Catalyzing Translational Innovation

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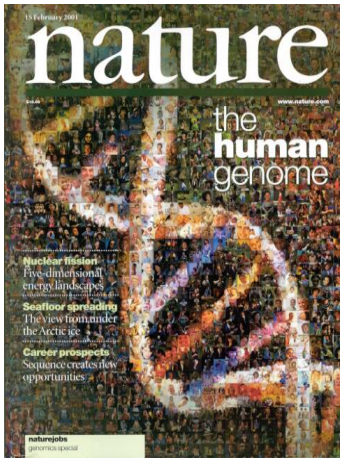
Professional Development Day

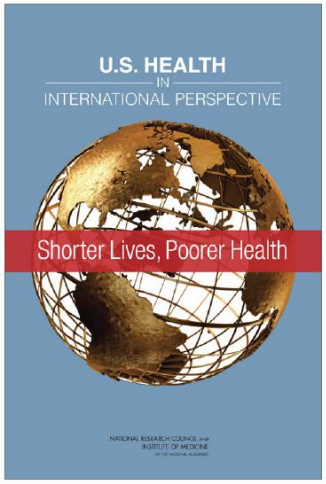
October 13, 2017

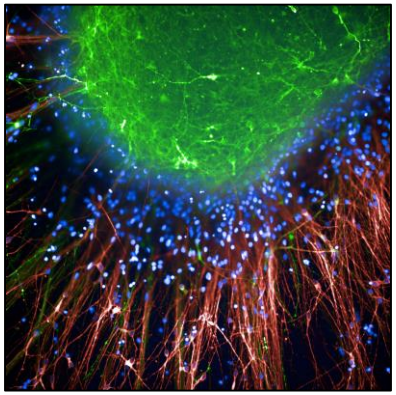
The Best of Times, the Worst of Times

Fundamental science unprecedentedly advanced, but:



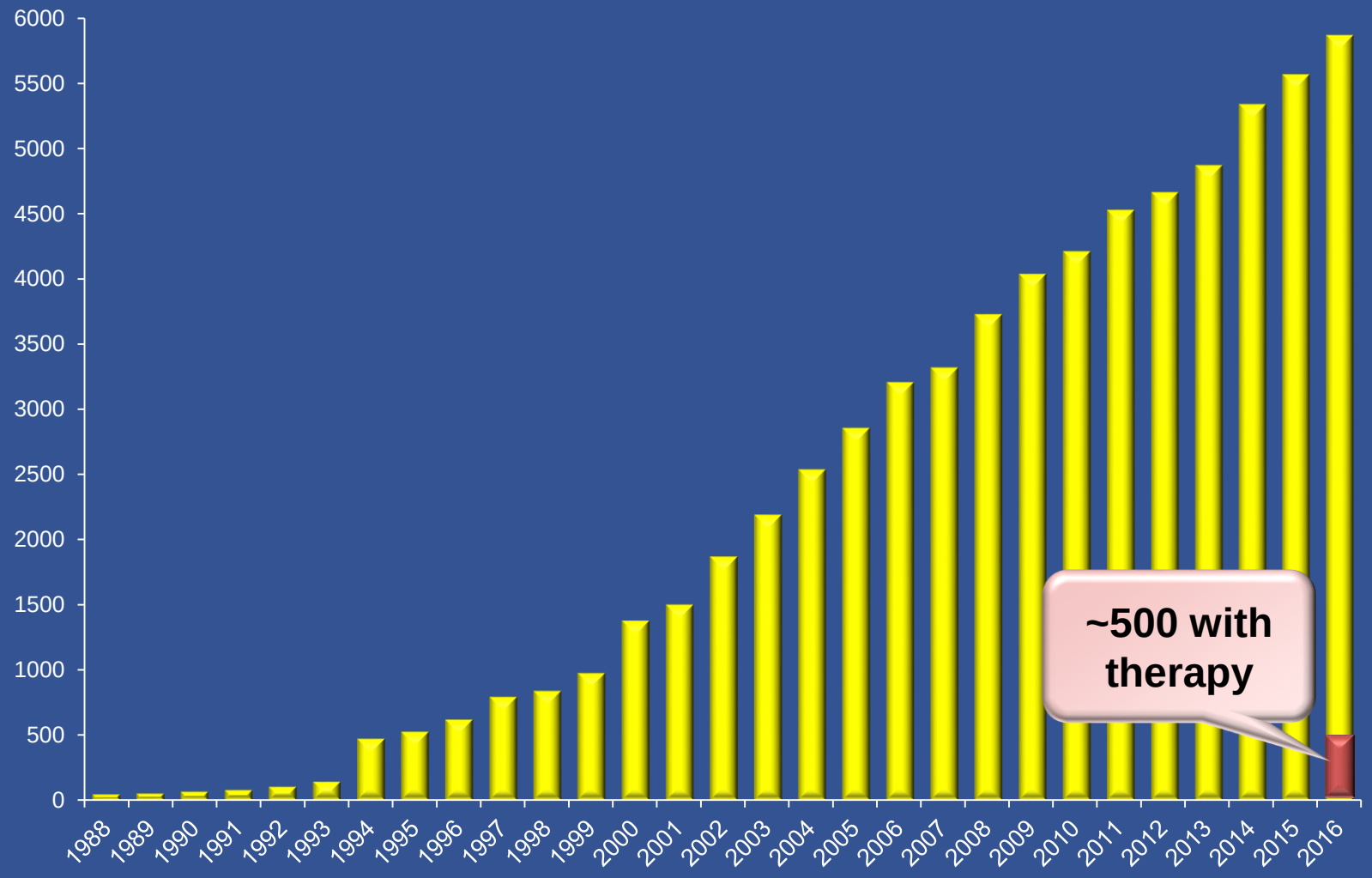
- Poor transition of basic or clinical observations into interventions that tangibly improve human health
- Intervention development failure-prone and expensive
- Poor adoption of demonstrably useful interventions





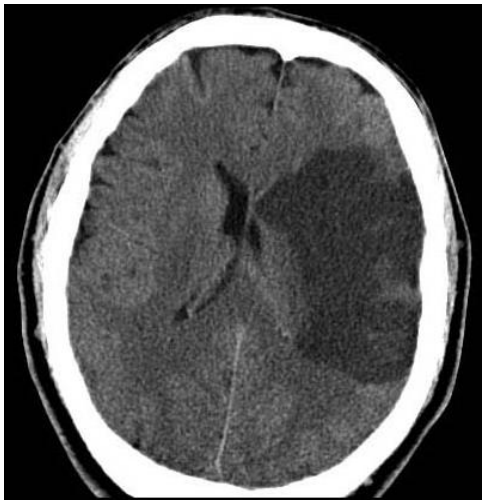
Enormous opportunity/need to deliver on promise of science for patients

Human Conditions with Known Molecular Basis



Source: Online *Mendelian Inheritance in Man*, Morbid Anatomy of the Human Genome

So…



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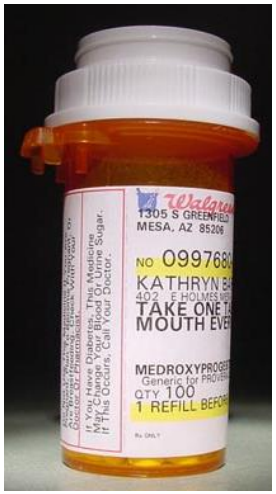

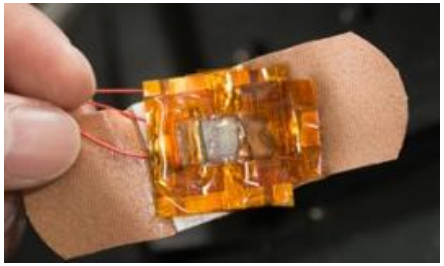

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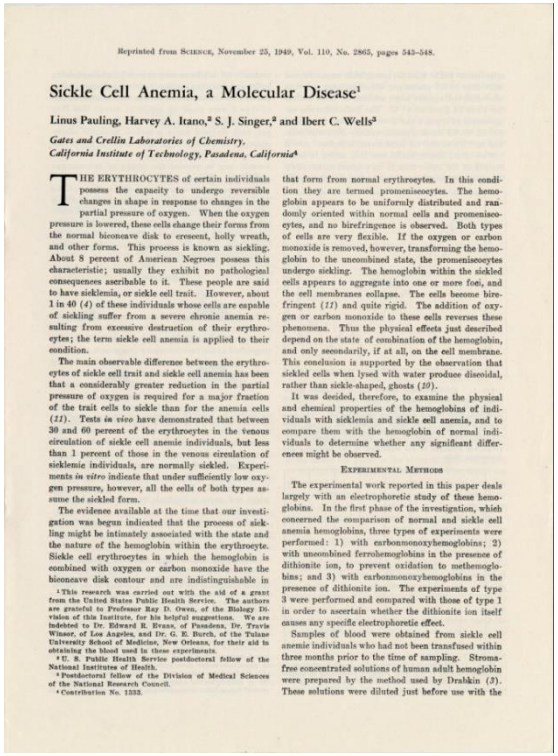

Reprinted from SCIENCE, November 25, 1949, Vol. 110, No. 2865, pages 543–548.

Sickle Cell Anemia, a Molecular Disease[1]

Linus Pauling, Harvey A. Itano,[2] S. J. Singer,[2] and Ibert C. Wells[3]

Gates and Crellin Laboratories of Chemistry,
California Institute of Technology, Pasadena, California[4]

THE ERYTHROCYTES of certain individuals possess the capacity to undergo reversible changes in shape in response to changes in the partial pressure of oxygen. When the oxygen pressure is lowered, these cells change their forms from the normal biconcave disk to crescent, holly wreath, and other forms. This process is known as sickling. About 8 percent of American Negroes possess this characteristic; usually they exhibit no pathological consequences ascribable to it. These people are said to have sicklemia, or sickle cell trait. However, about 1 in 40 (*4*) of these individuals whose cells are capable of sickling suffer from a severe chronic anemia resulting from excessive destruction of their erythrocytes; the term sickle cell anemia is applied to their condition.

The main observable difference between the erythrocytes of sickle cell trait and sickle cell anemia has been that a considerably greater reduction in the partial pressure of oxygen is required for a major fraction of the trait cells to sickle than for the anemia cells (*11*). Tests *in vivo* have demonstrated that between 30 and 60 percent of the erythrocytes in the venous circulation of sickle cell anemic individuals, but less than 1 percent of those in the venous circulation of sicklemic individuals, are normally sickled. Experiments *in vitro* indicate that under sufficiently low oxygen pressure, however, all the cells of both types assume the sickled form.

The evidence available at the time that our investigation was begun indicated that the process of sickling might be intimately associated with the state and the nature of the hemoglobin within the erythrocyte. Sickle cell erythrocytes in which the hemoglobin is combined with oxygen or carbon monoxide have the biconcave disk contour and are indistinguishable in that form from normal erythrocytes. In this condition they are termed promeniscocytes. The hemoglobin appears to be uniformly distributed and randomly oriented within normal cells and promeniscocytes, and no birefringence is observed. Both types of cells are very flexible. If the oxygen or carbon monoxide is removed, however, transforming the hemoglobin to the uncombined state, the promeniscocytes undergo sickling. The hemoglobin within the sickled cells appears to aggregate into one or more foci, and the cell membranes collapse. The cells become birefringent (*11*) and quite rigid. The addition of oxygen or carbon monoxide to these cells reverses these phenomena. Thus the physical effects just described depend on the state of combination of the hemoglobin, and only secondarily, if at all, on the cell membrane. This conclusion is supported by the observation that sickled cells when lysed with water produce discoidal, rather than sickle-shaped, ghosts (*10*).

It was decided, therefore, to examine the physical and chemical properties of the hemoglobins of individuals with sicklemia and sickle cell anemia, and to compare them with the hemoglobin of normal individuals to determine whether any significant differences might be observed.

EXPERIMENTAL METHODS

The experimental work reported in this paper deals largely with an electrophoretic study of these hemoglobins. In the first phase of the investigation, which concerned the comparison of normal and sickle cell anemia hemoglobins, three types of experiments were performed: 1) with carbonmonoxyhemoglobins; 2) with uncombined ferrohemoglobins in the presence of dithionite ion, to prevent oxidation to methemoglobins; and 3) with carbonmonoxyhemoglobins in the presence of dithionite ion. The experiments of type 3 were performed and compared with those of type 1 in order to ascertain whether the dithionite ion itself causes any specific electrophoretic effect.

Samples of blood were obtained from sickle cell anemic individuals who had not been transfused within three months prior to the time of sampling. Stroma-free concentrated solutions of human adult hemoglobin were prepared by the method used by Drabkin (*3*). These solutions were diluted just before use with the

[1] This research was carried out with the aid of a grant from the United States Public Health Service. The authors are grateful to Professor Ray D. Owen, of the Biology Division of this Institute, for his helpful suggestions. We are indebted to Dr. Edward R. Evans, of Pasadena, Dr. Travis Winsor, of Los Angeles, and Dr. G. E. Burch, of the Tulane University School of Medicine, New Orleans, for their aid in obtaining the blood used in these experiments.

[2] U. S. Public Health Service postdoctoral fellow of the National Institutes of Health.

[3] Postdoctoral fellow of the Division of Medical Sciences of the National Research Council.

[4] Contribution No. 1233.

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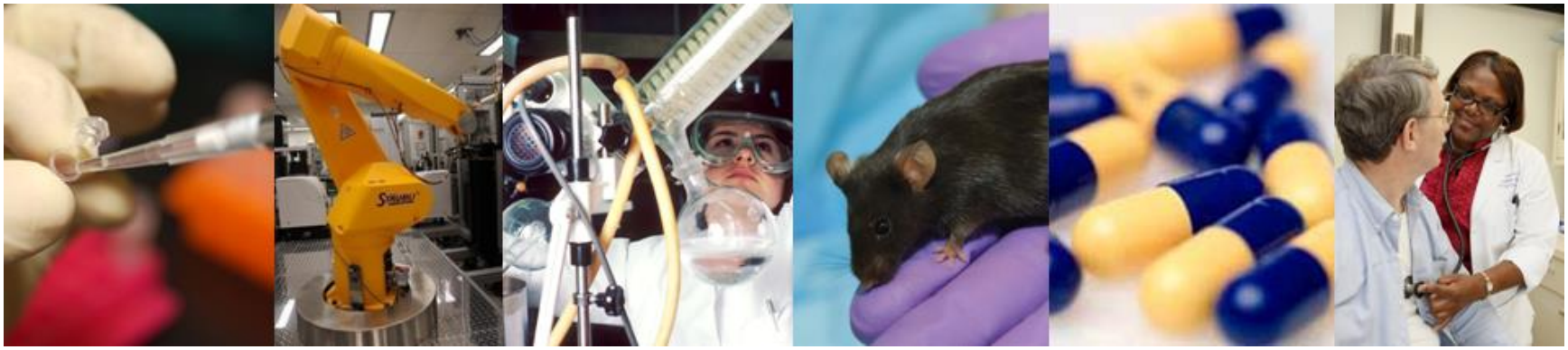
NCATS Mission



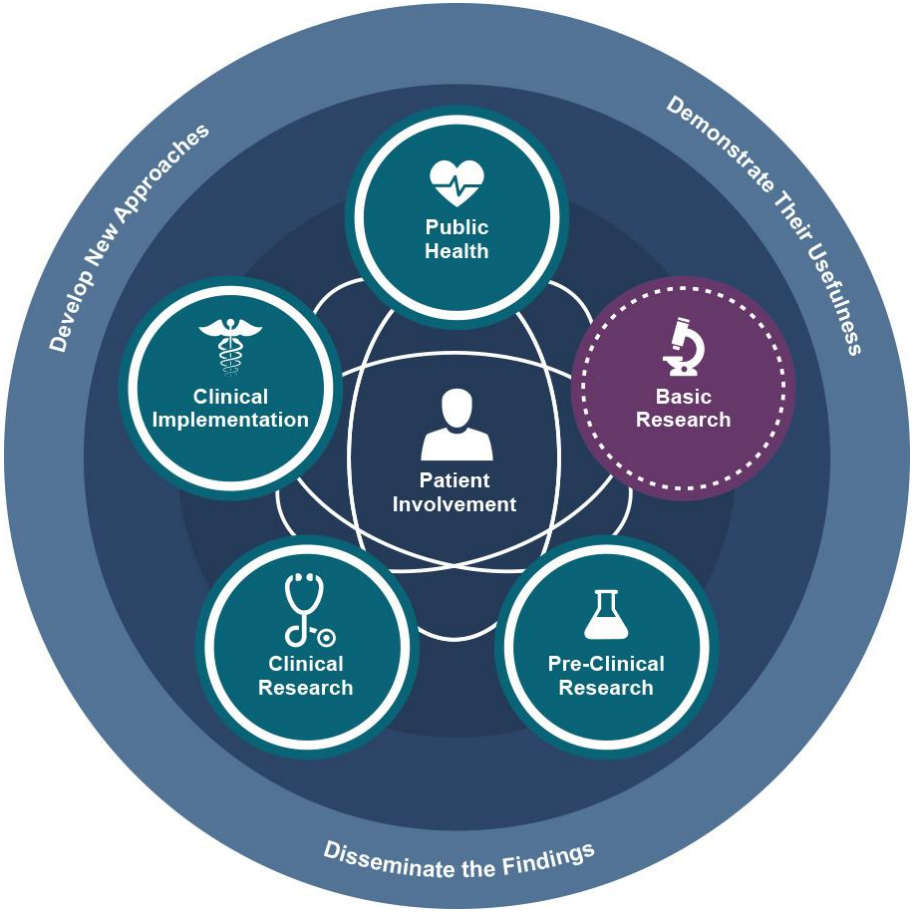
To catalyze the generation of innovative methods and technologies that will enhance the development, testing and implementation of diagnostics and therapeutics across a wide range of human diseases and conditions.

What is Translational Science?

Translational Science is the field of investigation focused on understanding the scientific and operational principles underlying each step of the translational process.

NCATS studies translation as a scientific and organizational problem.

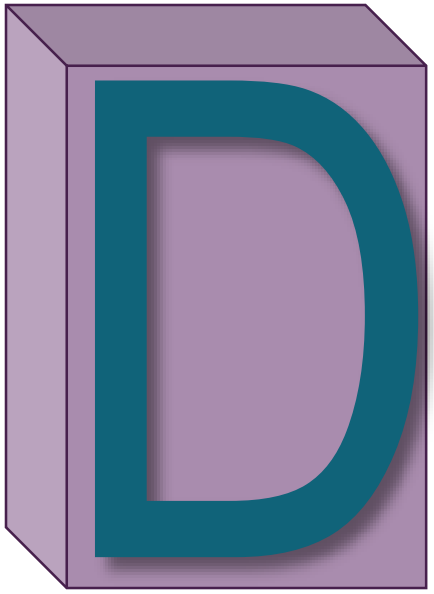
Translational Science Spectrum



Some of the Translational Problems on NCATS' To-do List

- Predictive toxicology
- Predictive efficacy
- De-risking undruggable targets/untreatable diseases
- Data interoperability
- Biomarker qualification process
- Clinical trial networks
- Patient recruitment
- Electronic Health Records for research
- Harmonized IRBs
- Clinical diagnostic criteria
- Clinical outcome criteria (e.g., PROs)
- Adaptive clinical trial designs
- Shortening time of intervention adoption
- Adherence
- Methods to better measure impact on health…

NCATS Modus Operandi: the “3D’s”



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The “Clinical Problem” writ large

“Clinical trials in this country take too long, cost too much, and too often don’t give us the answers we need to take care of our patients. Other than that, the system works great.”

- Rob Califf, M.D.
  Chair of Board, Patient-Centered Research Foundation
  Formerly: FDA Commissioner, Duke CTSA PI, Founding Director of Duke Clinical Research Institute and Clinical Trials Transformation Initiative (CTTI)

- *Cycle time for testing a clinical hypothesis (funding of clinical intervention concept to completion of test of therapeutic hypothesis) in an adequately powered study can easily be >10 years*
- *Time from approval/funding to start of clinical study can easily be two years*
- *Time for recruitment of participants can easily be 3-5 years*
- *Large percentage may be ultimately futile due to inability to recruit, and/or results being irrelevant by time study is completed due to science having moved on*

Enormous losses to health and lives of patients, careers of investigators, and advancement of science and medicine

Some of the scientific translational problems on NCATS' to-do list

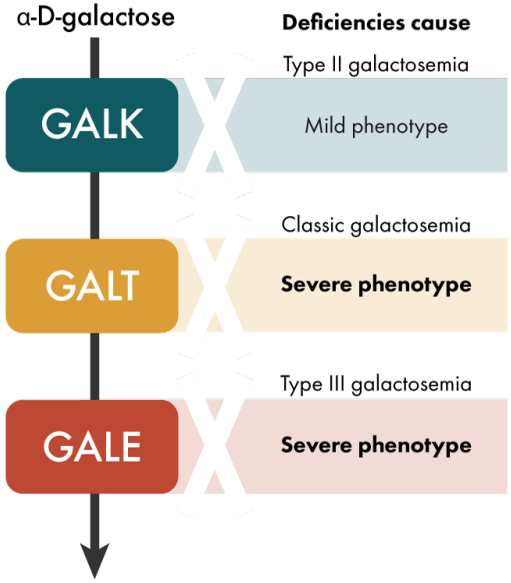
- Predictive toxicology
- Predictive efficacy
- Derisking undruggable targets/untreatable diseases
- Data interoperability
- Biomarker qualification process
- Harmonized IRBs
- Patient recruitment
- Flexible "JIT" clinical research studies
- Electronic Health Records for research
- Clinical diagnostic criteria
- Clinical outcome criteria (e.g., PROs)
- Adaptive clinical trial designs
- Shortening time of intervention adoption
- Methods to better measure impact on health (or lack thereof)

The Scope of Rare Diseases

- ~7000 diseases
  - ~80% Mendelian genetic
  - ~50% onset in childhood
  - ~250 new rare diseases identified each year
- Population prevalence ~8% (US ~25M; EU ~30M, World 350M)
- Definition of “rare disease” varies by country
  - Absolute prevalence: USA <200K; Japan <50K; S Korea <20K…
  - Percentage prevalence: EU <5 in 10K; Australia <1 in 2K…
- <5% of rare diseases have a regulatorily approved treatment
  - USA ~300 diseases
  - At current rate 3–5 newly treatable diseases/yr… >1000 yrs to all

First-in-class GALK Inhibitors for Classic Galactosemia

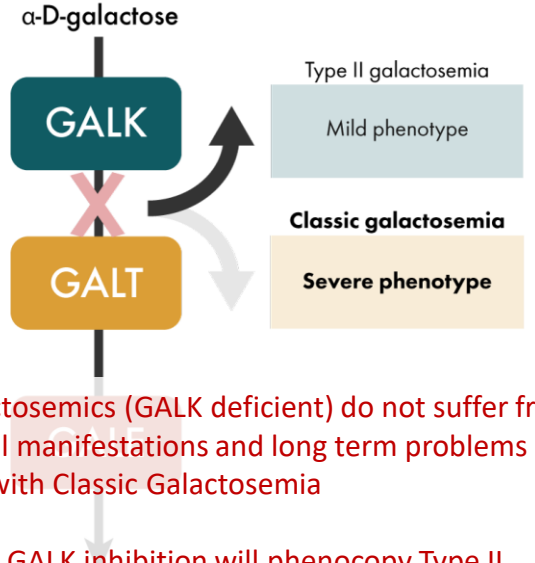
1 Galactosemias: Rare autosomal recessive disorders in which the body cannot properly metabolize galactose



Classic Galactosemia - most common & severe of the galactosemias (~1 in 30,000-60,000 births)

- Results from GALT deficiency
- Lethal without dietary galactose restriction
- Leads to mental deficits, ovarian dysfunction
- No current therapy

2 GALK as a drug target



Type II galactosemics (GALK deficient) do not suffer from same clinical manifestations and long term problems associated with Classic Galactosemia

Hypothesis: GALK inhibition will phenocopy Type II Galactosemia in Classic Galactosemics, leading to milder, more easily manageable disease

3 GALK high-throughput inhibitor screen

Screened 350,000+ compounds for human GALK inhibition → Performed med chem on top active scaffolds → Further refinement to improve ADME/PK

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Hit

GALK IC_{50}: **7.6 uM**
Solubility: **<1 ug/mL**

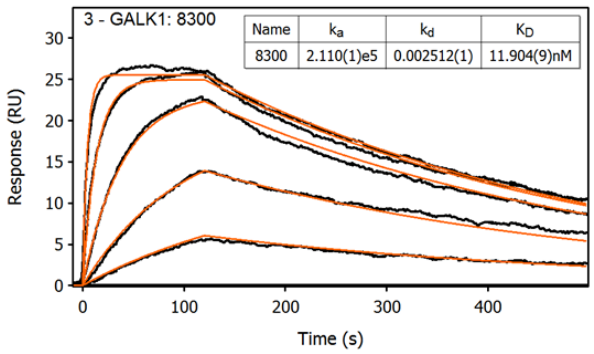
Lead

GALK IC_{50}: **330 nM**
Solubility: **64 ug/mL**

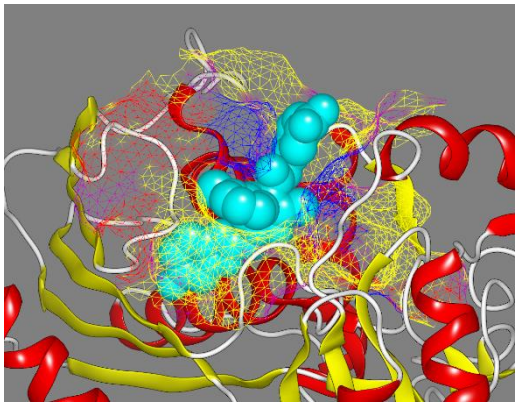
ADME:
Kin. Sol: 64 ug/mL
RLMS $t_{1/2}$: >30 min
MLMS: 93% rem @ 15 min

In vivo PK:
47 mg/kg, IP
$t_{1/2}$: 1.73 hr
Cmax: 226 uM
AUC_{inf} 28,358 h* ng/mL

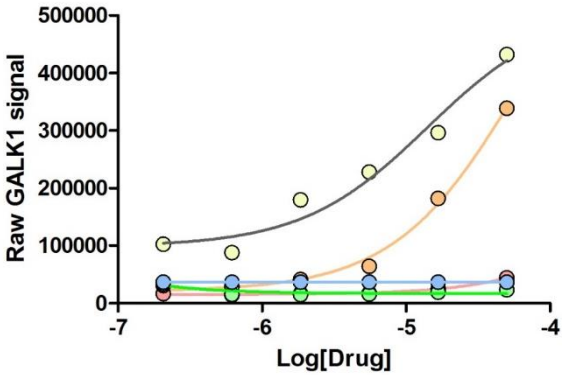
4 Lead characterization & cellular activity



SPR demonstrating high affinity GALK binding of lead

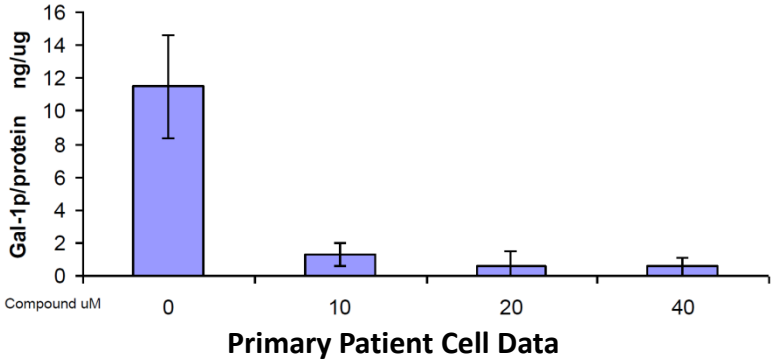


Human GALK co-crystal w/ lead



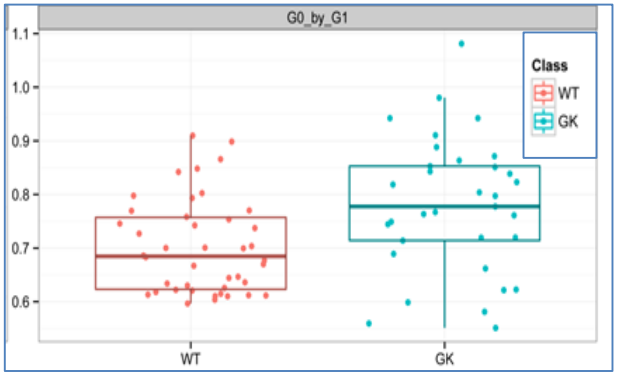
CETSA demonstrating on-target binding of GALK in cells

5 Patient cell activity and upcoming *in vivo* models



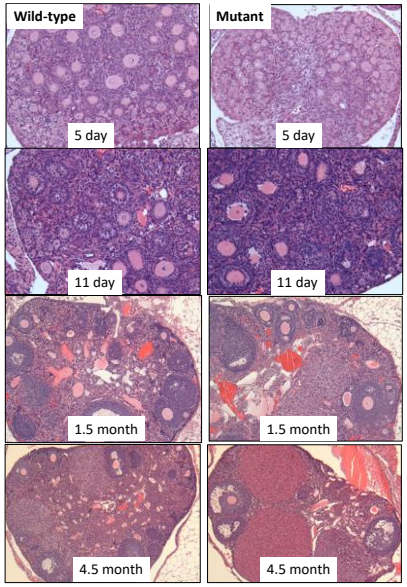
Compounds very effectively lower gal-1-p levels in Classic Galactosemia primary patient fibroblasts with no galactose challenge (clinically relevant assay)

GalT-gene trapped mice



Ratio of non-galactosylated IgG (G0) to mono-galactosylated IgG (G1) in wild type (red boxes) vs GalT-gene trapped (GalT-"knockout") (GK, blue boxes) mice

WT vs mutant mouse ovary histopathology



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Development of platform-based technologies

Example: Gene Therapy

- NCATS Therapeutics for Rare and Neglected Diseases (TRND) Program has established a portfolio of gene therapy development projects to develop and test solutions to common bottlenecks
- Some technologies under development:
  - Manufacturing
    - Plug-and-Play platform manufacturing processes for AAV serotypes
    - Compendium of standard analytical methods
    - Cell suspension technology
    - Cell potentiation method
  - Delivery toolbox
    - Devices for CNS delivery in infants

"Demonstration" Project
Aromatic L-Amino Acid Decarboxylase (AADC) Deficiency

Profound Developmental Failure

- Extremely limited muscle strength, control and movement
- Seizure-like symptoms (oculogyric crises)
- Lifelong care and frequent hospitalizations
- Severe forms have catastrophic course (average life expectancy of 4-8 yrs.)

A Significant, Underdiagnosed Disease

- Estimated global prevalence as high as 4,000-6,000 patients*
  - Founder Mutations in Asia Increase Prevalence
- Misdiagnosis results in likely under-diagnoses: differential diagnoses include cerebral palsy, seizure disorders

**Lee H-CH et al. Clin Chim Acta (2011) and Chien et al., 2016,*

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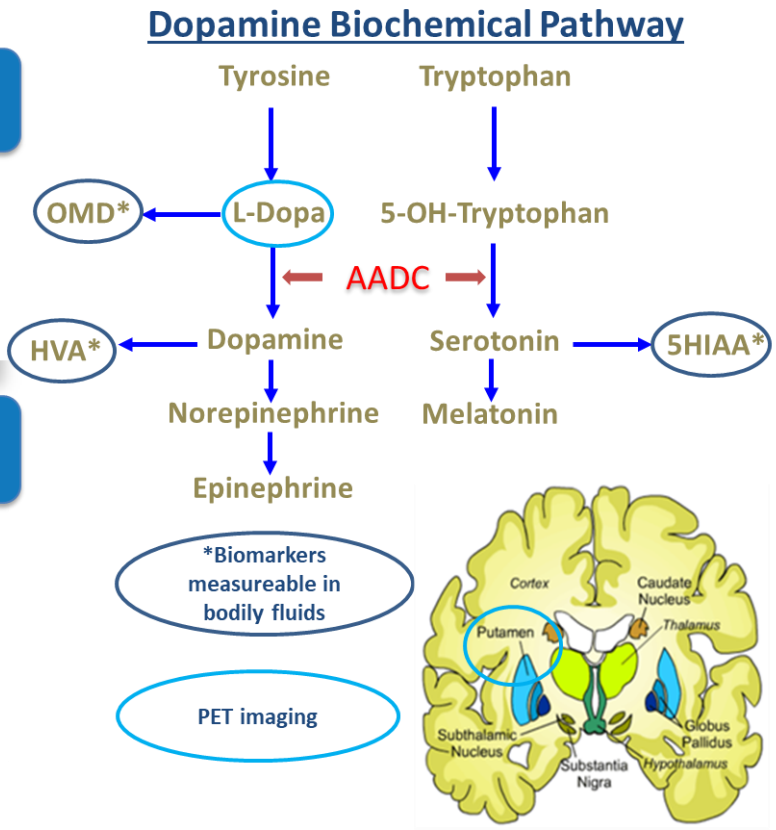
AADC Deficiency is a well-defined disorder amenable to gene therapy

Mutations in DDC Gene Cause Deficiency of the AADC Enzyme

- AADC makes dopamine and serotonin
- Deficiency impairs movement, mood, sleep and cognitive function
- The well-characterized nature of the gene supports use in gene therapy

Multiple Biomarkers for Imaging and CSF, Blood and Urine Tests

- Identify patients for clinical trials
- Monitor gene therapy efficacy
- Provide secondary endpoints for drug registration
- Expand patient population commercially



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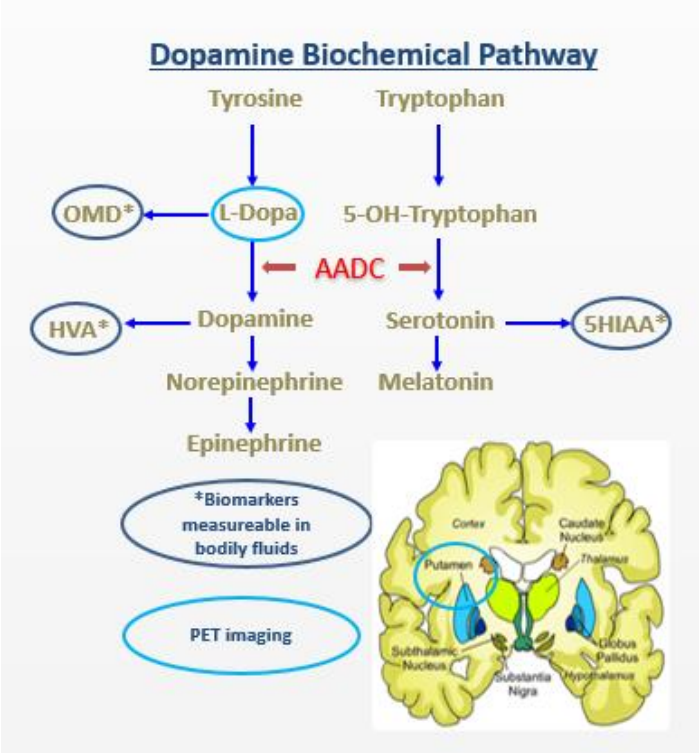
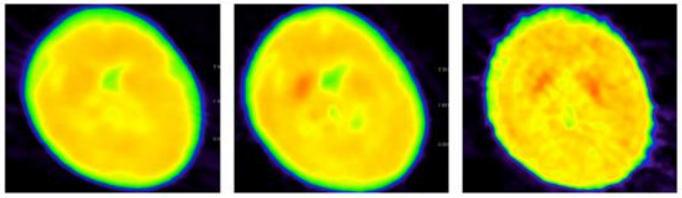
NCATS collaborative project to develop gene therapy for AADC Deficiency

- Collaborators: Agilis Biotherapeutics and NCATS TRND
- Intervention: single dose AAV-hAADC injection into brain (putamen)
- Challenges
  - Ultra-rare disease (underdiagnosed) – small market
  - Stereotactic surgery in infant brains
  - Regulatory: phase 1-2 human data outside of U.S.
  - 18 AADC patients received GT with some remarkable clinical responses
- TRND collaboration catalyzing development of AAV-AADC
  - NCATS collaboration started May 2016
  - GMP grade AAV-AADC manufacturing production
  - GLP animal bio-distribution and toxicology testing
  - Patient finding / epidemiology study
  - Device development
  - Regulatory milestone achieved: successful FDA EOP2 meeting, July 2017
    - No additional clinical studies required for Agilis to file for BLA, expected 2018
    - Currently most advanced CNS gene therapy in development



PET imaging demonstrates *de novo* dopamine production in putamen



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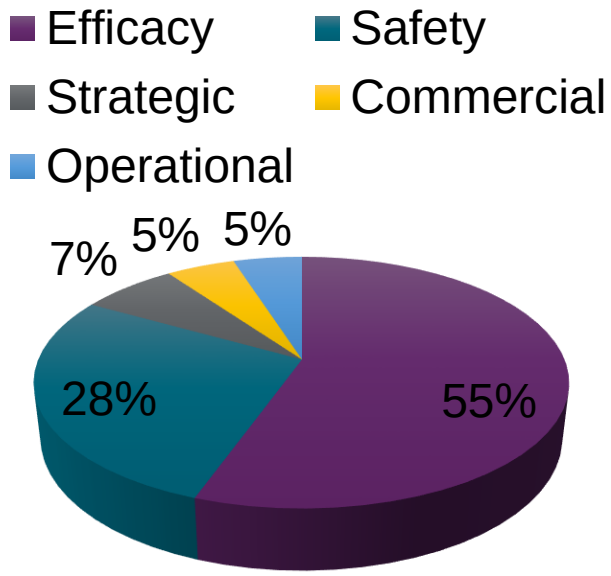
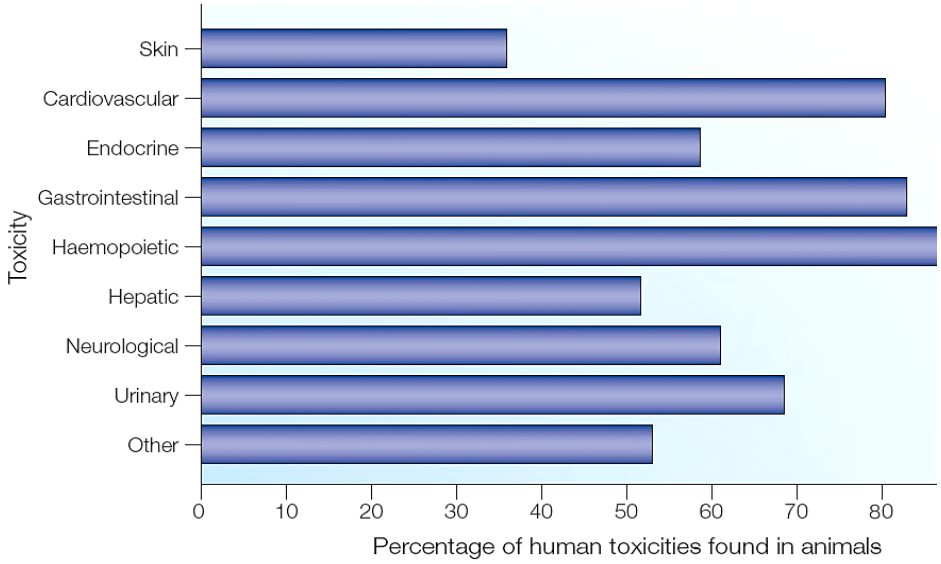
Why Drugs Fail in Development

Drug Failure Modes



Human Toxicities Found in Animals



Arrowsmith and Miller, Nature Reviews Drug Discovery, Volume 12, 569 (2013)

Cook et al., Nature Reviews Drug Discovery, Volume 13, 419 (2014)

Human Tissue Chip Program

Goal: develop biochips to test for safe, effective drugs

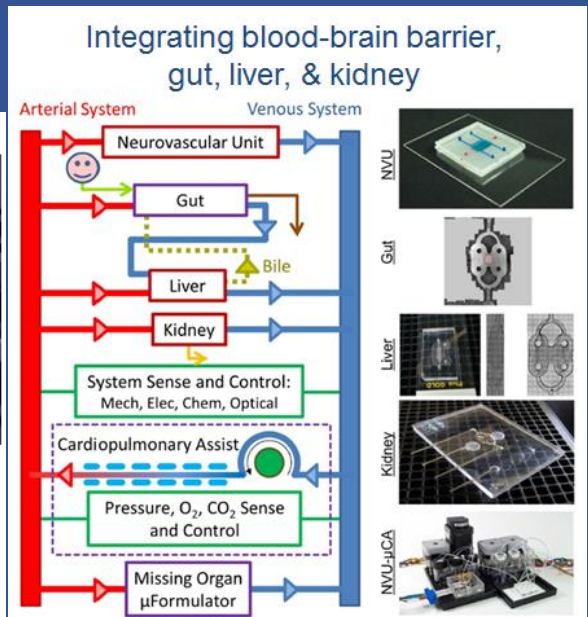
2012-13 → 2013-14 → 2014-15 → 2015-16 → **2016-17**

NIH

Phase 1:
Individual chips

Phase 2:
Cell incorporation and organ integration

- Current focus:
  - Integration (DARPA and NIH); insight/expertise (FDA); compound testing, validation
  - Partnerships (MTA: GSK; Pfizer; AZ; MOU: IQ Consortium)
  - Adoptions of the tech to the community



Barth Syndrome Heart on a Chip Model

ARTICLES

nature medicine

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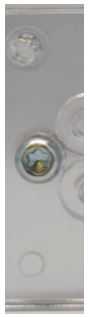

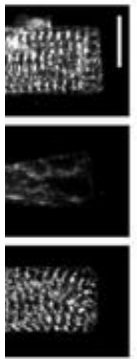

Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies

Gang Wang[1,14], Megan L McCain[2,14], Luhan Yang[2,3], Aibin He[1], Francesco Silvio Pasqualini[2], Ashutosh Agarwal[2], Hongyan Yuan[2], Dawei Jiang[1], Donghui Zhang[1], Lior Zangi[1], Judith Geva[1], Amy E Roberts[1,4], Qing Ma[1], Jian Ding[1], Jinghai Chen[1], Da-Zhi Wang[1], Kai Li[1], Jiwu Wang[5,6], Ronald J A Wanders[7], Wim Kulik[7], Frédéric M Vaz[7], Michael A Laflamme[8], Charles E Murry[8–10], Kenneth R Chien[11], Richard I Kelley[12], George M Church[2,3], Kevin Kit Parker[2,13] & William T Pu[1,13]

Study of monogenic mitochondrial cardiomyopathies may yield insights into mitochondrial roles in cardiac development and disease. Here, we combined patient-derived and genetically engineered induced pluripotent stem cells (iPSCs) with tissue engineering to elucidate the pathophysiology underlying the cardiomyopathy of Barth syndrome (BTHS), a mitochondrial disorder caused by mutation of the gene encoding tafazzin (*TAZ*). Using BTHS iPSC-derived cardiomyocytes (iPSC-CMs), we defined metabolic, structural and functional abnormalities associated with *TAZ* mutation. BTHS iPSC-CMs assembled sparse and irregular sarcomeres, and engineered BTHS 'heart-on-chip' tissues contracted weakly. Gene replacement and genome editing demonstrated that *TAZ* mutation is necessary and sufficient for these phenotypes. Sarcomere assembly and myocardial contraction abnormalities occurred in the context of normal whole-cell ATP levels. Excess levels of reactive oxygen species mechanistically linked *TAZ* mutation to impaired cardiomyocyte function. Our study provides new insights into the pathogenesis of Barth syndrome, suggests new treatment strategies and advances iPSC-based *in vitro* modeling of cardiomyopathy.

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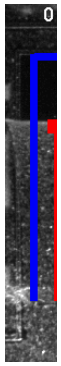

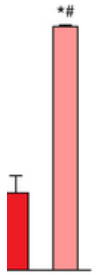

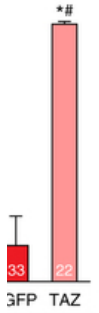

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