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

Samuel Wadsworth
Genzyme Corporation
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
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
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
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
Finding acceptable probe-primer set can be challenging in AAV

- Heterologous promoter
- Artificial intron
- Exon-intron boundaries
- polyA signal

~4600 bp
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
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
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
AAV vector infectivity assay

Ad helper

AAV helper plasmid

Shedding matrix with possible AAV vector

Human host cell

PCR to detect vector
AAV vector infectivity assay

Ad helper

AAV helper plasmid

Shedding matrix with possible AAV vector

Human host cell

PCR to detect vector
Discuss the data available on the relationship between bio-distribution and shedding

- Bio-distribution assays are performed preclinically, shedding assays less common
- Shedding assays are performed clinically with limited bio-distribution
- Overlap between bio-distribution and shedding data in preclinical and clinical settings can be informative
## Shedding and biodistribution results

<table>
<thead>
<tr>
<th>Clinical results</th>
<th>Subjects</th>
<th>Vector dose - DRP/pt</th>
<th>Positive samples (beyond target organ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic fibrosis - aerosol(^a)</td>
<td>CF pts</td>
<td>up to 10(^{13})</td>
<td>Blood - d 1, 1 pt Sputum - cleared by d 14</td>
</tr>
<tr>
<td>Cystic fibrosis - aerosol(^b)</td>
<td>CF pts</td>
<td>10(^{13})</td>
<td>Sputum - d 1, 90% pts; d 150, 18% pts</td>
</tr>
<tr>
<td>Hemophilia B - IM(^c)</td>
<td>Hemophilia B pts</td>
<td>up to 10(^{14})</td>
<td>Blood - 1 wk consistently, 1 pt sporadic to wk 12 Saliva - d 2 Urine - d 1</td>
</tr>
<tr>
<td>Hemophilia B - IHA(^d)</td>
<td>Hemophilia B pts</td>
<td>up to 2 x 10(^{12})</td>
<td>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</td>
</tr>
<tr>
<td>Preclinical results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inherited blindness - subretinal(^e)</td>
<td>Rats, LCA dogs</td>
<td>up to 3 x 10(^{12})</td>
<td>Muscle - sporadic in muscle (1-2 animals) out to 3 mo</td>
</tr>
<tr>
<td>Parkinson's - intra striatal(^f)</td>
<td>Non human primates</td>
<td>up to 10(^{12})</td>
<td>Spleen - 6 mo, 2/12 animals</td>
</tr>
<tr>
<td>Research study - IV(^g)</td>
<td>Non human primates</td>
<td>up to 10(^{13})</td>
<td>Spleen - 6 mo-1 yr, 8/8 PBMCs - 6 mo-1 yr 8/8 Semen - 180 d, 3 animals</td>
</tr>
</tbody>
</table>
General Conclusions from Biodistribution and Shedding Studies

- Vector biodistribution occurs largely via hematogenous 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
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)
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
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, e.g., 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?
Acknowledgements

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

- References cited in Shedding Table
  - a Aitken et al. Human Gene Therapy, 2001, 12 1907
  - b Moss et al. Chest, 2004, 125 509
  - 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