AAV specific issues pertaining to vector shedding in gene therapy clinical trials

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Workshop objectives

- Assess the impact of vector design on shedding (studies)
- Review available data on the relationship between bio-distribution and shedding of diverse vector systems
- Consider the potential shedding-associated safety concerns to be considered in clinical development

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What is the concern?

- "Shedding in the field of gene therapy means dissemination of the gene therapy product through excreta of the treated subject or patient"

- The potential concern has two components
  - Genetically altered viral vectors will go beyond treated subjects/patients
  - Such vectors will be biologically active with the potential to have deleterious effects on persons other than the study subject

- A few initial thoughts on this concern
  - Vectors do not replicate, continually diluted from the point of administration to potential sites of shedding
  - Shedding is limited by cell barriers and gauntlet of biological inactivation mechanisms
  - Even if shed, viral vectors do not propagate outside of cells

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- The impact of vector design on shedding

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The impact of vector design on shedding

- Two major technical aspects to vector shedding
  - qPCR
  - Bio assay, namely infectivity assay
- These assays are developed in parallel for
  - Biodistribution
  - Vector infectivity
  - Adapted for shedding studies
- qPCR/biodistribution generally more sensitive and robust
- Cell toxicity an issue in infectivity assays
- PCR or qPCR often used as an end point for infectivity assays

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Quantitative PCR (qPCR)

- "Taqman" qPCR assays are the norm
- Assay should be vector specific
  - Requires a primer set that distinguishes
    - Between natural therapeutic gene and that carried by vector
    - Between vector and parental virus
  - One primer within transgene and a second within either construct-specific or vector sequences
  - Challenge is that specificity and sensitivity are directly related to target size
  - AAV vectors at disadvantage due to size

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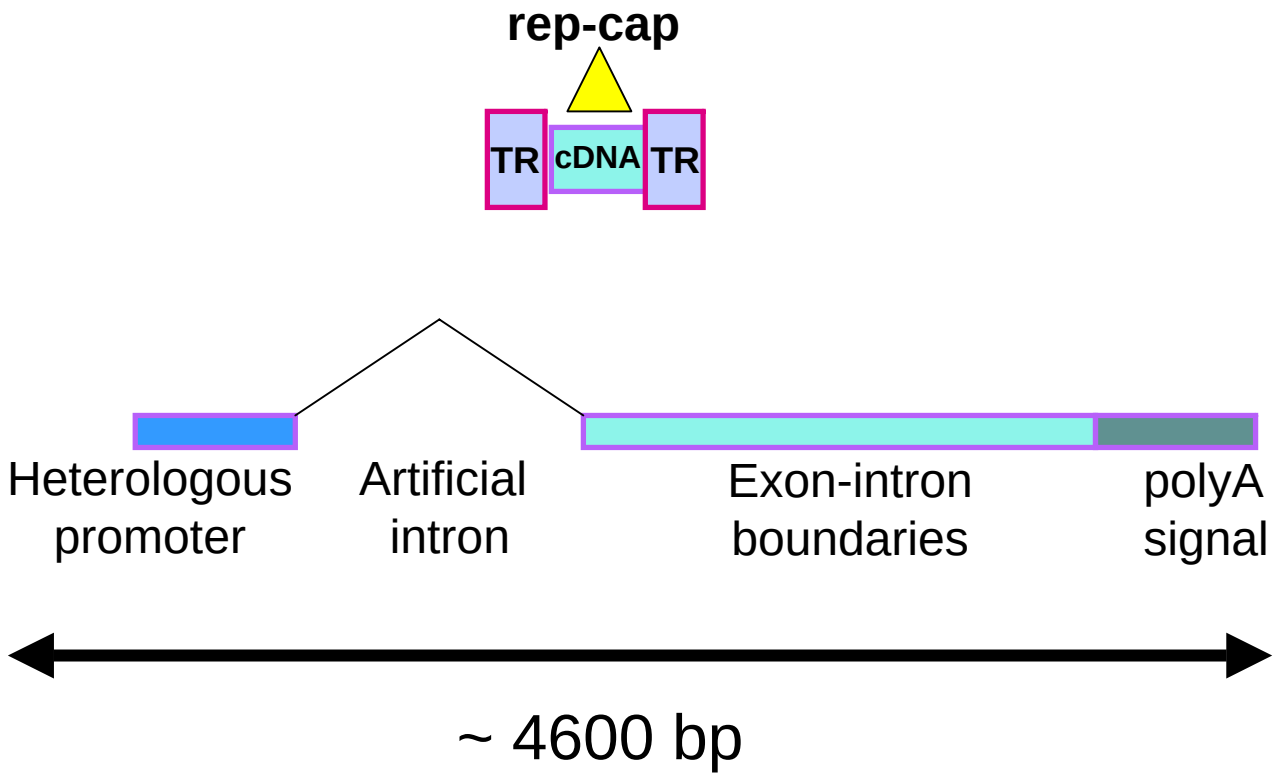
Finding acceptable probe-primer set can be challenging in AAV



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Limitations of qPCR assay

- Even when technically feasible, positive PCR results do not indicate biologically active vector
- Biological fluids can interfere with the assay
- Same assay may not work for follow-on expression studies, RT-PCR

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Technical points related to AAV

- AAV genomes are single stranded
- Single stranded standards can be “sticky”
- Thus PCR standards are commonly double stranded plasmids
- Linearized versions of plasmids should be used
- Ideally, single use, pre-diluted, QC’d standards should be used

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Bio assay

- AAV infectivity assays are challenging
- No plaques assay exists, detection is by PCR
- Cell toxicity of shedding matrices is a complicating factor
- Requires AAV helper genes and Ad helper genes

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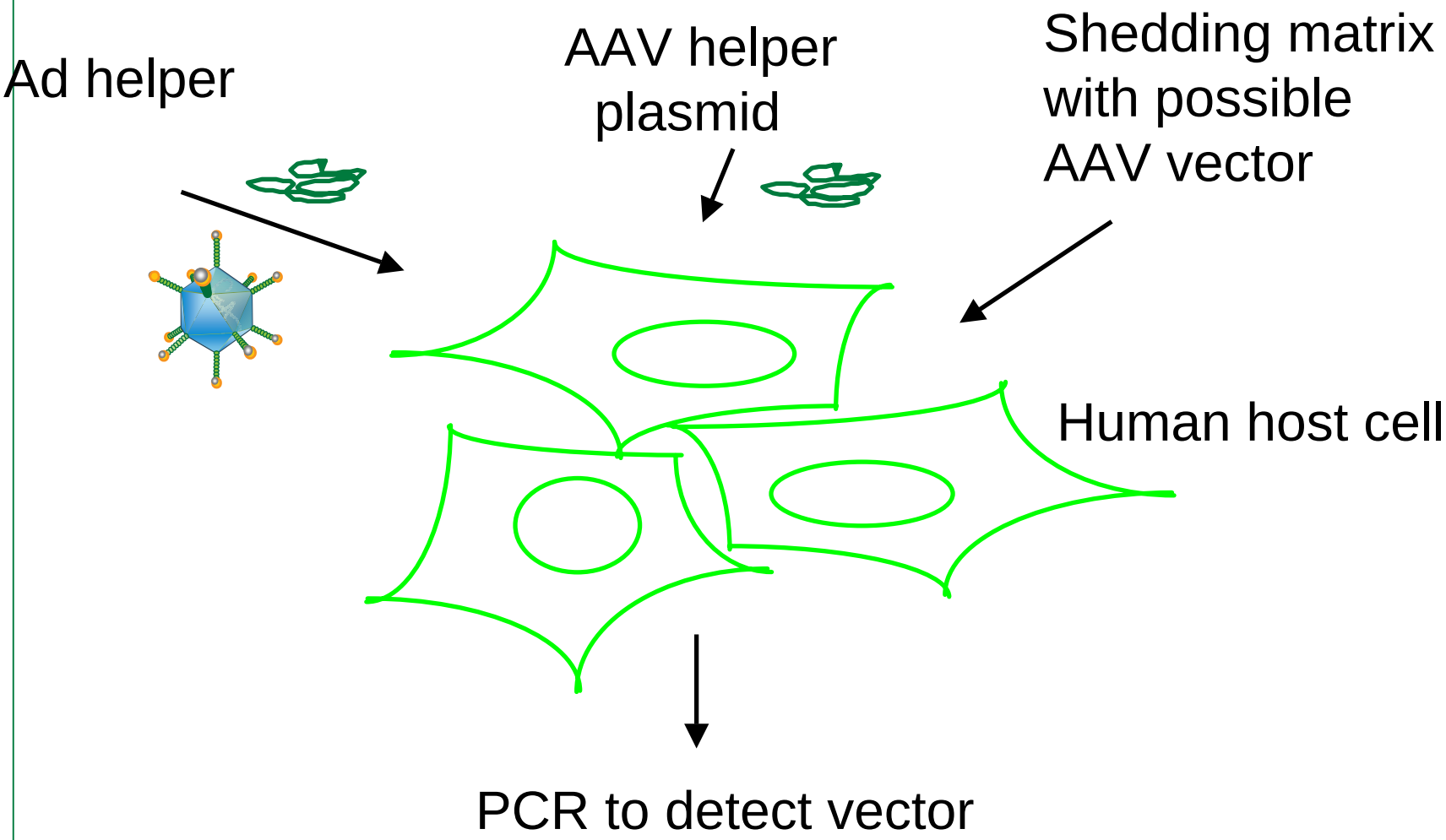
AAV vector infectivity assay



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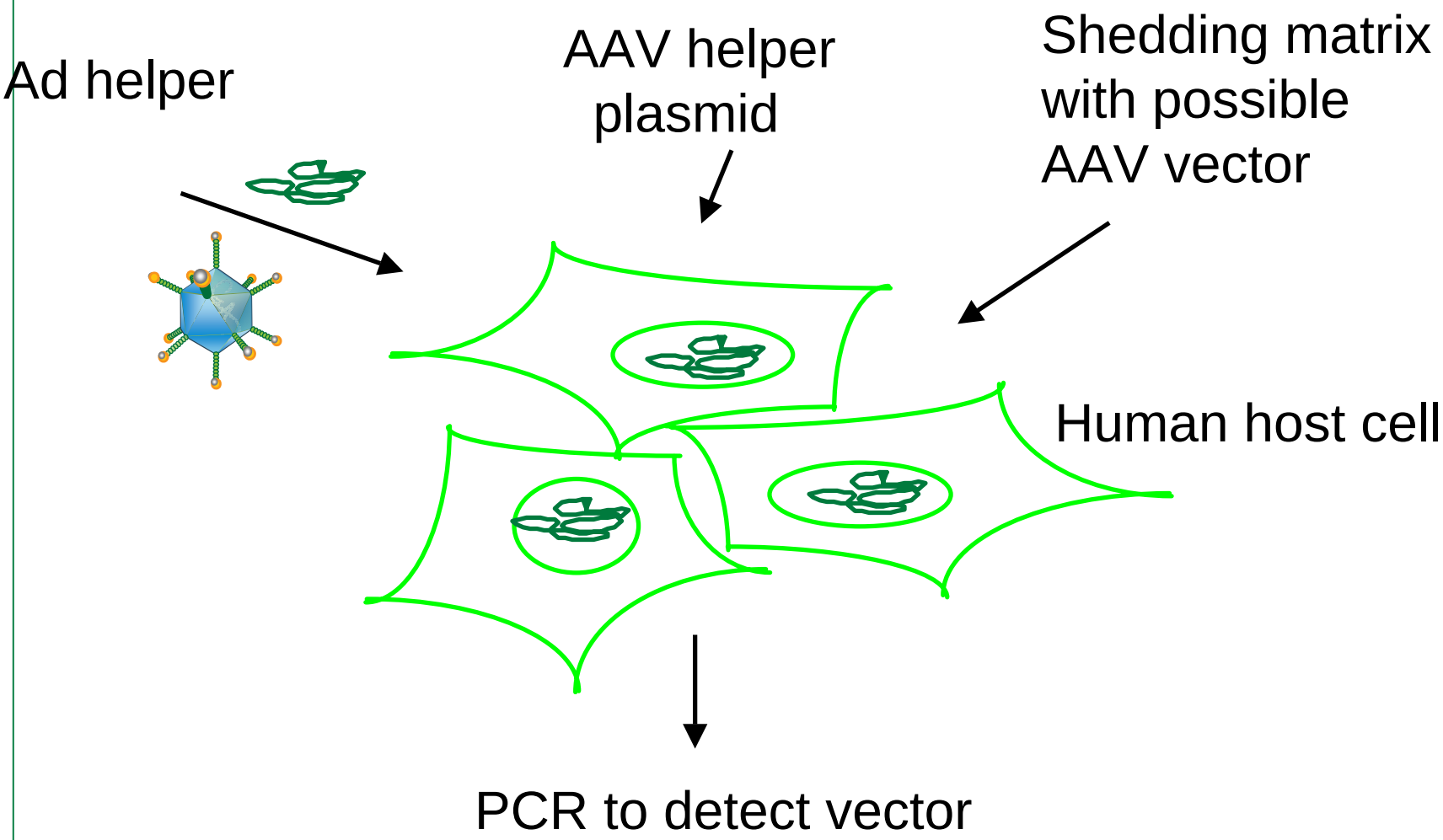
AAV vector infectivity assay



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- Discuss the data available on the relationship between bio-distribution and shedding
  - Bio-distribution assays are performed pre clinically, shedding assays less common
  - Shedding assays are performed clinically with limited bio-distribution
  - Overlap between bio-distribution and shedding data in pre clinical and clinical settings can be informative

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Shedding and biodistribution results

Clinical results	Subjects	Vector dose - DRP/pt	Positive samples (beyond target organ)
Cystic fibrosis - aerosol[a]	CF pts	up to 10^{13}	Blood - d 1, 1 pt Sputum - cleared by d 14
Cystic fibrosis - aerosol[b]	CF pts	10^{13}	Sputum - d 1, 90% pts; d 150, 18% pts
Hemophilia B - IM[c]	Hemophilia B pts	up to 10^{14}	Blood - 1 wk consistently, 1 pt sporadic to wk 12 Saliva - d 2 Urine - d 1
Hemophilia B - IHA[d]	Hemophilia B pts	up to 2×10^{12}	Blood - 1 week consistently, 1 pt sporadic to wk 14 PBMCs - wk 12, 5/7; wk 20, 1/7 Urine - d 2 Semen - 1 week consistently; 1 pt, w 16
Preclinical results			
Inherited blindness - subretinal[e]	Rats, LCA dogs	up to 3×10^{12}	Muscle - sporadic in muscle (1-2 animals) out to 3 mo
Parkinson's - intra striatal[f]	Non human primates	up to 10^{12}	Spleen - 6 mo, 2/12 animals
Research study - IV[g]	Non human primates	up to 10^{13}	Spleen - 6 mo-1 yr, 8/8 PBMCs - 6 mo-1 yr 8/8 Semen - 180 d, 3 animals

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General Conclusions from Biodistribution and Shedding Studies

- Vector biodistribution occurs largely via hematagenous spread
- Route of administration affects the level of vector in different compartments but not the pattern of spread
- Potential concerns are limited to a relatively short period of time post vector administration
- Longest persistence seen in blood, vector likely cell associated
- Shed levels of vector miniscule as compared to initial vector dose

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Potential shedding-associated safety concerns

- Viral vector cannot expand in the environment, dependent on cells
- Sponsors go to great lengths to demonstrate safety of high vector doses in experimental animals
- Unintended exposure of persons to vectors should be avoided, but data indicate amounts of vector in shed excreta will be very low
  - far below the detection limit for any biological activity in controlled experiments
  - non-intended contact between a person and vector will occur under non-optimal conditions (from the perspective of the vector)

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Potential AAV specific shedding-associated safety concerns

- AAV as a virus is not pathogenic
- For AAV vectors to replicate, two types of helper functions are required; AAV functions and Ad functions – all in the same cell
- Even a worst case scenario, presence of all helper functions plus AAV vector, would yield very low levels of additional vector

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Final Thoughts

- What is the real concern?
  - That large amounts of viral vectors are being shed?
  - That harm may come to others?
- Reminiscent of early days of the RAC
  - Originally general concerns about all recombinant plasmids
  - Refined to concern about recombinant plasmids with known risk, eg encoding toxins
  - Today, appropriately relaxed concern based on data
  - Primary role focused on concerns about vector effects on study subjects
- We know that shedding occurs but at very low levels
- Vector design, transgene, manufacturing methods unlikely to affect shedding
- We know that viral vectors are dependent on cells for expansion
- In view of the data, what more can we learn from continued emphasis on testing in clinical setting?
- Are there more critical issues we should be working on?

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Acknowledgements

- Genzyme Research
  - Scott Lonning
  - Jim Morris
  - Mike Lukason

- References cited in Shedding Table
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  - b Moss et al. Chest, 2004, 125 509
  - c Manno et al. Blood, 2003, 101 2963
  - d Manno et al. Nature Medicine, 12 342
  - e Jacobson et al. Molecular Therapy, 2006, 13 1074
  - f Avigen, unpublished data
  - g Genzyme, unpublished data

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