New technologies, novel therapeutics, and building companies to treat rare diseases: A personal history

Professor Harvey Lodish
Whitehead Institute for Biomedical Research
Departments of Biology and Biological Engineering,
Massachusetts Institute of Technology

Although I have helped start several successful biotechnology companies, at heart I am a cell and developmental biologist focused on understanding basic life processes

- 1979 Damon Biotech†
- 1979 BioInformation Associates
- 1981 Genzyme
  Sold to Sanofi for $20.2 billion
- 1983 Arris (now Axys) Pharmaceuticals
- 1993 Millennium Pharmaceuticals
  Sold to Takeda for $9 billion
- 2005 Allozyne†
- 2014 Rubius
- 2017 Tevard

From 2007 to 2016 I was the Founding Chair of the Scientific Advisory Board of the Massachusetts Life Sciences Center, the group charged with oversight of the state's 10- year $1 billion investment in the life sciences.

Since 2007 I have served on the Board of Trustees of Boston Children's Hospital and have been the Chair of the Board Research Committee

In aggregate rare diseases affect an estimated 25 to 30 million people in the United States

- Definition: Any disease or condition that affects fewer than 200,000 people in the United States
- There are more than 6,800 rare diseases
- Similar to the United States, Europe has approximately 30 million people living with rare diseases.
- It is estimated that 350 million people worldwide suffer from rare diseases

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Some examples of rare diseases: Most have been identified in Caucasians

- Cystic Fibrosis
- Phenylketonuria (PKU)
- Muscular Dystrophy
- Tay- Sachs
- Gaucher Disease
- Beta Thalassemia
- Familial Hypercholesterolemia
- Progeria
- Tourette Syndrome
- Severe Combined Immune Deficiency (Bubble boy disease)
- Dravet's Syndrome
- Kleefstra Syndrome

Most rare diseases are of genetic origin and appear early in life

- 80% of rare diseases are genetic in origin, and thus are present throughout a person's life, even if symptoms do not immediately appear
- Approximately 50% of the people affected by rare diseases are children

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Because of intermarriage, every ethnic group has its own constellation of rare genetic diseases.

Iceland:

Population 300,000; founded 1,100 years ago by between 8,000 and 20,000 people mainly from Scandinavia, Ireland and Scotland.

- Recessive frameshift mutation in *MYL4* (myosin essential light chain) causing early-onset atrial fibrillation.
- Mutation in ABDB4 (Multidrug resistance protein 3) increasing risk of gallstones

D. Gudbjartsson et.al., Nature Genetics 47, 435 – 444 (2015)

Finland:

- Mutation in SLC26A2 (Sulfate transporter) causing recessive multiple epiphyseal dysplasia (EDM4/rMED)

J. Hästbacka et. al., Cell 78: 1073 – 1087 (1994)

Many rare diseases can be prevented by prenatal screening of members of at-risk ethnic groups

- They test for common, incurable recessive diseases that present serious health issues or risk of fatality and for which there exists reliable testing methods with definitive carrier status results.
- They test for 16 diseases affecting Ashkenazic (Eastern European) Jews and 16 diseases common in Sephardic (Western European) Jews.
- Tay-Sachs and other genetic diseases have been virtually eliminated in these ethnic groups.

Most rare diseases currently lack appropriate treatment options

- According to the Kakkis EveryLife Foundation, 95% of rare diseases have not one single FDA - approved drug treatment
- Approximately 50% of rare diseases do not have a disease specific foundation supporting or researching the disease
- During the first 25 years of the U. S. Orphan Drug Act (passed in 1983), only 326 new drugs were approved by the FDA and brought to market for all rare diseases combined
- In general, large pharmaceutical companies are not pursuing new drugs for rare diseases, focusing instead on drugs for more common diseases such as diabetes, cancer, and cardiovascular disease.

New types of therapeutics are entering clinical practice and hold great promise for treating many rare diseases

- Small molecule drugs
- Proteins as therapeutics (~1980)
  - Monoclonal antibodies
  - Recombinant therapeutic proteins
- Cell therapies (~2010)
  - Replacement cells (e.g. pancreatic islets)
  - Engineered cells (e.g. red blood cells expressing new proteins; anti-cancer T cells)
- Nucleic acid therapies (~2010)
- Gene therapies (~2010)
- Gene editing (~2020?)

Policies of most U.S. research universities encourage faculty members to become entrepreneurs

- One day per week "Outside Professional Activity"
  - For - profit companies
  - Not - for - profit organizations
- Faculty can consult for and own stock in companies but cannot be an operating officer
- Clear conflict of interest rules

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- Rubius: A potential treatment for Phenylketonuria (PKU)
- Other novel treatments for rare diseases

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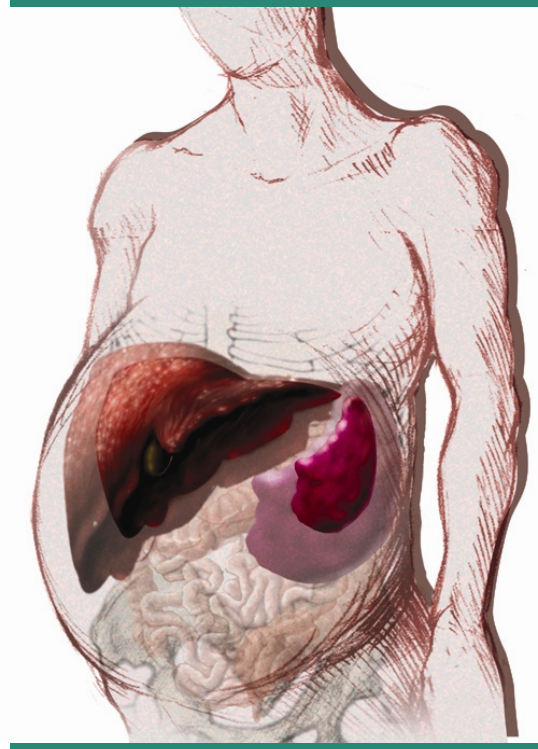
Type I Gaucher Disease

- A lysosome storage disease
- Type I Gaucher is a progressive, debilitating and sometimes life-threatening disease.
- Symptoms can include:
  easy bleeding and bruising, fatigue, anemia, weak bones, bone and joint pain, and enlargement of the spleen or liver.
- Symptoms can appear at any age.



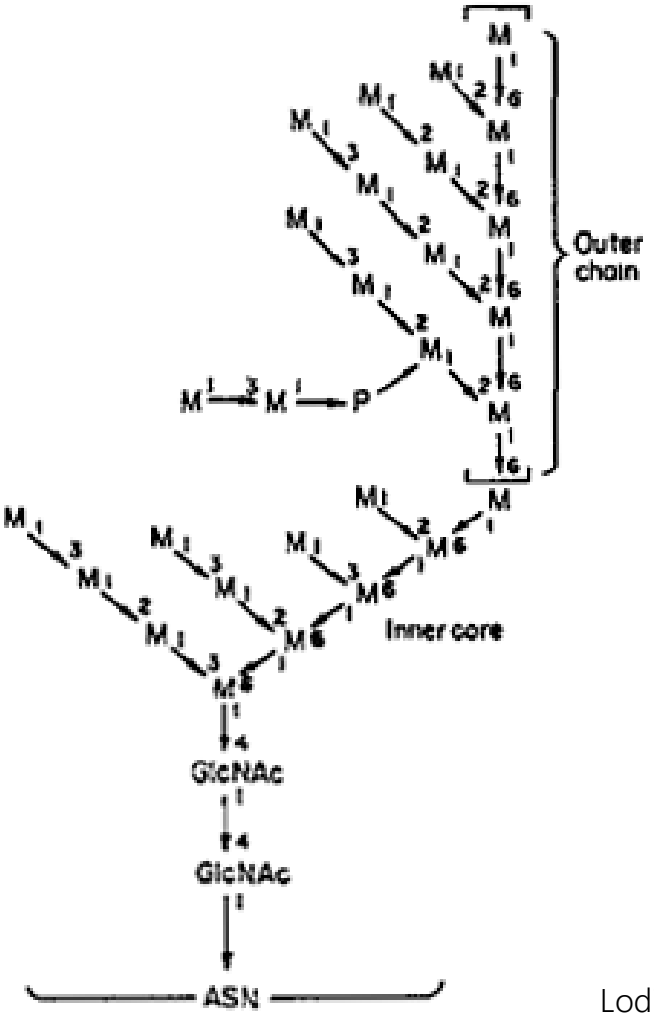
Gaucher Disease primarily affects macrophages

- Macrophage: from Greek *μακρος* (*makros*) = large, *φαγειν* (*phagein*) = to eat. The cells that digest outworn body cells, invading organisms, etc.
- Gaucher disease is the most common lysosomal storage disease – diseases in which one degradative enzyme is missing and the undegraded molecule accumulates in lysosomes
- Autosomal recessive – disease requires two bad copies of the gene
- Deficiency of the lysosomal enzyme β glucocerebrosidase (also called acid β glucosidase)
- Enzyme essential for the degradation of the glycolipid glucocerebroside
- When the enzyme is defective, glucocerebrosides accumulate, particularly in macrophages in the spleen, liver, kidneys, lungs, brain, and bone marrow, and cause symptoms of the disease.

Enzyme replacement therapy for Type I Gaucher Disease

- Replacement enzyme is targeted to macrophages *via* the macrophage mannose receptor, internalized by receptor- mediated endocytosis, and delivered to lysosomes

Structure of asparagine- linked oligosaccharides attached to yeast cell surface proteins

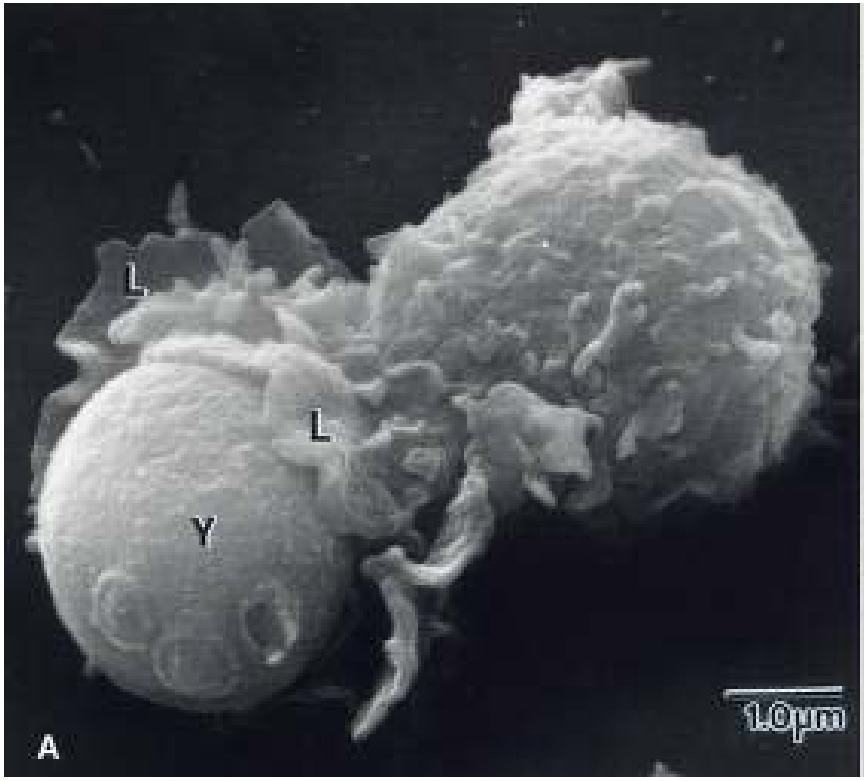


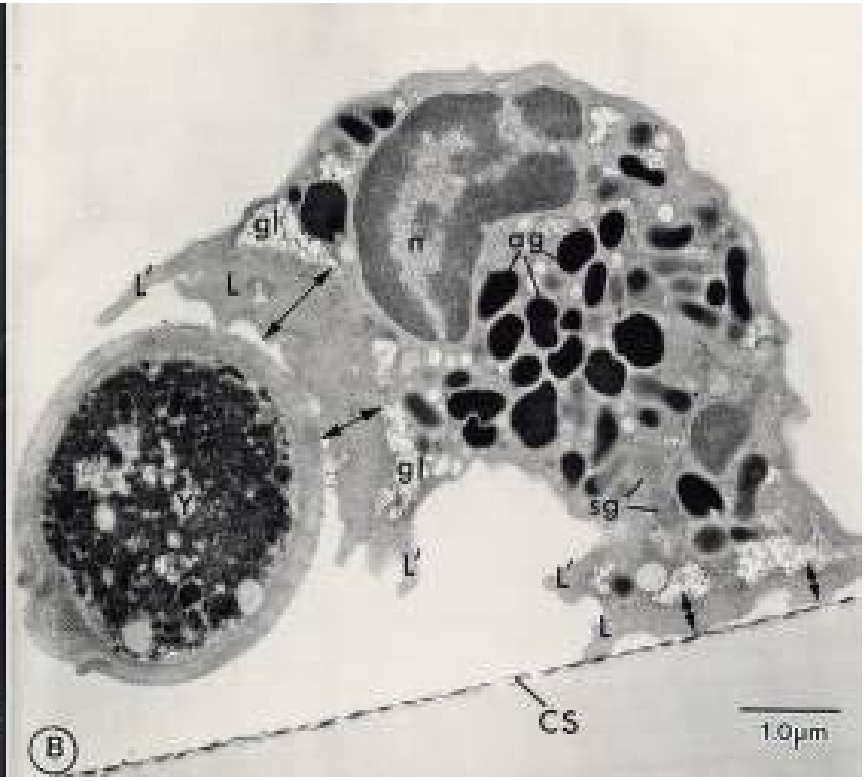
GlcNAc = N Acetyl glucosamine

M = Mannose

Lodish, H.F. Post-translational modification of proteins. Enzyme and Microbial Technology 3: 178-188 (1981).

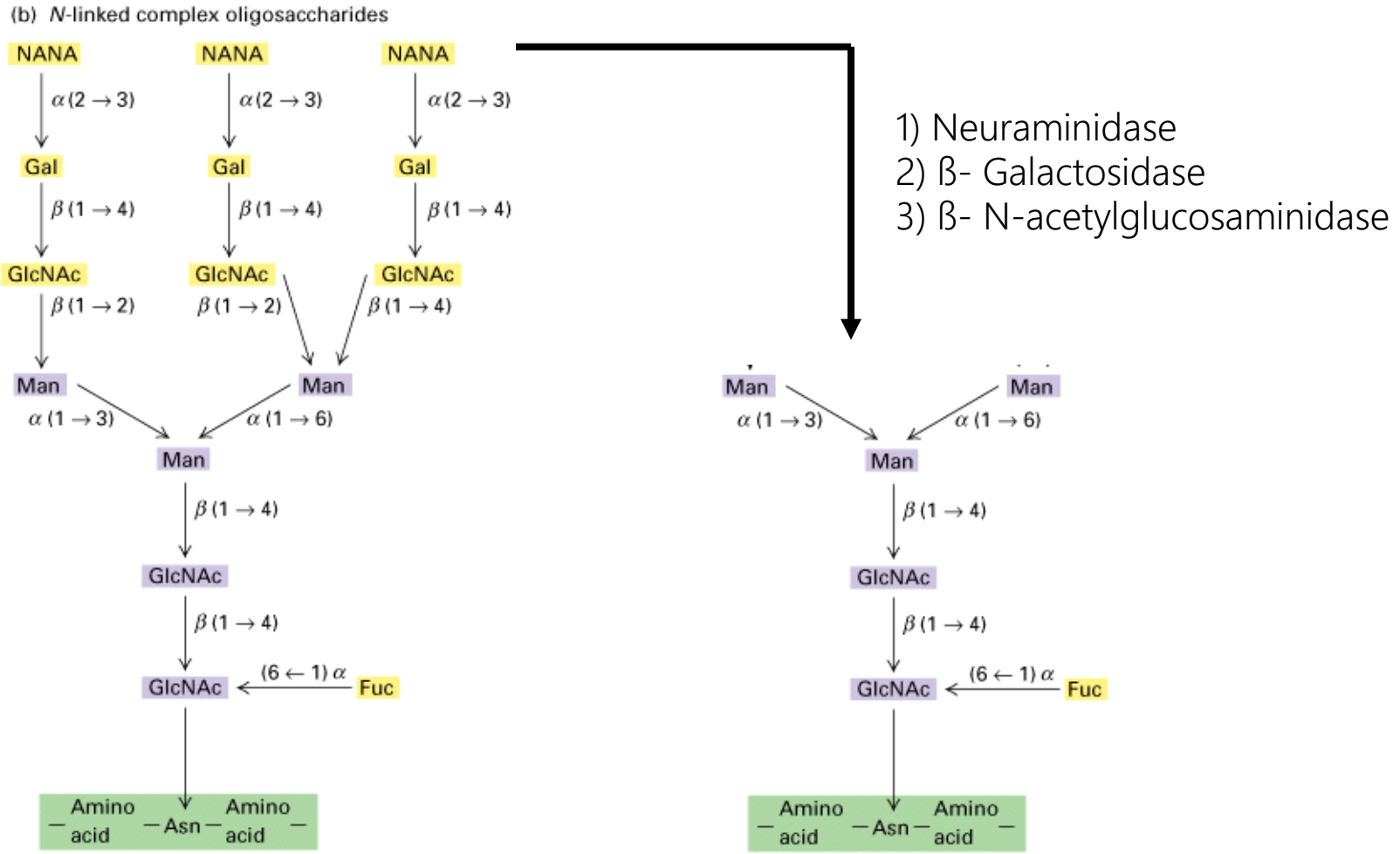
Phagocytosis of yeast cells by macrophages and transfer of the endocytosed particles to lysosomes utilizes macrophage cell surface mannose receptors





ASCB Image & Video Library

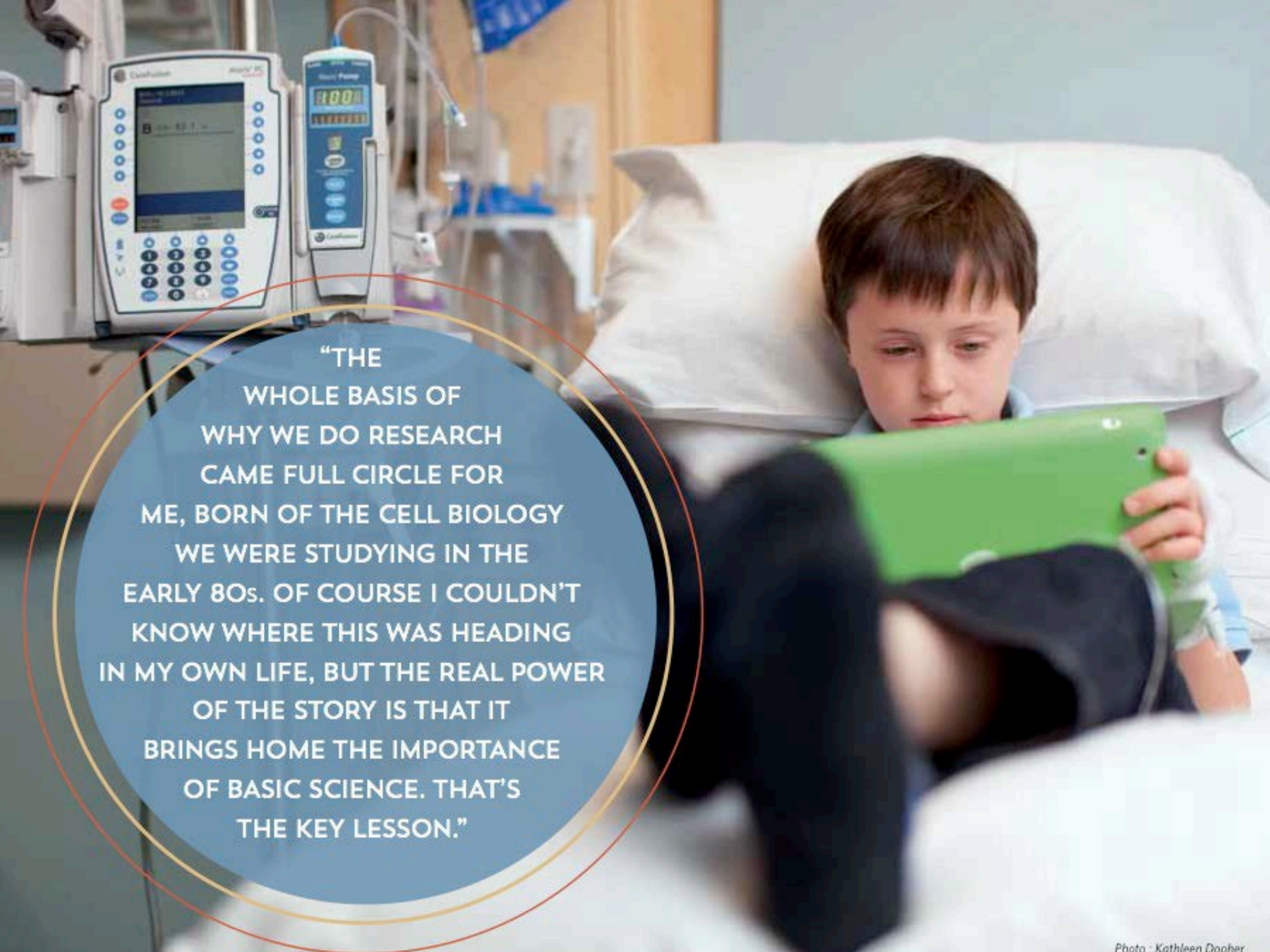
Enzymic formation of the modified mannose- terminal *N*-linked oligosaccharides on Ceredase™ and Cerezyme™ that target injected glucocerebrosidase protein to the macrophage mannose receptor, where it is internalized by endocytosis and transported to lysosomes



Cerezyme: novel technologies 1980- 1989

- A personalized medicine for a rare disease
- A recombinant protein
- A protein targeted to a specific type of cell
- Based on engineering sugars attached to proteins

One of my seven grandchildren has Gaucher Disease,
and is being treated with the Genzyme drug that his grandfather helped develop



"THE WHOLE BASIS OF WHY WE DO RESEARCH CAME FULL CIRCLE FOR ME, BORN OF THE CELL BIOLOGY WE WERE STUDYING IN THE EARLY 80s. OF COURSE I COULDN'T KNOW WHERE THIS WAS HEADING IN MY OWN LIFE, BUT THE REAL POWER OF THE STORY IS THAT IT BRINGS HOME THE IMPORTANCE OF BASIC SCIENCE. THAT'S THE KEY LESSON."

Photo : Kathleen Dooher

- Genzyme: An enzyme replacement for Gaucher Disease
- Rubius: A potential treatment for Phenylketonuria (PKU)
- Other novel treatments for rare diseases

A potential therapy for Phenylketoneuria (PKU) and many other diseases based on genetically modified red blood cells

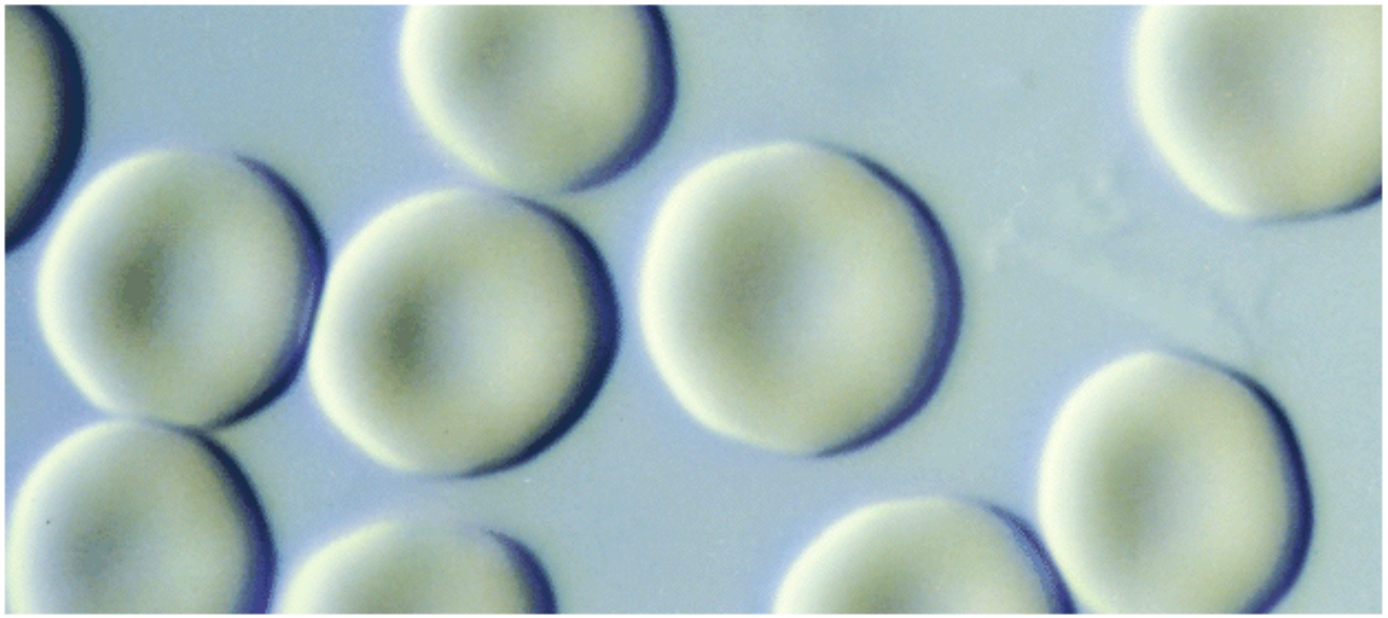
Rubius™ Therapeutics

A Flagship VentureLabs Company

Phenylketonuria (PKU) is an autosomal recessive enzyme deficiency that can lead to irreversible brain damage

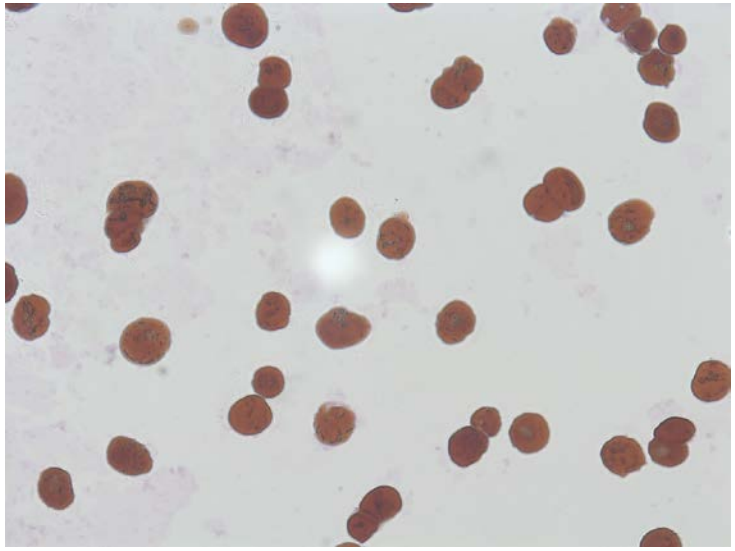
- Diagnosed at birth by a routine blood test
- An autosomal recessive genetic disease
- Deficiency of phenylalanine hydroxylase (PAH), an enzyme that breaks down phenylalanine
- If not properly managed results in nerve damage and intellectual disability
- Mainstay of therapy is dietary restriction of Phe through medical foods (cost: US$60,000 - $100,000/year)

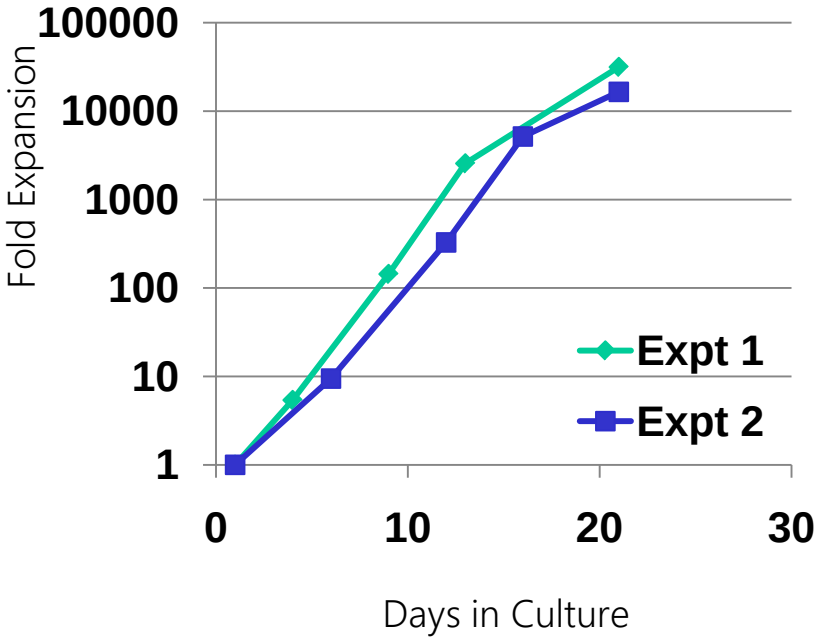
Red cells are attractive microparticles for introducing therapeutics & diagnostics into the human body



- Blood transfusion is a widely used therapeutic
- 7 μm diameter flexible biconcave discs
- Long lifespan: 120 days in blood stream
- Large cell surface area and excellent biocompatibility
- Cytosolic and membrane proteins and metabolism well-characterized
- Genes encoding foreign or chimeric proteins can be ectopically expressed at will in cultured erythroid progenitor cells
- Lack nucleus and mitochondria; no remnants of introduced DNAs

We developed a 21- day culture system for human bone marrow stem cells cells that generates millions of normal red blood cells



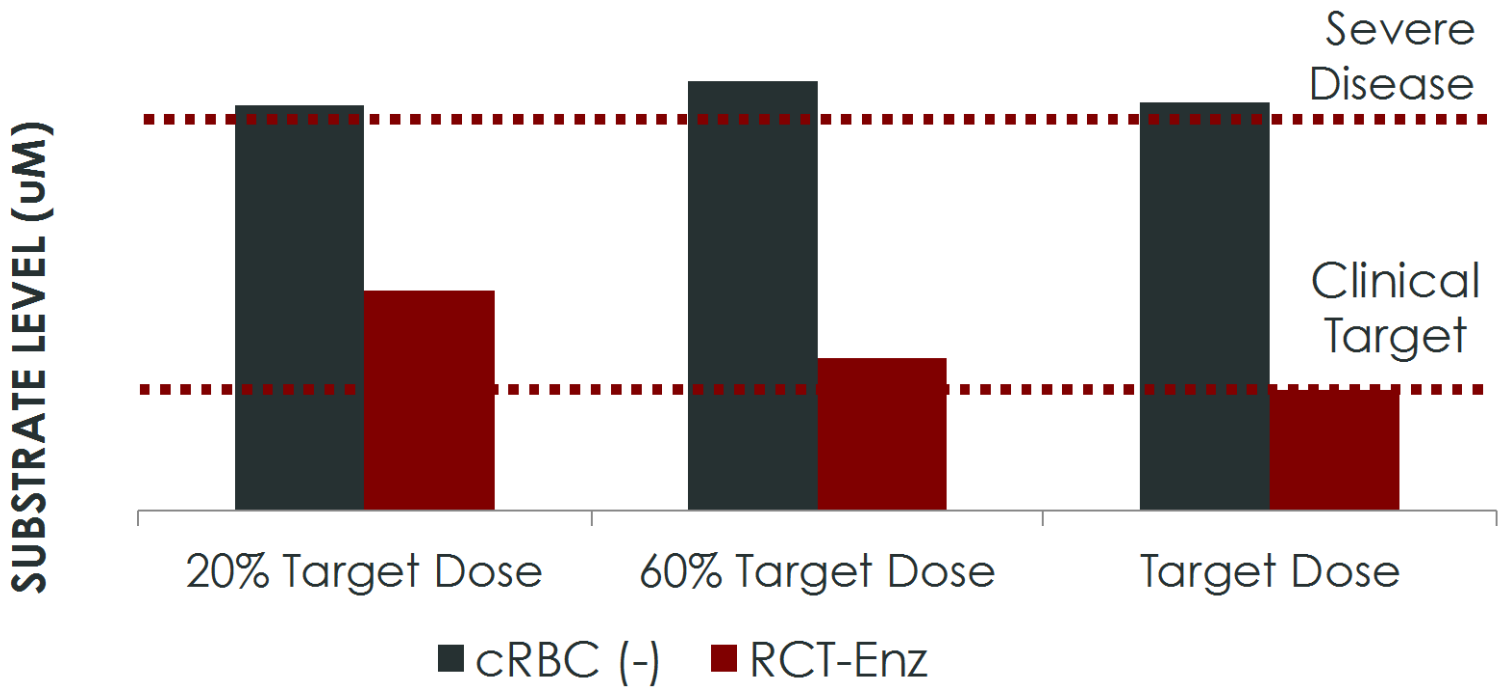


Lee et. al., Nature 522, 474–477 (2015)

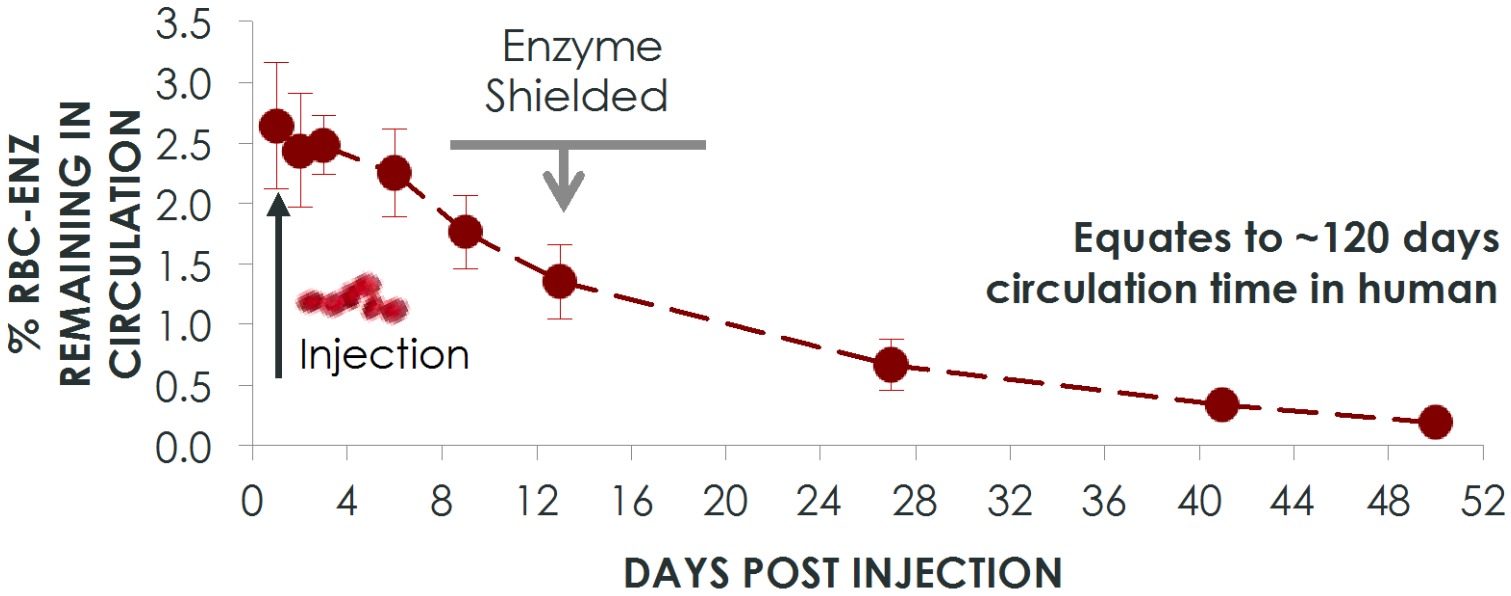
Rubius' solution

- Use recombinant DNA technology to introduce into blood stem cells the gene for a bacterial enzyme that degrades phenylalanine.
- Culture these blood stem cells under conditions where they divide many times and differentiate into otherwise normal red blood cells that contain the phenylalanine- degrading enzyme.
- Transfuse these red cells into a PKU patient.

Human RBC- ENZ degrades excess phenylalanine in human serum



Mouse RBC- ENZs have a normal lifetime in transfused mice



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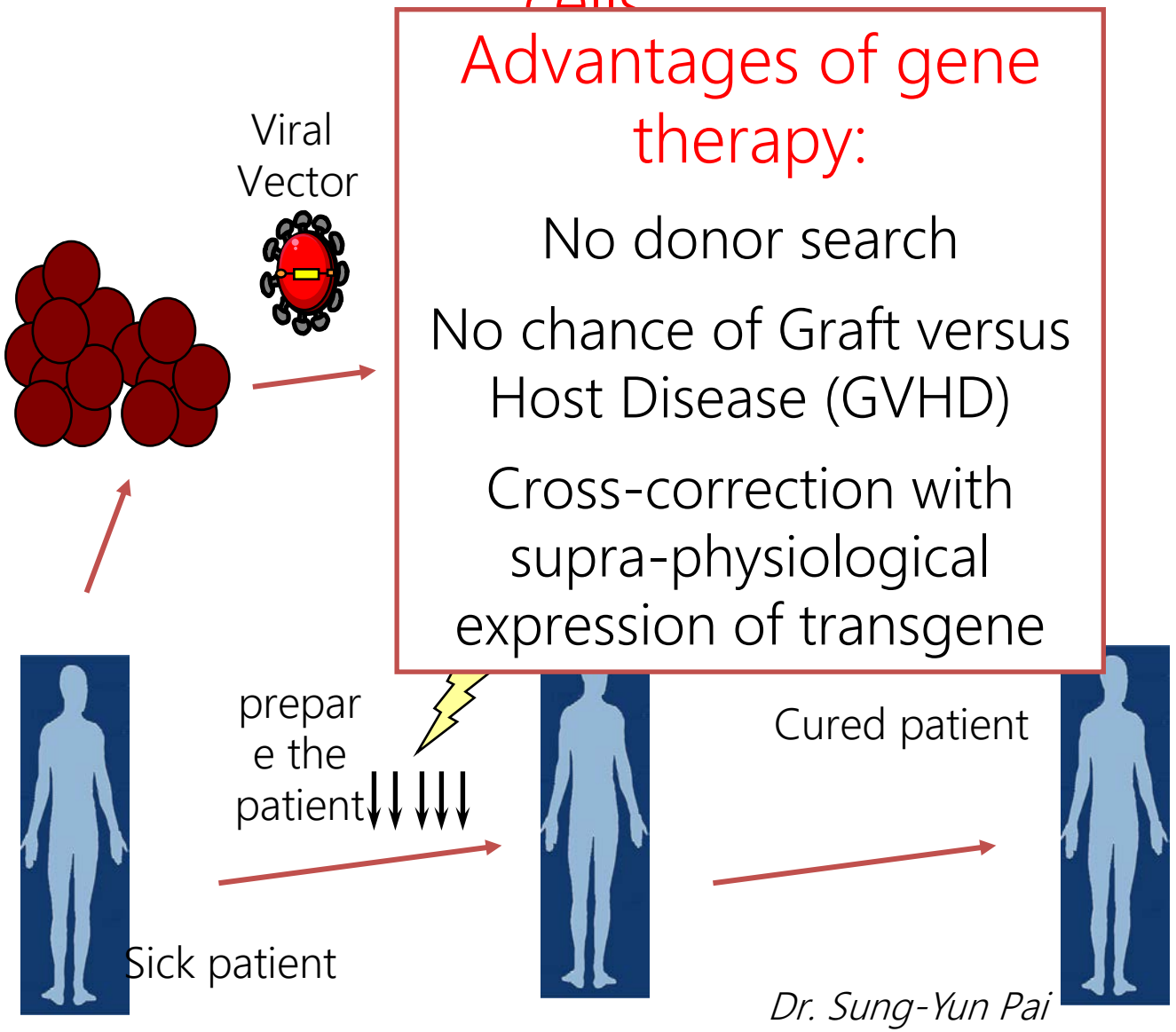
- Other novel treatments for rare diseases: Gene Therapies

Strategy for *ex vivo* gene correction of monogenic diseases using hematopoietic stem cells



Severe Combined Immune Deficiency (SCID)

ORIGINAL ARTICLE

A Modified γ-Retrovirus Vector for X-Linked Severe Combined Immunodeficiency

S. Hacein-Bey-Abina, S.-Y. Pai, H.B. Gaspar, M. Armant, C.C. Berry, S. Blanche, J. Bleesing, J. Blondeau, H. de Boer, K.F. Buckland, L. Caccavelli, G. Cros, S. De Oliveira, K.S. Fernández, D. Guo, C.E. Harris, G. Hopkins, L.E. Lehmann, A. Lim, W.B. London, J.C.M. van der Loo, N. Malani, F. Male, P. Malik, M.A. Marinovic, A.-M. McNicol, D. Moshous, B. Neven, M. Oleastro, C. Picard, J. Ritz, C. Rivat, A. Schambach, K.L. Shaw, E.A. Sherman, L.E. Silberstein, E. Six, F. Touzot, A. Tsytsykova, J. Xu-Bayford, C. Baum, F.D. Bushman, A. Fischer, D.B. Kohn, A.H. Filipovich, L.D. Notarangelo, M. Cavazzana, D.A. Williams, and A.J. Thrasher

ABSTRACT

BACKGROUND

In previous clinical trials involving children with X-linked severe combined immunodeficiency (SCID-X1), a Moloney murine leukemia virus–based γ-retrovirus vector expressing interleukin-2 receptor γ-chain (γc) complementary DNA successfully restored immunity in most patients but resulted in vector-induced leukemia through enhancer-mediated mutagenesis in 25% of patients. We assessed the efficacy and safety of a self-inactivating retrovirus for the treatment of SCID-X1.

METHODS

We enrolled nine boys with SCID-X1 in parallel trials in Europe and the United States to evaluate treatment with a self-inactivating (SIN) γ-retrovirus vector containing deletions in viral enhancer sequences expressing γc (SIN-γc).

RESULTS

All patients received bone marrow–derived CD34+ cells transduced with the SIN-γc vector, without preparative conditioning. After 12.1 to 38.7 months of follow-up, eight of the nine children were still alive. One patient died from an overwhelming adenoviral infection before reconstitution with genetically modified T cells. Of the remaining eight patients, seven had recovery of peripheral-blood T cells that were functional and led to resolution of infections. The patients remained healthy thereafter. The kinetics of CD3+ T-cell recovery was not significantly different from that observed in previous trials. Assessment of insertion sites in peripheral blood from patients in the current trial as compared with those in previous trials revealed significantly less clustering of insertion sites within *LMO2*, *MECOM*, and other lymphoid proto-oncogenes in our patients.

CONCLUSIONS

This modified γ-retrovirus vector was found to retain efficacy in the treatment of SCID-X1. The long-term effect of this therapy on leukemogenesis remains unknown. (Funded by the National Institutes of Health and others; ClinicalTrials.gov numbers, NCT01410019, NCT01175239, and NCT01129544.)

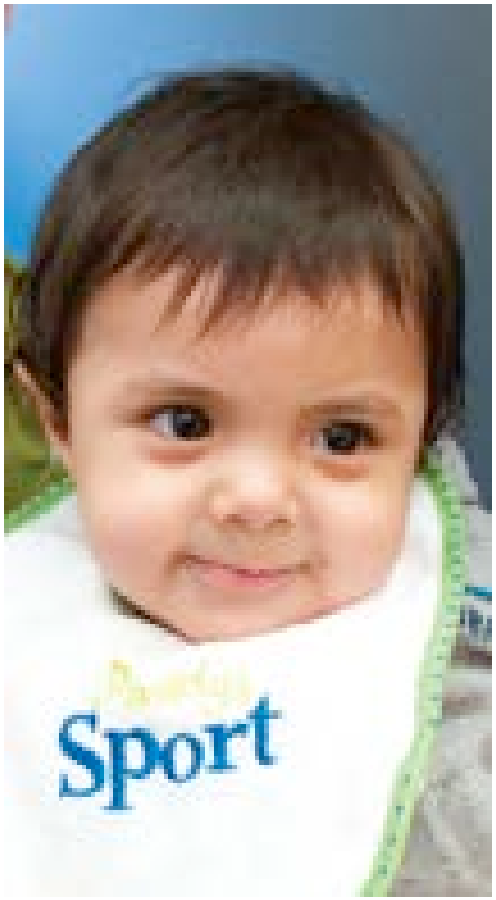
Successful gene therapy treatments for rare hematological diseases at Boston Children's Hospital

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Williams at Boston Children's Hospital, Dana–Farber/Boston Children's Cancer and Blood Disorders Center, 300 Longwood Ave., Karp 08125.3, Boston, MA 02115, or at dawilliams@childrens.harvard.edu; or to Dr. Fischer at Imagine Institute, Hôpital Necker–Enfants Malades, 24 Blvd. Montparnasse, 75014 Paris, France, or at alain.fischer@nck.aphp.fr.

Drs. Hacein-Bey-Abina and Pai and Drs. Bushman, Fischer, Kohn, Filipovich, Notarangelo, Cavazzana, Williams, and Thrasher contributed equally to this article.

N Engl J Med 2014;371:1407-17.
DOI: 10.1056/NEJMoa1404588

A boy with Severe Combined Immune Deficiency (SCID) from Argentina



A couple in Argentina had a boy with SCID, who died from a routine immunization.

They had another boy who was healthy, then A.C. was born June 2010 and diagnosed with SCID

No bone marrow matches in family or in ~17 million donors in the worldwide bank

His doctor reached out to Boston Children's for help and he was enrolled on a trial of gene therapy for X-linked SCID (D.A. Williams Sponsor, S.-Y. Pai PI)

A normal life after gene therapy

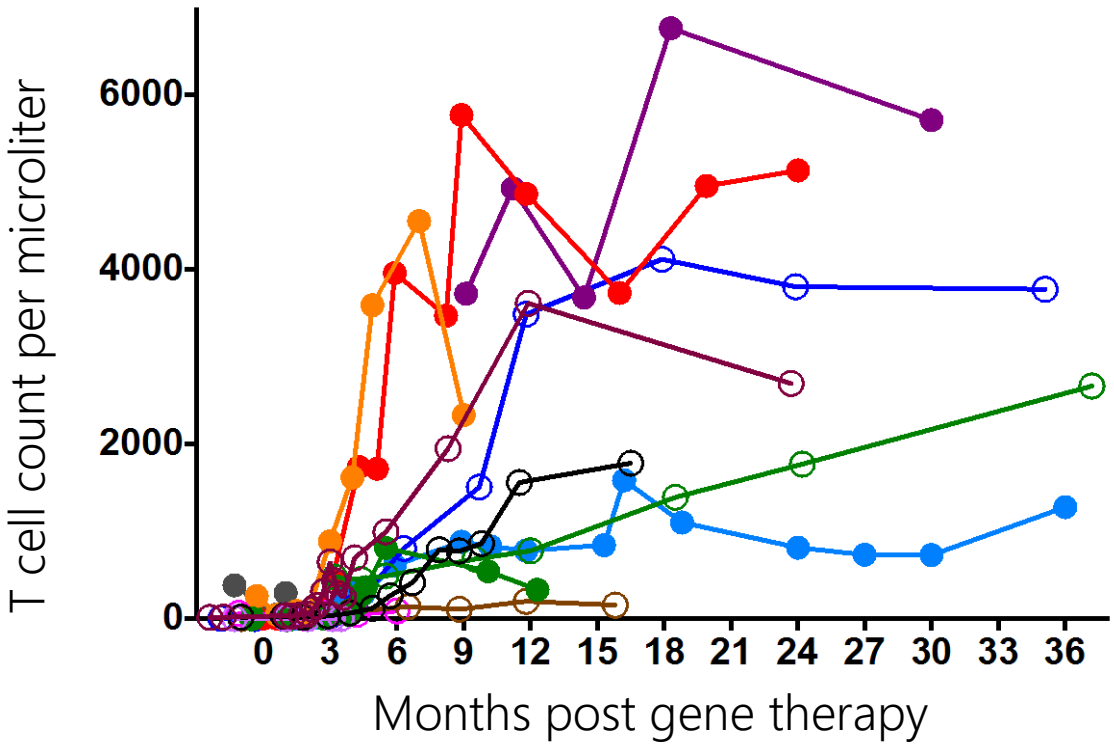
5 months post

2 years post

5 years post

In school, thriving, no serious infections

Gene Therapy for X-linked SCID
Safe and effective



12 of 13 alive
(1 died of pre-existing infection)

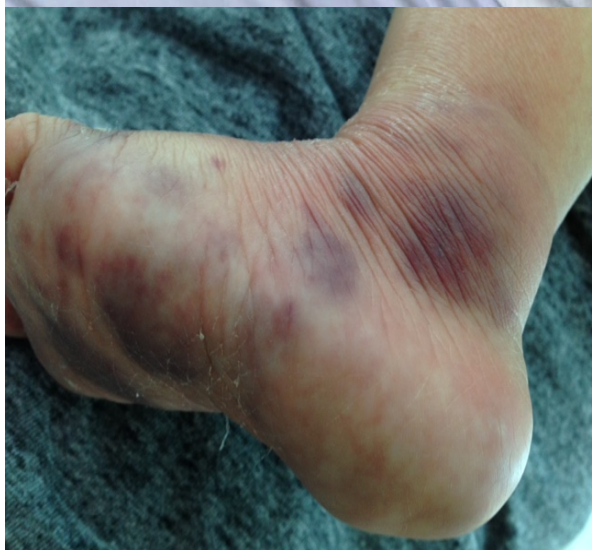
10 of 12 have T cells from gene therapy
(2 had transplant and are well)

No serious infections

No leukemia

2-6 years of follow-

A boy with Wiskott-Aldrich syndrome from Vietnam

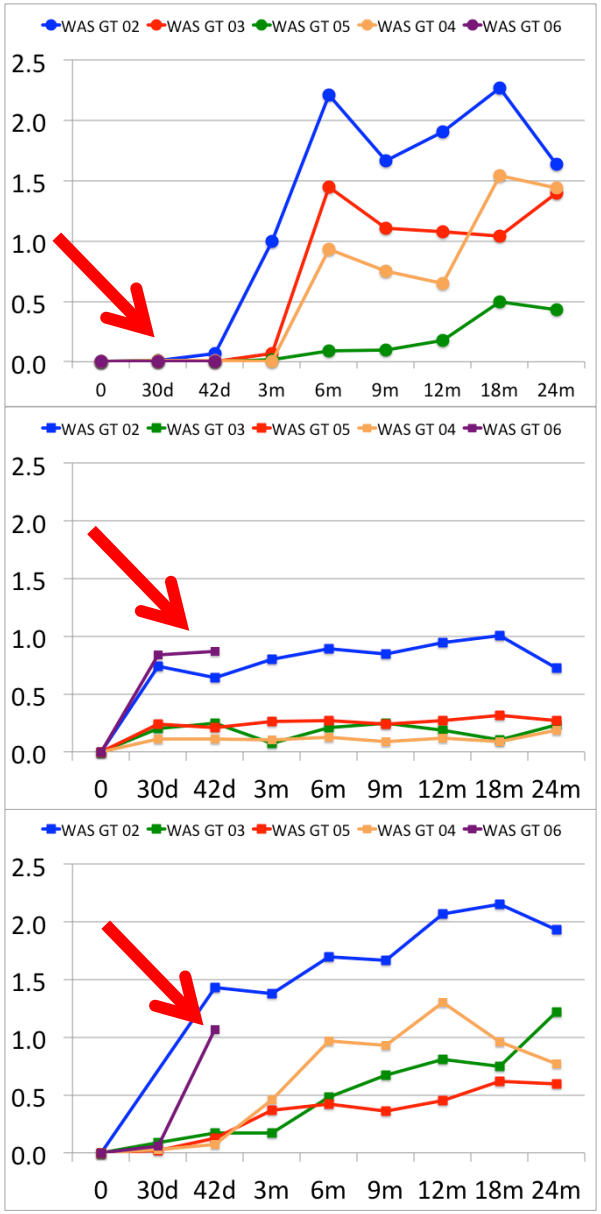


Only child of a physician and bank manager in Vietnam

Diagnosed with Wiskott-Aldrich syndrome, symptoms of GI bleeding, fevers, eczema and vasculitis on the feet, preventing him from walking due to pain and swelling

Enrolled as the 5th and last patient on trial of gene therapy for WAS (D.A. Williams, Sponsor, S.-Y. Pai, PI)

Successful gene therapy into stem cells



No GI bleeding
Platelet transfusion independent
Was off the growth curve, now on
Vasculitis controlled
Took first steps one month ago, 2 months post gene therapy

Among central nervous system rare diseases Dravet Syndrome is a catastrophic epilepsy

- Dravet Syndrome is a rare pediatric epilepsy syndrome encompassing a range of cognitive/developmental delays, drug resistant seizures, and high mortality rates
  - Monogenic disease caused in 75% of cases by a mutation in one copy (haploinsufficiency) of the SCN1a gene that encodes a voltage-gated sodium channel
  - Incidence of Dravet: 1:15,000 to 1:20,000 (7,500 – 15,000 cases in the US) (*Jenna Krueger et al, 2016*)

Most current therapies only try to reduce seizures instead of targeting the root cause of the disease

Tevard Bio is a company developing novel therapeutics for Dravet by targeting its underlying genetic cause

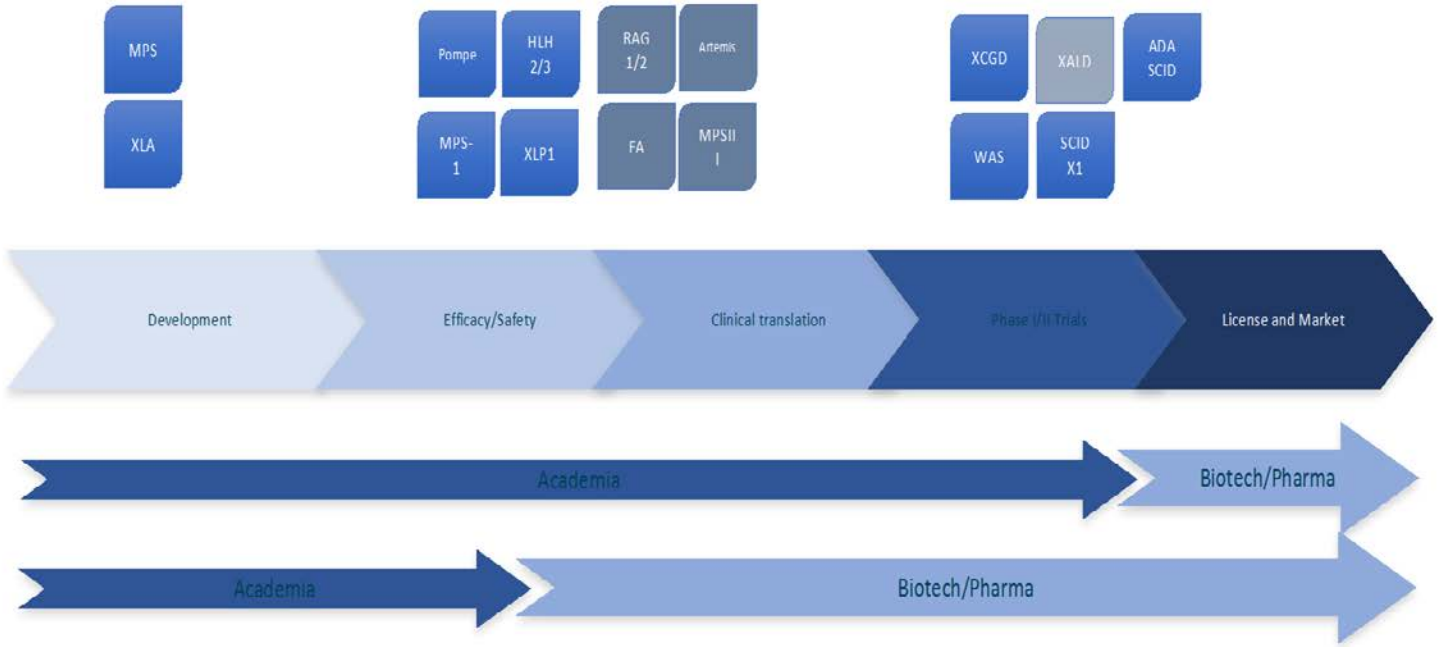
- Founded with my help by two businessmen fathers of children with Dravet Syndrome.
- Our goal is to identify and develop approaches that could potentially lead to permanently or semi-permanently increasing levels of SNC1a by either:
  - Increasing the expression of the healthy SCN1a allele; or
  - Fixing/replacing the mutated copy of SCN1a
- Partnering with world leaders to develop effective gene therapy approaches and delivery methods to the brain

Long term goal is to build a platform to deliver gene therapies for CNS disorders

To date we have identified 3 promising approaches that we are evaluating through pre-clinical testing

Approach	Description
Ectopic delivery of select tRNAs	• Restore expression of the diseased allele of SCN1a through a suppressor tRNA that encodes an amino acid at UGA stop codons • Stabilize the mRNA derived from the normal allele of SCN1A through over expression of several tRNAs
Base editing	• New approach that avoids a double stranded break in repairing base pairs. Greatly improves editing efficiency in cells Addresses point mutations using 3 part process. Converts A's to G's or T's to C's
Orally controlled gene therapy	• Increase the expression of the healthy SCN1a allele through Cas9 attached to a promoter. Activation of Cas9 is controlled through an oral drug (small molecule)

International academic collaborations are driving new medicines in cell and gene therapy



Boston Children's Hospital
Until every child is well

Cincinnati Children's Hospital Medical Center

Necker Enfants Malades

MANCHESTER 1824
The University of Manchester

Mattel Children's Hospital UCLA

MHH Hannover Medical School

Ospedale San Raffaele

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