Non-Clinical Development in Gene Therapy: Thoughts and Lessons from My Past 12 Years in the Trenches

Moderator – Jake McDonald, PhD

Vice President, Applied Sciences

Jake is Lovelace's Chief Scientific Officer, Vice President of Applied Sciences, and has led the gene therapy/toxicology program at Lovelace since its inception in 2007

Speaker – Janet Benson, PhD

Janet is one of Lovelace's longest tenured senior scientists, with 40 years of experience. For the last 13 years, Janet has been a leader in gene therapy preclinical studies and programs.

Role at Lovelace Biomedical – Study PI and Program Co-PI, Marketing, IACUC Member

Question & Answer Panel

Additional Key Staff at Lovelace

Sharla Birch,
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Outline

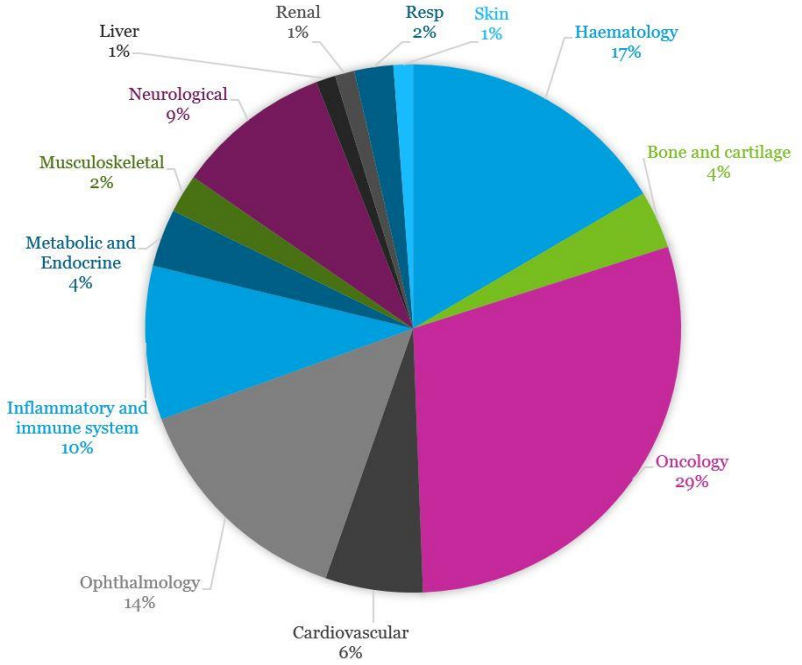
- Brief Overview of Gene Therapy
  - Focus on AAV based therapies
- Major Differences in Pre-Clinical Safety Evaluations: Small Molecule vs Gene Therapy
- Key Study Components – Input on Experience
- Conclusions

Gene Therapy Clinical Trials and Disease Areas

372 Clinical Trials as of Q1 2019

- **217 Phase II**
- **123 Phase I**

(440 as of Feb 2020)



https://report.nih.gov/categorical_spending_project_listing.aspx?FY=2018&ARRA=N&DCat=Gene%20Therapy

Guidance Documents/Resources

www.fda.gov

- Preclinical Assessment of Investigational Cellular and Gene Therapy Products. FDA, CBER, November 2013
- Long Term Follow-Up After Administration of Human Gene Therapy Products. FDA, CBER, January 2020

Other Useful Resources

- American Society for Gene and Cell Therapy www.asgct.org
- National Heart Lung and Blood Disease, Gene Therapy Resources Program (GTRP) www.gtrp.org
- National Gene Vector Biorepository (NGVB) www.ngvbcc.org

Basics

- Goal of gene therapy is to deliver a gene that codes for a critical protein missing or nonfunctional protein that results in disease
- Gene delivery
  - Naked DNA/plasmid
  - DNA enclosed in viral or non viral vector
  - Most common adeno-associated virus (AAV)
    - Gene, promoter, enhancer
- Viral doses needed for efficacy 10^{12} to 10^{13} virus particles/kg BW

AAV Serotype Application

AAV Serotype	Disease Entities
AAV1	Heart failure, Alpha-1 Antitrypsin deficiency, Hemophilia, Cystic fibrosis, Hearing disorders, Hyperlipidemia *(Glybera®)*
AAV2, AAV2.8, AAV2.5	Parkinson's disease, Pompe disease, Osteoarthritis, Retinal disease *(Luxturna®)*
AAV9	CNS disorders, Pompe disease, Muscular dystrophy *(Zolgensma®)*
AAV1, 2, 5, 6, 8, 9	Packaging and delivery of CRISPR components to cells

Domenger and Grim, Hum Mol Genet 2019, 28(R1):R3-R14
Lau and Suh, F1000Research 2017,6(F1000 Faculty Review):2153.

Gene Therapy Pre-Clinical Study Designs

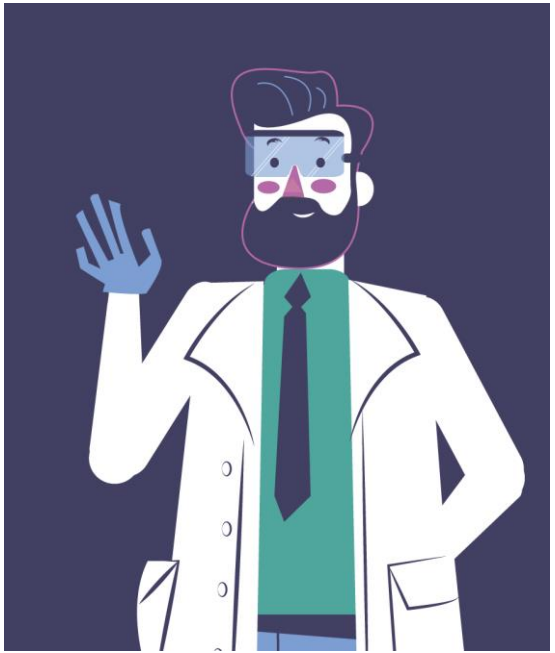
- "Office of Cell Therapy and Gene Therapy uses a flexible, science driven review process to address safety issues in a context that considers both the biology of the product and its intended clinical indication"
- Differences
  - Gene Therapy:
    - One time administration
    - Doses determined in proof of concept studies
    - Endpoints determined serially over time
    - Biodistribution to assess vector persistence and off target distribution
  - Small Molecule:
    - Repeated dosing
    - Dose and frequency evaluated in maximum tolerated dose/effective dose and PK studies
    - Usually a core and hold subgroups for endpoint evaluations

Other Differences

- Small Molecule: Rodent and large animal species
- Gene Therapy: “Best animal model”
  - Animal model of disease
    - Knockout mice
    - Chemically induced
    - Surgically induced
  - Normal/wild type animals

Many Pieces to the Puzzle

- **Test Article:**
  - GMP comparable
  - Well characterized – GLP COA
  - Enough?
  - Vialed to minimize waste
  - Numbered to facilitate inventory
  - Retention sample for stability



Puzzle Continued

- Test System: Justifiable Model
  - Different from animal model used for proof of concept evaluation?
  - Animal model of disease – available from vendor? Chemically or surgically induced? – timing
  - Sufficient animal numbers to cover all desired end points
    - Large animals: 2-3 per sex per dose and time point
    - Rats: 5M/5F per dose and time point
    - Mice: 10M/10F per dose and time point plus satellite groups for additional endpoints
  - Screen for pre-existing antibodies to AAV (depending on serotype may screen 2 – 3 times number needed)

Puzzle Concluded

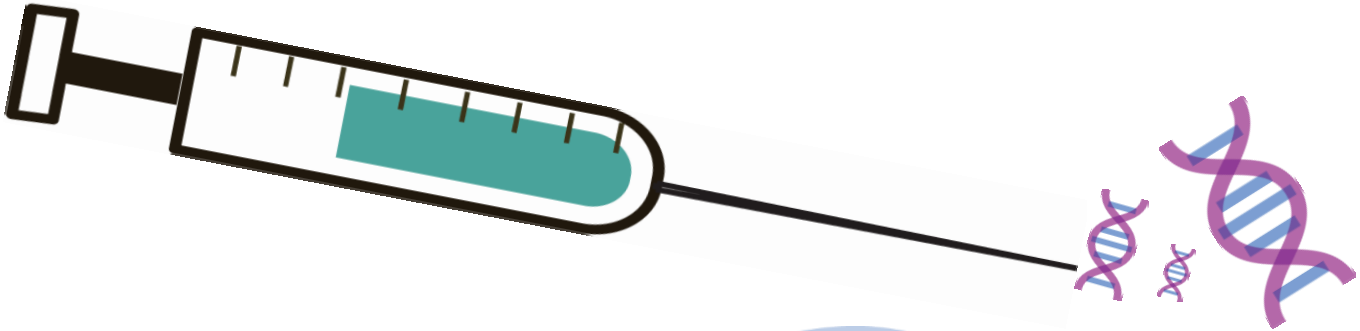
- Dose Delivery:
  - Same method as intended for clinic (with justification to FDA if not feasible)
  - Complexity
- Doses
  - At least 2 doses of therapy plus vehicle control
- Euthanasia Time Points
  - At least 2 time points (30, 90 days)
  - Increasingly common for 3 time points (up to 6 months) or more (up to 1 year)
- Endpoints – Product and Disease Specific:
  - Existing and transferable or performed by Sponsor?
  - Require development (validation and associate cost and time)?
  - Necropsy
    - Harvesting tissue for histopathology, biodistribution, other endpoints?
    - Clean collections for biodistribution/gene expression
- Quality Assurance

Endpoints

- Basic Safety Study Endpoints
- Some Unique to Gene Therapy
  - Humoral antibody response
  - Cell mediated immune response
  - Distribution of vector in the body (qPCR) and persistence at target
  - Transgene expression
- Other Parameters
    - Troponin (muscle damage)
    - Cytokines (inflammatory response)
    - Physiological/functional endpoints based on vector and disease entity
- Vector Shedding (FDA CBER, 2015)

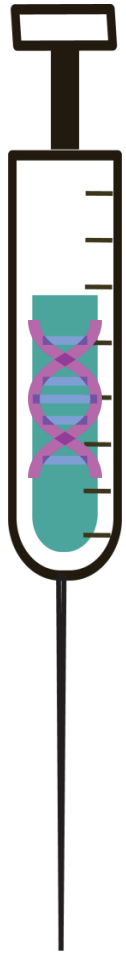
Delivery Considerations

- "The delivery device intended for use in the clinical studies should be used to administer the investigational product in the definitive toxicology studies; justification should be provided if the intended delivery device is not used". (USFDA, 2013)



Delivery – Systemic or Target Organ

Delivery Route	Consideration
Hydrostatic Vascular Infusions	Large animals – fluoroscope guidance, potential for muscle damage, systemic distribution of vector.
Intrapleural, Intraparenchymal	Large animal or rodent, surgical procedure. Systemic distribution.
Intra-articular Injection (knee capsule)	Focused delivery, validation of accurate delivery by x-ray.
Inhalation, Oral, Nose-only	Vector stability, line losses, targeting desired regions of respiratory tract.
Epicardial Painting	Surgical procedure, post surgery clinical monitoring.
Intraocular	Direct to target, little systemic distribution.
Intracranial and Intrathecal	Focused delivery to brain, spinal cord. Systemic distribution.
Intravenous and Intramuscular	Widespread vector distribution, control target site delivery with specific promotors. Retro-orbital useful for larger volume delivery in mice.



Respiratory Tract Delivery – Vector Sparing

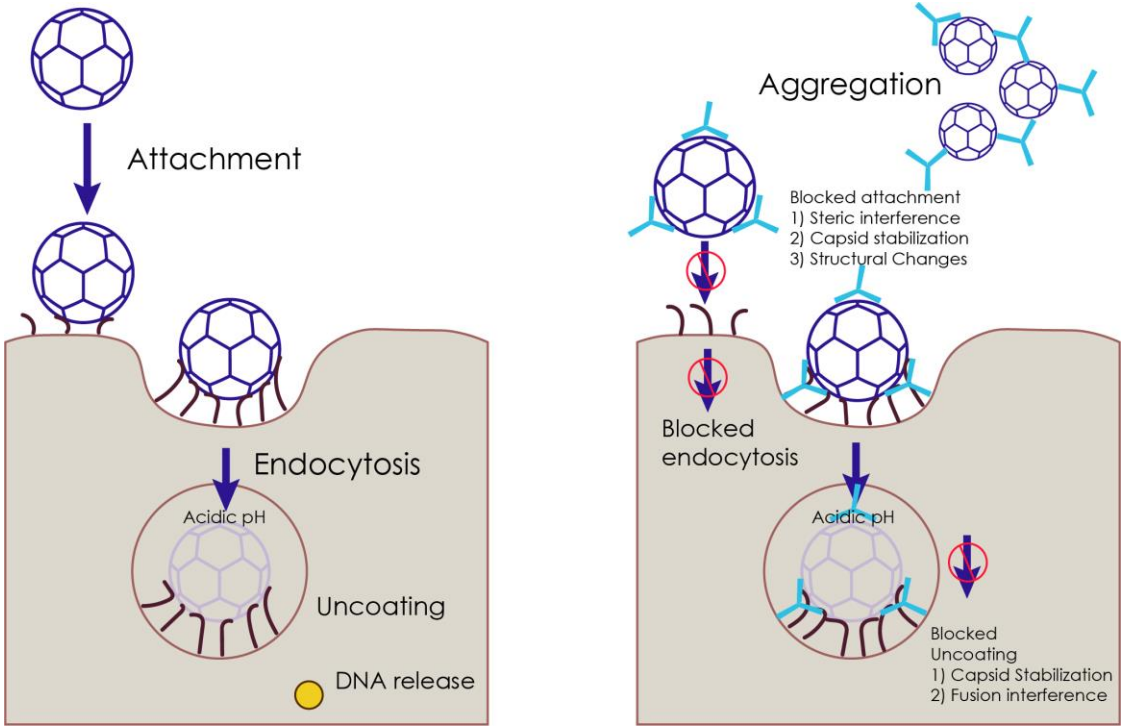
Route	Application	Advantages	Points to Consider
Oropharyngeal	Proof of concept in anesthetized rodents	Mimics "aerosol delivery", avoids nasal deposition, rapid, product sparing	Exact dose delivered to lung may not be known
Intratracheal Instillation	Proof of concept in anesthetized rodents, pivotal toxicology/ bio-distribution studies?	Avoids nasal deposition, known delivered dose, product sparing	Not an aerosol delivery, pulmonary distribution not as even as with aerosol.
Oral or intratracheal Aerosol Delivery	Proof of concept and pivotal studies	Avoids nasal deposition, known delivered dose, product sparing, mimics oropharyngeal delivery in humans	
Nose Only Aerosol	Multiple species	Simultaneous exposure to multiple animals	Stability, line losses, off target deposition

Study Examples at Lovelace[a]

Disease	Outcome	Animal Species	Dose Route
Alpha 1 antitrypsin deficiency	Emphysema, liver disease	NHPs, mice	IM, intrapleural, direct lung injection, intravascular limb infusion
Pompe Disease (acid alpha glucosidase deficiency)	Impaired respiration, heart function	GAA knockout mice	IV, IM
Cystic Fibrosis (mutated CFTR gene)	Impaired airway mucus transport, lung infections	NHP	Intratracheal inhalation
Osteoarthritis (Cytokine IL-1 mediated)	Degeneration of joint cartilage and bone	Rats (mono-iodoacetate induced arthritis)	Injection into knee capsule

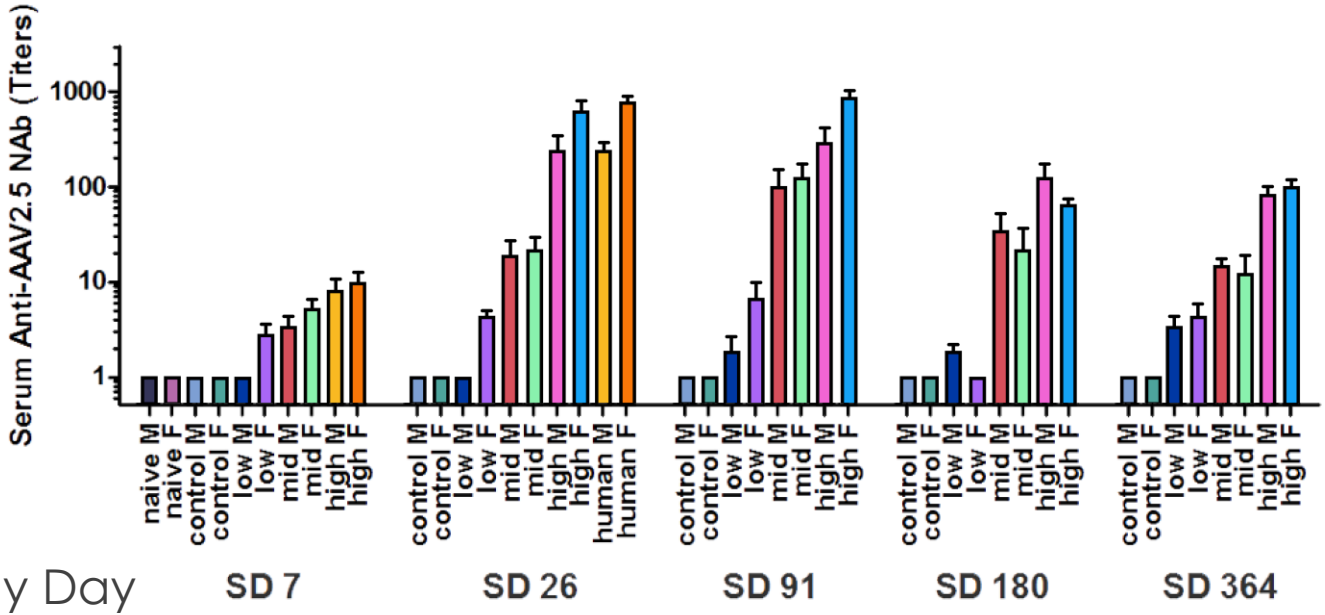
[a]Published in open literature. Funded by the NHLBI Gene Therapy Resources Program

Neutralizing Antibodies



Challenge of Immune Response

Nab responses dose route, dose dependent, and persistent



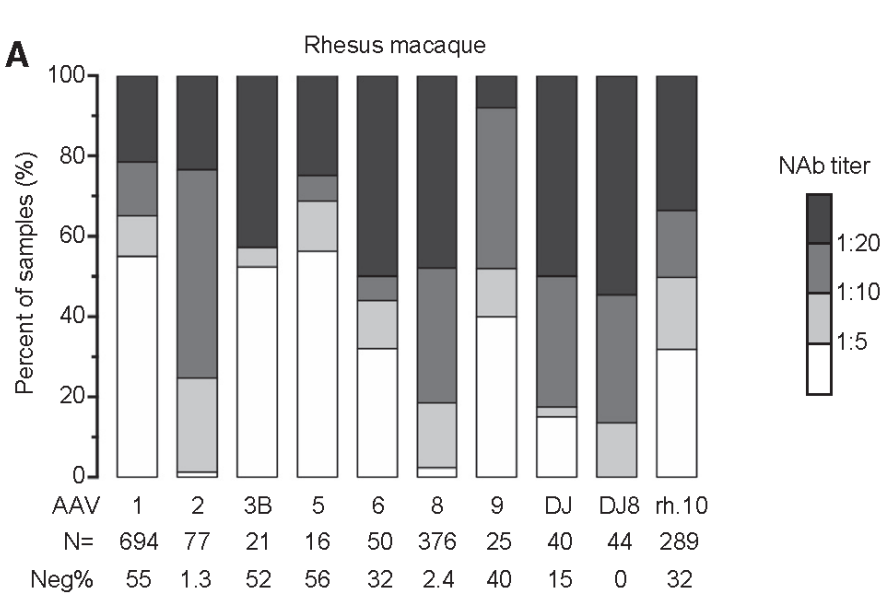
M = Male

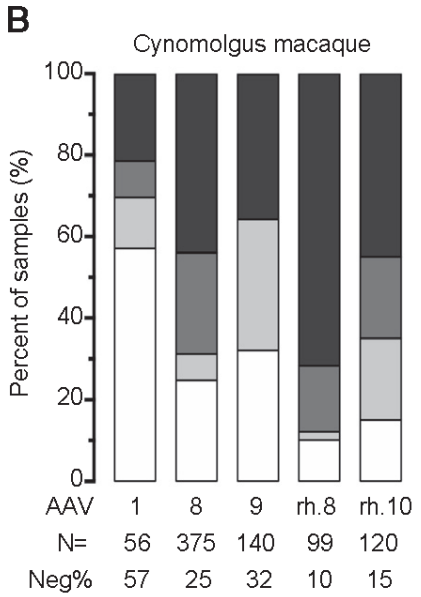
F = Female

Pre-existing Nabs Titers Serotype Dependent Rhesus Macaque

	Percent of Animals Tested (Neutralization in Huh7 cells)			
Titer	Anti AAV1 (n = 30/sex)		Anti AAV9 (n = 30/sex)	
	Males (%)	Females (%)	Males (%)	Females (%)
≤5	47	47	77	83
10	23	7	17	10
20	7	13	3	3
40	10	27	3	0
≥ 80	13	7	0	3

NHP Strain Differences in Nabs





Wang et al, Molecular Therapy, Methods & Clinical Development, 11, Dec. 2018

Minimizing Humoral Immune Response

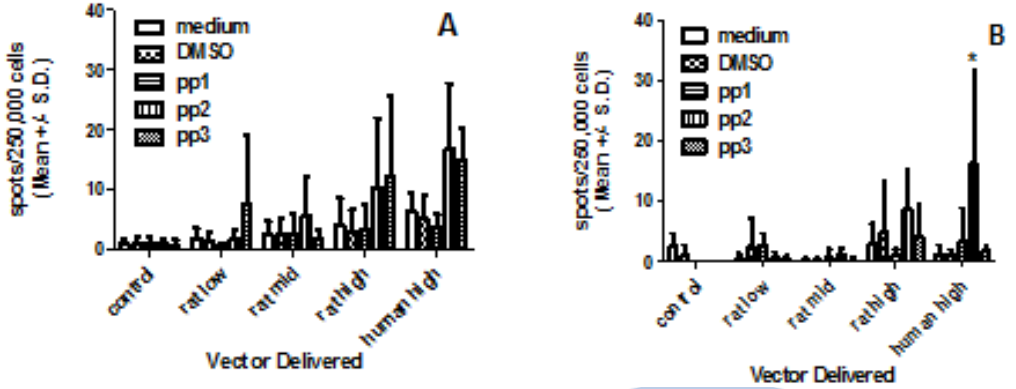
- For safety studies in rodents, deliver rodent gene to prevent immune response to human protein
- Administer immune suppressants – primarily NHP
  - Steroids (prednisolone/prednisone)
  - Rituximab infusion – (monoclonal antibody directed against B cells) – *timing important to prevent rebound of B cells*
  - Rapamycin (prevents B and T immune cell activation) – Meliani et al, *nature communications, 2018*
  - Antibody and steroid "cocktails"
- Engineer vector capsid for reduced immunogenicity

T-Cell - Mediated Immune Response ELISpot

- Looking for T cell mediated immune responses against capsid proteins (3) and/or expressed protein
- Response is organ and dose route specific
- Assay using peripheral blood monocytes (large animals –serial collections) or splenocytes (rodents at necropsy) 1 x 10^6cells/sample

Lymphocyte Mediated Immune Responses ELISpot – AAV2.5 IL-1rRa or AAV2.5IL-1hRa Capsid

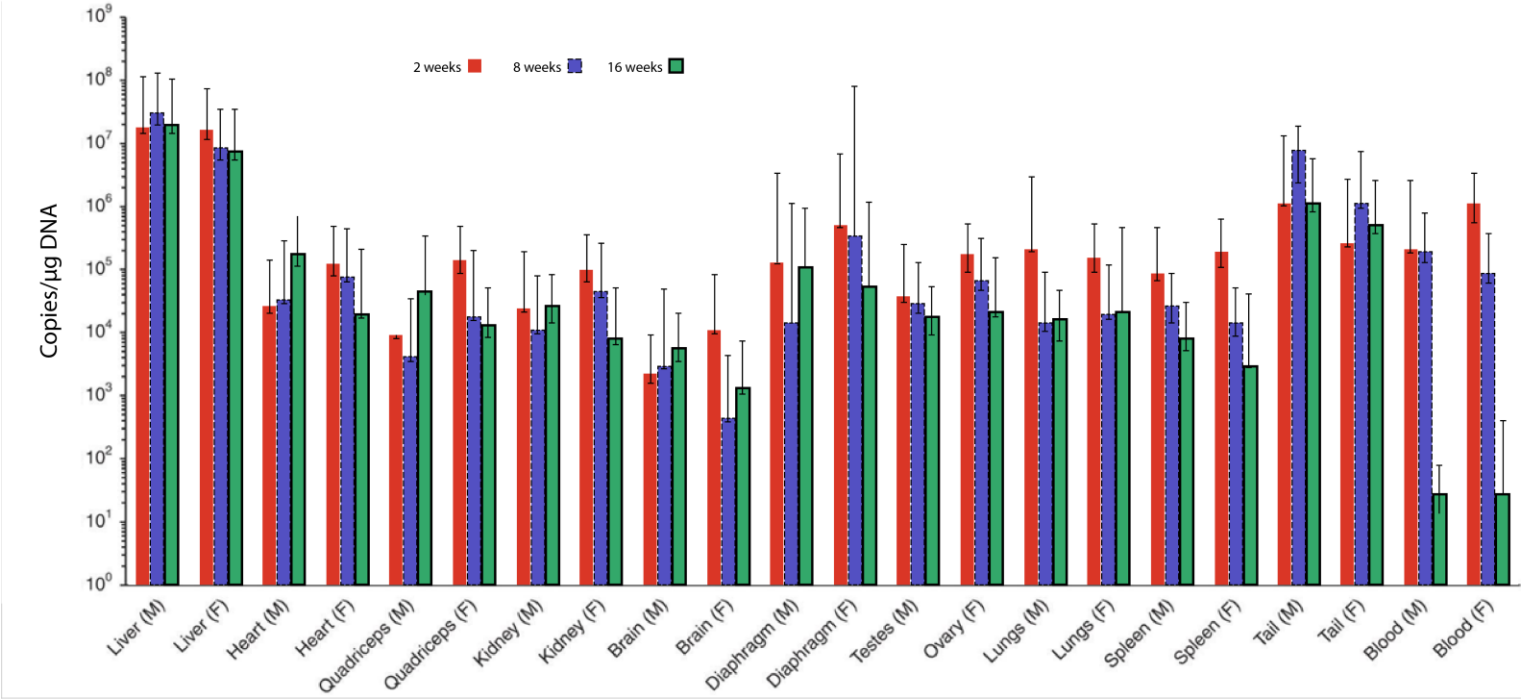
T Cell-Mediated Immune Response to AAV2.5 capsid 26 Days Post Vector Administration ; A = Females, B = Males



Biodistribution Assay Development qPCR

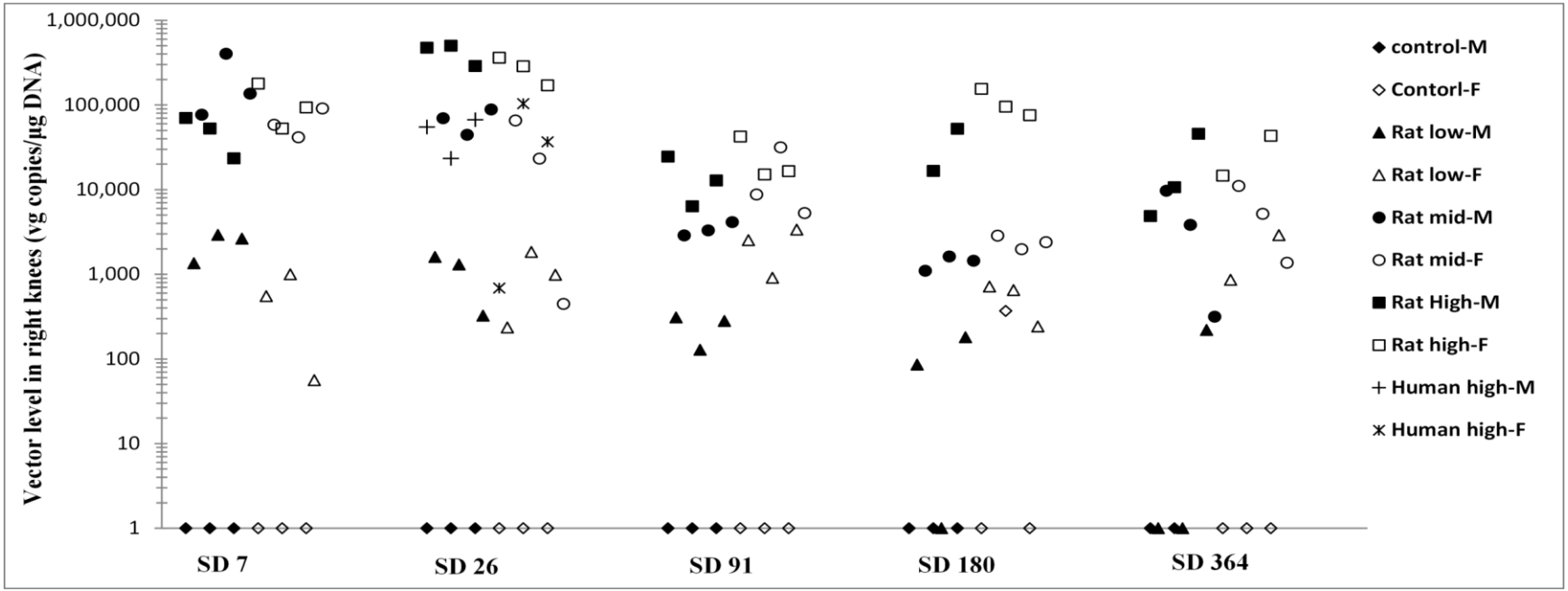
- Perform in conjunction with safety assessment or separately
- Assay qualification/validation - timing
  - Standard curve 8 point, including LLOQ
  - Run in matrix of genomic DNA (liver) or buffer?
  - FDA LLOQ – 50 vector genomes/µg genomic DNA
  - Positive and negative controls
  - Precision/accuracy
  - Selectivity of sample matrix
  - Vector short (extracted DNA) and long term (frozen tissue) storage stability
  - Pre-established acceptance criteria

Biodistribution of AAV2.8 (IV)



Wang G et al, Molecular Therapy – Methods and Clinical Development 2014 1, 14018;doi:1038/mtm.2014.18.

Vector Concentration in Knee



Off target distribution only to draining LNs, liver, spleen at SD 7.

Molecular Therapy — Methods & Clinical Development 2016 Jan 13;3:15052. doi: 10.1038/mtm.2015.52. eCollection 2016.

Gene Expression

- Direct measurement of transgene protein in serum by chemical analysis (Alpha-one antitrypsin, Acid alpha glucosidase)
- Immunohistochemistry (biopsy and terminal samples)
- RT-PCR for transgene mRNA (specify vector genome content of tissue to prioritize samples – LOD greater than for qPCR)

Vector Shedding

- Vector shedding assesses the presence of vector in excreta, saliva, and tears
- Concern – unintentional exposure to people and environment
- *Highly relevant in preventing cross contamination among animals in pre-clinical studies*

Conclusions

- Pre clinical IND-enabling studies for gene therapies are complex and require extensive thought and planning
- Base design as much as possible on outcome of proof of concept studies with the therapy
- Early contact with FDA through INTERACT and Pre-IND meetings encouraged

Thank you!

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