Cardiovascular Gene Therapy

Efficient gene delivery to the heart? What are the best targets?

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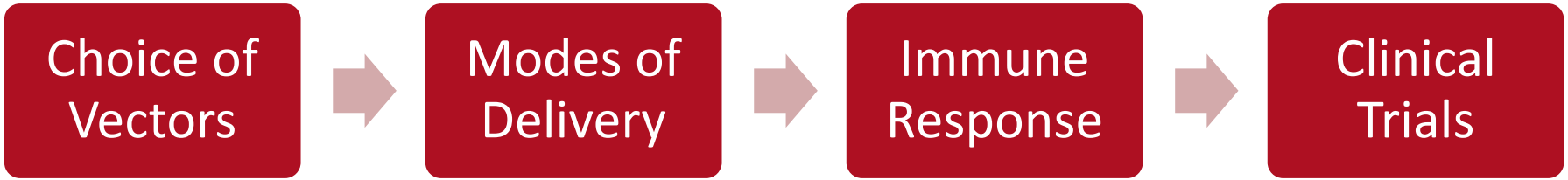
ESC
European Society of Cardiology

Disclosures

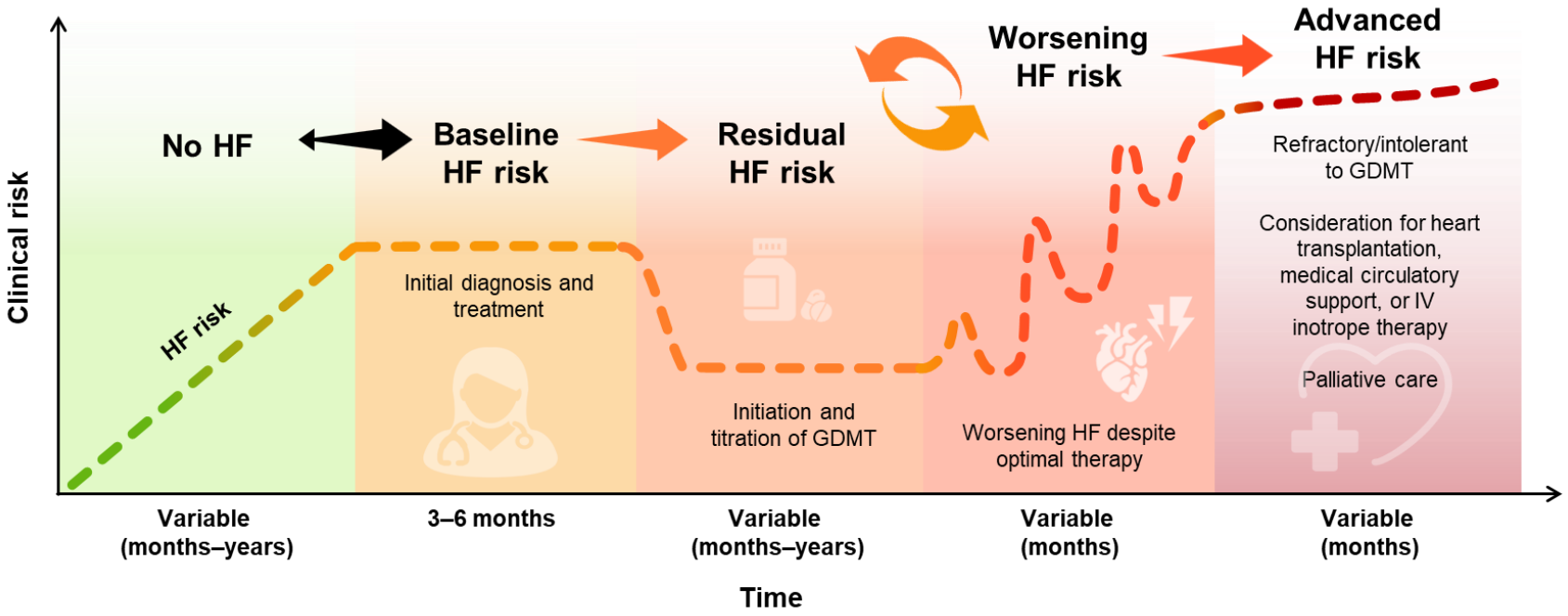
- **Co-founder: Nanocor/AskBio, Medera Biopharma, Ring Therapeutics, Sail Biomedicines, VJ Bio**
- **SAB/Consultant: RNATICs, Ampersand**

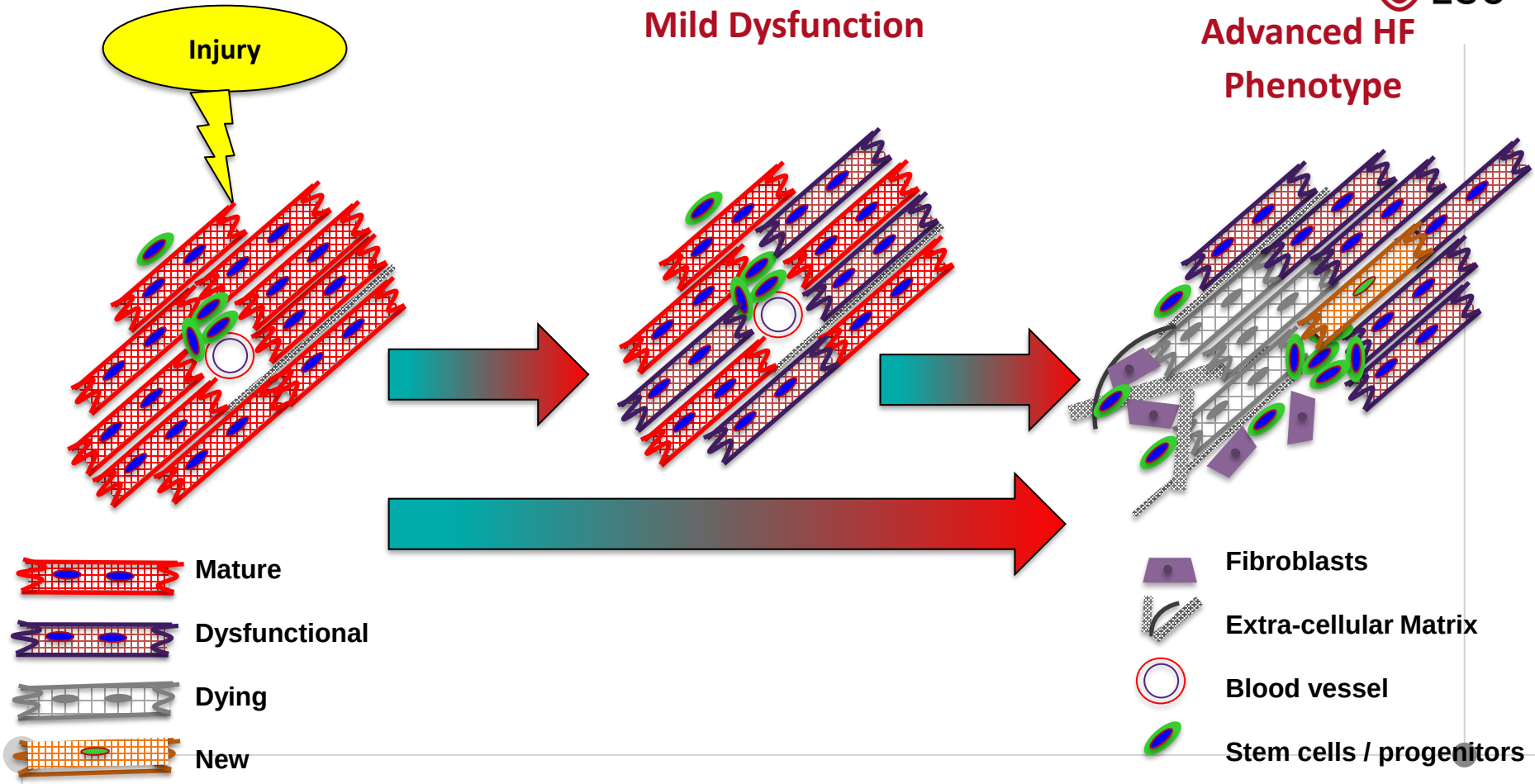
TARGETING CARDIAC DISEASES BY GENE THERAPY



Cardiomyopathies & Congestive heart failure in need of innovative therapies

Despite current standard of care, patients continue to progress to advanced HF




Injury
Mild Dysfunction
Advanced HF
Phenotype
Mature
Dysfunctional
Dying
New
Fibroblasts
Extra-cellular Matrix
Blood vessel
Stem cells / progenitors

Abnormal calcium cycling, Decreased SERCA2a and increased PP1 contribute to impaired cardiac contractility in failing hearts

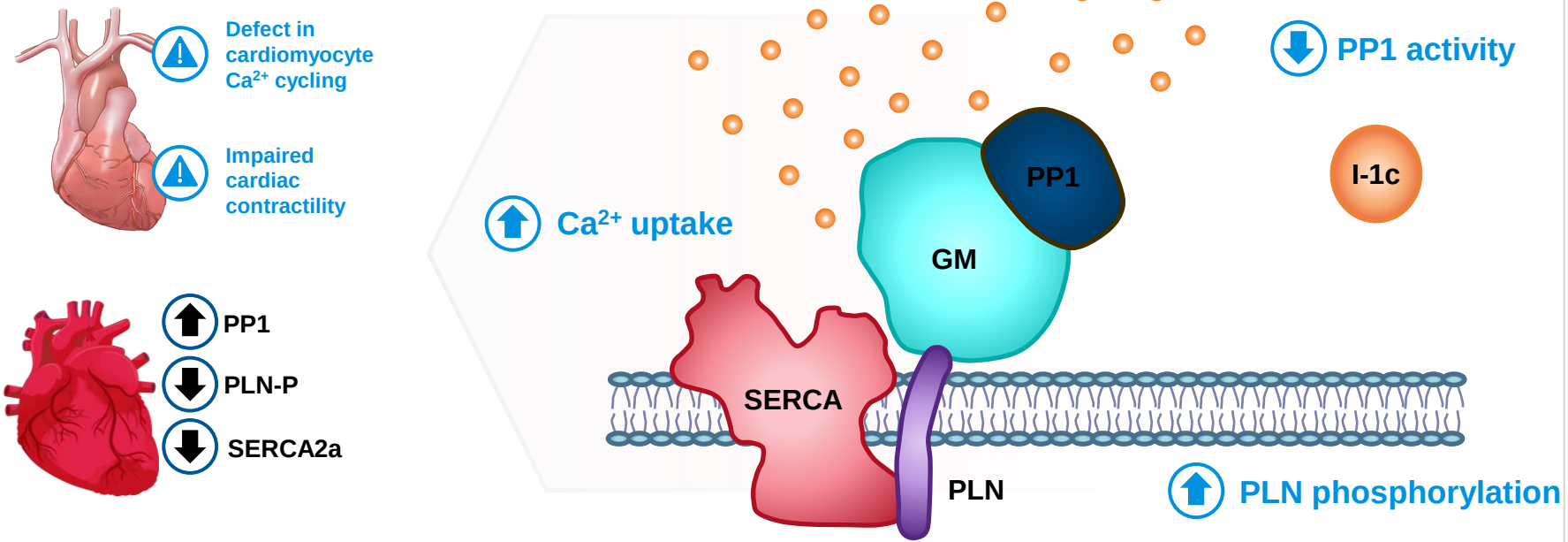
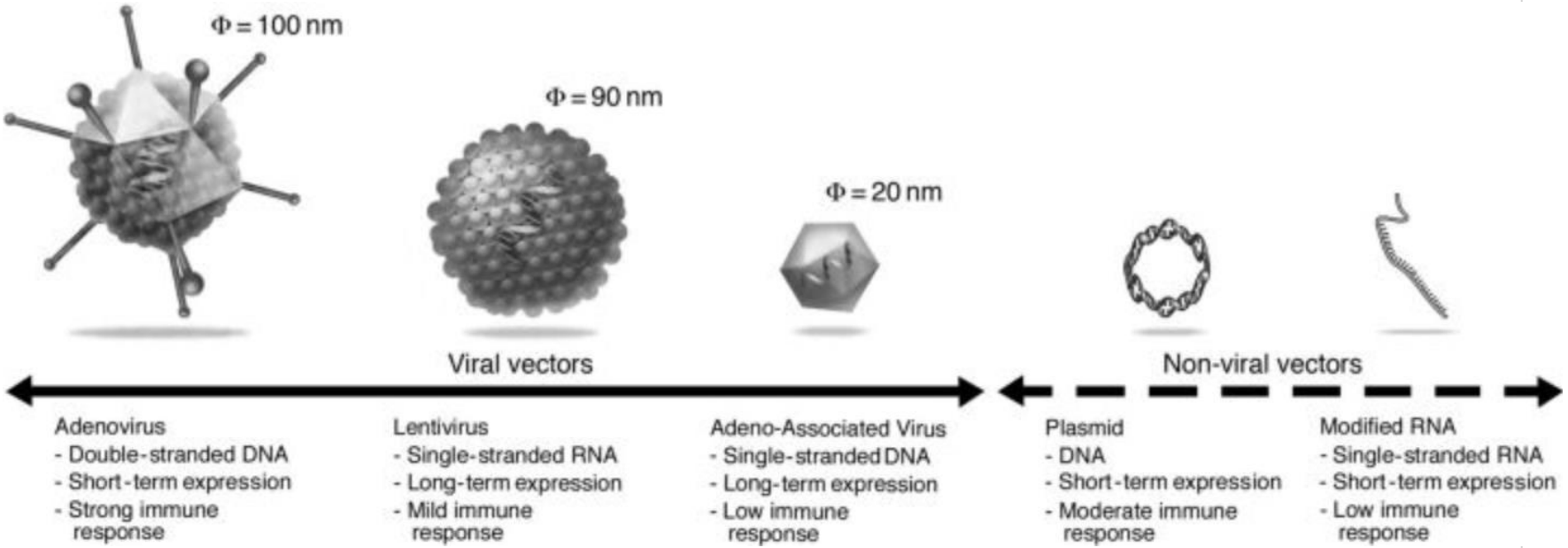


Figure adapted from Watanabe S, *et al. J Am Coll Cardiol* 2017;70:1744–1756

1. Watanabe S, *et al. J Am Coll Cardiol* 2017;70:1744–1756; 2. Marks AR. *J Clin Invest* 2013;123:46–52; 3. Nicolaou P & Kranias EG. *Front Biosci (Landmark Ed)* 2009;14:3571–3585

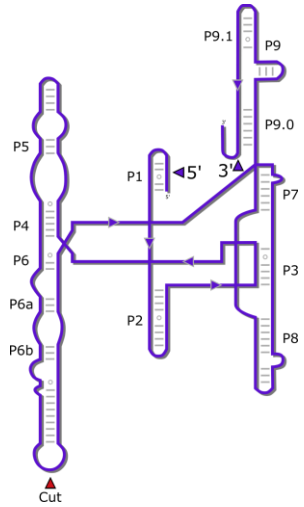
Vectors For Cardiovascular Applications

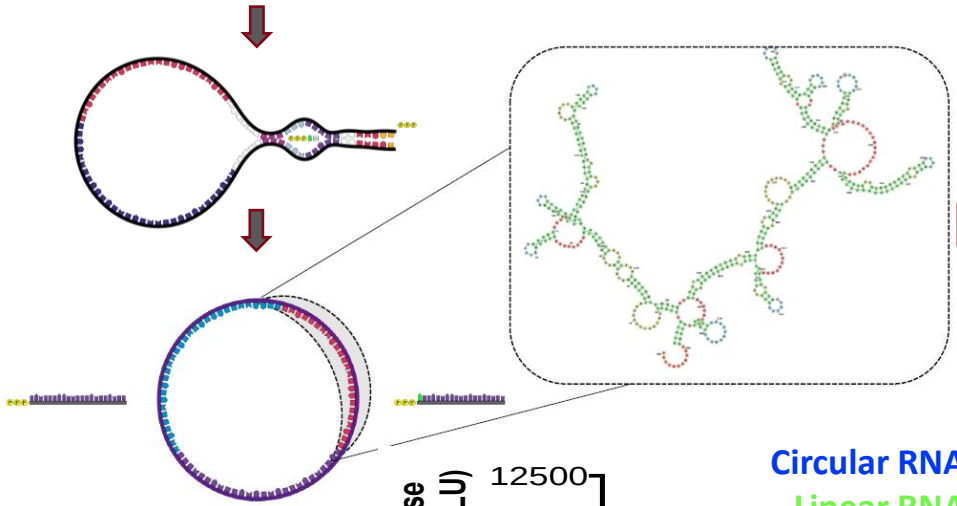


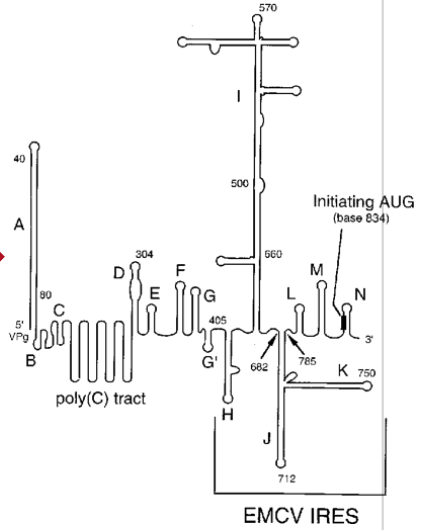
Hulot & Hajjar, EHJ 2016

Circular RNA

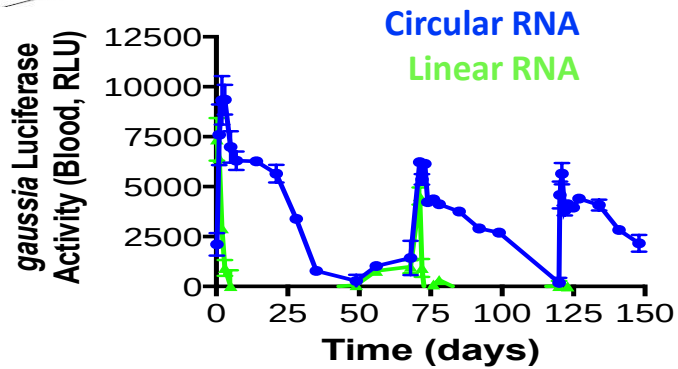
Translation is driven independently of a cap using a viral IRES



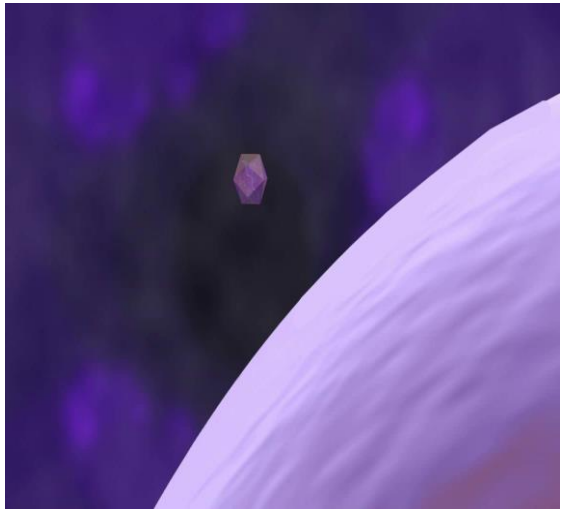




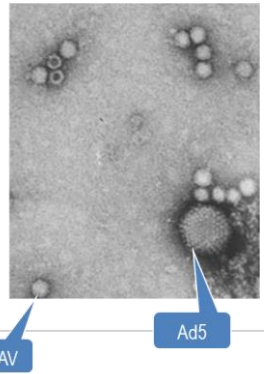
Circularization of a linear precursor is driven by an autocatalytic intron

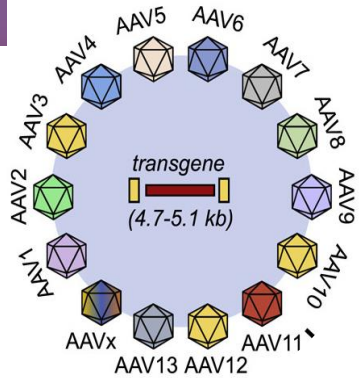


AAV Vectors



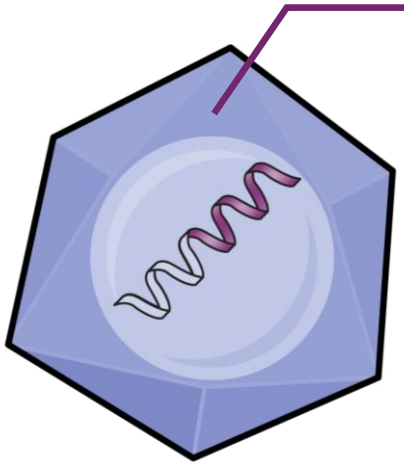
- Nonpathogenic
- Helper dependent parvovirus
- rAAV contains no viral genes
- 20 nm icosahedral particle
- Capsid composed of three related proteins
- Results in long-term expression
- Broad distribution to the myocardium
- Different serotypes have different tropisms

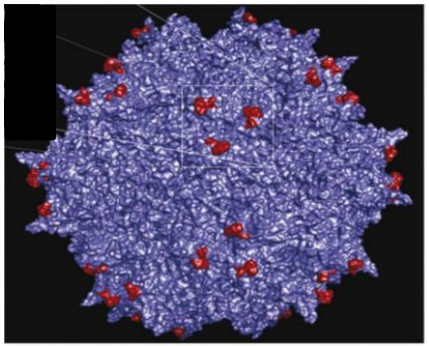




most commonly used serotype	Heart	Brain	Lung	Eye	Liver	Muscle	Pancreas
AAV1	*	*		*	***	***	*
AAV2		*		***	*		*
AAV5		*	***	*	*		
AAV6			**		*	*	
AAV7		*		*	**	***	
AAV8		**		***	***		*
AAV9	***	***	*	*	***	***	*

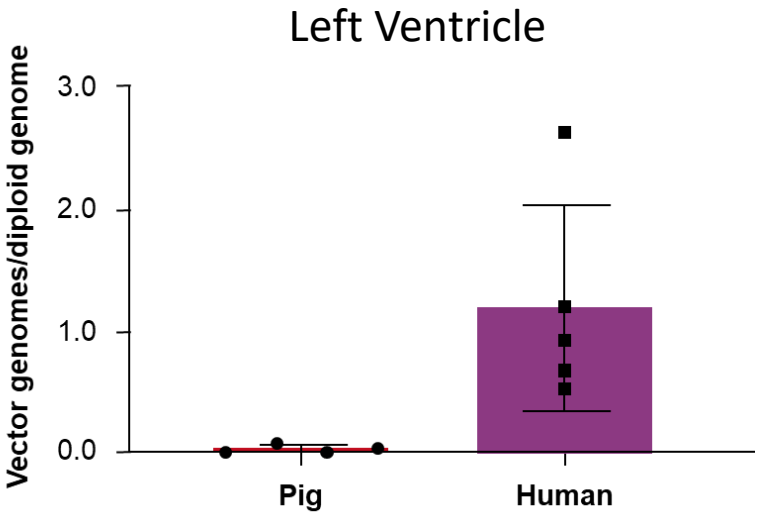
Novel AAV Capsid: High Cardiac Tropism





AAV2i8 VECTOR CAPSID

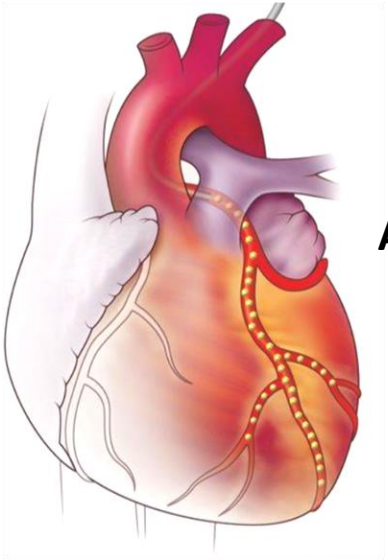
- Nonpathogenic, chimeric cardiotropic AAV2/AAV8 vector capsid
- Readily traverses the vasculature and selectively transduces cardiac muscle tissues with high efficiency in mice[2,3]
- Markedly reduced hepatic tropism[2]



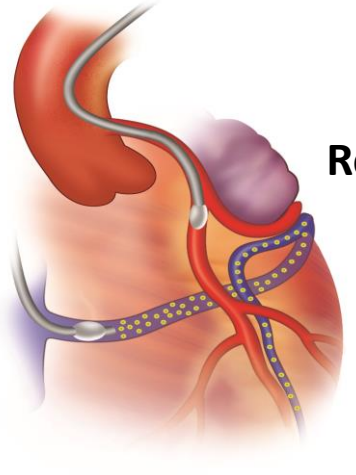
AAV2i8, adeno-associated virus AAV2/AAV8 vector capsid; CMV, cytomegalovirus; DNA, deoxyribose nucleic acid; I-1c, constitutively active inhibitor-1; EMA, European Medicines Agency; FDA, US Food and Drug Administration.

1. Pathak A, et al. *Circ Res*. 2005;96(7):756-766. 2. Asokan A, et al. *Nat Biotechnol*. 2010;28:79-82. 3. Ishikawa K, et al. *Circ Res*. 2018;123:601-613.

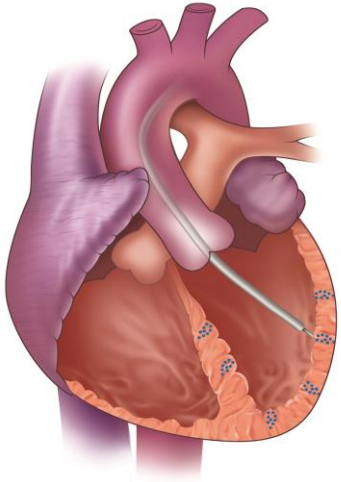
Delivery Methods



Antegrade

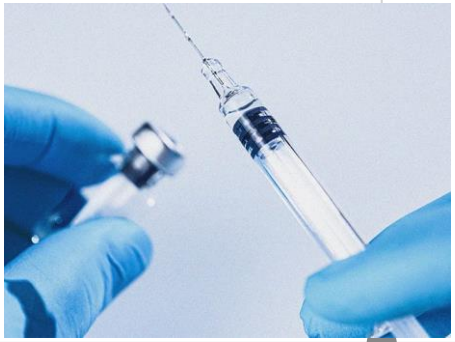


Retrograde



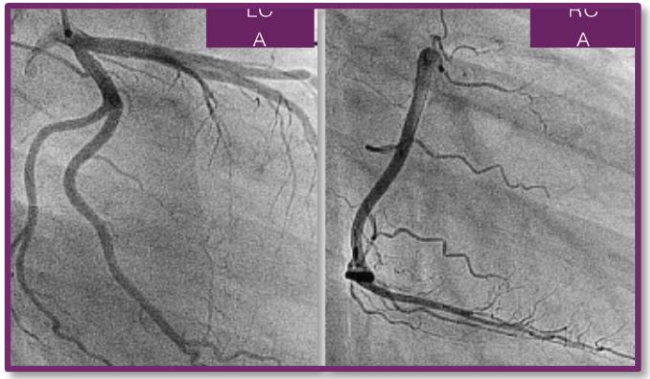
Intra-myocardial

Intravenous



Administration Via Percutaneous Intracoronary Artery Infusion

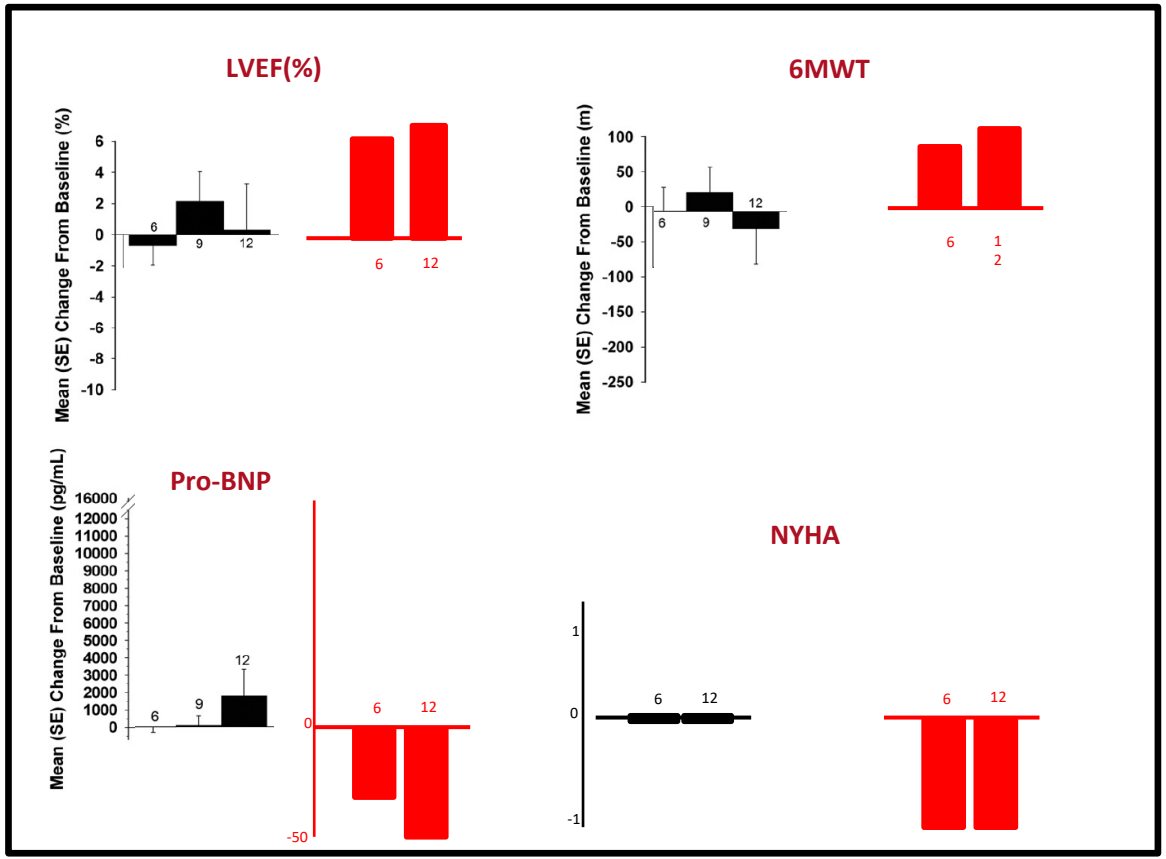
- Antegrade epicardial coronary artery infusion over 10 minutes
- 60 mL divided infusions based on dominance and coronary artery anatomy
- Delivered via commercially available angiographic injection system and guide or diagnostic catheters



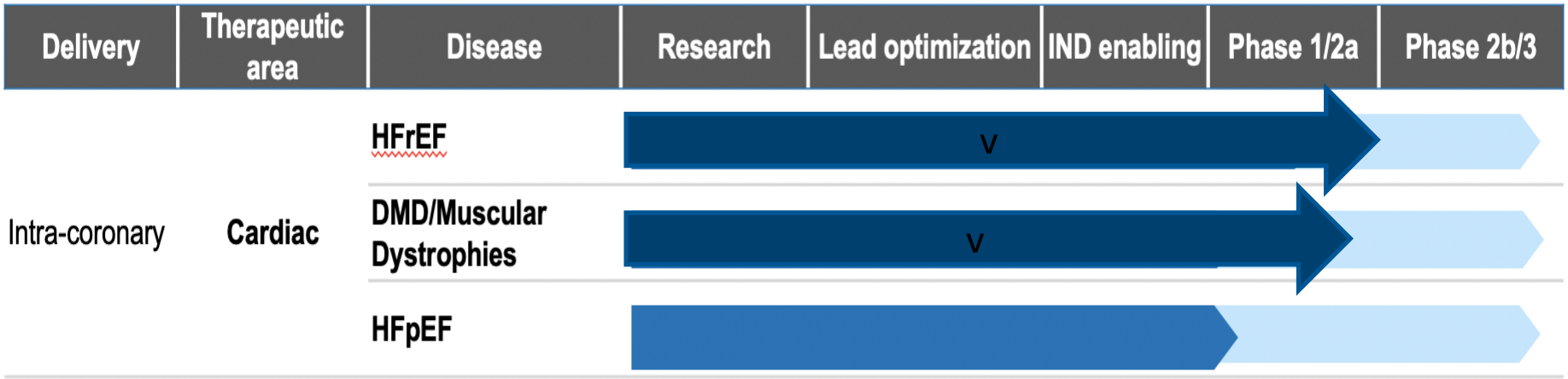
Significant Clinical Efficacy of AAV.SERCA2a in HF patients with Class III/IV & LVEF<35%: Effects of Dose Escalation

AAV1.SERCA2a

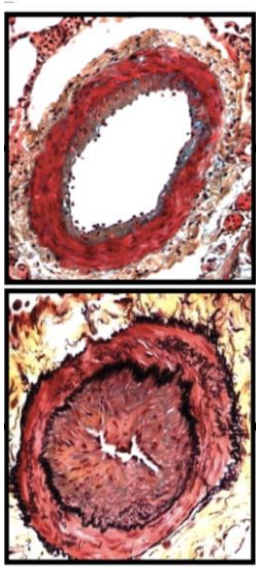
- 1x10^{13} vg/patient
- 3x10^{13} vg/patient

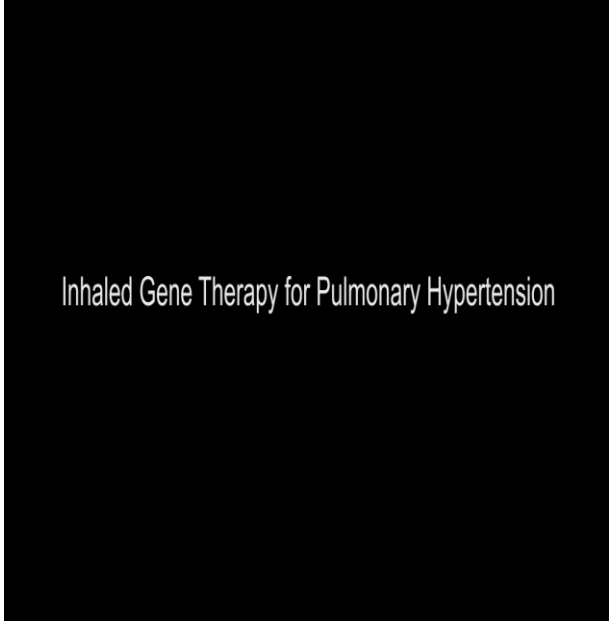


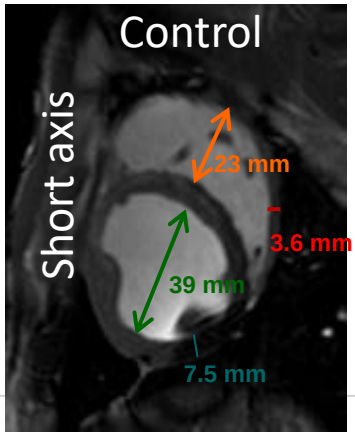
Three Ongoing Trials: Dose Escalation AAV1.SERCA2a

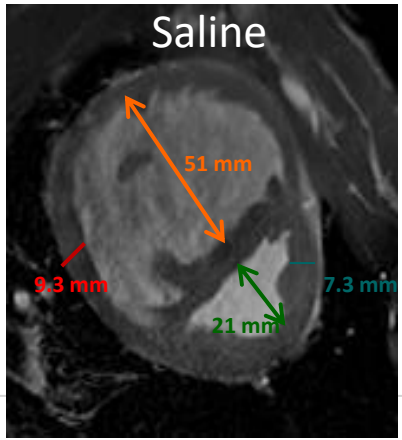


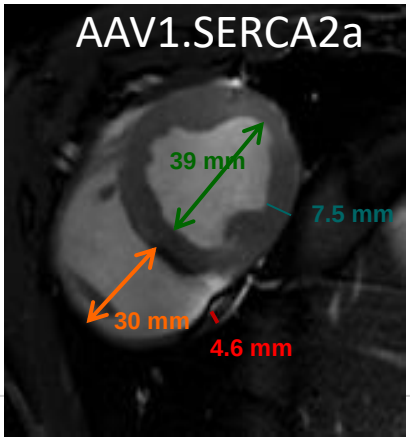
Gene Therapy for Pulmonary Hypertension



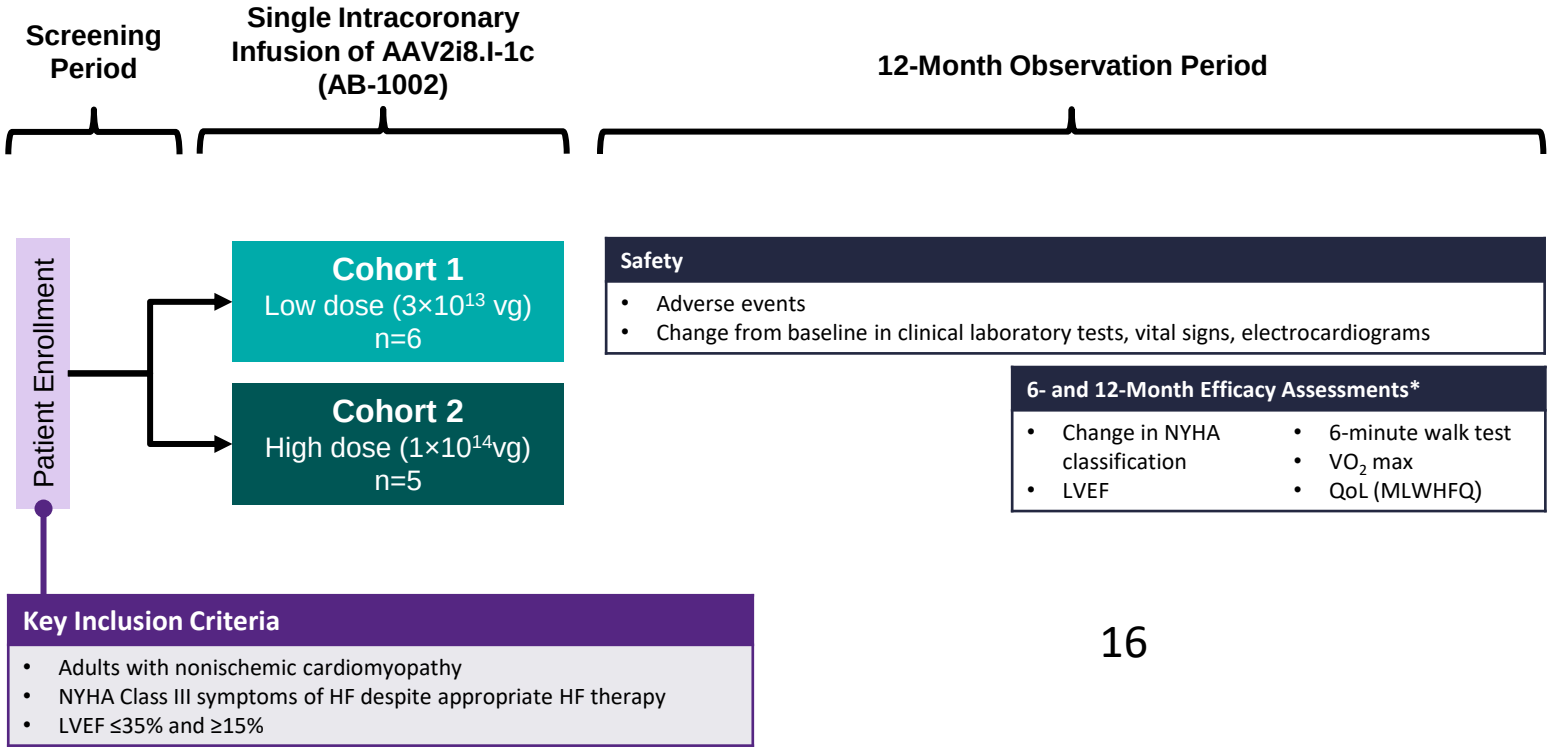








Phase 1 Clinical Gene Therapy Trial for the Treatment of Heart Failure Using AAV2i8 (NCT04179643)



*This phase 1 study was not powered to detect statistical significance for changes in these efficacy assessments. In Cohort 1, Patient 2 can be treated 30 days after Patient 1, and Patient 3 can be treated 14 days after Patient 2. In Cohort 2, patients are treated sequentially after 14 days. HF, heart failure; LVEF, left ventricular ejection fraction; MLHFQ, Minnesota Living With Heart Failure® Questionnaire; NYHA, New York Heart Association; QoL, quality of life; vg, viral genomes; VO_2 max, maximal myocardial oxygen consumption. Alvisi M, et al. Poster presented at: European Society of Gene & Cell Therapy 29th Annual Congress; October 11-14, 2022; Edinburgh, Scotland. Abstract P670.

Single-Dose Administration of AAV2i8.I-1c resulted in clinically meaningful improvements in Key efficacy parameters

		NYHA FC			LVEF			MLHFQ			VO_2 max			6MWT		
	Patient	BL	6 Mo	12 Mo	BL	6 Mo	12 Mo	BL	6 Mo	12 Mo	BL	6 Mo	12 Mo	BL	6 Mo	12 Mo
Cohort 1 Low dose	1	3	2	2	37.5	39.0	48.0	80.0	38.0	47.0	12.3	13.2	–	314.0	365.8	–
	2	3	1	1	29.0	44.5	51.0	–	–	–	14.3	14.0	15.8	381.0	321.5	419.0
	3	3	2	2	34.0	41.0	40.0	51.0	48.0	39.0	7.9	7.0	13.0	132.5	219.5	260.0
Cohort 2 High dose	4	3	NA	NA	18.0	NA	NA	43.0	NA	NA	19.8	NA	NA	454.0	NA	NA
	5	3	2	2	21.0	28.2	28.0	16.0	6.0	11.0	15.8	15.2	13.4	362.7	416.1	406.2
	6	3	2	2	19.0	35.0	35.0	79.0	46.0	26.0	25.1	20.5	20.6	438.9	457.2	466.3
	7	3	3	3	20.5	–	29.0	64.0	26.0	49.0	16.2	–	14.5	384.0	–	371.8
	8	3	3	3	25.0	31.0	42.0	88.0	85.0	93.0	18.0	19.1	19.6	325.0	326.1	255.0

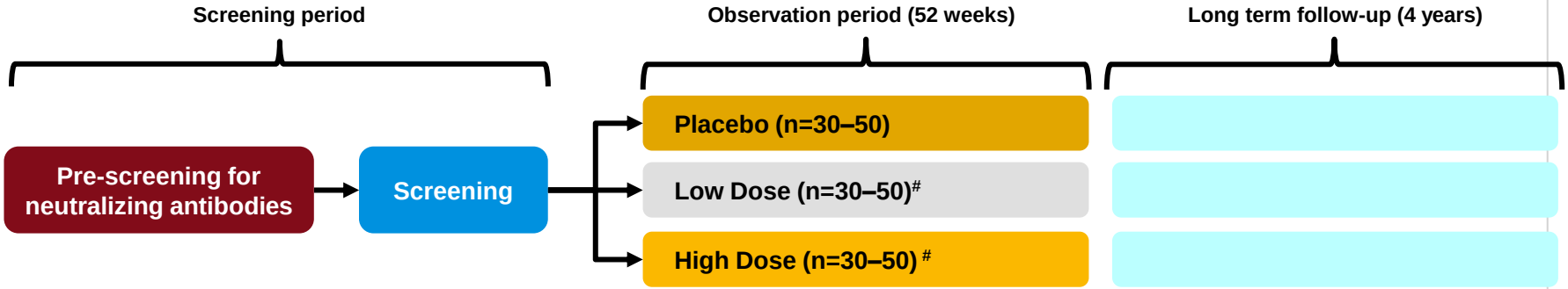
Clinically meaningful changes from baseline*

Worsening | Stabilization | Improvement

Henry et al, 2023, AHA

First Patient has been enrolled in GenePHIT is a phase II, adaptive, randomized, double-blind, placebo-controlled, multicenter trial

Objective: To explore the efficacy and safety of AAV2i8.I-1c vs placebo in patients with nonischemic cardiomyopathy and NYHA class III heart failure



Primary endpoint:

Hierarchical evaluation

1. CV death
2. NYHA functional class change from baseline
3. LVEF change from baseline
4. VO2 max change from baseline
5. 6MWT change from baseline

Key secondary endpoints:

- Functional status (NYHA classification, LVEF, VO2 max, 6MWT) and hospitalizations (number of and time to first admission)
- Echocardiographic assessments and NT-proBNP
- QoL assessments
- Incidence of cardiac transplantation
- Incidence of LVAD implantation
- Number of HF hospitalizations
- All-cause mortality

Pathway Cardiac Diseases Leveraging AAV or Adenoviral Vectors

Disease	Phenotype	Overall Prevalence	Target Gene	AAV	Mode of Delivery	Clinical Trial
HFrEF (non-Ischemic)	NYHA Class III, LVEF<35%	~0.4 M US ~0.4 M EU	I-1c	AAV2.8	Intracoronary	Dose escalation Phase 1b trial completed Phase 2 trial started
HFrEF (ischemic & non-ischemic	NYHA Class III & IV, LVEF<35%	~0.6 M US ~0.6 M EU	SERCA2a	AAV1	Intracoronary	Dose Escalation Phase 1b Trial
HFpEF	LV>40%, PCWP>25 mmHg	~0.8M in US ~0.8M in EU	SERCA2a	AAV1	Intracoronary	Dose escalation Phase 1b Trial
Chronic Ischemic heart disease	Chronic angina	~1M in US ~1M in EU	VEGF	Adenovirus	Surgical direct Intramyocardial	Dose escalation Phase 1 Trial completed

Hereditary Cardiac Indications Leveraging AAV Vectors

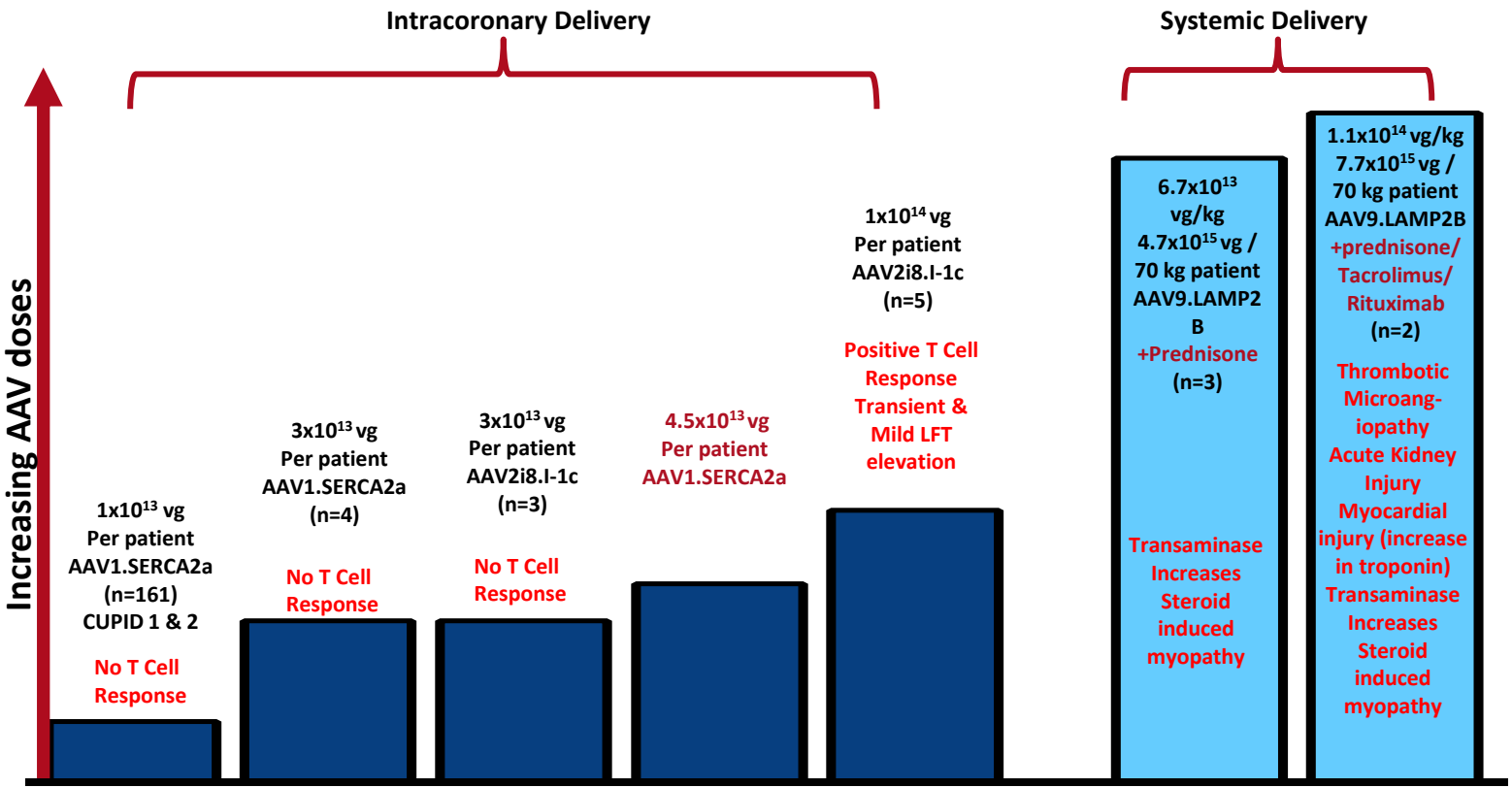
ESC

Disease	Phenotype	Overall Prevalence	Causative Gene	AAV	Mode of Delivery	Clinical Trial
Danon	X-linked recessive Hypertrophic cardiomyopathy	15-30k (US+EU)	LAMP2	AAV9	Intravenous	Phase 1 completed with two high doses in adult and pediatric patients Pivotal Phase 2 to start
Genetic Hypertrophic Cardiomyopathy	Severe Hypertrophy of the walls of the ventricles	>500k (US, total HCM)	MYBPC3	AAV9	Intravenous	Phase 1 Trial intravenous. High dose
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	Arrhythmias and right ventricular dyskinesis. Majority due to PKP2 mutations	~100K in US	PKP2	AAVrh10 AAVrh74	Intravenous	Dose escalation Phase 1 Trial intravenous. High dose
Freidreich's Ataxia	Autosomal Recessive neurological & muscular Disorder affecting the heart	~6,000 in US	FXN	AAVrh10	Intravenous	Dose escalation Phase 1 Trial intravenous. High dose
Duchenne Cardiomyopathy	Autosomal Dominant, LVEF<40%	~25,000 in US	SERCA2a	AAV1	Intracoronary	Dose escalation Phase 1 Trial

AAV Related Toxicities

TOXICITY	Adverse Events	AAV serotype	Mode of Delivery
Hepatotoxicity	Elevated Liver Function Tests	All serotypes	Systemic, Intracoronary
	Serious Liver Injury	AAV8, AAV9	Systemic
	Liver Failure	AAV8, AAV9	Systemic
Thrombotic Microangiopathy	Thrombocytopenia	AAV9	Systemic
	Hemolytic Anemia	AAV9	Systemic
	Acute Kidney Injury	AAV9	Systemic
	Complement Activation	AAV9	Systemic
	Thrombotic Microangiopathy	AAV9	Systemic
Neurotoxicity	Dorsal Root ganglia Neuronal Loss	AAV9	Intrathecal
	Procedure related Neuronal Injury	AAV2	Intracranial
	Abnormal T2 hypersensitivities on MRI	AAV2, AAV9	Intracranial

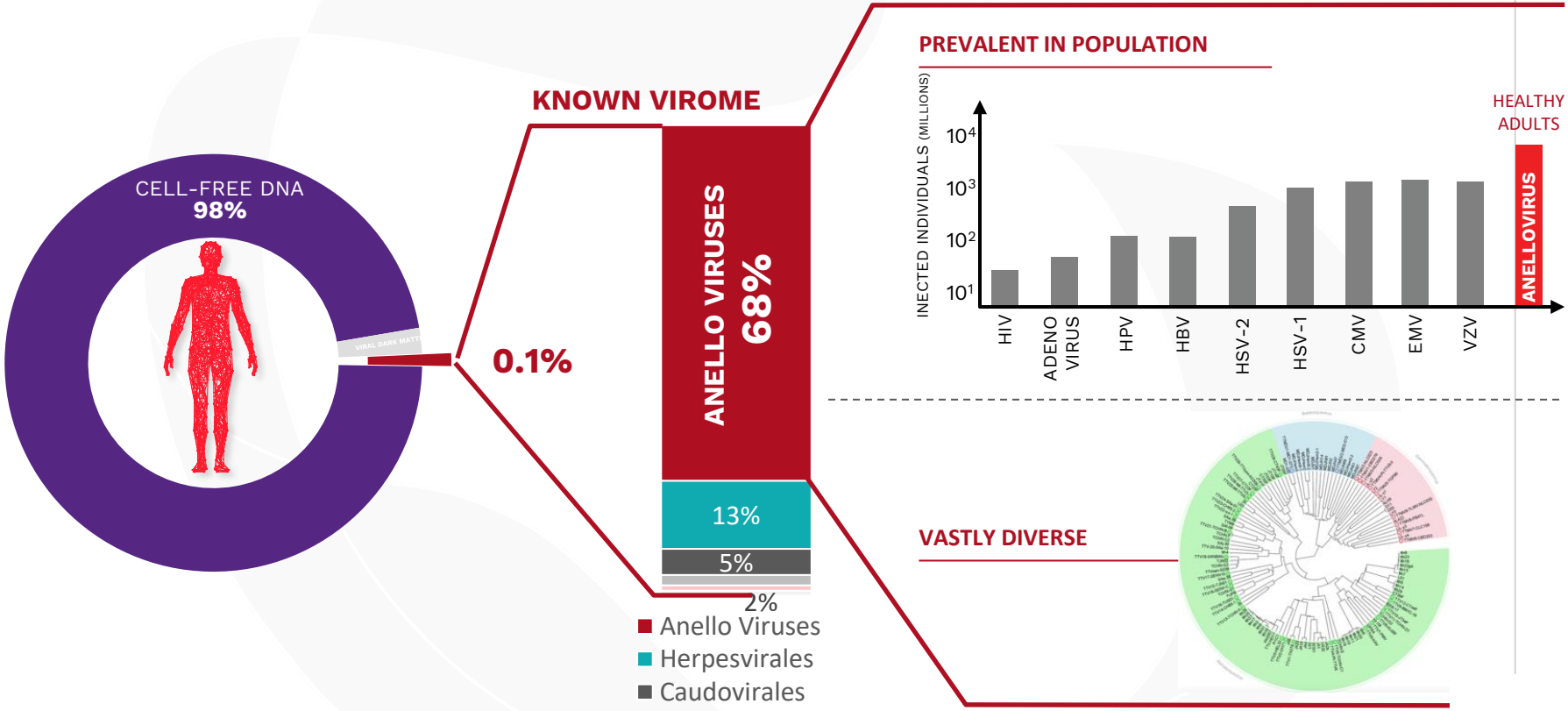
Delivery of AAV and T Cell Response



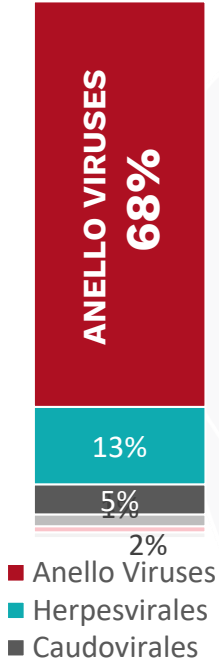
NEXT Generation vectors

- Capsids with high cardiac tropism
- De-targeting the liver
- Resistant to antecedent neutralizing antibodies
- Novel vector systems

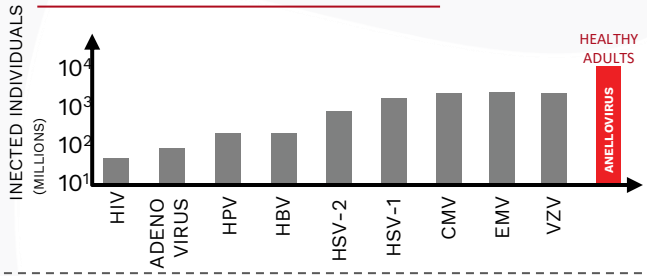
Anelloviruses constitute a key component of the human virome



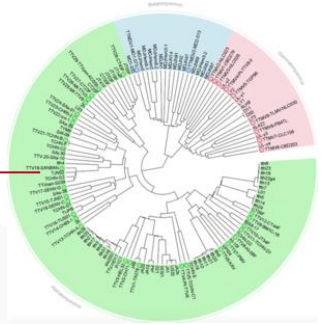
Anelloviruses constitute a key component of the human virome



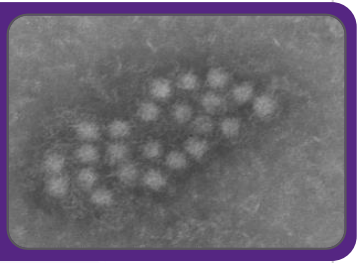
PREVALENT IN POPULATION



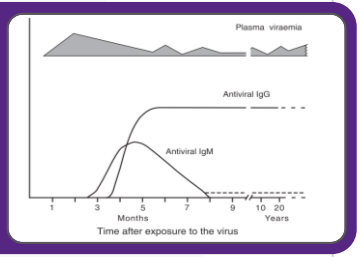
VASTLY DIVERSE



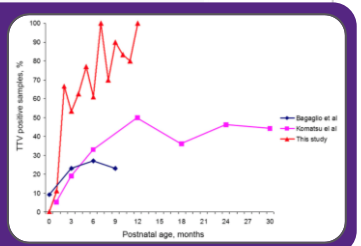
Ubiquitous[1]



Immune responses to anelloviruses are slow and fail to clear the virus

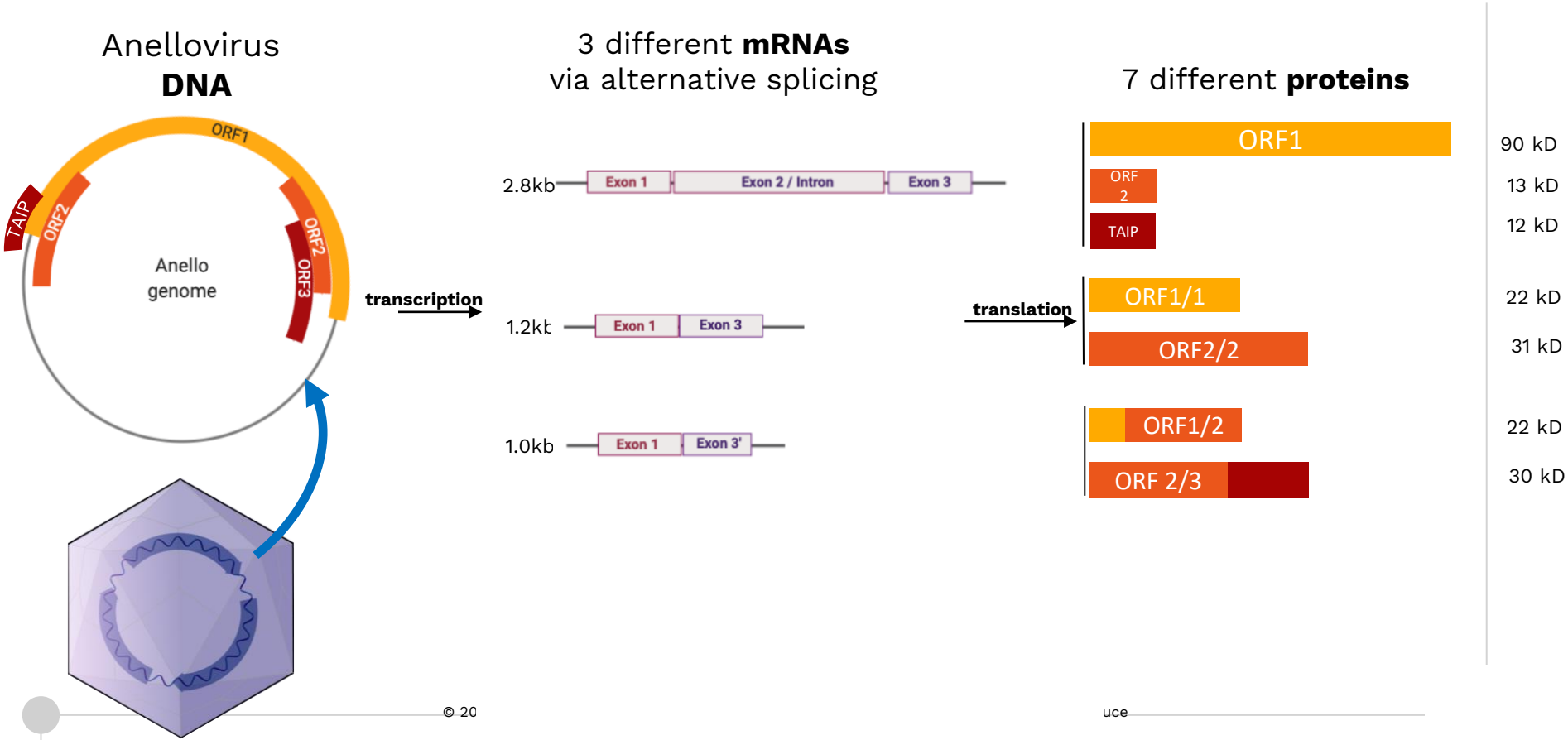


Acquired by everyone in infancy[2]



1. Itoh et al., Biochemical and Biophysical Research Communications 279, 718–724 (2000)
2. Tyschik et al. Virol J 2018 May 30;15(1):96.
3. Rezahosseini et al Transplantation Reviews; Volume 33, Issue 3, July 2019, Pages 137-144
4. Maggi and M. Bendinelli, 2009 TT Viruses: The Still Elusive Human Pathogens, © Springer Verlag Berlin Heidelberg 2009

Anatomy of the anellovirus genome



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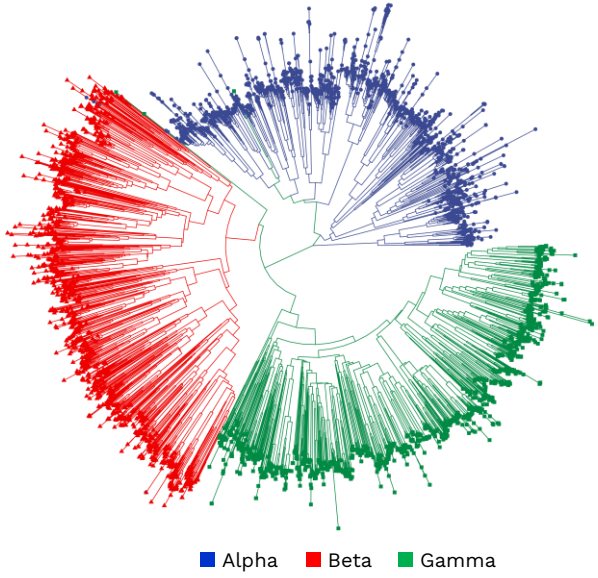
Discovery of anelloviruses with unprecedented number & diversity

Explosion of the ANV catalogue

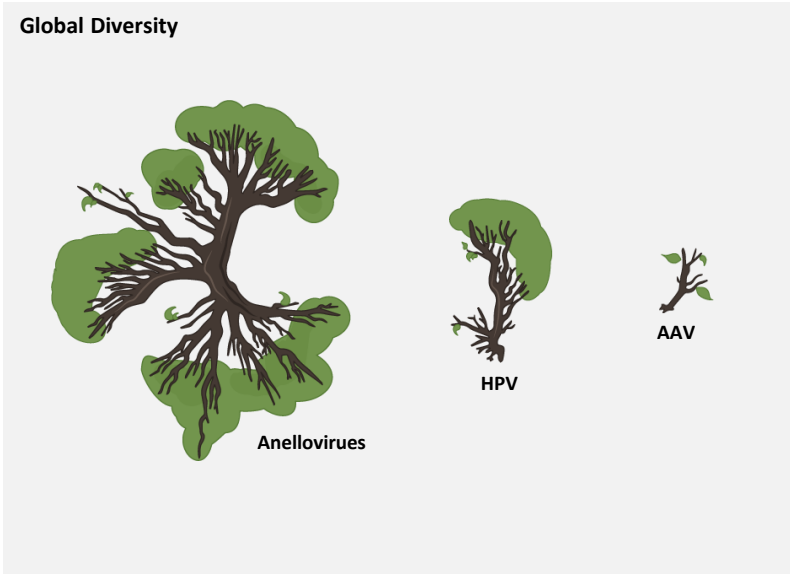
>6,000 Anellovirus ORF1 identified across all tissues



▲ Phylogentic analysis of Anellovirus catalogue late 2018 to early 2022.

Unprecedented diversity

3-4x more diverse than other representative viruses

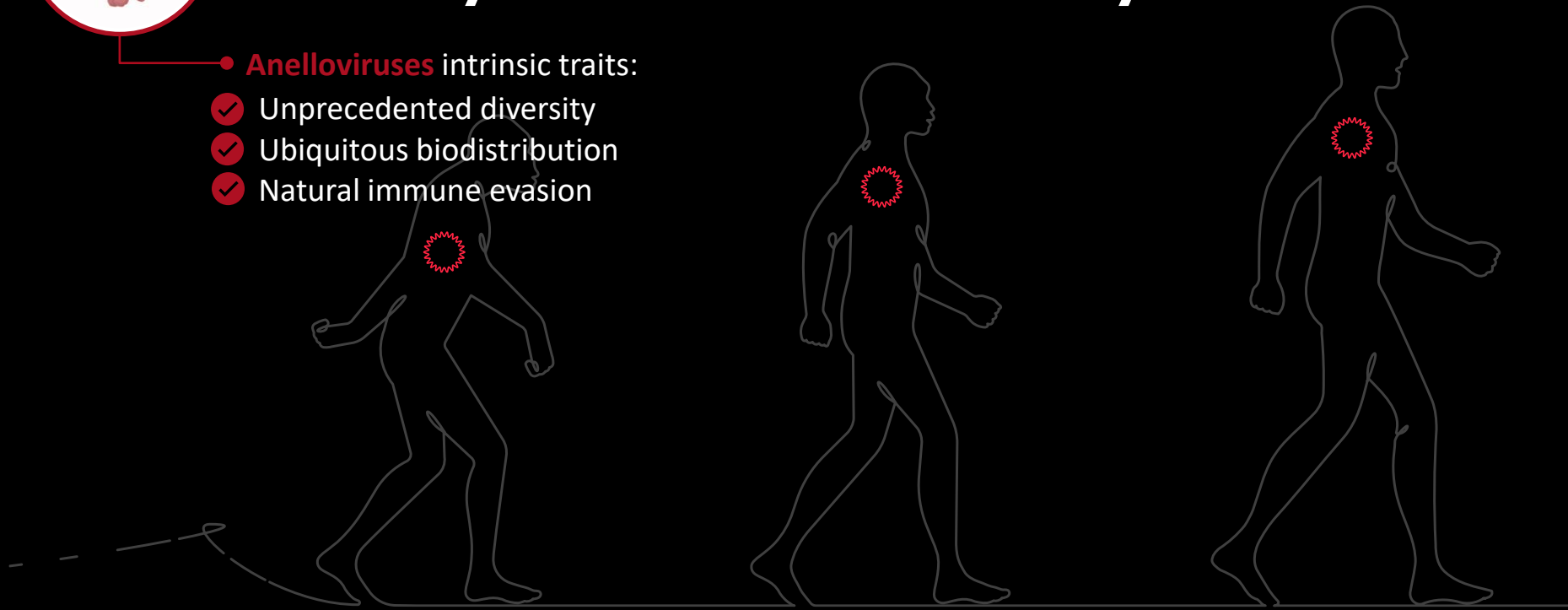


Abbreviations: AAV, adeno-associated virus; ANV, anellovirus; HPV, human papillomavirus; MDS, multidimensional scaling; ORF, open reading frame.

Anelloviruses have evolved and lived in harmony with us for millions[1] of years.

Anelloviruses intrinsic traits:

- Unprecedented diversity
- Ubiquitous biodistribution
- Natural immune evasion



1. Kaczorowska and van der Hoek, *FEMS Microbiology Reviews*, 2020.

Anellovector Platform

AnelloScope

Anellovirus discovery engine to mine sequences from human tissues

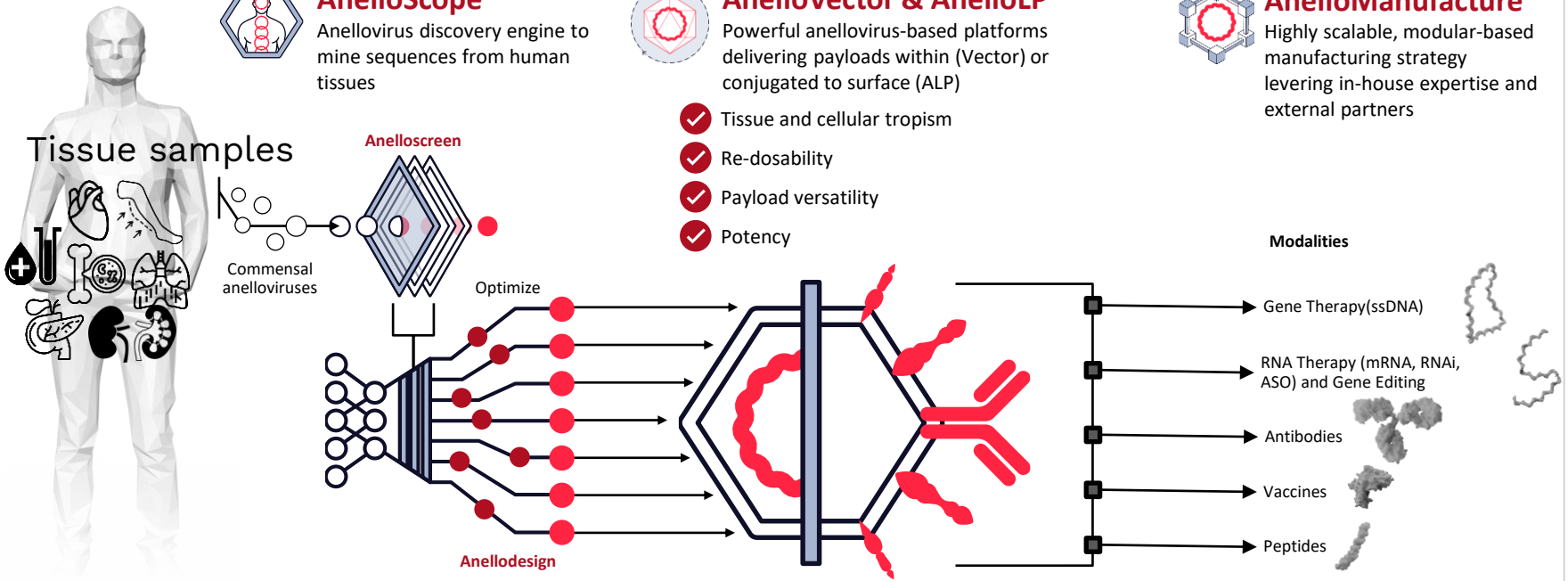
AnelloVector & AnelloLP

Powerful anellovirus-based platforms delivering payloads within (Vector) or conjugated to surface (ALP)

- Tissue and cellular tropism
- Re-dosability
- Payload versatility
- Potency

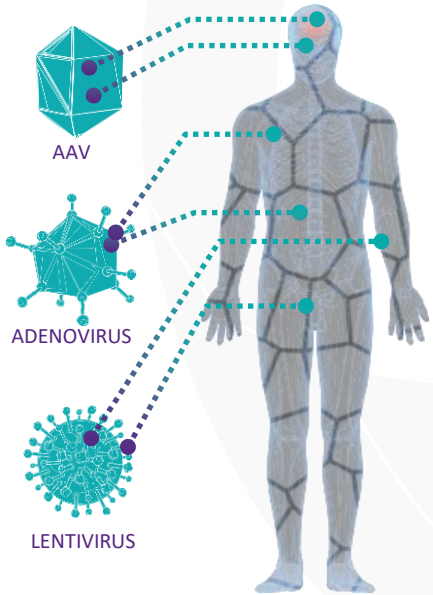
AnelloManufacture

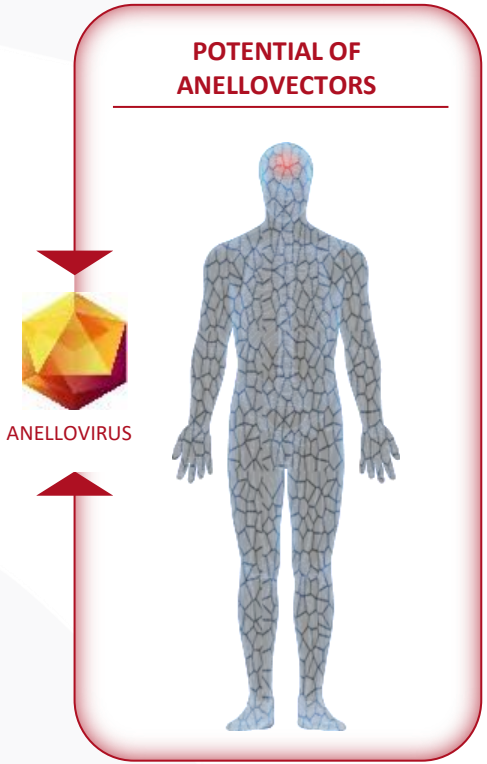
Highly scalable, modular-based manufacturing strategy levering in-house expertise and external partners



Abbreviations: ANV, anellovirus (commensal virus), ALP, anello-like particle

Gene Therapy: Promise of New Vector Platform





POTENCY
Lower doses, less toxicity

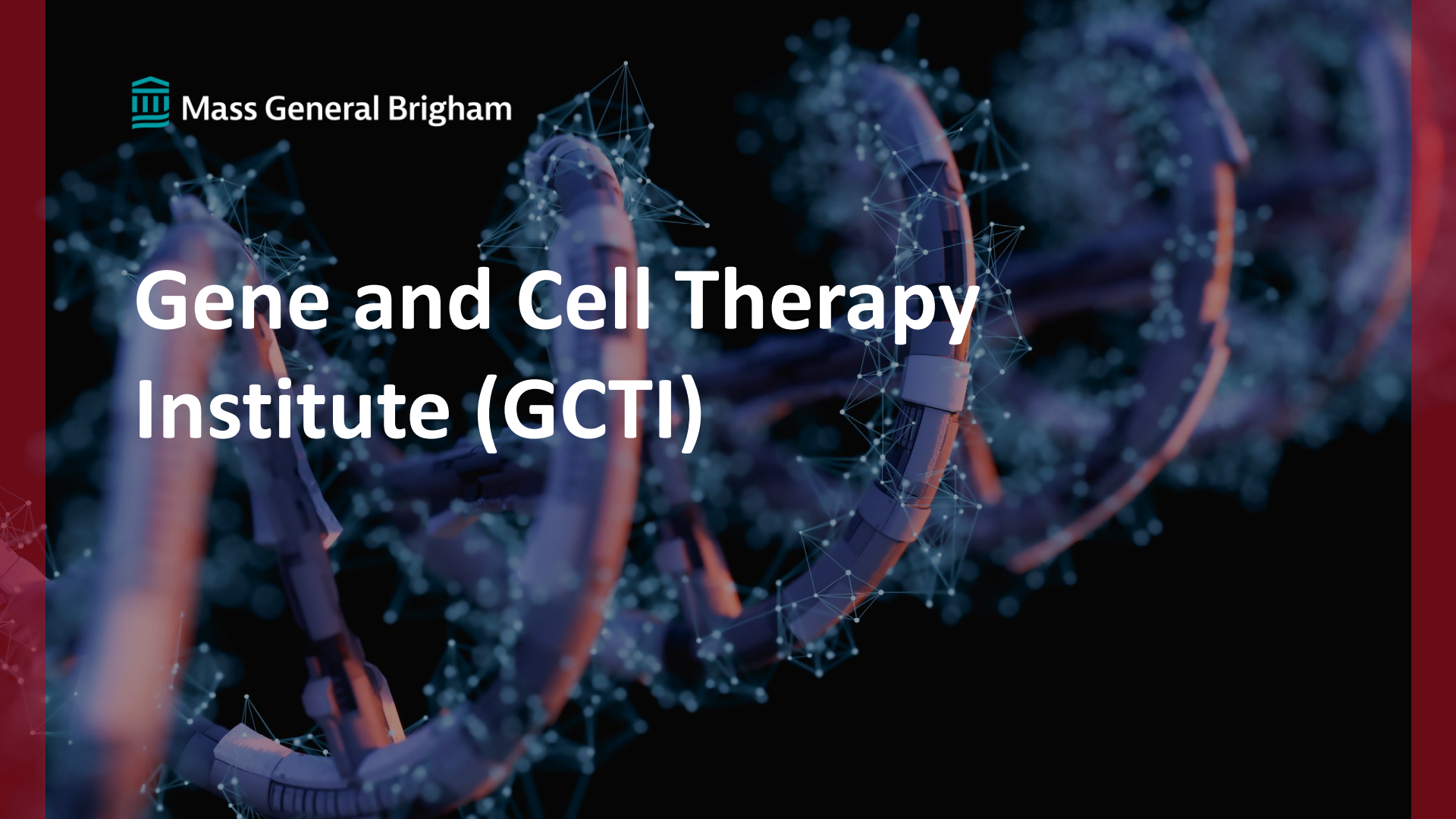
PRODUCTION
Lower doses require lower amounts

TROPISM
All organs in body

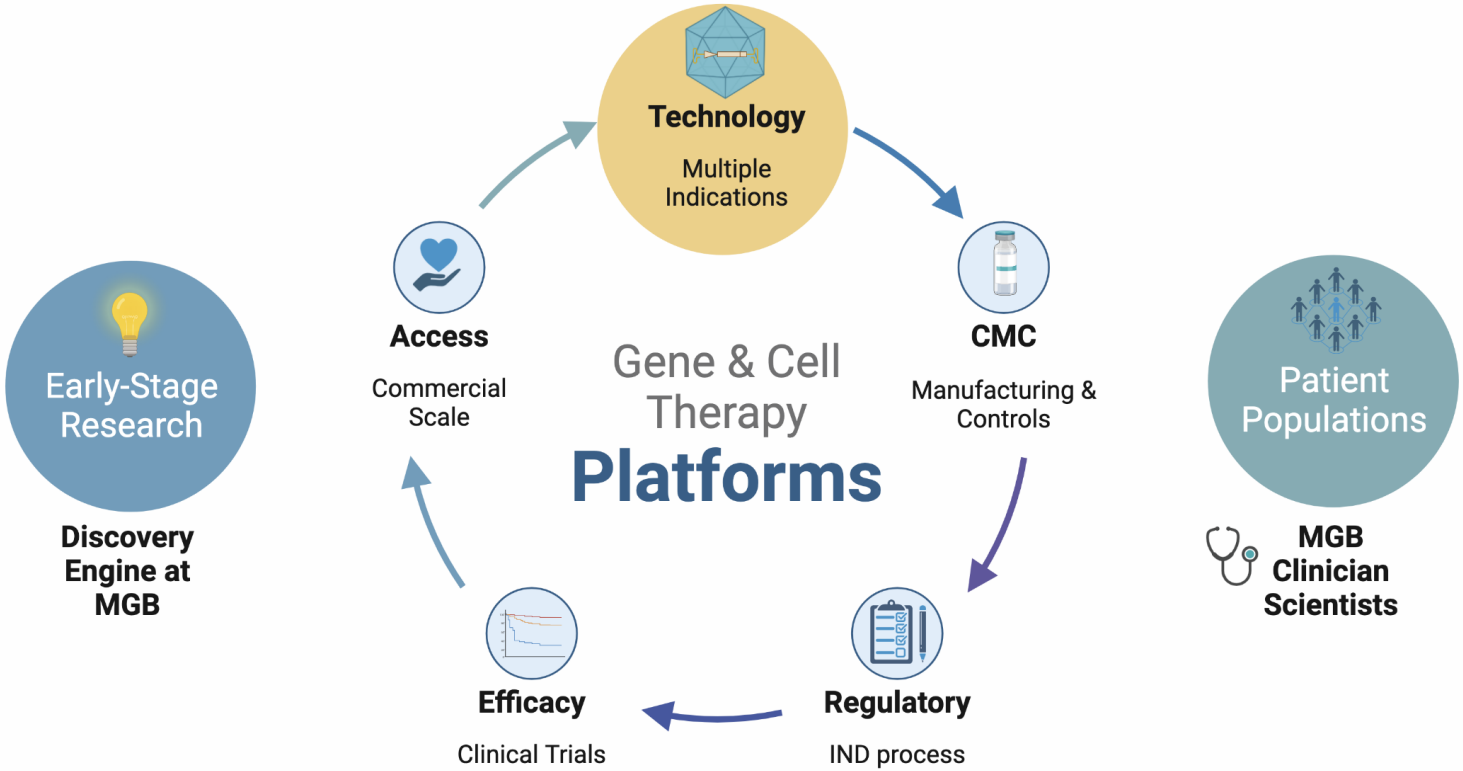
EPISOMAL
No insertional mutagenesis

IMMUNE PROFILE
No evidence of neutralizing antibodies against anelloviruses

RE-DOSING
Repeat dosing


Mass General Brigham
Gene and Cell Therapy Institute (GCTI)

Academic Centers accelerators for GCT Programs



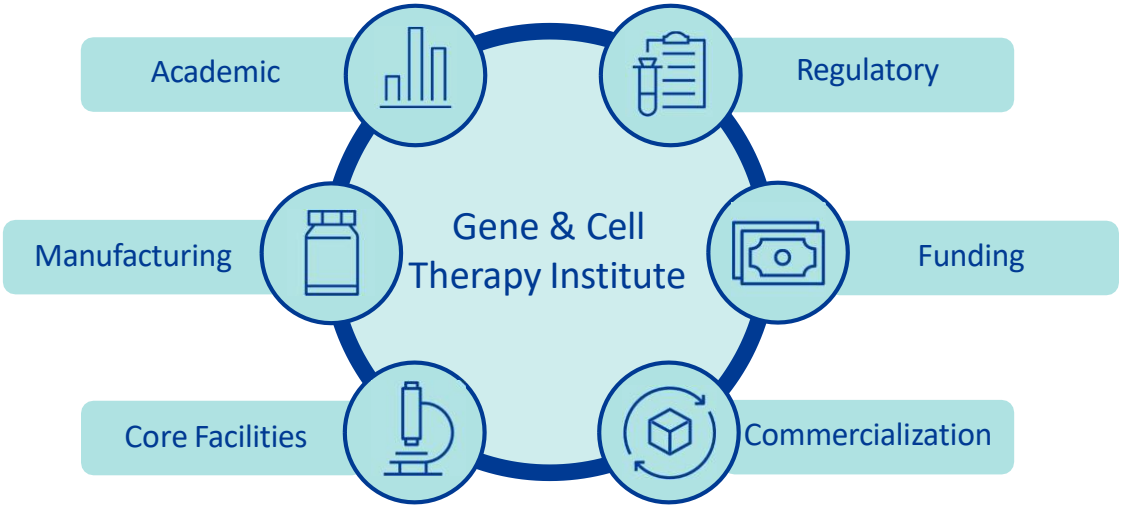
Why Academic Medical Institutes?

GCT is transforming the treatment paradigm

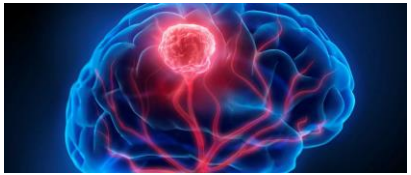
Treatment Approach	Potentially **curative or preventative**, **one-time treatment** that targets underlying genetic causes of disease
Discovery and Development	2-4x faster than traditional pharma, **3-5 years of clinical trials** on small, disease-based population Academic medical center-led, with close **physician-researcher-patient** collaborations
Clinical Trial Success Rates	GCT **success rates are typically higher** than traditional pharma due to higher target specificity **Earlier detectable viability** lowers investment risk and increases cost efficiency

Launched in 2022, MGB's Gene & Cell Therapy Institute has grown into a hub of innovation and collaboration

Integrated Team and Established Structure



Promising Areas of Focus



Novel glioblastoma gene therapy first-in-human trial

Pioneering Glaucoma cell replacement therapy strategy

Thank you

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