



AUDENTES ➔

Experiences in Scaling Gene Therapy Manufacturing

John T. Gray, PhD
CSO, Audentes Therapeutics

Courageous Patients. Bold Effort.

Except for statements of historical fact, any information contained in this presentation may be a forward-looking statement that reflects the Company's current views about future events and are subject to risks, uncertainties, assumptions and changes in circumstances that may cause events or the Company's actual activities or results to differ significantly from those expressed in any forward-looking statement. In some cases, you can identify forward-looking statements by terminology such as "may," "will," "plan," "expect," "estimate," "anticipate," "intend," "goal," "strategy," "believe" and similar expressions and variations thereof. Forward-looking statements include statements regarding the Company's business strategy; potential growth opportunities; clinical development activities; the timing and results of Investigational New Drug application and Clinical Trial Authorisation submissions; the timing and results of preclinical studies and clinical trials; the nature and timing of potential regulatory approvals; and the likelihood of future commercialization of product candidates. Although the Company believes that the expectations reflected in such forward-looking statements are reasonable, the Company cannot guarantee future events, results, actions, levels of activity, performance or achievements, and the timing and results of biotechnology development and potential regulatory approval is inherently uncertain. These forward-looking statements are subject to a number of risks, uncertainties and assumptions, including those described under the heading "Risk Factors" in documents the Company has filed with the SEC. These forward-looking statements speak only as of the date of this presentation, and the Company undertakes no obligation to revise or update any forward-looking statements to reflect events or circumstances after the date hereof.

Certain information contained in this presentation may be derived from information provided by industry sources. We believe such information is accurate and that the sources from which it has been obtained are reliable. However, we cannot guarantee the accuracy of, and have not independently verified, such information.

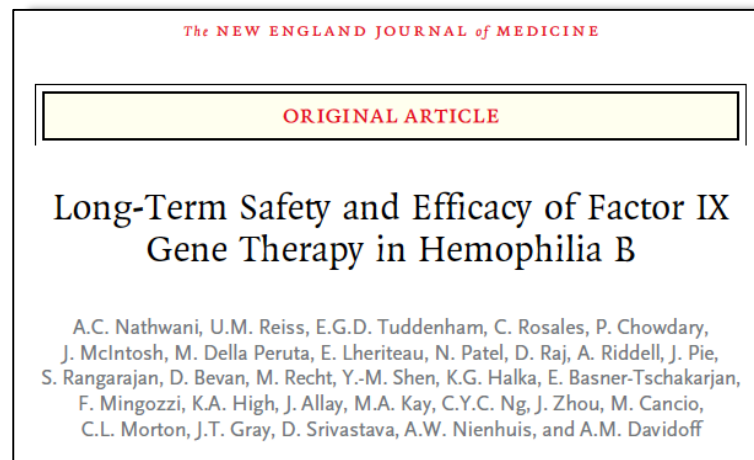
The trademarks included herein are the property of the owners thereof and are used for reference purposes only. Such use should not be construed as an endorsement of such products.

Evolution of Gene Therapy Manufacturing Platforms

Clinical success drives a need for scalability in manufacturing

➤ Hemophilia B, Human Factor IX gene therapy

- NEJM, November 2014, Vol 371, p1994



➤ SCID-X1, Human common gamma chain gene therapy

- Science Translational Medicine, 20 Apr 2016, Vol 8, Issue 335

Lentiviral hematopoietic stem cell gene therapy for X-linked severe combined immunodeficiency

Suk See De Ravin^{1,*}, Xiaolin Wu², Susan Moir³, Lela Kardava³, Sandra Anaya-O'Brien¹, Nana Kwatemma¹, Patricia Littell¹, Narda Theobald¹, Uimook Choi¹, Ling Su², Martha Marquesen¹, Dianne Hilligoss¹, Janet Lee¹, Clarissa M. Buckner³, Kol A. Zarembler¹, Geraldine O'Connor⁴, Daniel McVicar⁴, Douglas Kuhns², Robert E. Throm⁵, Sheng Zhou⁵, Luigi D. Notarangelo⁶, I. Celine Hanson⁷, Mort J. Cowan⁸, Elizabeth Kang¹, Coleen Hadigan³, Michael Meagher⁵, John T. Gray⁹, Brian P. Sorrentino^{5,†} and Harry L. Malech^{1,*†}

➤ Hemophilia A, Human Factor VIII gene therapy

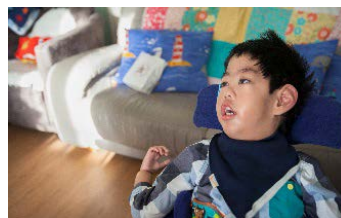
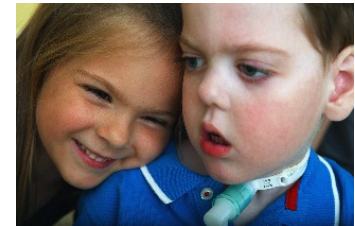
- <http://investors.biomarin.com>

BioMarin Announces Plans to Progress Both the 6e13vg/kg and 4e13 vg/kg Doses of BMN 270, its Investigational Gene Therapy for Hemophilia A, into Phase 3 Studies

Audentes Therapeutics

Developing gene therapy products to transform the lives of rare disease patients with limited or no treatment options

- Rare disease and AAV gene therapy strategic focus
- Multi-product pipeline based on focused selection criteria
- Internal cGMP manufacturing provides core strategic advantage
- Proven leadership team with significant experience
- Preliminary clinical data from lead programs expected in 2017

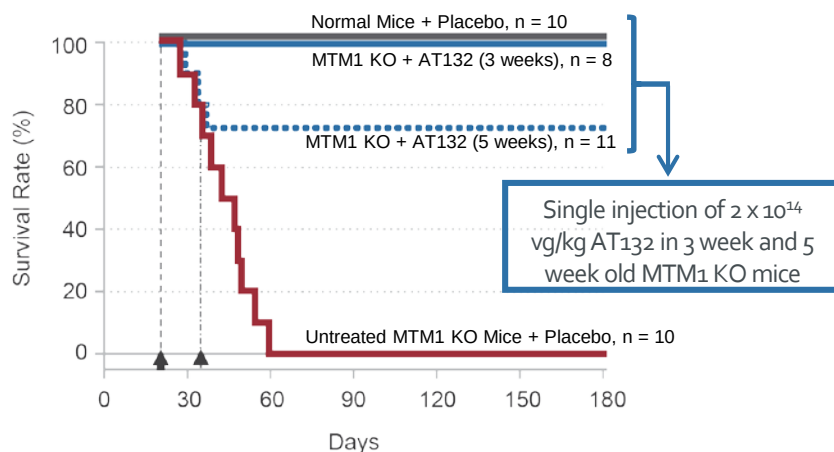


Robust Pipeline

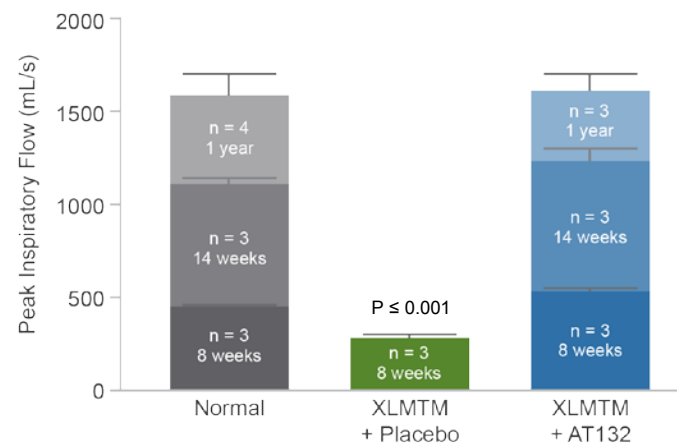
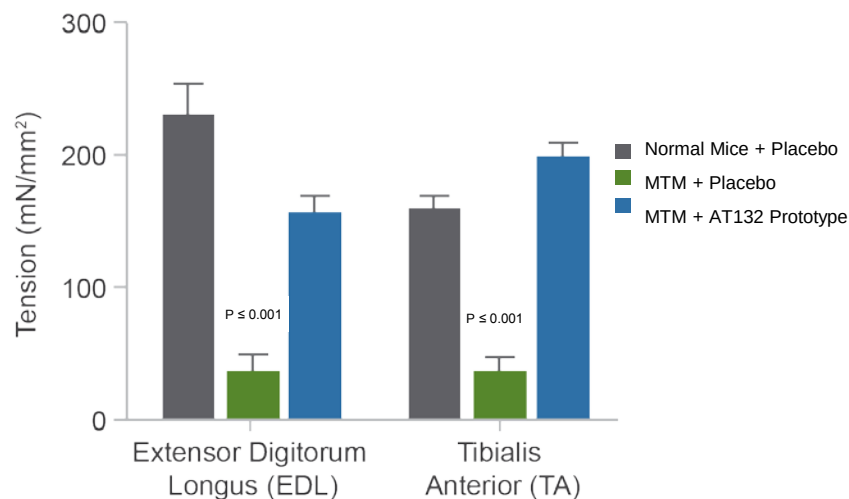
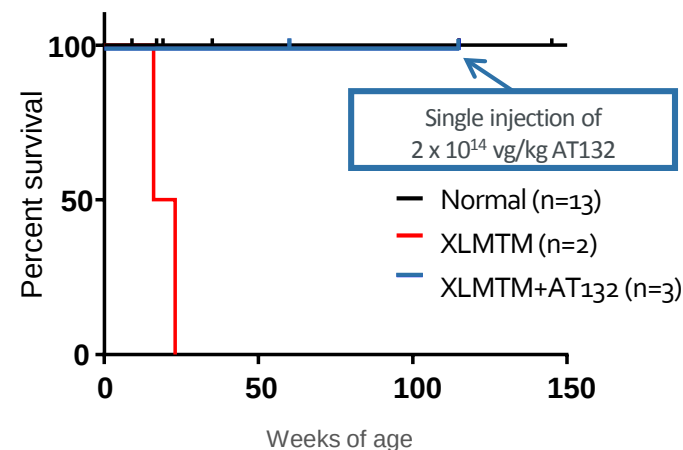
Indication	Stage of Development			Commercial Rights
	Proof-of-Concept	IND Enabling	Phase 1/2	
XLMTM	AT132			AUDENTES THERAPEUTICS
Crigler-Najjar Syndrome	AT342			AUDENTES THERAPEUTICS
Pompe Disease	AT982			AUDENTES THERAPEUTICS
CASQ2-CPVT	AT307			AUDENTES THERAPEUTICS

A Single Treatment with AT132 Results In Dramatic Improvement & Survival in XLMTM Mouse and Dog Models

MTM1 KO Mouse Model



XLMTM Canine Model



M. K. Childers et al., *Sci Transl Med*, 2014; Buj-Bello, et al. *Human Molecular Genetics*, 2008; Note: vector dosing numbers have been adjusted from publication as a result of improvements in titration methodology

Treatment with AT132 Results in Dramatic Improvement and Survival in XLMTM Dogs

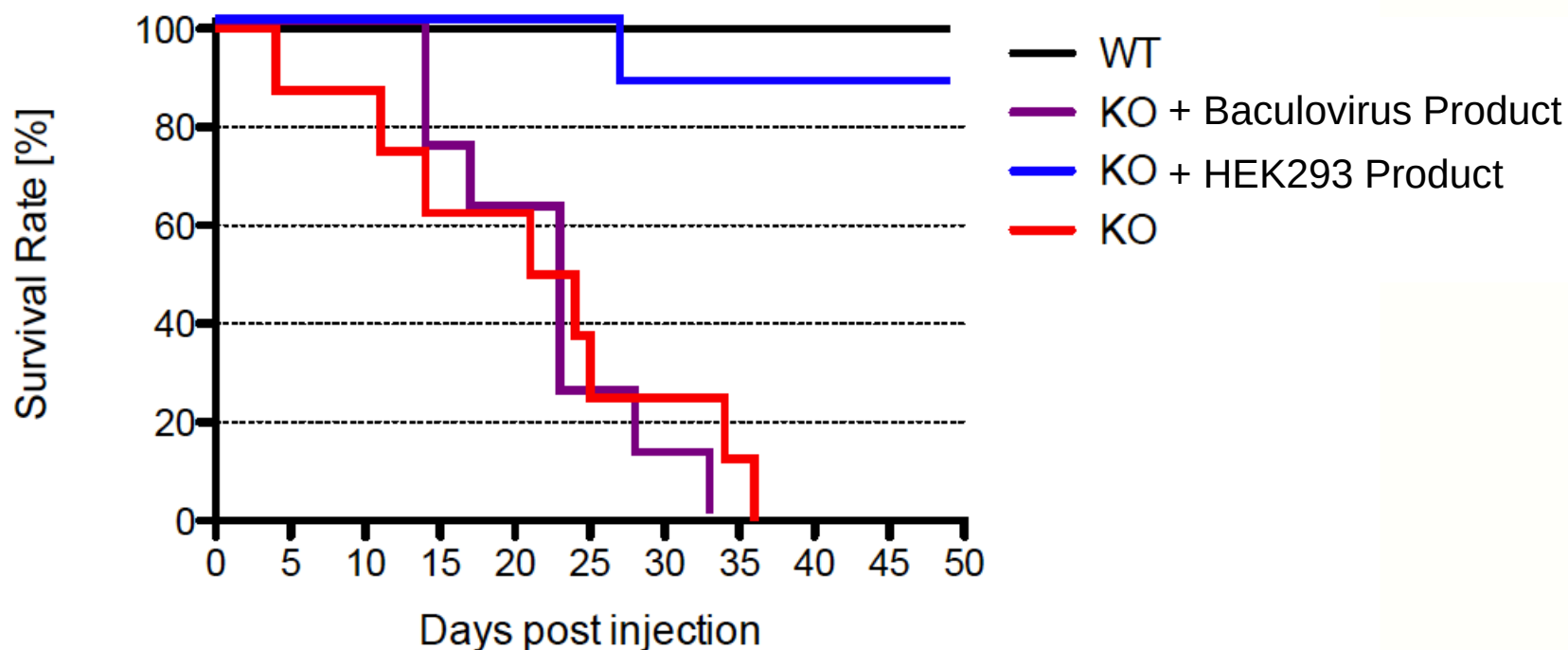


*Dogs 3, 4 and 5, the three dogs treated in the proof-of-concept canine study, achieved a statistically significant improvement in survival.

Manufacturing System Migration During Early Development

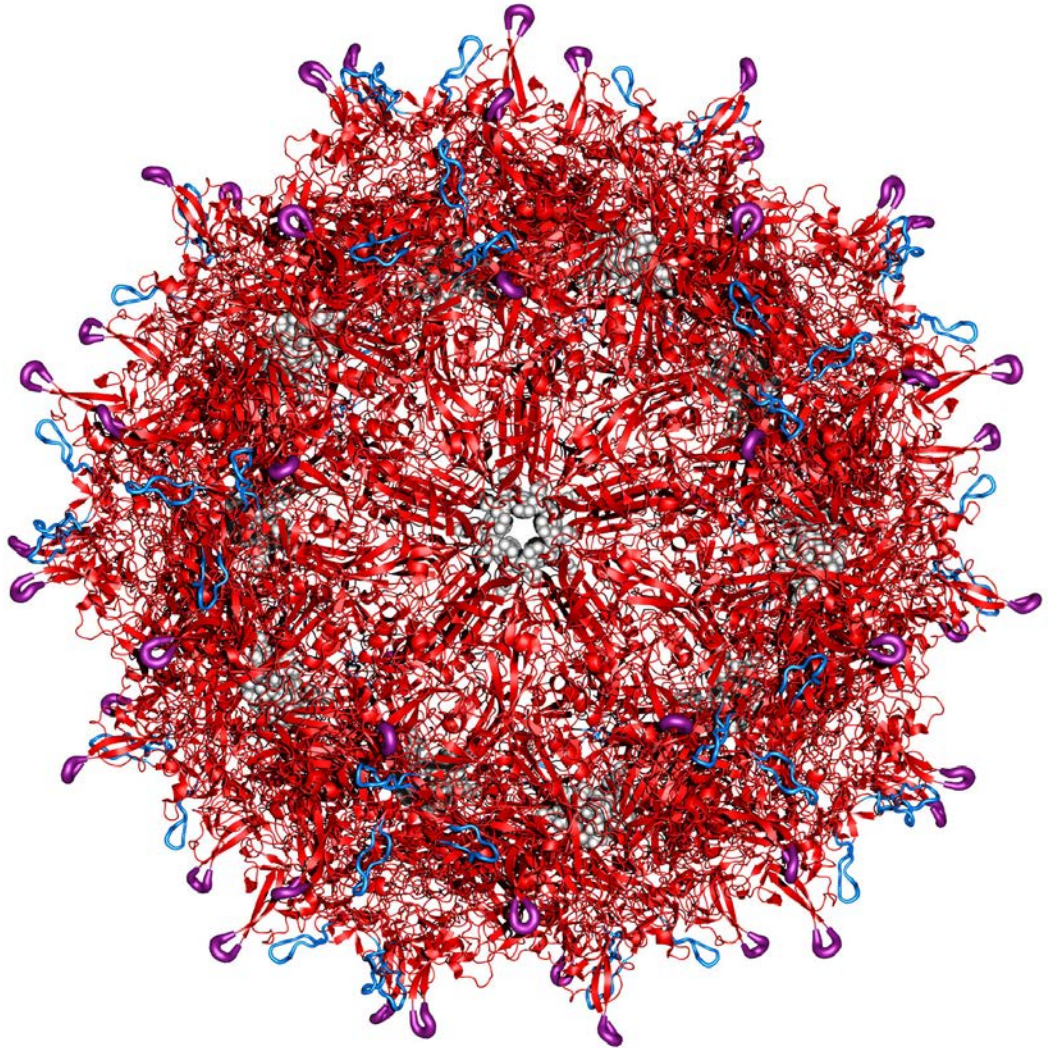
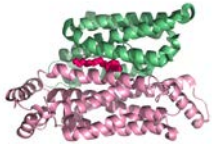
- Initial Mouse Studies
 - HEK293 (Human) cells, small scale, mouse gene
- Dog Studies
 - Sf9 (Insect) cells with Baculovirus, small and large (200L) scale, dog gene
- NHP Toxicity Study
 - Sf9 (Insect) cells with Baculovirus, 200L scale, human gene

Large Scale Human cDNA Vector from Baculovirus System Fails Potency

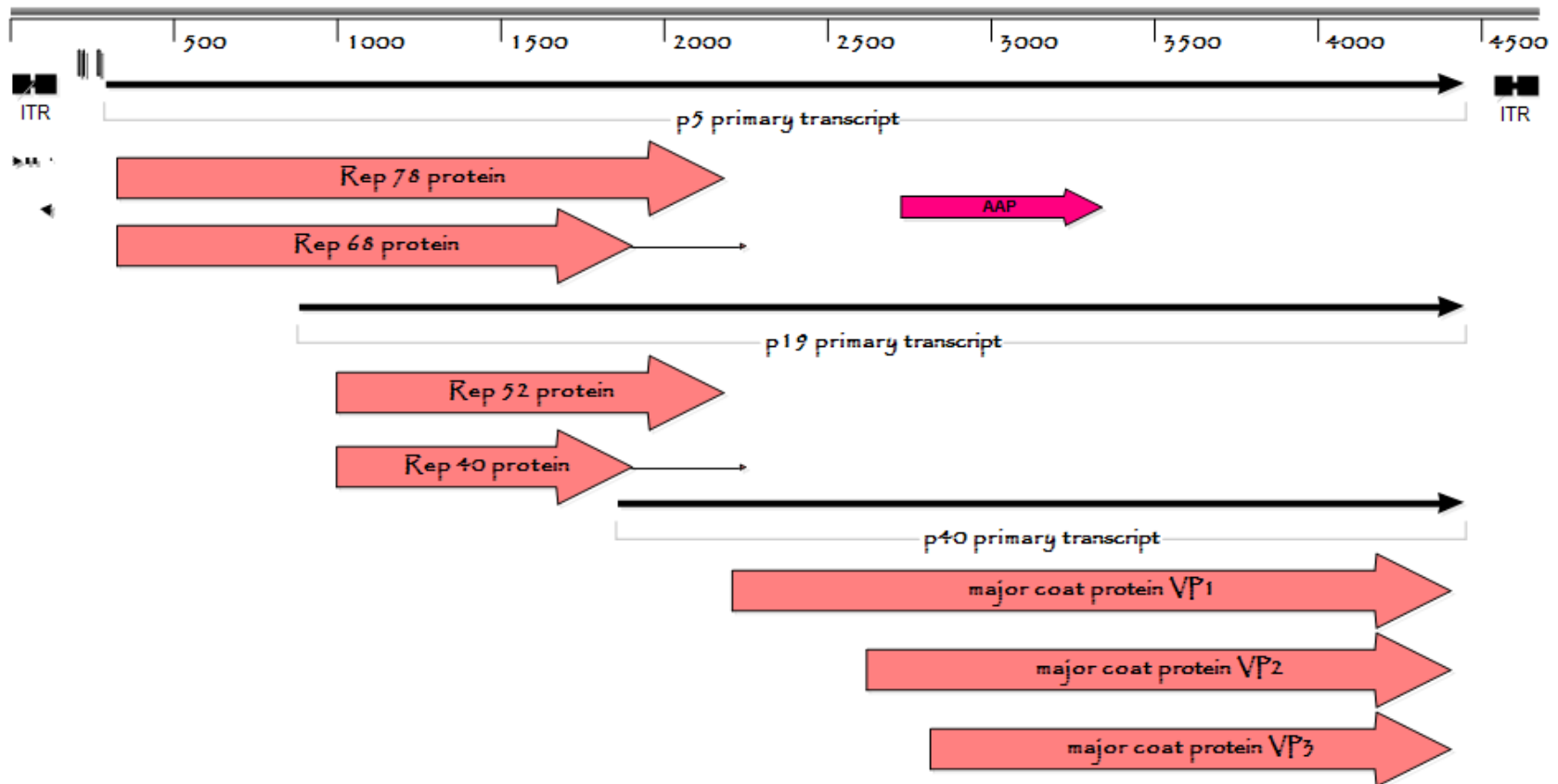


What Is AAV, and How Does It Get Broken?

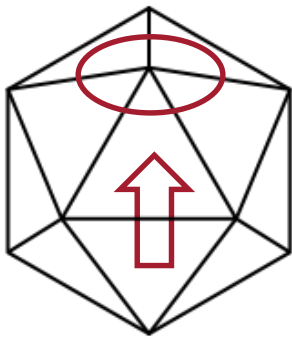
A little more complexity than your standard biologic...



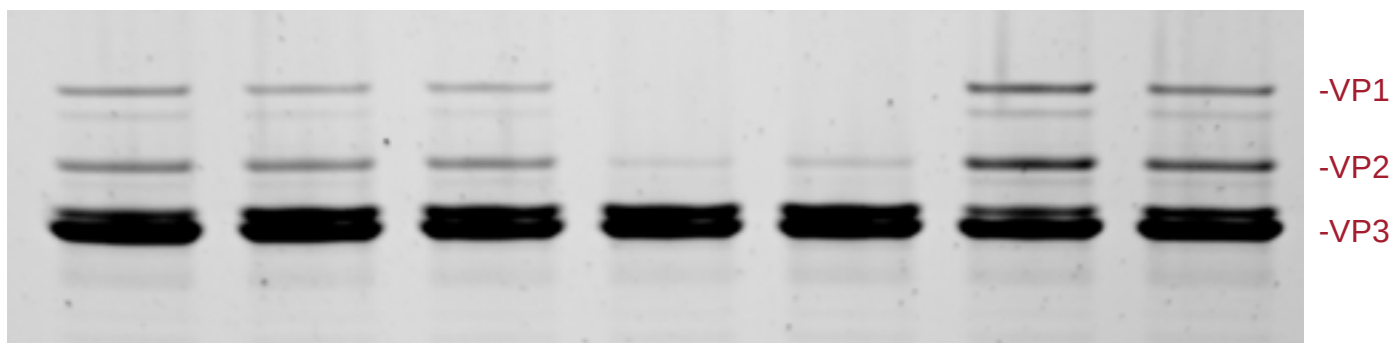
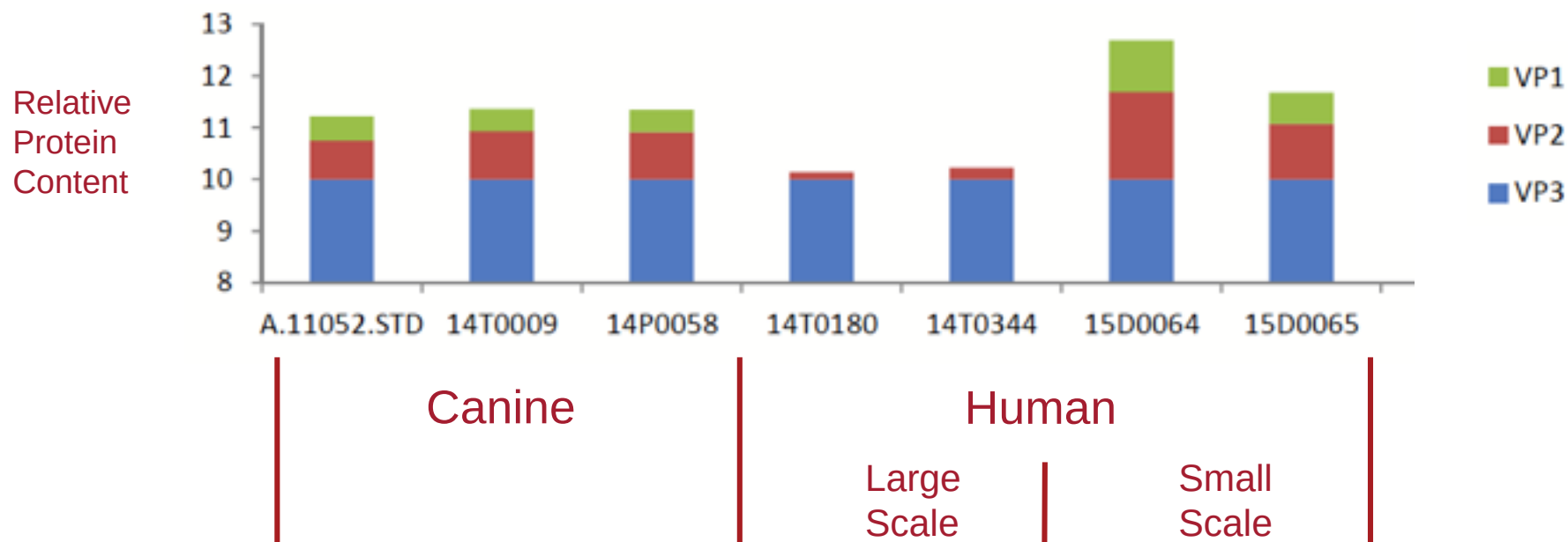
AAV Virus Genome Encodes 3 Capsid Protein Genes



60 Viral Protein Subunits Assemble to Form the Capsid

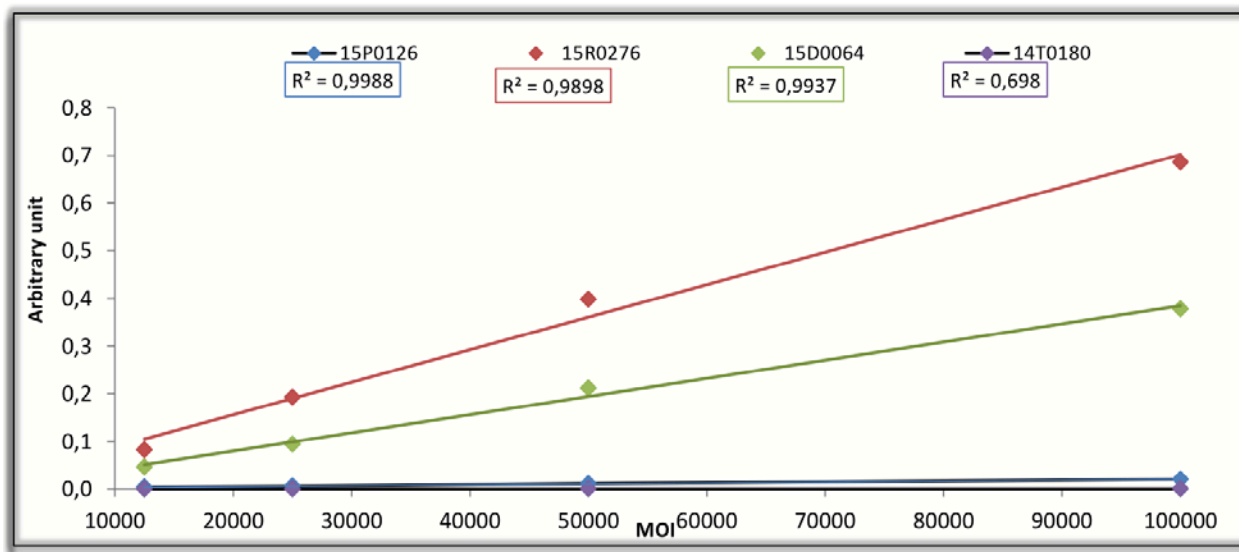


AAV8 Produced in Baculovirus System Can Show VP1 Deficits

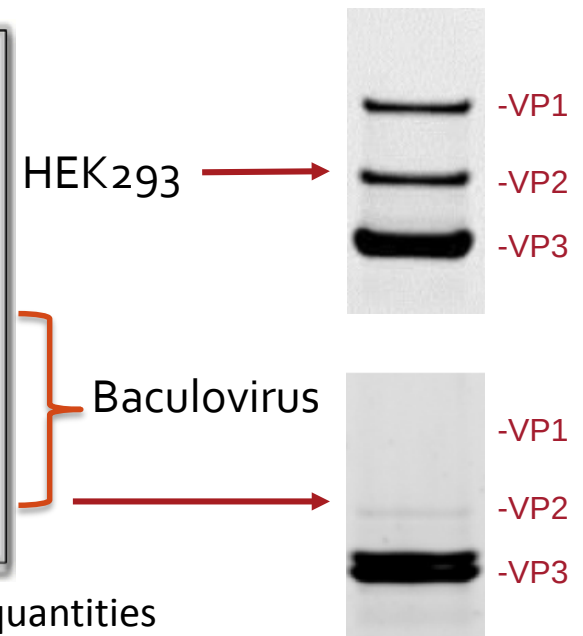


Mammalian Cell-Based Production Improves Potency

- Insect cell proteins are not native to the AAV replication complex – can lead to high levels of contaminating DNA
- AAV produced in ‘natural host’ may have lower production variability, higher purity and better potency
- Insect cell proteins and DNA may be more likely to be recognized as foreign
- *For AT132, mammalian cells generated superior VP1 content*



mRNA expression measured in cultured cells after exposure to increasing quantities of AAV vector produced in mammalian or insect cell system



Audentes Developed Internal GMP Manufacturing

- Based on transient transfection of HEK293 cells in suspension
 - Proprietary plasmid genetics
 - Scalable column chromatography purification
- cGMP Manufacturing facility established and on line
 - 38,000 square foot cGMP manufacturing facility in South San Francisco
 - 2 x 500L bioreactor scale in first GMP suite
 - In-house QC lab for critical release assays
 - Additional capacity (~5,000L) possible in existing lease footprint
 - Planned to be capable of commercial production



Internal Manufacturing Supports Robust IND-Enabling Studies

Murine and NHP studies of AT132 performed with material produced at 500L

COMPLETED STUDIES	KEY CONCLUSIONS
Murine model Dose Ranging and Biodistribution Doses: 2.5×10^{13} to 2.0×10^{14} vg/kg	Clinically meaningful and durable efficacy
Canine model Dose Ranging and Biodistribution Doses: 3×10^{13} to 5×10^{14} vg/kg	Well-tolerated up to 8×10^{14} vg/kg
Non-human Primates Toxicology and Biodistribution Dose: 8×10^{14} vg/kg	Robust biodistribution and protein expression

- ASPIRO Phase 1/2 initiating this quarter using material from our facility

Mack et al. Molecular Therapy. 2017



Lessons Learned

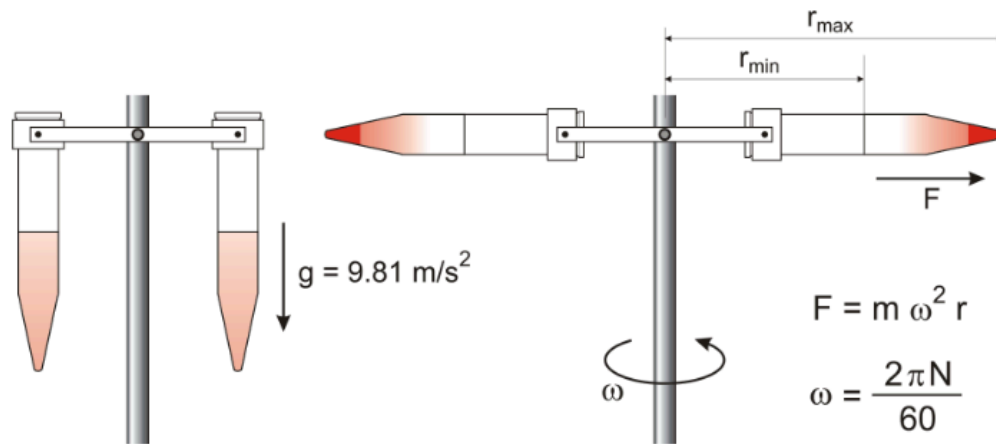
- With more complexity comes more ways in which products can have defects
- Deficiencies in viral and cell based products are not always straightforward to measure
- Internal control over manufacturing, supported by the appropriate expertise, can accelerate a company's ability to transition products into clinical and commercial phases
- With complex gene therapy products, some CMC development issues may only need to be solved once



AUDENTES ➤

Courageous Patients. Bold Effort.

Density Gradient Centrifugation Separates Empty and Full AAV Particles



$$F = m \omega^2 r$$
$$\omega = \frac{2\pi N}{60}$$

m = mass of particle

r = distance of particle from axis of rotation

ω = average angular velocity (rad/s)

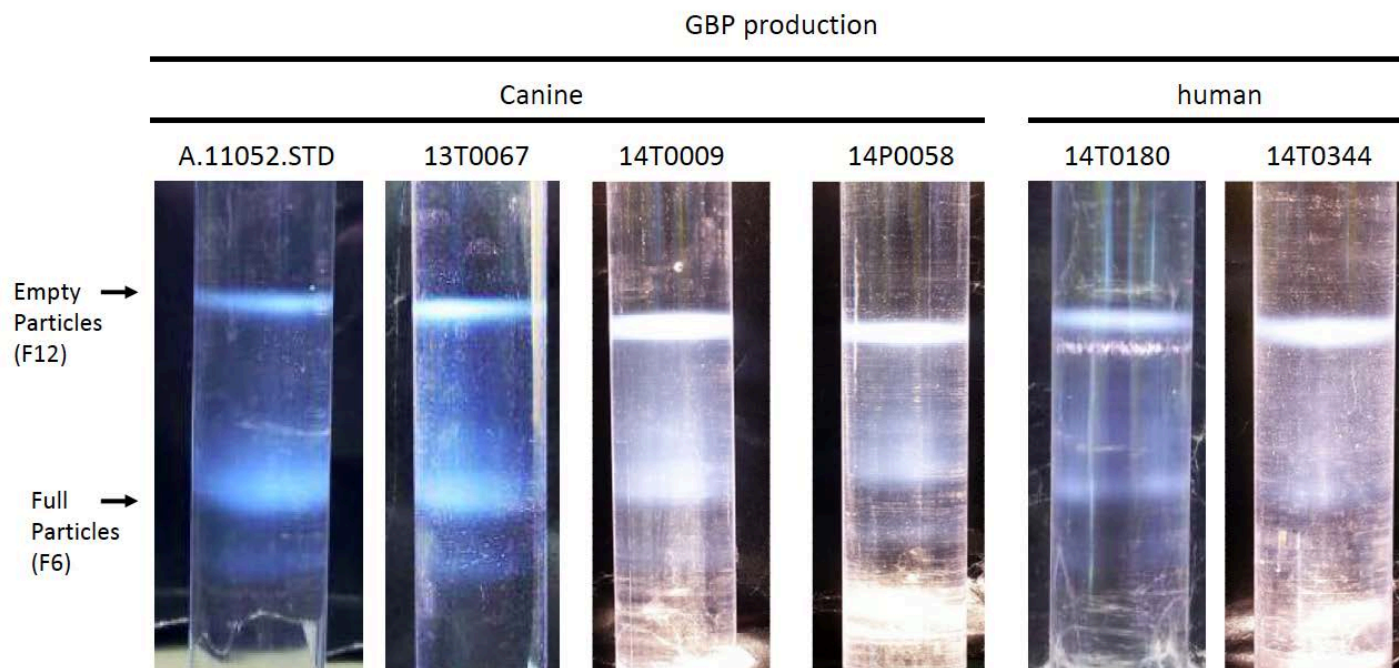
N = revolution per minute, r.p.m.



- Maximum density of medium > densities of any particles.
- Particles move towards bottom, but stop when they reach position where density of medium = density of particle.
- Sometimes referred to as *equilibrium density gradient centrifugation*.

Isopycnic Centrifugation

CsCl Gradient Centrifugation to Isolate Full particles

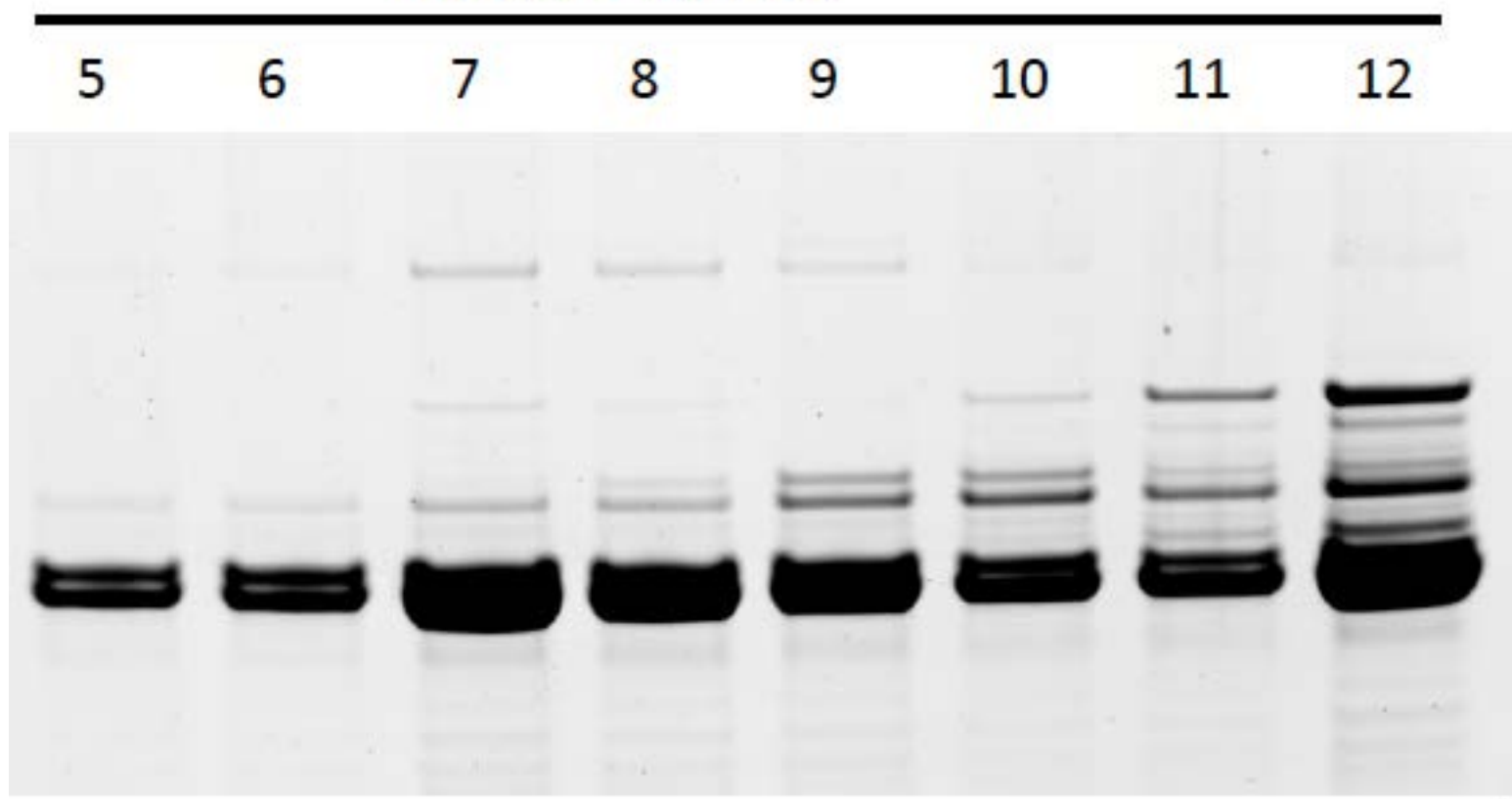


Selective VP1 Deficiency in Full Particles

Bottom

Fractions after CsCl

Top



-VP1
-VP2
-VP3