual downstream purification material (Caesium, Iodixanol, chromatography ligands). Methods: MS, Western blot, etc
        - Residual agents to prevent flocculation. Methods: HPLC, etc

Drug Substance characterization

7. **Characterization of impurities**:

   b) **Process related impurities**:

   - ***From starting materials***:
     - Residual HC-Protein (i.e. HEK293, SF-9). Methods: Cell type-specific ELISA, etc
     - Residual HC-DNA. Methods: qPCR, etc
     - Plasmid DNA. Methods: qPCR, etc
     - Baculovirus manufacturing system:
       - baculovirus-DNA. Methods: qPCR, etc
       - infectious baculovirus. Methods: qPCR infectivity assay, etc
     - Determination of DNA co-packaged and/or purified. Methods: qPCR of rep- and cap- genes, helper sequences (e.g. E1A/E1B specific sequences), antibiotic resistance genes, etc

5.

rAAV release specifications

rAAV release specifications

Group	Parameter	Method	Acceptance criteria	Comments
Identity	Vector genome identity	ddPCR, qPCR, NGS	Confirmed	DS and DP
	Capsid protein identity	ELISA, WB, SDS-PAGE, CE-SDS	Confirmed / Comparable to RS	DS and DP
	rAAV protein capsid content: Ratio VP1 : VP2 : VP3	CE-SDS, CGE, RP-HPLC	Ratio similar to 1: 1: 10 Comparable to RS	DS
Content	Vector genome titer (vg or gc/ mL)	qPCR, ddPCR	Range including the established concentration	DS and DP Dosing is on vg titer rather than infectious titer
	Vector particle concentration (vp/mL)	ELISA, SEC-HPLC, AUC	Range	DS and DP
	Infectious titer (iu/mL)	$TCID_{50}$	Range	DS and DP

rAAV release specifications

Group	Parameter	Method	Acceptance criteria	Comments
Purity	% monomeric particles and aggregates	AUC, DLS, SEC-HPLC		DP
	% Full and empty capsids	AUC		DS
	Ratio vector particles titer / genome copy titer	Calculated values or AUC	Should be ~ 1 vp/ gc (100% full capsids)	DS and DP
Potency	Infectivity: ratio genome copy titer / Infectious titer gc / iu	ddPCR, qPCR / $TCID_{50}$	Range to assure consistency between batches	DS and DP
	Transgene activity	Bioassay	Range to assure consistency between batches	DS and DP
	Transgene expression level	*In vitro* assay	Range to assure consistency between batches	DS and DP. In clinical trials when no Bioassay has been developed

rAAV release specifications

Group	Parameter	Method	Acceptance criteria	Comments
Impurities	Starting material: HC-DNA, HC-proteins, residual plasmid…	Ph Eur if mandatory	ng/mL < xxx ng/mL to assure consistency between batches	DS
	Raw materials: residual detergents, enzimes, colum ligands…	Ph Eur if mandatory	ng/mL < xxx ng/mL to assure consistency between batches	DS
Safety	rcAAV	Bioassay	Non detected / Negative / Below de LOD	DS
	Bioburden	Ph Eur 2.6.12	Complying with Ph Eur	DS
	Bacterial endotoxins	Ph Eur 2.6.14	Dependent on dose and indication. Complying with Ph Eur	DS
	Sterility	Ph Eur 2.6.1	No growth	DP
	Mycoplasma	Ph Eur 2.6.7	No growth	Tested on Unprocessed bulk: highest chance of detection

rAAV release specifications

Group	Parameter	Method	Acceptance criteria	Comments
General Tests	Appearance: • Clarity • Colour	Ph. Eur. 2.2.1 Ph. Eur 2.2.2	Complying with Ph Eur	DP and/or DS
	pH	Ph. Eur 2.2.3	Product dependent, around 7	DS and DP
	Osmolality	Ph Eur 2.2.35	Formulation buffer dependent	DS and DP
	Visible particles	Ph Eur 2.9.20	Essentially free of visible particles	DP
	Subvisible particles	Ph Eur 2.9.19	≥ 10 µm, ≤ 6000 parcicles/container ≥ 25 µm, ≤ 600 parcicles/container	DP
	Extractable volume	Ph Eur 2.9.17	No less than specifc amount or volume labelled	DP
	Concentration of preservatives	[Sucrose] : Ph Eur 2.2.35 [Polysorbate]: HPL-CAD	Range covering the established concentration	DP DS maybe

6.

Final remarks

General remarks

- rAAV are one of most widely used viral vectors for gene therapy: safe and efficient
- One limitation is the size of the transgene (< 4,4 Kb)
- Different process impurities depending on the manufacturing system used
- Optimization of production systems to eliminate unproductive particles (empty particles and residual encapsulation)
- The development of a standard method for rcAAV is desirable.
- Development of biological assays (potency) may be complex and require the use of subrogate potency tests
- The specifications are only a subset of the total control strategy and not all CQAs need to be included in the DS/DP specification
- DS/DP specifications could complement each other, unless e.g. parameters may be impacted by manufacturing downstream of DS

7.

Guidelines and Ph. Eur monographs relevant for AAVs

Guidelines and Ph. Eur monographs relevant for AAVs

EMA guidelines specific for ATMPSs and AAV:

- Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014)
- Guideline on the quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells EMA/CAT/GTWP/671639/2008 Rev. 1
- Reflection paper on quality, non-clinical and clinical issues relating specifically to recombinant adeno-associated viral vectors (CHMP/GTWP/587488/07)
- Reflection paper on design modifications of gene therapy medicinal products during development (EMA/CAT/GTWP/44236/2009)
- Questions and answers on gene therapy (EMA/CAT/80183/2014)
- DRAFT Guideline on quality, non-clinical and clinical requirements for investigational ATMPs in clinical trials (EMA/CAT/123573/2024)
- Q&A on Comparability considerations for Advanced Therapy Medicinal Products (EMA/CAT/499821/2019)
- Q&A on the principles of GMP for the manufacturing of starting materials of biological origin used to transfer genetic material for the manufacturing of ATMPs EMA/246400/2021

Guidelines and Ph. Eur monographs relevant for AAVs

EMA guidelines specific for ATMPSs and AAV:

- Q&A on comparability considerations for ATMPs EMA/CAT/499821/2019
- GL on core SmPC, Labelling and Package Leaflet for ATMPs containing genetically modified cells EMA/CAT/CHMP/158266/2021
- GL on potency testing of cell-based immunotherapy medicinal products for the treatment of cancer EMA/CHMP/BWP/271475/2006 rev.1 (2016)
- RP on management of clinical risks deriving from insertional mutagenesis EMA/CAT/190186/2012
- GL on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to ATMPs EMA/CAT/CPWP/686637/2011
- CHMP/CAT position statement on Creutzfeldt-Jakob disease and ATMPs EMA/CHMP/BWP/353632/2010
- RP on stem cell-based medicinal products EMA/CAT/571134/2009
- RP on design modifications of GTMPs during development EMA/CAT/GTWP/44236/2009
- GL on xenogeneic cell-based medicinal products EMEA/CHMP/CPWP/83508/2009
- Q&A on gene therapy EMA/CHMP/GTWP/212377/2008 (under revision)

Guidelines and Ph. Eur monographs relevant for AAVs

Ph. Eur:

- Ph. Eur. 3186 Gene therapy medicinal products for human use (Ph. Eur.)
- Ph. Eur. 5.2.12 Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

Other Ph. Eur. texts relevant to AAV

- 5.1.7 Viral safety
- 5.2.3 Cell substrates for the production of vaccines
- 2.6.16 Tests for extraneous agents in viral vaccines
- 2.6.1 Sterility
- 2.6.27 Microbiological control of cellular products
- 5.1.6 Alternative methods for control of microbiological quality
- 2.6.7 Mycoplasmas
- 2.6.14 Bacterial endotoxins
- 2.7.23 Numeration of CD34/CD45+ cells in haematopoietic products
- 2.7.28 Colony-forming cell assay for human haematopoietic progenitor cells
- 2.7.29 Nucleated Cell Count and Viability

Guidelines and Ph. Eur monographs relevant for AAVs

Other Ph. Eur. texts relevant to AAV

- 2.6.21 Nucleic Acid Amplification Techniques
- 2.7.27 Flow Cytometry
- 2323 Monograph on human haematopoietic stem cells
- 5.15 Functionality-related characteristics of excipients

Guidelines and Ph. Eur monographs relevant for AAVs

ICH quality guidelines relevant to AAV

- ICH Q5B: Analysis of the expression construct in cell lines used for production of r-DNA derived protein products
- ICH Q5D: Derivation and characterisation of cell substrates used for production of biotechnological/ biological products
- ICH Q5A: GL on viral safety evaluation of biotechnology products derived from cell lines of human/animal origin
- ICH Q5E: Comparability of biotechnological/biological products
- ICH Q5C: Stability testing of biotechnological/biological products
- ICH Q6B: Specifications: Test procedures & acceptance criteria for biotechnological/biological products
- ICH Q2: Validation of analytical procedures: text and methodology
- ICH Q7: GMP for active pharmaceutical ingredients
- ICH Q8: Pharmaceutical development
- ICH Q9: Quality risk management
- ICH Q10: Pharmaceutical quality system
- ICH Q11: Development and manufacture of drug substances

Thank you!

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