Screening & Treatment of Pompe Disease: Clinical Advances in a Changing Landscape

Tahseen Mozaffar, MD, FAAN

Supported by an educational grant from Spark Therapeutics, Inc.

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1

Titles & Affiliations

Tahseen Mozaffar, MD, FAAN
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2

Disclosure

- Consulting Fee (eg, Advisory Board): Abbvie, Alexion, Amicus, Argenx, Arvinas, Audentes, AvroBio, Maze Therapeutics, ML Bio, Momenta, Ra Pharmaceuticals, Sanofi-Genzyme, Spark Therapeutics, UCB, Zolgenix
- Speaker Bureau: Sanofi-Genzyme
- Medical Advisory Board: Myositis Association, Neuromuscular Disease Foundation, Myasthenia Gravis Foundation of California, Myasthenia Gravis Foundation of America
- Research Funding: Myositis Association, Muscular Dystrophy Association, National Institutes for Health; Alexion, Amicus, Argenx, Audentes, Bristol-Myers-Squib, Cartesian Therapeutics, Momenta, Ra Pharmaceuticals, Sanofi-Genzyme, Spark Therapeutics, UCB, Ultragenyx
- Serves on data safety monitoring board for Acceleron, Avexis, Sarepta, NIH

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3

Learning Objectives

- Describe the pathogenesis and diagnostic algorithm of Pompe disease
- Summarize the impact of Pompe disease on quality of life, and the unmet need for patients
- Outline the progressive nature of Pompe disease and the importance of early initiation of treatment
- Review current treatment options for Pompe disease
- Discuss emerging therapies and the changing treatment landscape regarding Pompe disease

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4

Joannes Cassianus Pompe (1901-1945)

Over idiopathische hypertrophie van het hart.

CARDIOMEGALIA GLYCOGENICA

ACADEMISCH PROEFSCHRIFT TER VERKRIJGING VAN DEN GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE UNIVERSITEIT VAN AMSTERDAM, OP GEZAG VAN DEN RECTOR-MAGNIFICUS, Dr. W. F. C. ZEEMAN, HOOGLERAAR IN DE FACULTEIT DER GENEESKUNDE, IN HET OPENBAAR TE VERDEDIGEN IN DE AULA DER UNIVERSITEIT, OP VRIJDAG 13 MEI 1938, DES NAMIDDAGS TE 2½ UUR.

DOOR

JOANNES CASSIANUS POMPE

- Studied medication at Utrecht, Netherlands
- Described first case of infantile Pompe disease
- On December 27, 1930, Dr Pompe carried out a postmortem on a 7-month-old girl who died of pneumonia
- He found the enlarged heart, now known to be characteristic of infantile form of the disease and demonstrated glycogen deposition

Pompe J-C. *Ned Tijdscr Geneeskd*. 1932;76:304.

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5

Pompe Disease

- Synonyms:
  - Glycogen storage disease type II (GSD II)
  - Acid maltase deficiency (AMD)
- Deficiency of acid α-glucosidase (GAA), which leads to the accumulation of lysosomal glycogen
- Autosomal recessive disorder: GAA 17q25.3 gene mutations
- More than 300 mutations have been described in GAA
- Broad spectrum of illness

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6

Table 2. Findings suggestive of Pompe disease

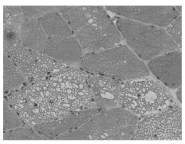
Assessment	Findings
Neurologic testing	
Manual (MRC) or quantitative muscle testing	Pattern of weakness: usually proximal greater than distal
Electromyography*	Markedly increased muscle membrane irritability Myotonic discharges (typical or atypical, may be observed only in the paraspinal muscles) Brief MUAPs (small amplitude and short duration)
Nerve conduction studies	Normal
Respiratory	
FVC seated and supine	≥10% drop in FVC from seated to supine testing†
Laboratory	
Creatine kinase	Ranges from normal to up to 15 times the upper limit of normal

MRC, Medical Research Council; MUAP, motor unit action potential; FVC, forced vital capacity.
**Findings may vary, depending on clinical involvement and muscles sampled.*
†This finding is suggestive of diaphragm weakness.

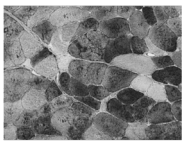
American Association of Neuromuscular & Electrodiagnostic Medicine. *Muscle Nerve.* 2009;40:149-160.

7

Pompe Disease



Cryostat H&E



Cryostat PAS

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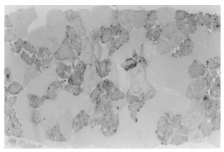
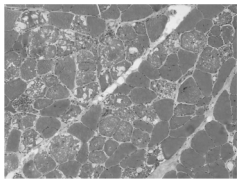
8

Diagnosis: Muscle Biopsy



PAS: excess glycogen and acid phosphatase



H&E: multiple vacuoles of various sizes in single muscle fibers

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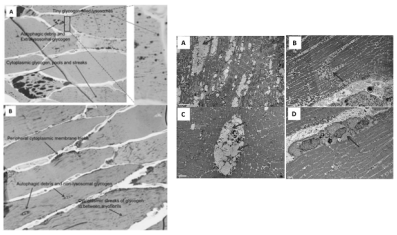
9

Presence of Lysosomal-Bound and Free Cytoplasmic Glycogen and Autophagic Material in Skeletal Muscle



van der Ploeg. *Mol Genet Metab*. 2016;119:115-123.

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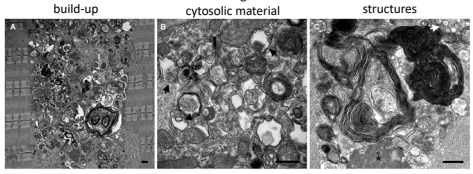
10

Pathophysiology: EM Images in GAA-KO Mouse

Autophagic build-up

Double membrane autophagosomes with undigested cytosolic material

Multimembrane structures



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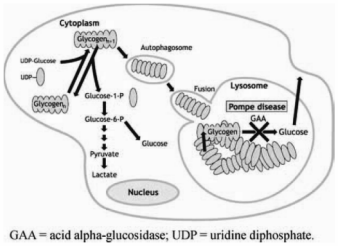
11

Pathophysiology of Pompe Disease



GAA = acid alpha-glucosidase; UDP = uridine diphosphate.

Cupler. *Muscle Nerve*. 2011;45:319-333.

12

Pathophysiology

- Progressive expansion of glycogen-filled lysosomes in multiple tissues
- Skeletal and cardiac muscle most affected
- Enzyme replacement therapy (ERT) successful in reversing cardiac but not skeletal muscle abnormalities.
- Abnormal autophagy: Engel 1970
- Autophagic build-up:
  - Causes loss of contractility and muscle mass
  - Affects the trafficking and delivery of the recombinant enzyme
  - The drug is diverted away from its intended target the lysosome, and instead ends up in autophagic area = sink for the recombinant enzyme

13

Pompe Disease

- Incidence ~ 1/40,000
- Infantile form: 1 in 35,000 to 1 in 138,000
- Late onset form: 1 in 57,00
- In USA: all forms frequency as high as 1 in 40,000

- Certain populations are at higher risk:
  - African Americans
  - Taiwanese
  - Dutch

14

Late Onset Pompe Disease (LOPD)

Andrew G. Engel, MD

Brain (1970) 93, 599–616.

ACID MALTASE DEFICIENCY IN ADULTS: STUDIES IN FOUR CASES OF A SYNDROME WHICH MAY MIMIC MUSCULAR DYSTROPHY OR OTHER MYOPATHIES[1]

BY

ANDREW G. ENGEL

(From the Mayo Clinic and Mayo Foundation: Section of Neurology and Neuromuscular Research Laboratory, Rochester, Minnesota)

The spectrum and diagnosis of acid maltase deficiency

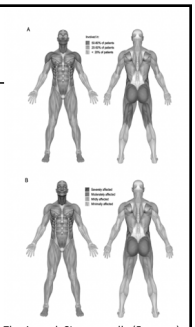
Andrew G. Engel, M.D., Manuel R. Gomez, M.D., Marjorie E. Seybold, M.D., and Edward H. Lambert, M.D., Ph.D.

Engle. *Neurology*. 1973:23:95-106.

15

Clinical Picture of LOPD

- Clinical spectrum much broader than initially recognized
- Onset of symptoms at any age, ranging from infancy to late adulthood
- Limb-girdle muscle weakness
- Less familiar features such as ptosis, bulbar weakness, and scapular winging
- Respiratory insufficiency found in the majority of patients
- Neck drop and camptocormia is quite common
- Infraspinatus muscle weakness very common, even in the absence of scapular winging



Thesis work CI van capelle (Erasmus)

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16

Table 1. Differential diagnosis of late-onset Pompe disease.

Disorder type	Diagnoses
Dystrophies	• Limb-girdle muscular dystrophy • Dystrophinopathies (Duchenne and Becker muscular dystrophy) • Myofibrillar myopathy • Myotonic dystrophy type 2 • Scapuloperoneal syndromes • Danon disease • X-linked myopathy with excessive autophagy • Facioscapulohumeral muscular dystrophy
Inflammatory myopathies	• Polymyositis • Inclusion body myositis
Congenital myopathies	• Nemaline rod myopathy • Central core and multiminicore myopathy • Centronuclear myopathy • Hyaline body myopathy • Other congenital myopathies
Other metabolic myopathies	• Debranching enzyme deficiency • Branching enzyme deficiency • McArdle disease (late-onset) • Mitochondrial myopathy • Lipid disorder myopathies
Motor neuron disorders	• Spinal muscular atrophy types II and III • Kennedy disease • Amyotrophic lateral sclerosis
Neuromuscular junction disorders	• Myasthenia gravis • Congenital myasthenic syndromes • Lambert-Eaton syndrome

American Association of Neuromuscular & Electrodiagnostic Medicine. *Muscle Nerve.* 2009;40:149-160.

17

Illustrative Case

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18

Illustrative Case

- 23 year old Hispanic graduate student at UC Irvine seen in 2003
- 2 year history of progressive proximal weakness in the hip girdle and now shoulder girdle muscles (limb-girdle pattern)
- No pain
- Has lately noticed dyspnea on exertion
- No skin rash
- No family history

19

Case (continued)

- Examination showed proximal muscle weakness
  - Deltoids, biceps, triceps symmetric 4+ weakness
  - Hip flexors, glutei, hip adductors 4- weakness
  - No skin rash
  - Forced vital capacity: 87% sitting; 63% lying
  - CK elevated at 1,230 iu/l
  - Myositis autoantibody panel negative
  - Nerve conduction studies with no clear abnormalities
  - EMG examination with profuse fibrillations and myotonic discharges especially in the paraspinal muscles; motor units small amplitude, with narrow duration and early recruitment

20

Case

What do we do next?

21

Case (continued)

- Underwent a quadriceps muscle biopsy
- No specific findings
- Small number of degenerating and regenerating fibers
- No inflammation
- No vacuoles

22

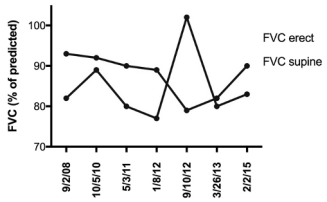
Case (continued)

- Whole blood acid alpha glucosidase (GAA) very low (1.5 pmol/mm punch/hr)
- Muscle GAA assay preformed: 15% of normal
- DNA analysis confirms 2 heterozygote mutations in the GAA gene, including the common Caucasian leaky splice site mutation (c.-32-13T>G) and c.1841C>A

- **Late onset (noninfantile) Pompe disease (acid maltase disease)**

23

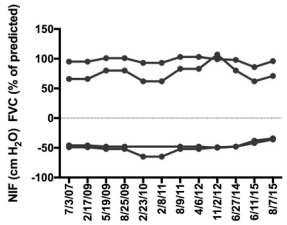
Differential Drop in FVC



Mozaffar. Unpublished clinical data.

24

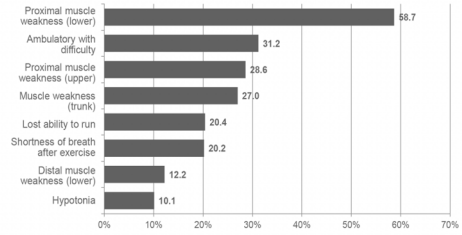
Stabilization of FVC and NIF with Treatment



Mozaffar. Unpublished clinical data.

25

Proximal muscle weakness is the most common clinical feature of LOPD



Kishnani. *Am J Med Genet A*. 2013;161:2431-2443.

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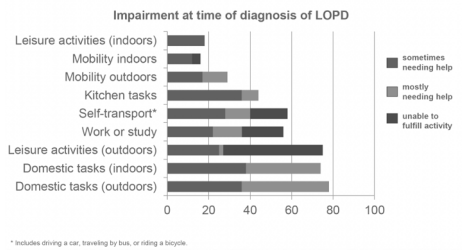
26

Most patients with LOPD have severely impaired health status at diagnosis



* Includes driving a car, traveling by bus, or riding a bicycle.

Rigter. *Mol Gen Metab*. 2012;107:448-455.

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27

Patterns of Weakness

Clinical presentation (%)

- Isolated hyperCKemia (12%)
- HyperCKemia + generalized LGMW + ventilation (61%)
- HyperCKemia + shoulder LGMW (9.5%)
- HyperCKemia + pelvic LGMW (14.8%)
- HyperCKemia + ventilation (2.7%)

Lukacs. *Neurology*. 2016;87:295-298.

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28

Unmet Needs in Pompe Disease #1

- Significant delay in diagnosis and thus development of irreversible disease
- Significant morbidity develops before patients get diagnosed

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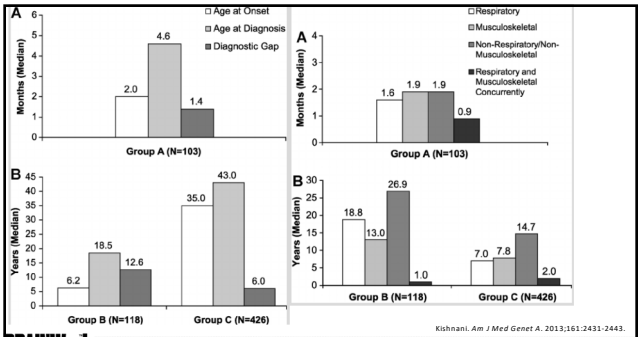
29



Kishnani. *Am J Med Genet A*. 2013;161:2431-2443.

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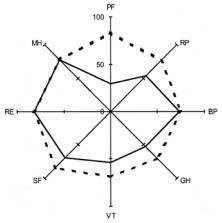
30

Quality of Life in LOPD: Reduced, Affects All Domains



Hagemans. *Neurology*. 2004;63:1688-1692.

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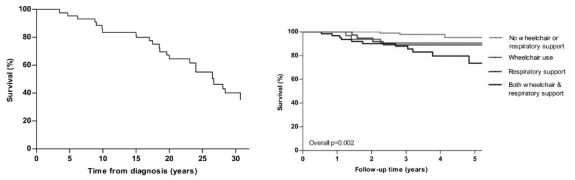
31

Mortality: Increased in LOPD



Analysis	Median follow-up time (range)	Observed no. deaths (O)	Expected no. deaths (E) *	Ratio (O/E)	P -value
1	2.3 (<1 month–7 years)	5	2.3	2.2	0.09
2	3.3 (<2 months–7 years)	9	2.8	3.2	0.002

Güngör. *Orphanet J Rare Dis*. 2011;6:34.

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32

Diagnosis

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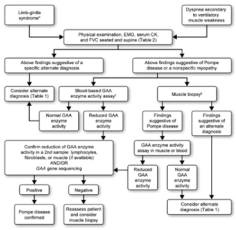
33

Diagnostic Algorithm to Diagnose LOPD



American Association of Neuromuscular & Electrodiagnostic Medicine. *Muscle Nerve.* 2009;40:149-160.

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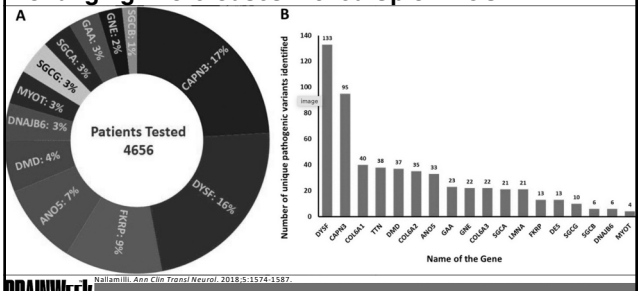
34

The Paradigm to Diagnose LOPD May Be Changing: More Cases Picked Up on NGS



Nallamilli. *Ann Clin Transl Neurol.* 2018;5:1574-1587.

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35

Table 1. Summary of *GAA* variants identified in late-onset Pompe patients. Identification of 28 patients with two *GAA* pathogenic variants indicates the increased prevalence of late-onset Pompe disease in this study. (AOP: Adult-onset Pompe)

Patient ID	Gender	Age	Gene	Variant 1	Variant 2
AOP1	Female	61	*GAA*	c.-32-13T>G	c.1124G>T (p.R375L)
AOP2	Female	79	*GAA*	c.-32-13T>G	c.2140delC
AOP3	Female	33	*GAA*	c.-32-13T>G	c.525delT
AOP4	Male	71	*GAA*	c.-32-13T>G	c.1912G>T (p.G638W)
AOP5	Unknown	54	*GAA*	c.-32-13T>G	c.2512C>T(p.Q838X)
AOP6	Male	66	*GAA*	c.-32-13T>G	c.2481 + 102_2646 + 31del (Exon 18 deletion)
AOP7	Male	70	*GAA*	c.-32-13T>G	c.2481 + 102_2646 + 31del (Exon 18 deletion)
AOP8	Female	44	*GAA*	c.-32-13T>G	c.2481 + 102_2646 + 31del (Exon 18 deletion)
AOP9	Male	18	*GAA*	c.-32-13T>G	c.2481 + 102_2646 + 31del (Exon 18 deletion)
AOP10	Male	40	*GAA*	c.-32-13T>G	c.2238G>A(p.W746X)
AOP11	Male	59	*GAA*	c.-32-13T>G	c.1655T>C(p.L552P)
AOP12	Male	70	*GAA*	c.736delC	c.546G>A(p.T183T)
AOP13	Female	53	*GAA*	c.-32-13T>G	c.1841C>A(p.T614K)
AOP14	Male	68	*GAA*	c.-32-13T>G	c.1143delC
AOP15	Female	40	*GAA*	c.853C>T(p.P285S)	c.2560C>T(p.R854X)

Nallamilli. *Ann Clin Transl Neurol.* 2018;5:1574-1587.

36

What Is the Prevalence of LOPD in Neuromuscular Clinics?

- Prospective study of 924 patients presenting to academic tertiary neuromuscular neurology practices with complaints of proximal muscle weakness, isolated hyperCKemia, or neck flexor weakness
- **1%** of patients were found to have LOPD
- Another 1% of patients were found to have pseudodeficiency state

Investigating Late-Onset Pompe Prevalence in Neuromuscular Medicine Academic Practices

The IPaNeMA Study

Marie Wencel, BS, Aziz Shaibani, MD, Namita A. Goyal, MD, Mazen M. Dimachkie, MD, Jaya Trivedi, MD, Nicholas E. Johnson, MD, Laurie Gutmann, MD, Matthew P. Wicklund, MD, Sankar Bandyopadhay, MD, Angela L. Genge, MD, Miriam L. Freimer, MD, Neelam Goyal, MD, Alan Pestronk, MD, Juliane Florence, PhD, Chafic Karam, MD, Jeffrey W. Ralph, MD, Dnah Rasheed, MD, Melissa Hays, BA, Steve Hopkins, BS, and Tahseen Mozaffar, MD, on behalf of the IPaNeMA Study Group

Neurol Genet 2021;7:e623. doi:10.1212/NXG.0000000000000623

Table 4 Diagnosed Pompe Patient Characteristics (n = 9)

Age (y)	52.2	(20-5)
	N	**%**
Sex		
Female/Male	6/3	66.7/33.3
Ethnicity		
Hispanic/Not Hispanic	1/8	11.1/88.9
White/Caucasian	8	88.9
Black/African American	1	11.1
Inclusion criteria		
Proximal weakness	9	100
Neck weakness	5	55.6
High creatine kinase	7	77.8

Wencel. *Neurol Genet*. 2021;7:e623.

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37

Newborn Screening for Pompe

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38

The Case for Newborn Screening for Pompe Disease: Advantages

- A treatable condition
- Treatment is effective for reversing or preventing cardiomyopathy and respiratory insufficiency/ventilator dependence
- Prevalence is generally higher than estimated
- Early treatment is more effective
- Presymptomatic patients can be identified and monitored
- Gain knowledge on how the disease progresses and whether carriers are affected in any way or not

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39

The Case for Newborn Screening for Pompe Disease: Drawbacks and Challenges

- Late onset forms are much more common than infantile forms
- These late onset forms may not manifest disease until in their teens, and some even much later
- No clear agreement on how to monitor these subjects and when to initiate treatment
- Detection of a genetic disease that may not have manifestations until adult life may result in unnecessary anxiety/depression, modification/restrictions of diet and lifestyle and unnecessary treatment and testing
- Discrimination for life insurance and long-term disability

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40

Newborn Registry Experiences So Far

Country/State	Taiwan	Taiwan	Austria	Hungary	Missouri	Washington	Illinois	New York
Number of Samples	473,738	191,786	34,737	40,024	237,000	111,544	128,876	390,000
Follow up	Clinical	Clinical	Molecular Genetics	Molecular Genetics	Clinical	Molecular Genetics	Clinical	Molecular Genetics
Prevalence	1 in 16,919	1 in 11,987	1 in 8,684	1 in 4,447	1 in 5,463	1 in 27,886	1 in 14,213	1/165,000
Cases of Pompe	28	16	4	10	23	4	8	75
Percentage of LOPD cases	67%	64%	100%	20%	73%	100%	75%	90%
Carriers	?	?	?	25	25	7	6	0
Pseudodeficiency	14.5%	?	?	?	20	?	9	14

Modified from Matern D, Seminars in Perinatology 2015, dx.doi.org/10.1053/j.semperi.2015.03.005

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41

Results from US Newborn Screening (NBS) Programs for Pompe Disease*

Illinois: 1:21,979

Missouri: 1:11,229

New York: 1:165,000**
(data includes IOPD cases only based on NY state reporting)

Washington (pilot): 1:31,000

*Burton. *J Pediatr*. 2017 Nov; 190:130-135; Bodamer. *Pediatrics*. 2017 Jul; 140 (Suppl):S4-S13.
**Includes data from select states; see www.newsteps.org for current list of states screening.

| 42

42

GAA Levels Cannot Differentiate Between IOPD and LOPD

From: Y-H Chien. J Pediatr 2011;158:1023-7

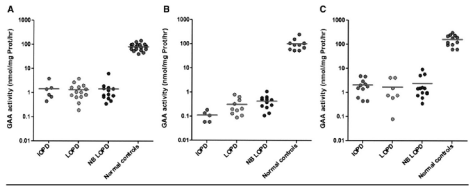

Figure 2. GAA activity of newborns with infantile-onset Pompe disease *(IOPD)*, patients with later-onset Pompe disease *(LOPD)*, newborns with later-onset Pompe disease *(NB LOPD)*, and control subjects. **A**, Lymphocyte GAA activity assayed with 4-methylumbelliferyl-α-D-glucopyranoside *(4MU)*; **B**, fibroblast GAA activity assayed with 4MU; **C**, fibroblast GAA activity assayed with glycogen.

Yang. *Am J Med Genet A*. 2014;164A(1):54-61.

43

Unmet in LOPD #2

- For the cases of Pompe picked up on NBS, what is the optimal monitoring scheme, and who pays for it?
- What are the best tests to confirm that disease has set in?
- What is the optimal time for starting treatment with ERT?
- Can other strategies be used, as glycogen substrate reduction, to delay disease/symptom onset?
- What is the reason for the disconnect between the higher incidence rate of LOPD and low prevalence of LOPD cases in the neuromuscular cases?

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44

The Caucasian Dilemma

- The *GAA* c.336-13T>G mutation has a residual enzyme activity of 20%-40% and is present in around 71% of people with late-onset Pompe disease worldwide
- This mutation has not been identified in patients with infantile-onset Pompe
- There is significant phenotypic variability among individuals with the IVS1-3T>G mutation which appears to be dependent upon the nature of the paired mutation
- Homozygosity for this mutation results in typical late-onset Pompe disease with age of onset between 12-55 years
- 1 patient at age 49 only had hyperCKemia

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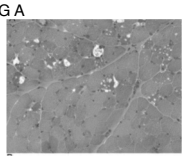
45

Making the Diagnosis of Pompe Disease at a Presymptomatic Stage: To Treat or Not to Treat?

- 21 yo man, active healthy
- 13 months had 1.5x CK-emia, dx with LOPD
  - GAA mutations c.-32–13T>G and c.655G A (p.Gly219Arg)
  - Normal echo
  - Muscle biopsy at 19 months
- Yearly exams (echo)
- @ 16 years: more assessments,
  - Standardized, PFTs, MRI, in vivo NMR



BRAINWEEK

46

Neurology® The Official Journal of the American Academy of Neurology

Editorials

"I'm fine; I'm just waiting for my disease"

The new and growing class of presymptomatic patients

Jennifer M. Kwon, MD and Robert D. Steiner, MD

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Neurology August 9, 2011 vol. 77 no. 6 522-523

THE WALL STREET JOURNAL.

Genetic Testing Leaves More Patients Living in Limbo

So-called patients-in-waiting have genes for disease but no symptoms

47

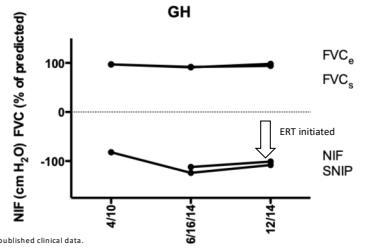
Management of LOPD Includes a Multidisciplinary Approach

Mozaffar. Unpublished clinical data.

BRAINWEEK

48

PFTs Useful to Decide When to Intervene with ERT



Mozaffar. Unpublished clinical data.

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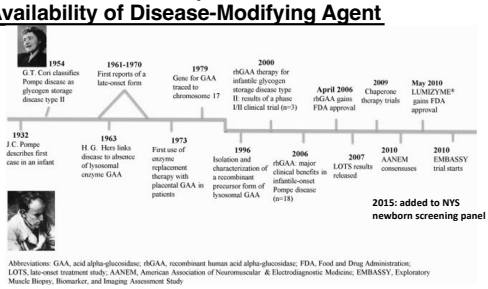
49

Timeline for Pompe Disease: 75 Years from Description to Availability of Disease-Modifying Agent



Abbreviations: GAA, acid alpha-glucosidase; rhGAA, recombinant human acid alpha-glucosidase; FDA, Food and Drug Administration; LOTS, late-onset treatment study; AANEM, American Association of Neuromuscular & Electrodiagnostic Medicine; EMBASSY, Exploratory Muscle Biopsy, Biomarker, and Imaging Assessment Study

50

Yuan-Tsong Chen, MD, PhD
Duke

Priya Kishnani, MD
Duke

Arnold J. Reuser, PhD
Erasmus

Ans van der Ploeg, MD, PhD
Erasmus

Enzyme Replacement Therapy

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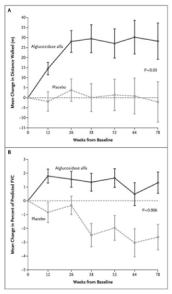
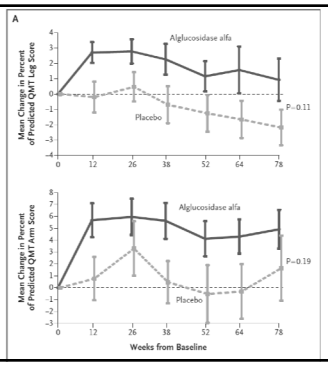
51

Enzyme Replacement in Late Onset Pompe Disease



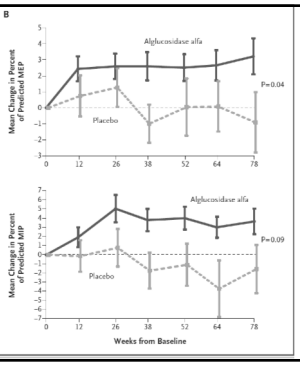
van der Ploeg. *N Engl J Med*. 2010;362:1396-1406.

52



van der Ploeg. *N Engl J Med*. 2010;362:1396-1406.

53



van der Ploeg. *N Engl J Med*. 2010;362:1396-1406.

54

Unmet Needs in LOPD #3

- Treatment with ERT "stops" working after 2-3 years
- Only 1% of the enzyme makes it into the target organ (muscle or cardiac muscle)
- The enzyme is "unprotected" in circulated and some, if not most of it, may be denatured by the time it reaches target organ
- Some patients develop antibodies to the drug with prolonged treatment. It is not clear if these anti-GAA antibodies are neutralizing antibodies and probably an epiphenomenon in LOPD
- When do you stop treatment in LOPD, if a patient is not responding?

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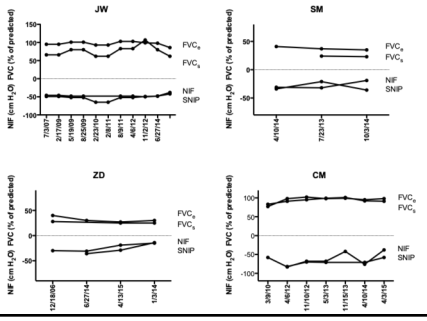
55

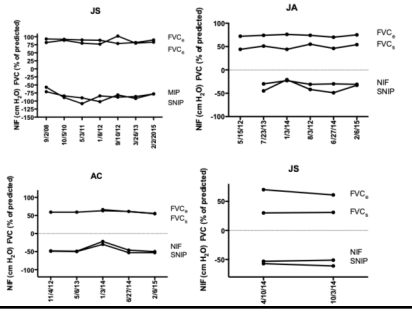
Pompe – Worsening PFTs



van der Ploeg. *N Engl J Med.* 2010;362:1396-1406.

56

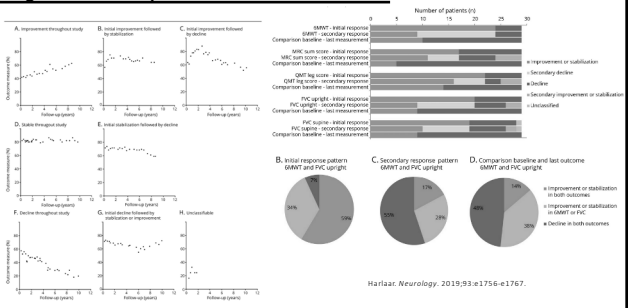
Pompe – Stable PFTs



Mozaffar. Unpublished clinical data.

57

Long Term Follow-Up of ERT-Treated LOPD Patients



Harlaar. *Neurology*. 2019;93:e1756-e1767.

58

When do you stop treatment in LOPD?

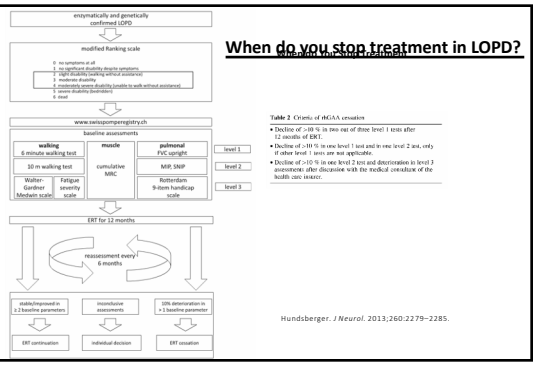


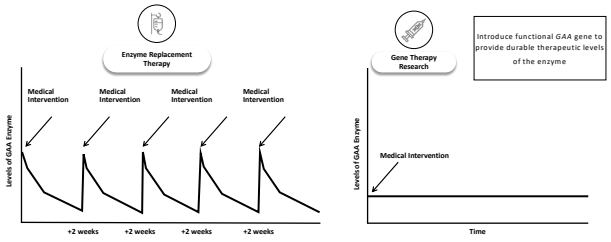
Table 2 Criteria of rhGAA cessation

- Decline of >10 % in two out of three level 1 tests after 12 months of ERT.
- Decline of >10 % in one level 1 test and in one level 2 test, only if other level 1 tests are not applicable.
- Decline of >10 % in one level 2 test and deterioration in level 3 assessments after discussion with the medical consultant of the health care insurer.

Hundsberger. *J Neurol*. 2013;260:2279–2285.

59

The Objective of Gene Therapy Research: Enable a Single Administration that Leads to Sustained Enzyme Expression, as Secreted by the Liver

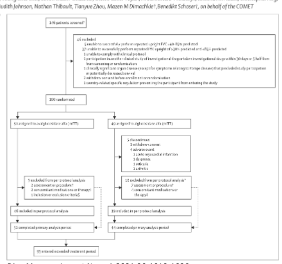


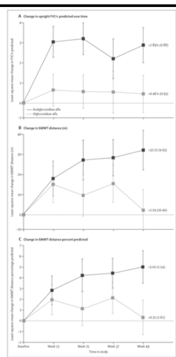
Kishnani. *Pediatr Res*. 2009;66:329-335. Chen. *Cochrane Database Syst Rev*. 2017;11:1-26. Kishnani. *Mol Genet Metab*. 2010;99:26-33. Ronzitti. *Ann Transl Med*. 2019;7(13):287-303. Zhu. *Mol Ther*. 2009;17:954-963. Wu. *Mol Ther*. 2008;16(2):280-289.

60

Safety and efficacy of avalglucosidase alfa versus alglucosidase alfa in patients with late-onset Pompe disease (COMET): a phase 3, randomised, multicentre trial

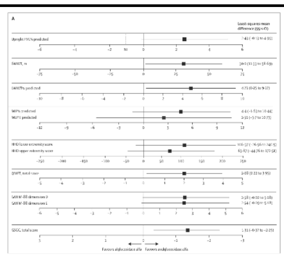
Jordi Diaz-Manera, Priya S Kishnani*, Hani Kushlaf, Shafeeq Ladha, Tahseen Mozaffar, Volker Straub, Antonio Toscano, Ans T van der Ploeg, Kenneth I Berger, Paula R Clemens, Yin-Hsiu Chien, John W Day, Sergey Illarioshkin, Mark Roberts, Shahram Attarian, Joao Lindolfo Borges, Francoise Bouhour, Young Chul Choi, Sevim Erdem-Ozdamar, Ozlem Goker-Alpan, Anna Kostera-Pruszczyk, Kristina An Haack, Christopher Hug, Olivier Huynh-Ba, Judith Johnson, Nathan Thibault, Tianyue Zhou, Mazen M Dimachkie†, Benedikt Schoser†, on behalf of the COMET Investigator Group*

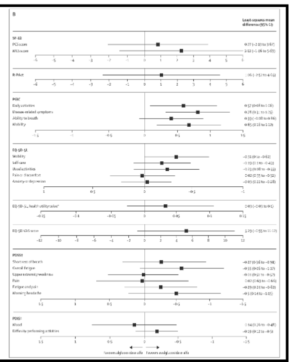




Diaz-Manera. *Lancet Neurol.* 2021;20:1012-1026.

61





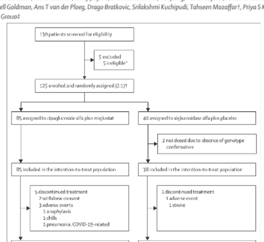
Diaz-Manera. *Lancet Neurol.* 2021;20:1012-1026.

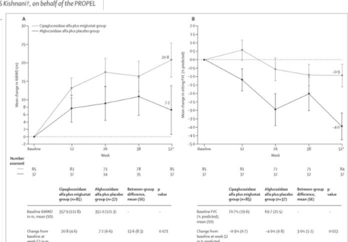
62

Safety and efficacy of cipaglucosidase alfa plus miglustat versus alglucosidase alfa plus placebo in late-onset Pompe disease (PROPEL): an international, randomised, double-blind, parallel-group, phase 3 trial

Benedikt Schoser, Mark Roberts*, Barry J Byrne, Sheela Sitaraman, Hai Jiang, Pascal Laforêt, Antonio Toscano, Jeff Castelli, Jordi Díaz-Manera, Mitchell Goldman, Ans T van der Ploeg, Drago Bratkovic, Srilakshmi Kuchipudi, Tahseen Mozaffar†, Priya S Kishnani†, on behalf of the PROPEL Study Group‡*

Schoser. *Lancet Neurol.* 2021;20:1027-1037.





63

	Endpoint hierarchy	Cipaglucosidase alfa plus miglustat group (n=85)		Alglucosidase alfa plus placebo group (n=37)		Least square mean difference (95% CI)
		Baseline, mean (SD); n	Change from baseline at week 52, mean (SD, SE); n	Baseline, mean (SD); n	Change from baseline at week 52, mean (SD, SE); n	
Motor function						
6MWD, m	Primary	357·9 (111·8); 85	20·8 (42·8, 4·4); 85	353·0 (121·3); 37	7·2 (40·3, 6·6); 37	13·7 (−1·2 to 28·5)
GSGC total score	Key secondary	14·5 (5·3); 74	−0·5 (2·5, 0·3); 72	14·5 (4·7); 32	0·8 (3·8, 0·9); 30	−1·4* (−2·5 to −0·4)
10-m walk, m	Other secondary	9·7 (7·6); 84	−0·5 (1·8, 0·4); 80	9·6 (5·1); 35	1·9 (5·4, 1·0); 35	−2·7* (−5·0 to −0·3)
Four-stair climb, s	Other secondary	14·1 (29·1); 79	−8·1 (19·1, 2·3); 78	8·7 (9·1); 35	0·3 (5·7, 1·0); 34	−3·1 (−6·3 to 0·4)
Gower's manoeuvre, s	Other secondary	10·8 (7·5); 61	−0·3 (5·8, 0·7); 61	10·8 (11·2); 27	−2·2 (8·6, 1·4); 25	1·6 (−1·5 to 4·7)
Rising from chair, s	Other secondary	13·4 (39·1); 77	−10·2 (84·7, 9·7); 77	4·5 (3·2); 33	−0·5 (3·5, 0·7); 33	−0·8 (−2·4 to 0·7)
Timed up-and-go, s	Other secondary	12·9 (19·1); 75	−0·3 (8·5, 1·0); 75	12·8 (9·6); 32	−0·8 (2·7, 0·5); 32	−0·5 (−3·4 to 2·4)
6MWD, % predicted	Other secondary	57·8% (15·8); 85	4·1% (7·0, 0·8); 85	56·0% (17·1); 37	1·6% (8·0, 1·4); 37	2·4% (−0·3 to 5·0)
Pulmonary function						
Sitting FVC, % predicted	Key secondary	70·2% (19·6); 85	−0·9% (6·1, 0·7); 84	69·2% (21·5); 37	−4·0% (4·9, 0·8); 37	2·7%* (0·4 to 5·0)
Sitting SVC, % predicted	Other secondary	69·6% (17·9); 85	−1·3% (8·6, 1·0); 83	68·6% (20·7); 36	−1·9% (8·6, 1·5); 35	1·8% (−0·8 to 4·5)
Maximum vital capacity, % predicted	Other secondary	72·3% (19·1); 85	−1·2% (6·0, 0·7); 84	70·9% (20·9); 37	−3·9% (5·0, 0·8); 37	2·4%* (0·2 to 4·7)
IVJV, % predicted	Other secondary	61·8% (26·7); 85	2·1% (19·7, 2·1); 84	59·6% (23·6); 37	−2·7% (10·6, 2·8); 37	4·7% (−3·4 to 13·8)
MEP, % predicted	Other secondary	70·7% (32·6); 85	0·6% (25·5, 2·6); 84	65·1% (29·5); 37	−9·6% (32·8, 2·1); 37	3·9% (−5·6 to 13·3)
SNIP, % predicted	Other secondary	69·7% (23·3); 85	2·2% (15·0, 1·6); 84	65·2% (28·6); 37	2·8% (23·8, 3·9); 37	−2·0% (−10·2 to 5·9)
Muscle strength						
Lower MMT score	Key secondary	28·6 (5·1); 84	1·6 (3·8, 0·4); 80	27·7 (9·2); 37	0·9 (2·6, 0·4); 34	1·0 (−0·5 to 2·4)
Upper MMT score	Other secondary	34·3 (3·4); 84	1·5 (3·4, 0·4); 82	34·7 (4·5); 37	0·7 (3·6, 0·6); 37	0·9 (−0·2 to 2·1)
Total MMT score	Other secondary	63·1 (8·7); 84	3·1 (6·1, 0·7); 80	62·4 (9·7); 34	1·4 (4·4, 0·8); 34	2·7 (−0·1 to 4·5)
Total QMT score, kg	Other secondary	265·6 (68·9); 82	6·9 (33·4, 6·1); 81	138·0 (80·4); 36	0·2 (32·2, 5·2); 36	3·0 (−15·7 to 22·7)
Patient-reported outcomes						
PROMIS—physical function score	Key secondary	66·6 (17·3); 84	1·6 (7·5, 0·8); 84	68·0 (13·1); 37	0·7 (10·8, 1·8); 37	1·9 (−1·5 to 5·3)
PROMIS—fatigue score	Key secondary	20·3 (8·1); 85	−2·6 (5·8, 0·6); 85	21·1 (6·1); 37	−1·7 (4·6, 1·1); 37	0·0 (−2·1 to 2·2)
Biomarkers						
Urinary Hex4, mmol/mol creatine	Pharmacodynamic	6·6 (3·4); 84	−1·6 (2·4, 0·3); 84	6·0 (6·9); 37	3·2 (4·4, 0·7); 37	−2·5* (−3·7 to −1·3)
Serum creatine kinase, U/L	Pharmacodynamic	447·0 (339·5); 85	−110·5 (211·2, 23·1); 85	527·0 (426·9); 37	69·2 (159·5, 26·2); 37	−179·0* (−244·4 to −107·6)

Schoser. *Lancet Neurol.* 2021;20:1027-1037.

64

Gene Therapy

65

Evaluation of the Impact of Investigational Gene Transfer on Enzyme Levels in Patients with Pompe Disease

- Gene transfer is a potential gene therapy strategy currently being investigated for Pompe disease and other LSDs[1]
- AAV-based gene transfer is the most commonly used gene therapy approach[1,2]
- Investigational gene transfer approaches are being evaluated to determine their impact on the levels of functional enzyme in target tissues involved in Pompe disease[1,3]
- Gene transfer for Pompe disease is still subject to clinical investigation, and its safety has yet to be established

Primarily Affected in Infants[4]

- Cardiac muscles
- Skeletal muscles
- Respiratory muscles
- Central nervous system

Primarily Affected in Children and Adults[4]

- Proximal skeletal muscles (especially in the trunk and lower limbs)
- Respiratory muscles
- Central nervous system

1. Ronzitti. *Ann Transl Med*. 2019;7(13):287-303. 2. Kotterman. *Annu Rev Biomed Eng*. 2015;17:63-89.
3. Kishnani. *Genet Med*. 2006;8:267-288. 4. Korlimarla. *Ann Transl Med*. 2019;7(13):289.

66

Examples of Investigational Gene Therapies Under Development to Treat Pompe Disease

Current as of July 2021

Company[1]	Gene Therapy Vector[1]	Transgene[1]	Target Tissue[1]	Development Status
Actus[2,3,a]	AAV2/8 (in vivo)	*GAA*	Liver	Phase 1/2
Spark[4,b]	AAV *SPK3006* (in vivo)	Secretable *GAA*	Liver	Phase 1/2
Audentes[5,6]	AAV8 (in vivo)	*GAA*	Muscle	Phase 1/2
AvroBio[7,8]	LV (ex vivo)	GILT-*GAA* fusion	CD34+ HSCs	IND submission
Amicus[9]	AAV (in vivo)	*In development*	*In development*	Preclinical
Regeneron	AAV (in vivo)	CD63-*GAA* fusion	Liver	Preclinical
Sarepta[c]	AAV (in vivo)	*GAA*	CNS	Preclinical

[a]Therapeutic candidate in-licensed from Duke University. [b]Therapeutic candidate in-licensed from Genethon. [c]Therapeutic candidate in-licensed from Lacerta, Inc.

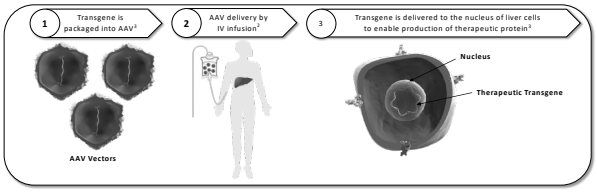
AAV, adeno-associated virus; CD34+, cluster of differentiation 34-positive; CD63, cluster of differentiation 63; HSCs, hematopoietic stem cells; GILT, glycosylation-independent lysosomal targeting; IND, investigational new drug; LV, lentivirus.

1. Ronzitti. *Am Transl Med.* 2019;7(13). 2. Asklepios Biopharmaceutical – Pipeline. Accessed 3/2021. www.askbio.com/gene-therapy-pipeline. 3. ClinicalTrials.gov. AAV2/8-LSPhGAA in Late-Onset Pompe Disease. Accessed 6/2021. clinicaltrials.gov/ct2/show/NCT03533673 4. ClinicalTrials.gov. A Gene Transfer Study for Late-Onset Pompe Disease (RESOLUTE). Accessed 6/2021. clinicaltrials.gov/ct2/show/NCT04093349 5. Audentes – Pipeline. Accessed 3/2021. www.audentestx.com/pompe-disease. 6. ClinicalTrials.gov - Gene Transfer Study in Patients With Late Onset Pompe Disease (FORTIS). Accessed 6/2021. clinicaltrials.gov/ct2/show/NCT04174105. 7. AvroBio – Pipeline. Accessed 3/2021. www.avrobio.com/our-pipeline. 8. AVROBIO Presents New Preclinical Data on Lentiviral Gene Therapy Program for Pompe Disease at ASGCT 2020. Accessed 6/2021. investors.avrobio.com/news-releases/news-release-details/avrobio-presents-new-preclinical-data-lentiviral-gene-therapy. 9. Amicus Therapeutics – Development Pipeline. Accessed 3/2021. www.amicusrx.com/programs-pipeline/pipeline.

67

Rationale for Liver-Directed AAV Investigational Gene Therapy for Pompe Disease

- Delivered systemically via IV infusion[1]
- Use of an AAV vector with natural tropism to the liver and a liver-specific promoter (LSP), with the objective of expressing GAA specifically in hepatocytes[1,2]



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68

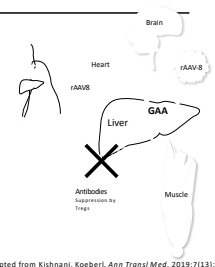
Proposed Mechanism of Action of Liver-Directed Investigational AAV Gene Therapy for Pompe Disease

The selected AAV serotype with naturally high tropism to the liver transfects hepatocytes, creating a depot in the liver

Hepatocytes secrete GAA enzyme into the bloodstream

GAA enzyme is taken into target tissues (eg, skeletal, cardiac muscle) via mannose-6-phosphate (M6P) receptor–mediated uptake

Once inside the cell, the GAA enzyme may be processed further to become fully functional to clear glycogen



rAAV-8, recombinant adeno-associated virus 8; Tregs, regulatory T cells. Figure adapted from Kishnani, Koeberl. *Ann Transl Med.* 2019;7(13):288

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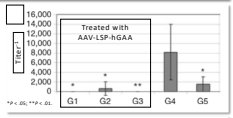
69

Considerations for Liver-Directed Approach to Investigational Gene Therapy for Pompe Disease

Liver-Specific Characteristics

- AAV vectors have a very high tropism for the liver[1]
- The liver is immunologically tolerant to transgene products[2]
  - Presentation of AAV antigens by hepatic APCs induces antigen-specific immune tolerance[2]
- Subject to clinical investigation; safety of investigational gene therapy for Pompe disease has yet to be established



Inverse titers of anti-hGAA antibodies at week 10 following AAV-LSP-hGAA infusion in Pompe disease mice and rhGAA immune challenge[3]

Liver-Specific Considerations

- Potential for T-cell mediated immunity to destroy AAV-transduced hepatocytes[4]

APCs, antigen presenting cells; hGAA, human acid alpha-glucosidase; rhGAA, recombinant human acid alpha-glucosidase.
1. Salabarria. *J Neuromuscul Dis*. 2020;7(1):15-31. 2. Ronzitti. *Ann Transl Med*. 2019;7(13):287-303. 3. Figure adapted from Sun. *Mol Ther*. 2009;18(2):353-360. 4. Mingozzi. *Hum. Blood*. 2013;122(1):23-36. 5. Kishnani. *Ann Transl Med*. 2019;7(13):288.

BRAINWEEK

70

Liver-Directed Approach to Investigational Gene Therapy for Pompe Disease: Preclinical Findings

Preclinical Results

- Biochemical correction of glycogen accumulation in muscle and the central nervous system, and reduced cardiac hypertrophy[1,2]
- Functional improvement in skeletal and respiratory muscles[1]
- Secreted GAA demonstrated lower immunogenicity than nonengineered/native GAA[1]
- Similar immunogenicity was seen in nonhuman primates[1]
- This data is hypothesis-generating and informs further evaluation in the clinic

1. Puzzo. *Sci Transl Med*. 2017;9(418):eaam6375. 2. Kishnani. *Ann Transl Med*. 2019;7(13):288.

BRAINWEEK

71

Rationale for and Proposed Mechanism of Action of Muscle-Directed AAV Investigational Gene Therapy

Intramuscular (IM) Delivery

- Delivered directly to muscles via injection to local muscle fibers[1]
  - Example: Intrapleural injections to respiratory muscles[2,3]
- Muscle-specific promoter (eg, MHCK7) may enhance GAA expression in target muscles[2,4]

AAV vectors are taken up directly by muscle cells, which then produce functional enzyme to mitigate the effects of lysosomal glycogen accumulation[3]

Intravenous (IV) Delivery

- An AAV vector with a muscle-specific serotype and muscle-specific promoter, delivered IV[3]

AAV vectors are delivered IV into systemic circulation and then taken up directly by muscle cells, which produce functional enzyme to mitigate the effects of lysosomal glycogen accumulation[3]

MHCK7, hybrid α-myosin heavy-chain creatine kinase. 1. Costa Verdera. *Mol Ther*. 2020;28(3):723-746. 2. Salabarria. *J Neuromuscul Dis*. 2020;7(1):15-31. 3. Ronzitti. *Ann Transl Med*. 2019;7(13):287-303. 4. Kishnani. *Ann Transl Med*. 2019;7(13):288.

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72

Muscle-Directed IM Approach to Investigational Gene Therapy for Pompe Disease: Preclinical Findings

IM Investigational Gene Therapy

Muscle-Specific Characteristics

- Directly targets distinct muscle units[1]
  - Liver-directed AAV indirectly targets muscle
- Targeting local muscles showed promising results in preclinical studies[1]
  - **Intralingual** rAAV-GAA in GAA-/- mice demonstrated the potential for treating lingual dysfunction[1]
  - **Intradiaphragmatic** AAV-GAA administration addressed respiration in GAA$^{-/-}$ mice, supporting the concept that vector has potential ability to transduce phrenic motor neurons that innervate diaphragm[2,3]

Muscle-Specific Considerations

- Transduction of muscle fibers is limited to the surrounding area of the injection[1,2]
  - Injections at multiple sites may potentially increase transgene expression[1,2]
- Intramuscular injections have been associated with increased transgene immunogenicity[2]

rAAV, recombinant adeno-associated virus.
1. Kishnani. *J Neuromuscul Dis*. 2020;7(1):15-31. 2. Ronzitti. *Ann Transl Med*. 2019;7(13):287-303. 3. Mavilio. *J Neurosci Methods*. 2006;157(2):249-260.

BRAINWEEK

73

Dosing Ranges Explored in Preclinical Studies of Muscle-Directed Investigational AAV Gene Therapies

Recombinant AAV9, MHCK7 Promoter[1,a]

- IM effective dose: At least >4 x 10^{12} vg/kg
- Liver depot effective dose: 2 x 10^{12} vg/kg

Effective doses substantially cleared lysosomal glycogen in skeletal muscle in the same strain of adult Pompe disease mice

Recombinant AAV9, Desmin (DES) Promoter[1]

- IM effective dose: 4 x 10^{13} vg/kg

Dose demonstrated similar efficacy to rAAV9–containing MHCK7 promoter[1,2]

rAAV-9, recombinant adeno-associated virus 9; vg/kg, vector genomes per kilogram of body weight. [a]MHCK7 promoter can induce supraphysiologic GAA activity in skeletal muscle and clear substantial lysosomal glycogen.
1. Kishnani. *Ann Transl Med*. 2019;7(13):288. 2. Salabarria. *J Neuromuscul Dis*. 2020;7(1):15-31.

BRAINWEEK

74

Muscle-Directed IV Approach to Investigational Gene Therapy for Pompe Disease: Clinical Considerations

IV Investigational Gene Therapy

Muscle-Specific Characteristics

Systemically bathes muscle tissue with AAV vector[1]

Muscle-Specific Considerations

Approach requires a high vector dose, which may further increase the risk of anti-GAA antibodies associated with muscle-specific GAA expression[2]

Preclinical Doses for AAV8 with Muscle-Specific Promoter[1,3]

1 x 10^{14} vg/kg IV

3 x 10^{14} vg/kg IV

These doses substantially cleared lysosomal glycogen in the diaphragm, heart, and quadriceps in Pompe disease mice, achieving lysosomal glycogen clearance comparable to wild-type mice[1]

1. Mavilio. Abs 263. *WORLDSymposium* 2020. 2. Ronzitti. *Ann Transl Med*. 2019;7(13):287-303. 3. ClinicalTrials.gov. Gene Transfer Study in Patients With LOPD. Accessed 7/2021. clinicaltrials.gov/ct2/show/NCT04174105.

BRAINWEEK

75

Conclusions

- It is an exciting period for patients with Pompe disease given the recent advances in newer therapeutics for Pompe and the prospect of gene therapies
- The newborn screening program has raised many new questions but also developed new opportunities for understanding disease progression in Pompe disease
- The incidence of Pompe is much higher than previously estimated, but the neuromuscular clinics don't appear to be seeing all these patients. Is this a misdiagnosis problem?
- Much work needs to be done to address anti-GAA immunity that develops in LOPD patients as well as capsid immunity that may preclude AAV-based gene therapy or redosing in AAV-based gene therapy

BRAINWEEK

76