# Development of an Oral Treatment for Galactosemia

## EDUCATIONAL EVENT

- Thursday, October 17, 2019
- 12:45pm – 2:00pm

# Welcome

- Lunch boxes are available in the Foyer

# Introduction

**Riccardo Perfetti, MD, PhD**
Chief Medical Officer | Applied Therapeutics Inc.

# Today's program

| | | |
|---|---|---|
| 12:30pm | Lunch | |
| 12:45pm | Introduction | Riccardo Perfetti |
| 12:50pm | Clinical presentation of Classic Galactosemia | Jerry Vockley |
| 1:10pm | Biology and biochemistry of Classic Galactosemia | Gerard Berry |
| 1:25pm | Preclinical evidence and clinical development of a novel oral compound to prevent complications of Classic Galactosemia | Riccardo Perfetti |
| 1:40pm | Questions & Answers | All |
| 1:50pm | Conclusions | Riccardo Perfetti |

# Our faculty

**Riccardo Perfetti, MD, PhD**
Applied Therapeutics Inc.

**Jerry Vockley, MD, PhD**
UMPC Children's Hospital,
University of Pittsburgh

**Gerard T. Berry, MD**
Boston Children's Hospital and
Harvard Medical School

# Clinical Presentation of Galactosemia

**Jerry Vockley, M.D., Ph.D.**
Director, Center for Rare Disease Therapy
Chief Division of Medical Genetics
University of Pittsburgh Medical Center – Children's Hospital Pittsburgh

## Galactosemia

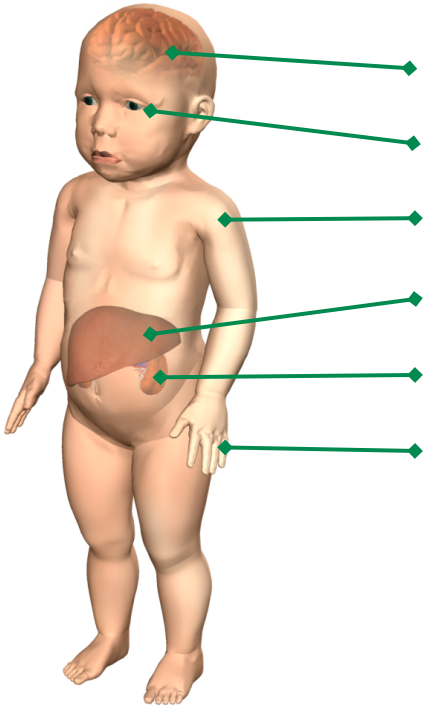
- Rare autosomal recessive disorder which impacts normal metabolism of the sugar galactose, a component of lactose (normally further metabolized to glucose)
- Cause: Mutation/deletion in one of three enzymes that are involved in the normal metabolism of galactose to glucose (GALK, GALT, GALE)
- Incidence: ~1 in 60,0000. Estimated 2,800 individuals in US
- Consequence: Supra-physiologic levels of galactose, galactitol and Gal-1-Phosphate (Gal-1P)

## Clinical Presentation

- **Acute** – Neonatal Galactosemia (newborns and infants)
- Potentially life threatening if not identified and managed immediately
- **Chronic** – (childhood through adulthood)

- Result of long-term exposure to galactose and metabolites despite restriction of dietary lactose
- Endogenous production of galactose maintained
- Long term neurologic, cognitive deficits and other pathologies

# Acute Galactosemia – (Neonatal period)



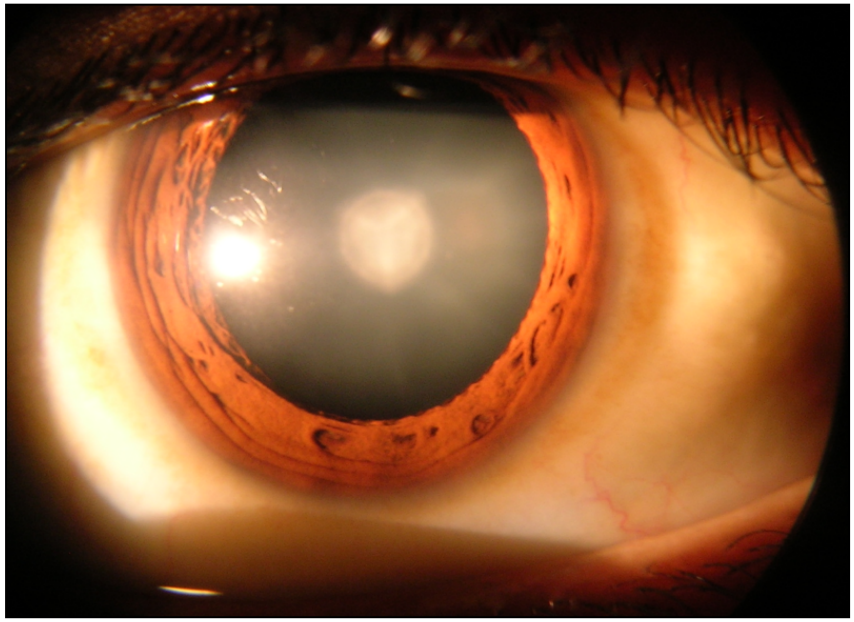
- **Brain damage, pseudotumor cerebri**
- **Cataracts**
- **Jaundice**
- **Enlarged liver and liver failure**
- **Kidney damage** (renal Fanconi syndrome)
- **± Hemolysis**

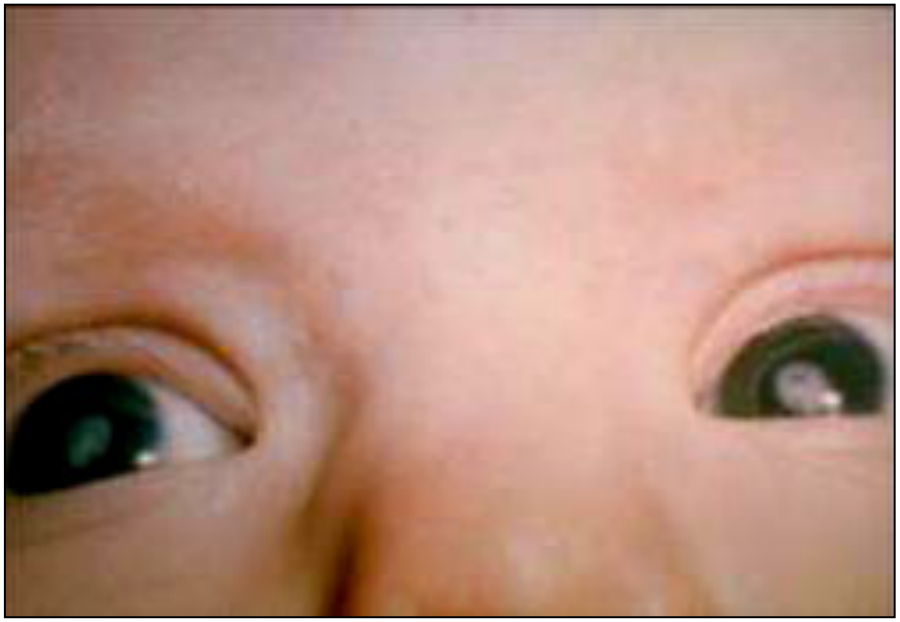
Gram negative sepsis

## Neonatal (Acute) Galactosemia

- Symptoms triggered by lactose/galactose in diet
- Severe forms may present prior to newborn screening
- Stop milk feeding immediately in an ill infant in whom you are considering Galactosemia
- Replace milk with a galactose-free diet

# Acute Galactosemia - Cataracts





# Clinical Case Study: Neonatal Galactosemia

- Birth: Full-term white female, birth weight = 2,400g
- Day #1–4
  - Alert and active
  - Feeding well
  - Breastfeeding + Enfamil
- Day #4
  - Jaundice = 12.0 mg% Bilirubin (205.2 µmol/L) & direct = 1.0 mg% (17.1 µmol/L)
- Day #5
  - Sepsis work-up:
  - ↓ feeds,
  - emesis post feeds
- Day #6
  - Formula changed to Similac. Jaundice persists, feeds poorly, IV fluids continued

# Clinical Case Study: Neonatal Galactosemia

- Day #8
  - Listless, appears dehydrated despite IV fluids only off 6 hours
  - Liver edge down 3 cm
  - Palpable spleen tip
- **What test can be done at the bedside?**
  - Urinalysis
  - Urine reducing substances

STOP galactose and check for spot test

## Subsequent Course

- Sepsis evaluation repeated — WBC 20,000 (20 x 109/L)
- Total bilirubin 18 mg%, direct 10 mg% (total 308 µmol/L, direct 171)
- SGOT 375 U/L (6.3 µkat/L)
- Urine reducing substances positive
- Blood culture from 3 days ago now positive E. coli
- Slit lamp exam positive — lenticular opacities

# Diagnosis

- Galactosemia
  - RBC Gal-1P elevated
  - Gal-1P uridyltransferase activity low
- Newborn screen reported positive at 10 days for increased RBC Gal/ Gal-1P and equivocal GALT
- Repeat testing requested
- NBS Follow up
  - GALT activity
  - Metabolite analysis (galactitol and Gal-1-P
  - Gene sequencing

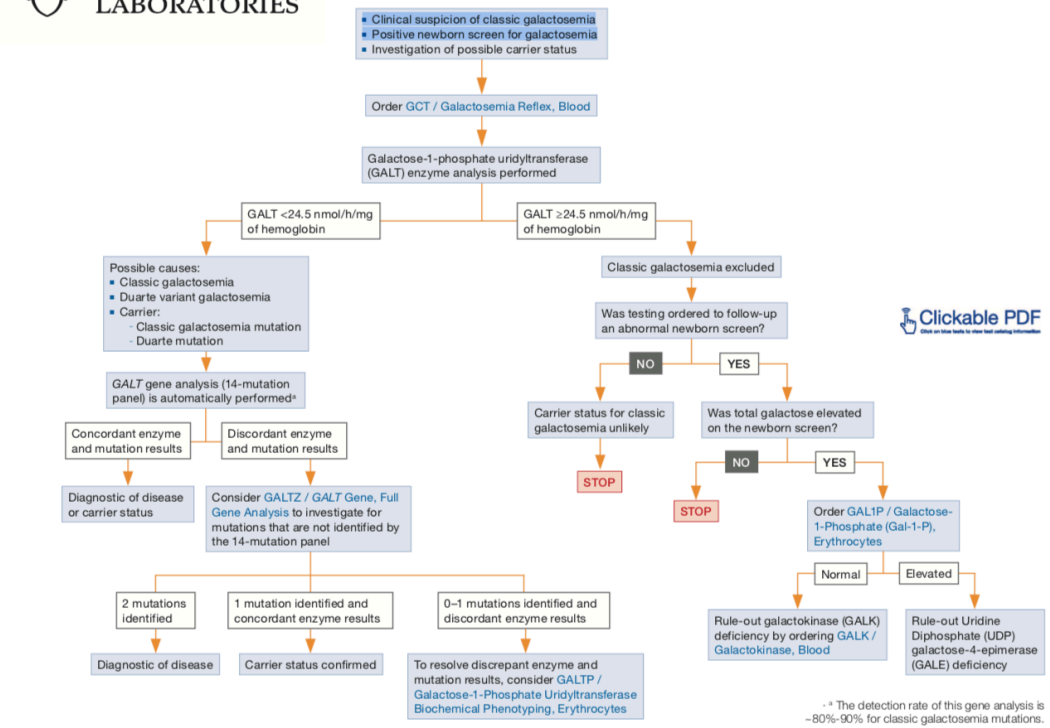
# Galactosemia Gene Analysis

- **Useful For**
  - Second-tier test for confirming a diagnosis of Galactosemia (indicated by enzymatic testing or newborn screening)
  - Carrier testing family members of an affected individual of known genotype (has mutations included in the panel)
  - Resolution of Duarte variant and Los Angeles (LA) variant genotypes
- **Testing Algorithm**
  - Tests for the presence of the following 14 mutations in the GALT gene:
  - -119_-116delGTCA, D98N, S135L, T138M, M142K, F171S, Q188R, L195P, Y209C, K285N, N314D, Q344K, c.253-2A>G, and 5 kb deletion.
  - Gene sequencing

https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/55071 (accessed 9/26/19)

MAYO CLINIC LABORATORIES

# Galactosemia Testing Algorithm




Clickable PDF

- [a] The detection rate of this gene analysis is ~80%-90% for classic galactosemia mutations.



08/2015

# Galactosemia (GALT Enzyme Assay) Newborn Screening[1]

**Limitations of Newborn Screening:**

- False positives and negatives[1]
- Delay in reporting vs onset of neonatal symptoms
- Treatment may confound
- Newborn screening incidence in USA: **~1/60,000**

[1]Pyhtilia, BM, Shaw, KA, Neumann, SA, Fridovich-Keil, JL, *JIMD Reports* 2015;15:79-93. doi: 10.1007/8904_2014_302. Epub 2014 Apr 1

## Acute Galactosemia: Clinical Outcomes

- Neonatal liver disease
- Increased risk of neonatal sepsis
- Cataracts
- Renal proximal tubule dysfunction
- Neurologic outcomes (highly variable)
- Decreased bone density is common
- Pseudotumor cerebri

# Chronic Galactosemia – Long Term Complications in Children and Adults

## Early and Chronic Speech motor pathologies

- Identifiable by ~ 1 year (recommend screening 7-12 months)

## Long term neurologic / CNS related abnormalities[1]

- Below average IQ (72%)
- Tremor (46%)
- Ataxia (15%)
- Leukodystrophy
- Difficulties in spatial orientation and visual perception

*•Still unclear whether CNS and other long-term complications are progressive or static over lifetime of patients – many patients may require caregiver support throughout life and may face intellectual limitations that will impact QOL*

## Cataracts[1] (>21%)

## Ovarian Insufficiency (almost all females)

[1]Waisbren, S.E., Potter, N.L., Gordon, C.M., RC Green, *et al. J Inherit Metab Dis* (2011) 35: 279.

## Neurologic Outcomes – Chronic Galactosemia

- Speech difficulties are common
- Difficulties in spatial orientation and visual perception
- Motor difficulties
- Cognitive outcome
- Leukodystrophy in adults
- Tremor
- Seizures

# Ovarian Failure – Chronic Galactosemia

- Hypergonadotropic hypogonadism[1,2]
- No apparent effect of diet
- Timing of signs and symptoms very variable
- A few pregnancies reported

[1]Rubio-Gozalbo ME, Haskovic M, Bosch A, Burnyte B, et al. *Orphanet J Rare Dis*. 2019; 14: 8
[2]Forges T, Monnier-Barbarino PB, Leheup B, .Jouvet P *Human Reproduction Update*, 2006: Vol.12, No.5 pp. 573–584

# Standard of Care / Current Management

- **Neonatal**
  - Lactose dietary restrictions (soy based or elemental formula)
  - Medical management of acute symptoms
- **Chronic**
  - Dietary management – restriction of dairy intake
    - Diet will not address endogenous galactose production
  - Appropriate cognitive, neurological and speech assessment evaluation and treatment

- *No enzyme replacement options currently approved to treat galactosemia*
- *No currently approved drug therapies for acute or long-term treatment*

# Chronic Galactosemia Patient Management[1]

- Diet[1]
  - Avoid Lactose milk disaccharide: Glu+Gal
  - Soy formula (sucrose-based) during infancy
  - Always work with a dietitian!!!
- $Ca^{++}$ and vitamin D supplement
- Monitoring for compliance
- Clinical follow-up of speech and fertility issues

[1]Welling L, Bernstein, L, Berrry, G, Burlina, A, et. al *J Inherit Metab Dis* (2017) 40:171–176

## Galactose Reduction in Diet

- Endogenous galactose synthesis occurs
- Complete restriction no longer recommended
- Galactose is required for proper glycosylation
- Liberalization of the small amounts of galactose contained in fruits, vegetables and medications is recommended
- There is no evidence correlating cognitive outcomes with higher degrees of dietary restriction

## Summary

- Galactosemia, while a rare disease, remains a health challenge and has significant and devastating acute and long-term consequences
- Awareness, screening and appropriate dietary and medical management are critical in the acute (neonatal) disease phase
- Long term sequelae include speech pathologies, cognitive challenges, tremors, ovarian insufficiency (females). The severity of these disease outcomes can vary between patients
- While dietary restriction of lactose remains the standard of care for the management of Galactosemia, the long-term impact of dietary restriction in terms of disease modification has yet to be fully established

# The Biology of Galactosemia A Molecular and Genetic Perspective

**Gerard T. Berry, M.D.**

Division of Genetics and Genomics

The Manton Center for Orphan Disease Research

Boston Children's Hospital

Harvard Medical School, Boston, MA, USA

# Estimated US Disease Prevalence for Type I and Type II Galactosemia

- Genetic screening of newborns for Galactosemia
  - 1960's initiation of screening
  - 2004 – Mandatory in all states
- Prior to screening, majority of patients with severe enzyme deficient Galactosemia did not survive the newborn period
- Based on genetic frequency for classic Galactosemia and birth rates since screening, the estimated number of newborn- adult individuals with CG is estimated to be approximately 1200 + patients older than 15 years US (yearly birth rate of 80 patients/year) US individuals.

# Enzyme Deficiencies Identified in Galactosemia

- **GALT (Type I) (Galactose Transferase Deficiency)**
  - Severe (Classic Galactosemia -CG) - < 1% activity (dietary galactose restriction required)
  - Moderate (Clinically Variant –CV) 1-10% enzyme activity – (dietary galactose restriction required)
  - Mild (Duarte ) ~15-35% enzyme activity – (no dietary intervention required)
  - Incidence of classic disease – 1: 40,000 – 1: 60,000 (Ireland: 1:16,000)
- **GALK (Type II) (Galactose Kinase Deficiency)**
  - Severe disease
  - Incidence – highly variable by region
    - Eastern European (Romani)
    - UK / Ireland (Irish Traveler)
    - 1:100,000 (US), 1: 40,000 (Germany)
- **GALE *(Type III) (Galactose Epimerase Deficiency)**
  - Systemic disease form - extremely rare
  - Hypomorphic disease – rare
  - Asymptomatic form – African Americans

# GALT and GALK Enzyme Deficiencies

- **GALT Deficient (Type I Galactosemia)**

  Characterized by:

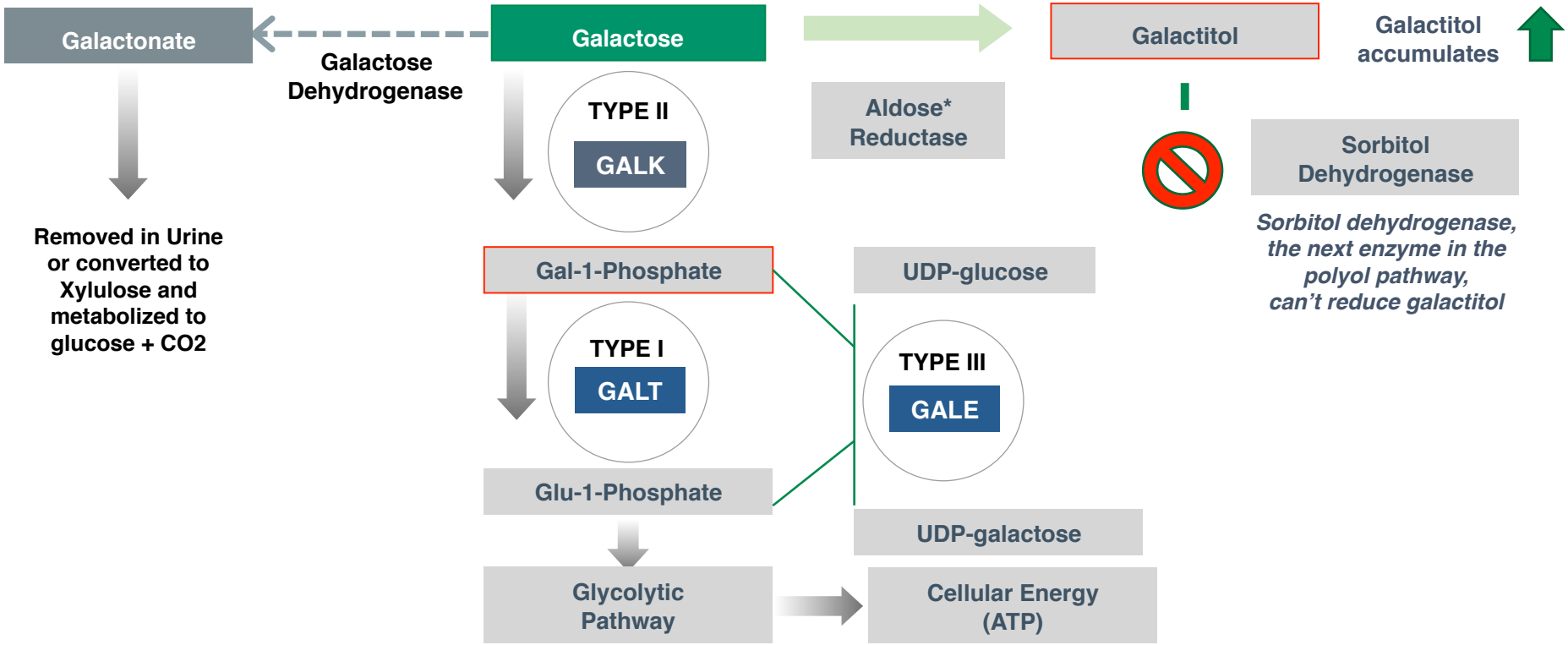
  - Elevated levels of galactose-1- phosphate (Gal-1P)
  - Elevated levels of erythrocyte Galactitol (8x normal),
  - Elevated levels of Galactitol are also observed in brain tissue via MRI
  - (Galactitol levels are increased in individuals with GALT deficiency due to increased flux through the aldose reductase pathway)

- **GALK Deficient (Type II Galactosemia)**

  Characterized by:

  - Normal levels of galactose-1- phosphate (Gal-1P)
  - Elevated levels of erythrocyte Galactitol

# Key Metabolic Intermediates in Galactosemia



# Variability in Measured Galactose Levels: Children versus Adults

- Galactose Appearance Rate (GAR) in patients with GALT deficiency
  - Infants and children, 1 month – 14 years old:
    1.34 +/- 0.53 mg / kg / h (n=17)
    (7.4 umol/kg/h)
  - Adults, 19 – 33 years old:
    0.56 +/- 0.01 mg / kg / h (n=5)
    (3.1 umol/kg/h)

# Age Dependency of Endogenous Galactose Production

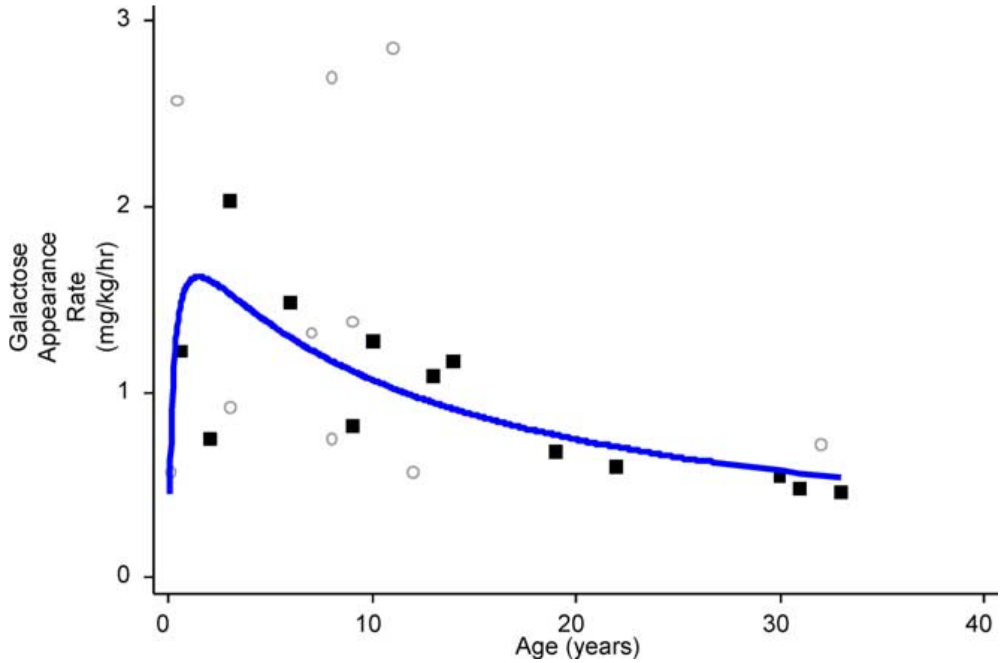
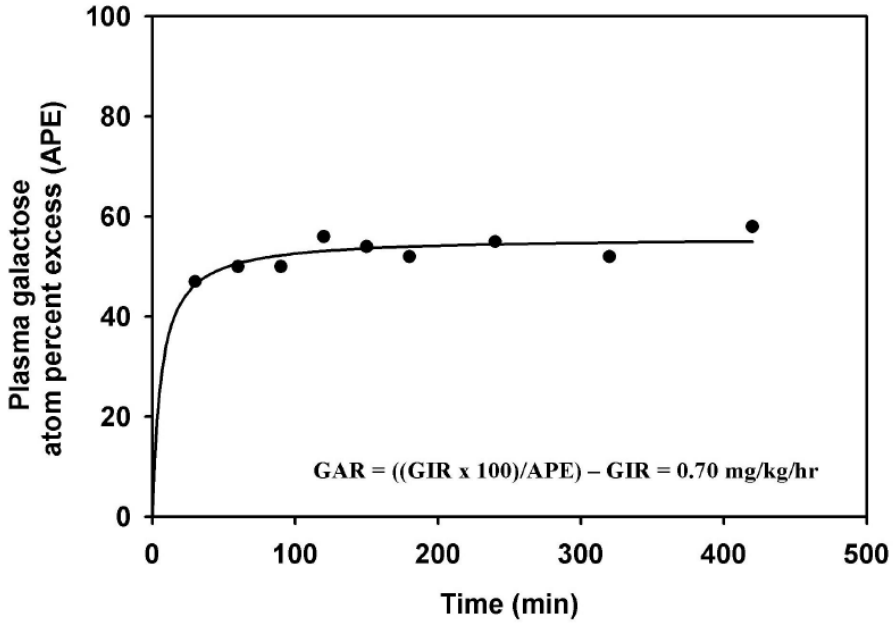


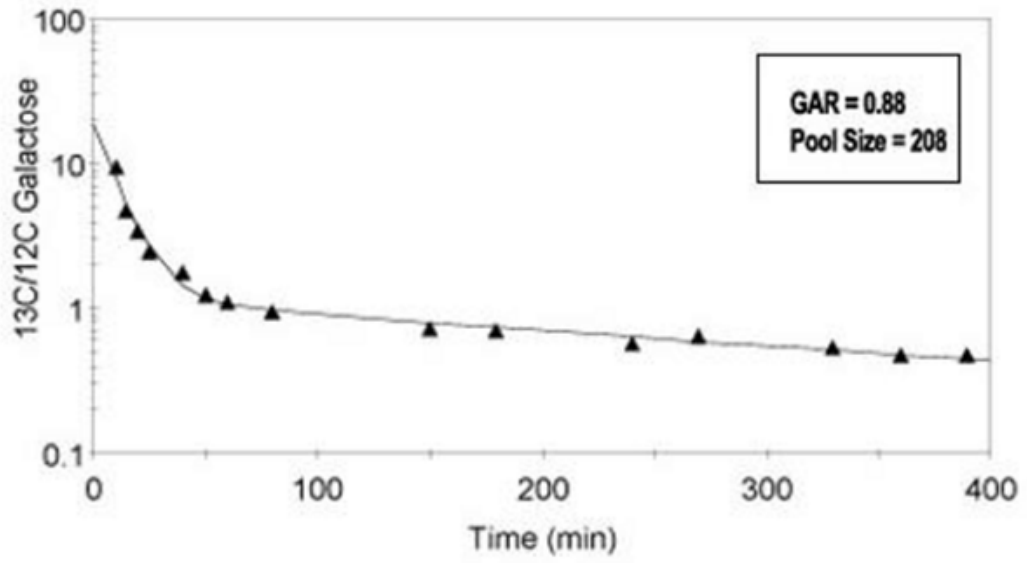

Fig. 1. The GAR in patients with galactosemia of different ages determined by the continuous intravenous infusion method. The patients with a Q188R/Q188R genotype are shown as the ■ symbols while patients with other genotypes are shown as ○ symbols.

# [1-13C] Galactose Continuous Infusion in a 33 Year Old Male with Q188R/Q188R Genotype




Berry GT *et al. Mol Genet Metab.* 2004 Jan;81(1):22-30

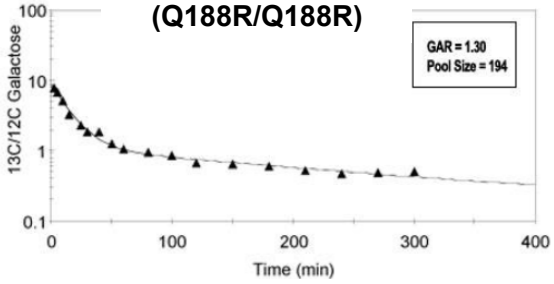
# Single Bolus Method with [1-13C] Galactose in a 30 Year Old Male with Q188R/Q188R Genotype



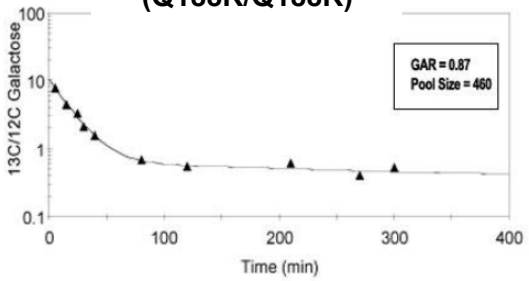
# Galactose Disposition as Measured by Stable Isotope Studies

**Single Bolus Studies with [1-$^{13}$C] Galactose**

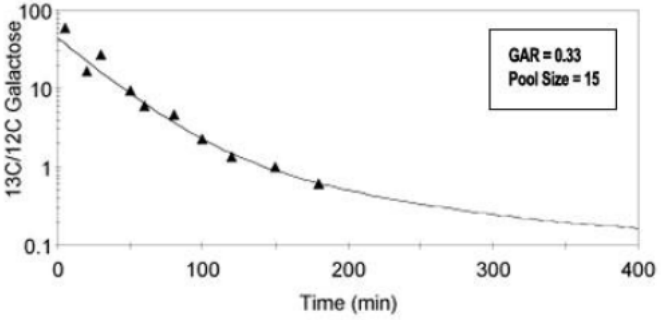
**22 Year old Female (Q188R/Q188R)**



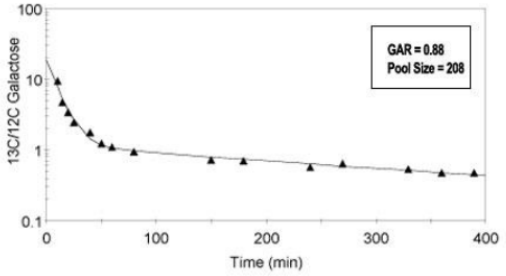

**31 Year old Female (Q188R/Q188R)**



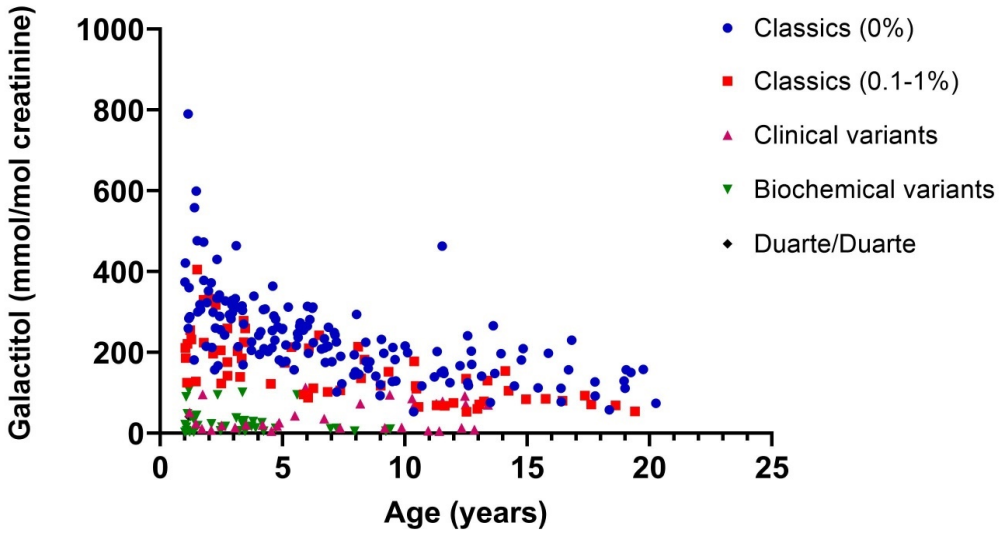

**25 Year old Female (normal/normal)**




**30 Year old Male (Q188R/Q188R)**



# Urine Galactitol Levels Never Normalize - Even When on a Lactose Restricted Diet

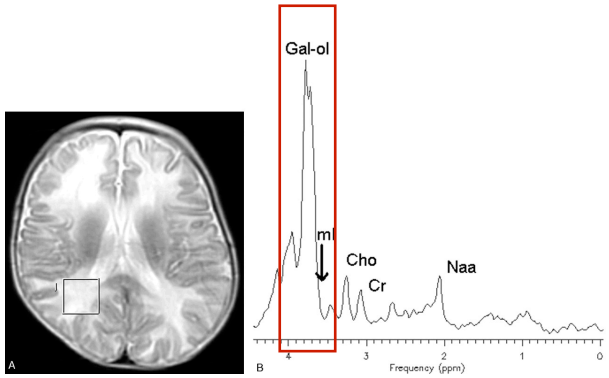


# Galactose, Galactitol and GAL1P Metabolite Levels in Healthy vs. Classic (Type I) Galactosemia Patients

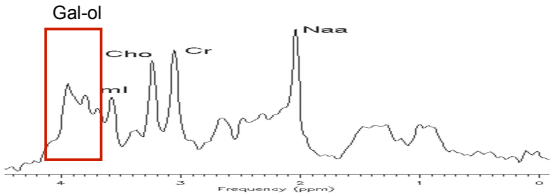
| Galactose and Its Metabolites | Healthy Subjects; Unrestricted Diet | Subjects with CG; Galactose-restricted Diet |
|---|---|---|
| **Plasma (µmol/L)** | | |
| **Galactose** | 0.1 – 6.3[1] GC/MS | 0.6 – 4.0[1] GC/MS Q188R/Q188R |
| **Galactitol** | Undetectable[2] GC | 8.4 – 15.1[2] Q188R/Q188R (n=8) GC<br>9.2-15.98[1] Q188R/Q188R (n=15) GC/MS |
| **Erythrocytes (µmol/L)** | | |
| **Galactitol** | 0.3-1.3[3] n=19, GC/MS | 4.0 – 7.9[3] n=17, Q188R/Q188R, GC/MS |
| **Gal-1p** | Undetectable – 15.3[3] n=19 GC/MS | Q188R/Q188R<br>77.8 – 214.3[3], n=17, (2.02-5.57[3] (mg/dL)) GC/MS<br>72-425 [1] (n=12) enzymatic |
| **Urine (mmol/mol Cr)** | | |
| **Galactitol** | <1 year old <2-78 (46)[2] GC<br>1-6 year old <2-36 (28)[2] GC<br>>6y <2-19 (21)[2] GC | Q188R/Q188R, GC<br><1 year old 183-909(38)[2]<br>1-6 year old 194-620 (38)[2]<br>>6y 98-282 (32)[2] |

[1]Ning C, *et al. Metabolism*. 2000 Nov;49(11):1460-6. [2]Palmieri M, *et al. Metabolism*. 1999 Oct;48(10):1294-302.
[3]Yager CT, *et al. Mol Genet Metab*. 2003 Nov;80(3):283-9.

# Elevated Galactitol Levels in Human Brain Tissue Observed via Magnetic Resonance Spectroscopy



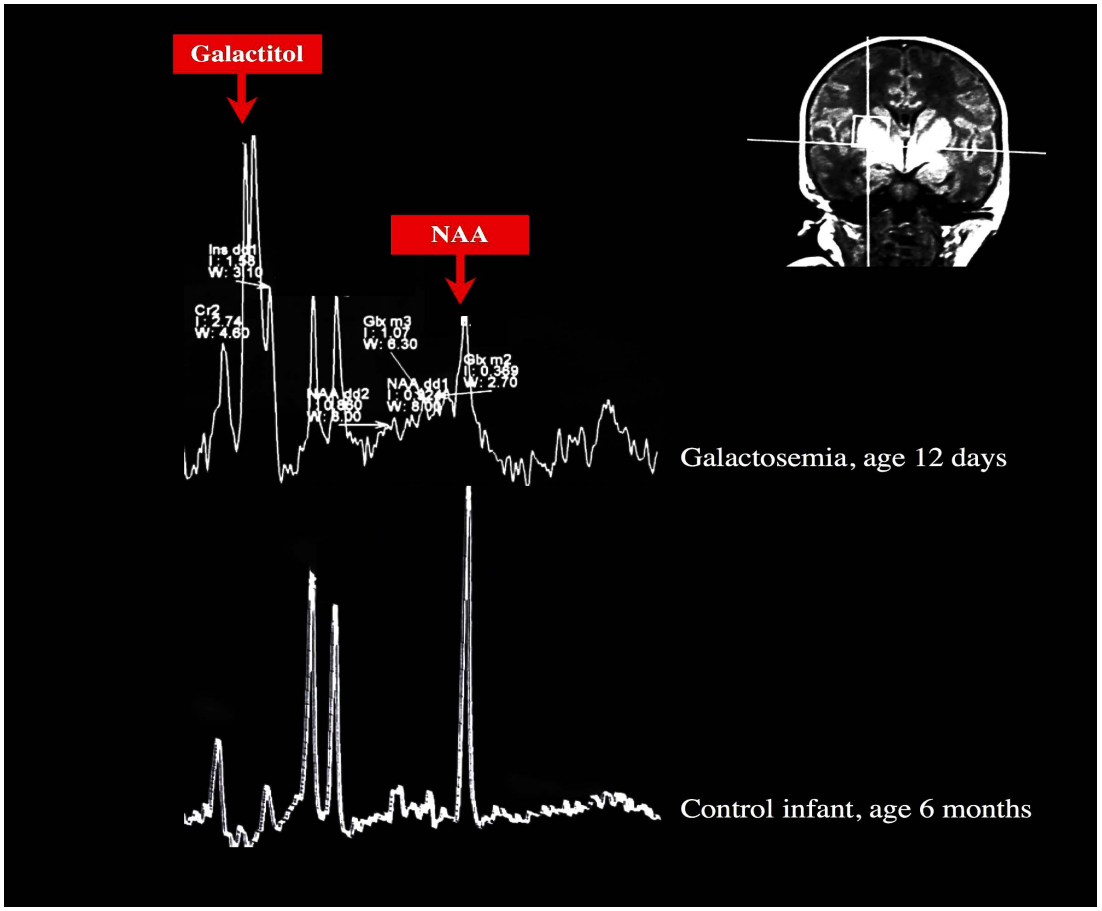

Two years txt with intensive dietary lactose restriction




Axial localizer T2-weighted image showing the MR spectroscopy voxel location (A). STEAM (TE/TR, 30/1500 milliseconds) (B) in vivo 1H-MR spectroscopy spectrum of the patient before treatment.

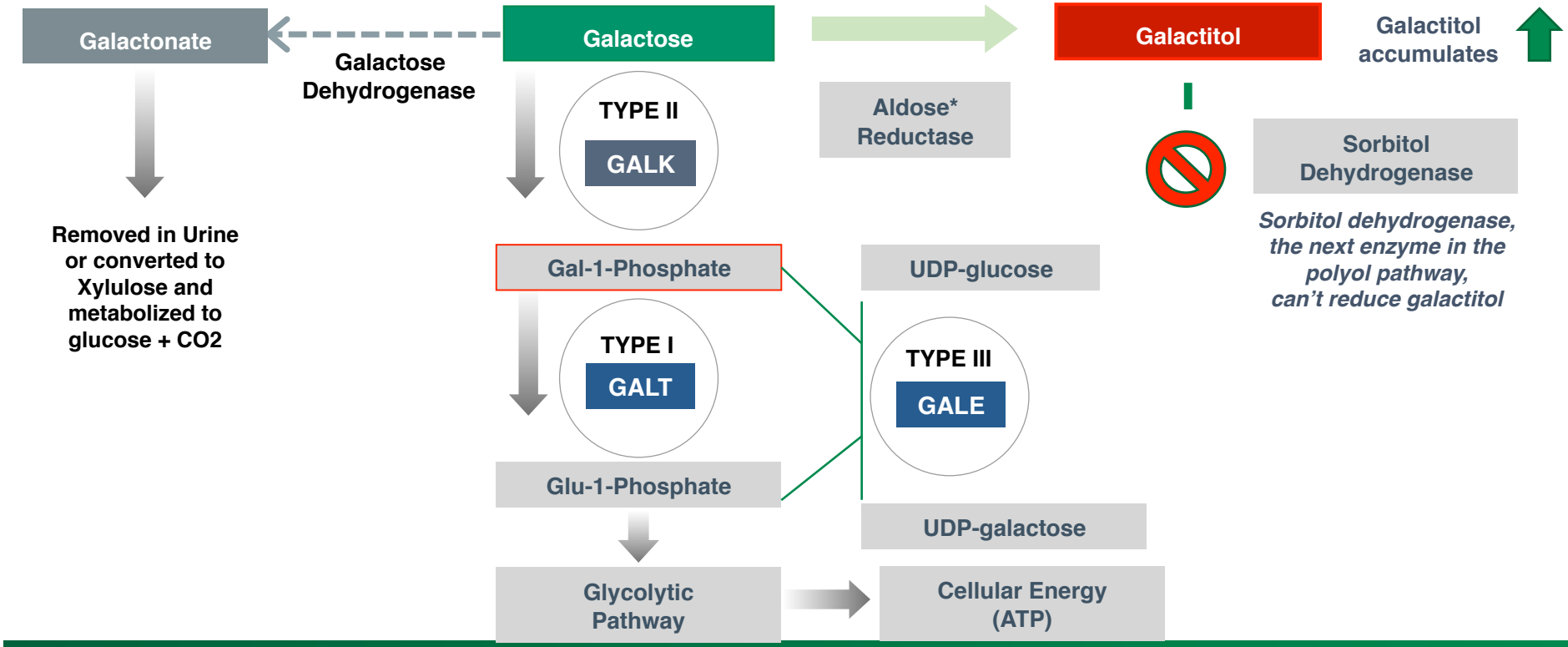
STEAM (TE/TR, 30/1500 milliseconds) in vivo 1H-MR spectroscopy spectrum of the patient after treatment

**Proton MR Spectroscopy and Imaging of a Galactosemic Patient before and after Dietary Treatment**




From Strauss,K& Morton, H, CSC

# Key Metabolic Intermediates in Galactosemia



# Preclinical evidence and clinical plan for a novel oral compound to prevent complications of Galactosemia

**Riccardo Perfetti, MD, PhD**
**Chief Medical Officer, Applied Therapeutics**

APPLIED THERAPEUTICS

# AT-007 for Galactosemia

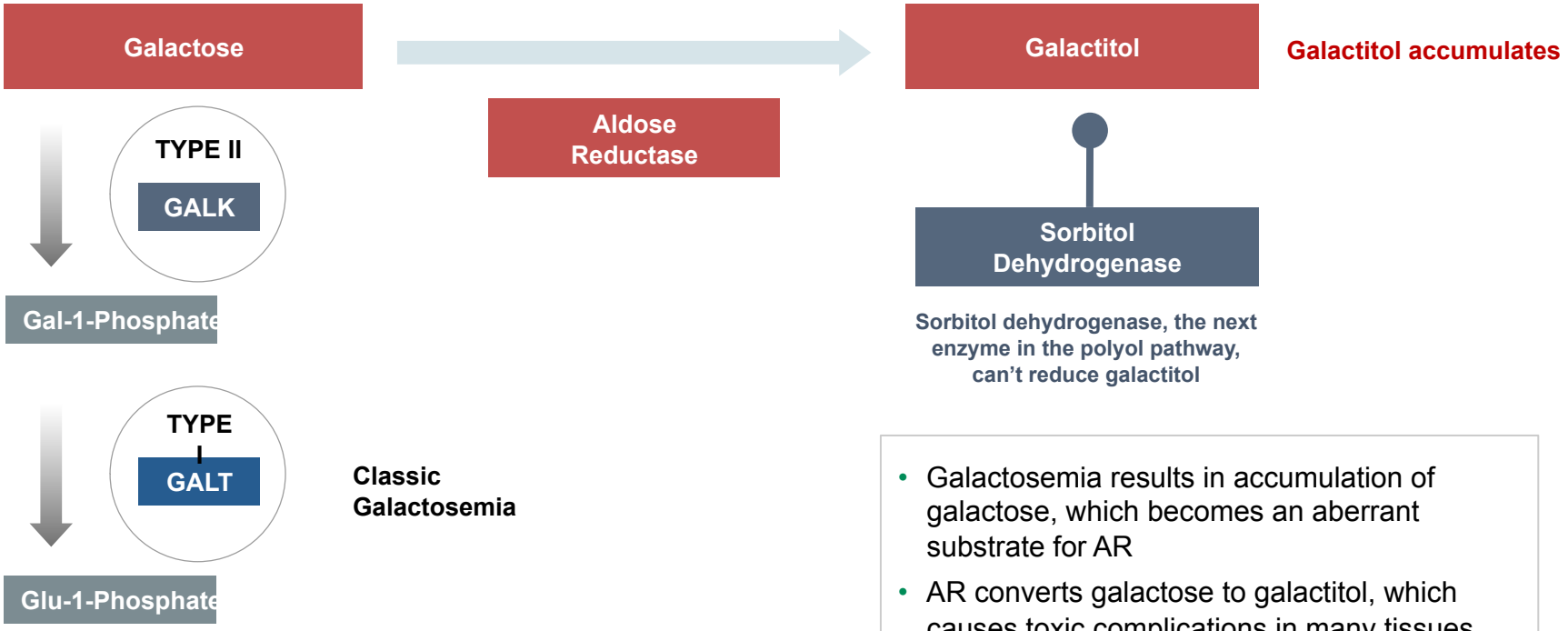
## Burden of Disease

- Rare genetic metabolic disease caused by inability to break down galactose
  - Metabolite of lactose
  - Produced de novo by cells
- Even with strict dietary restriction of external lactose, endogenous galactose is produced within the body, leading to toxic build-up of galactitol
- Long-term consequences of disease include: Frequent pre-senile cataracts, significant motor, speech, cognitive, and psychiatric impairments, seizures, and ovarian insufficiency

## Standard of Care

- Mandatory newborn screening in the US/EU; potentially fatal if undetected in first weeks of life and infant is exposed to lactose in breast milk or formula
- No approved therapies
- Standard of care is strict dietary restriction of lactose and galactose, which prevents fatalities, but **does not prevent long term consequences of disease**
- Greatly impacts children's development potential and quality of life (causes severe and permanent cognitive, intellectual and speech deficiencies)
- In adults, frequent cataracts due to galactitol build up in the eye; many develop persistent tremors

# Aldose Reductase Activity Causes Toxic Accumulation of Galactitol in Galactosemia




- Galactosemia results in accumulation of galactose, which becomes an aberrant substrate for AR
- AR converts galactose to galactitol, which causes toxic complications in many tissues

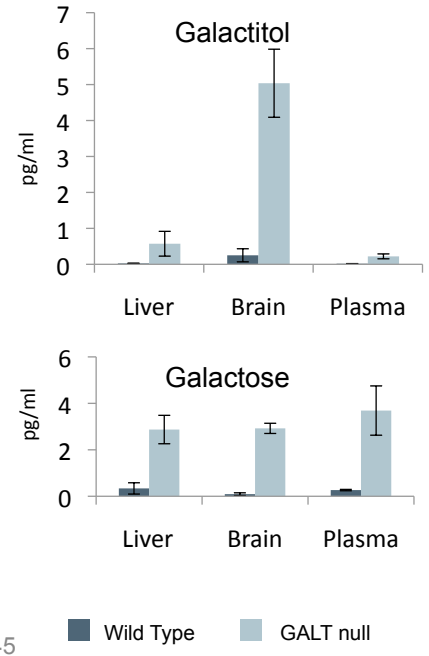


APPLIED THERAPEUTICS

# GALT Deficient Rat Model Closely Mirrors Human Disease

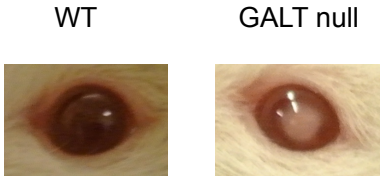
## Biochemical Effects

*GALT null rats have exponentially higher levels of galactose and galactitol, as well as Gal1p*



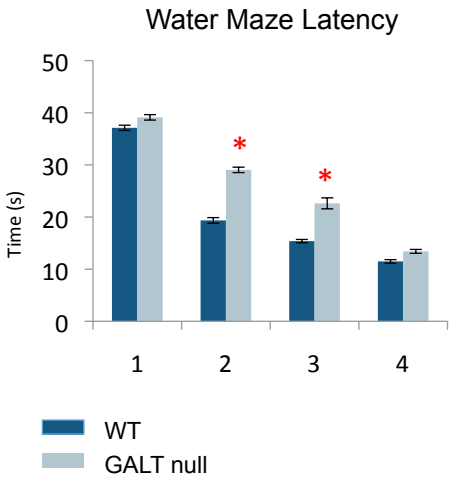

## Tissue Deposition of Galactitol

*All GALT null rats display cataracts (caused by galactitol deposition in the eye) vs. none of the WT rats*




## CNS Outcomes

*GALT null rats display deficiencies in learning, cognition, and motor skills as measured by rotarod and water maze*



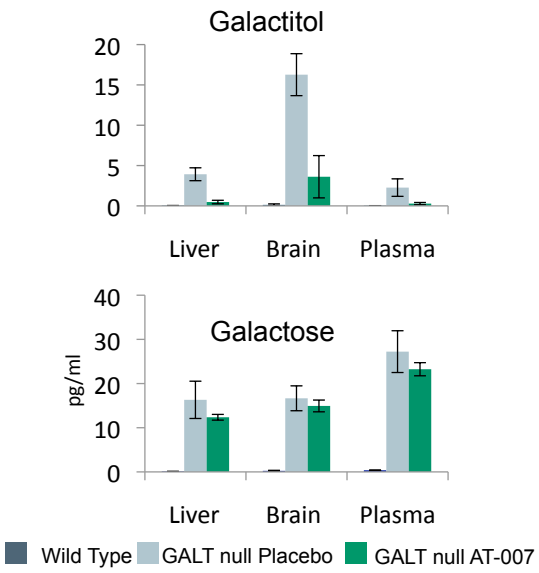



APPLIED THERAPEUTICS

# AT-007 Treatment Corrects All 3 Aspects of Disease in the Galactosemia Rat Model

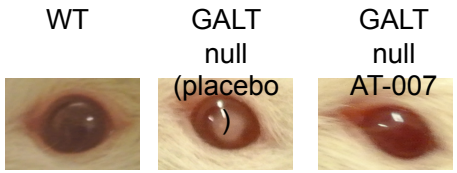
## Biochemical Effects

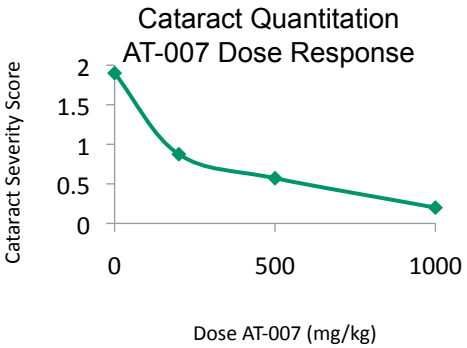
*AT-007 treatment significantly reduced galactitol levels in all tissues without increasing galactose or Gal1p*




## Tissue Deposition of Galactitol

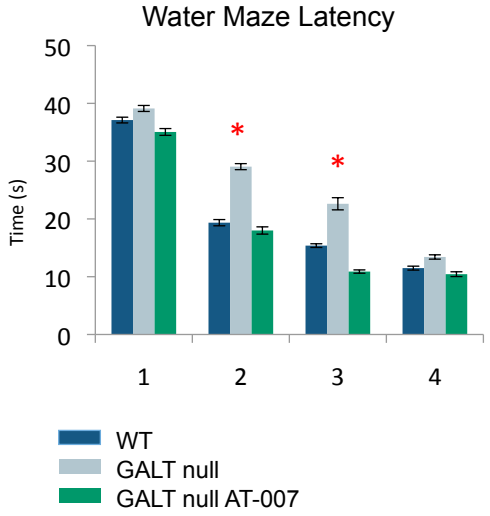
*AT-007 treatment prevented galactitol accumulation in tissues, resulting in absence of cataracts*




## CNS Outcomes

*AT-007 treatment normalized CNS outcomes on both water maze and rotarod*



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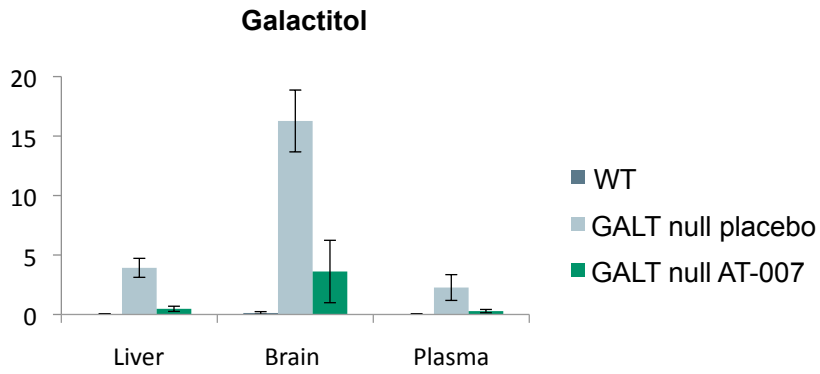
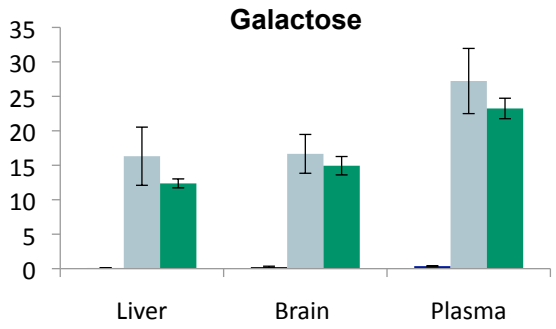
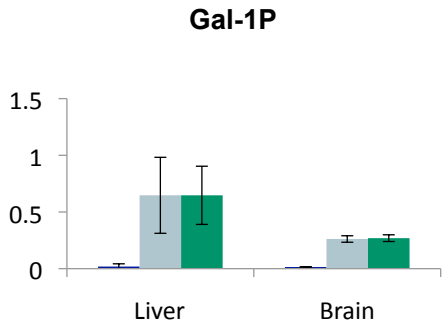
# A Closer Look: AT-007 Significantly Reduces Galactitol Levels in all Target Tissues Without Increasing Galactose or Gal-1P




- AT-007 treatment from neonatal Day 1 to Day 10 significantly reduced galactitol in liver, brain and plasma
- Treatment did not increase galactose or Gal1P levels; similar results seen at Day 22 and age 5 months



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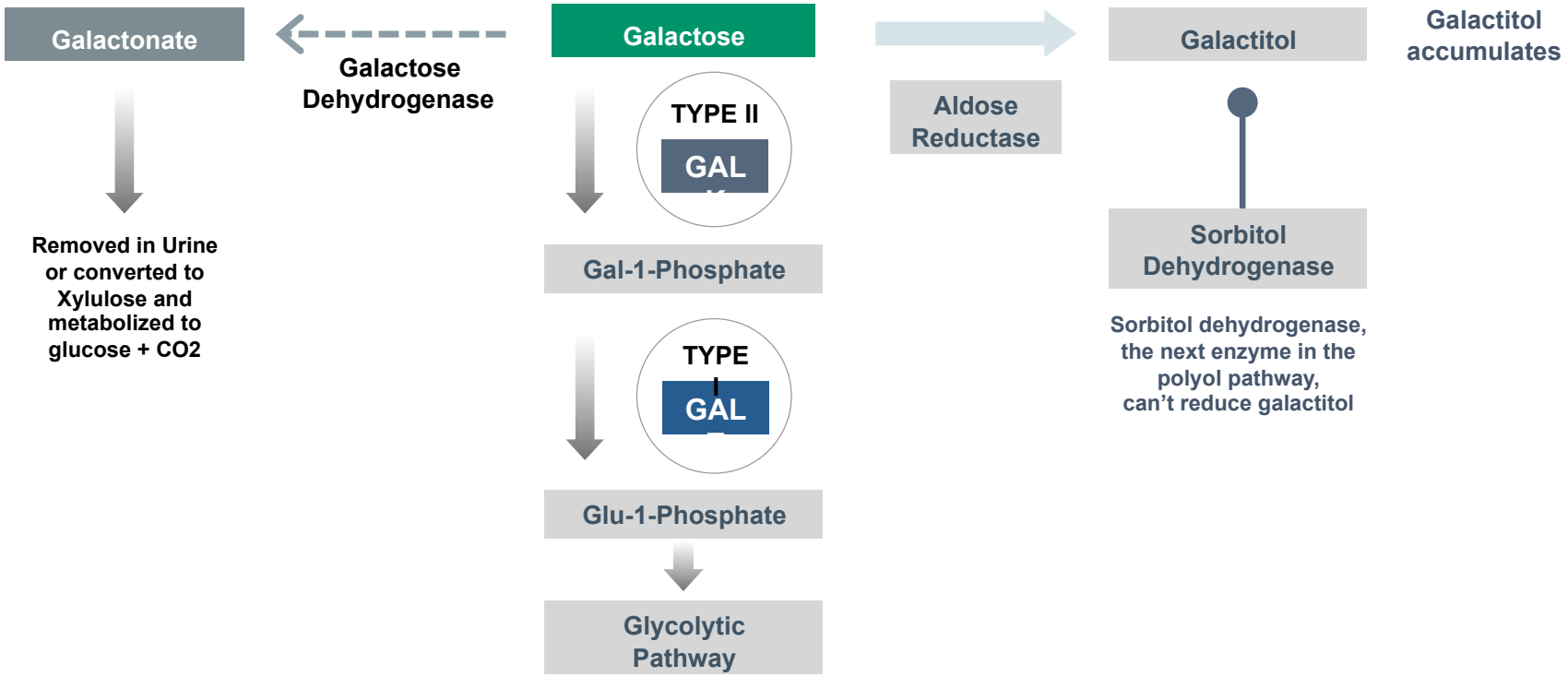
# If Blocking AR Doesn't Increase Galactose or Gal-1P…..
# Where Does the Extra Substrate Go?



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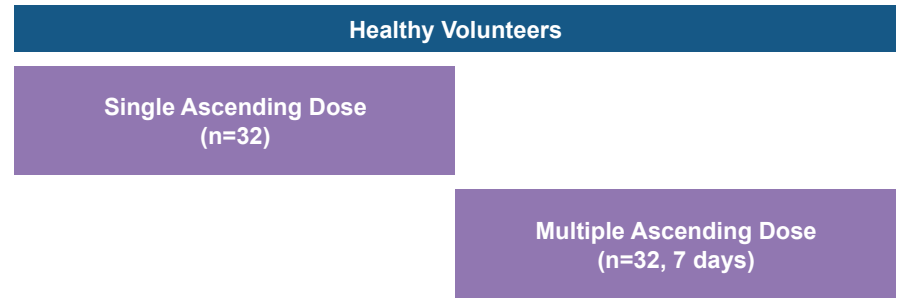
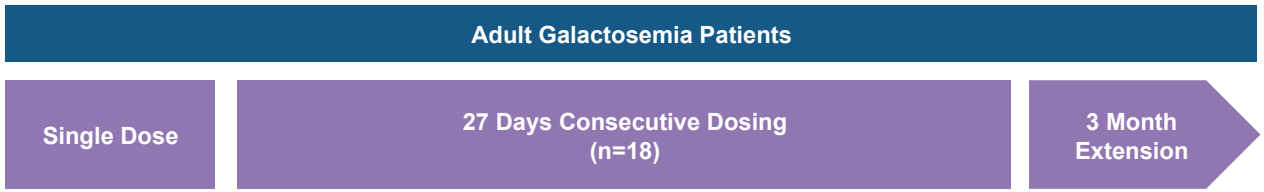
# Galactosemia Phase 1/2 Registrational Study (ACTION-Galactosemia)

Multi-Center Placebo-Controlled Study in Healthy Volunteers & Adult Galactosemia Patients




Healthy Volunteer Endpoints:

- Safety
- Pharmacokinetics
- Pharmacodynamics




Galactosemia Endpoints:

- Safety
- Pharmacokinetics/ Pharmacodynamics
- **Efficacy Biomarker - Galactitol**



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# Galactosemia Phase 1/2 Registrational Study (ACTION-Galactosemia)

Multi-Center Placebo-Controlled Study in Healthy Volunteers & Adult Galactosemia Patients

**Healthy Volunteers**

**Single Ascending Dose (n=32)**

**Healthy Single Ascending Dose Results:**

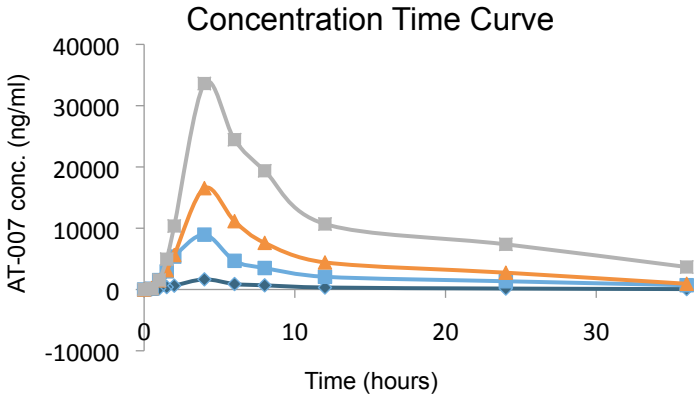
No drug-related safety issues at any dose tested

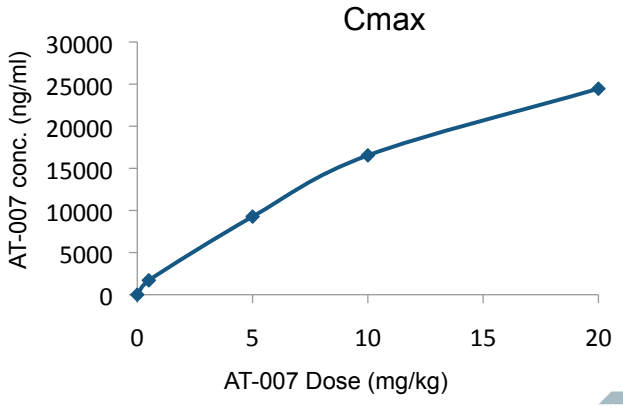
Dosing: 0.5, 5.0, 10, 20mg/kg

PK consistent with once daily dosing (half-life ~12 hours)

Consistent exposure across patients

Linear dose response



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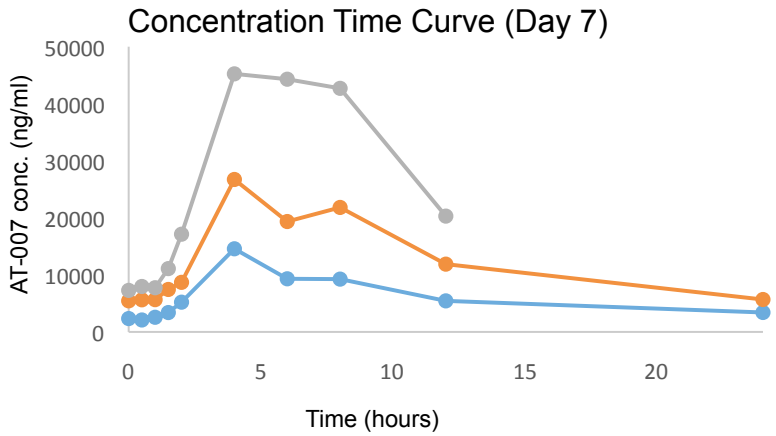
# Galactosemia Phase 1/2 Registrational Study (ACTION-Galactosemia)

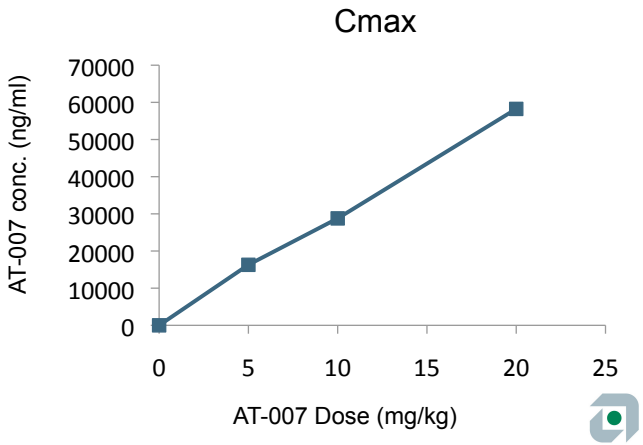
## Multi-Center Placebo-Controlled Study in Healthy Volunteers & Adult Galactosemia Patients

**Healthy Volunteers**

**Single Ascending Dose (n=32)**

**Healthy Single Ascending Dose Results:**
No drug-related safety issues at any dose tested
Dosing: 5.0, 10, 20mg/kg
PK consistent with once daily dosing (half-life ~12 hours)
Consistent exposure across patients
Linear dose response
No accumulation, no first passage clearance

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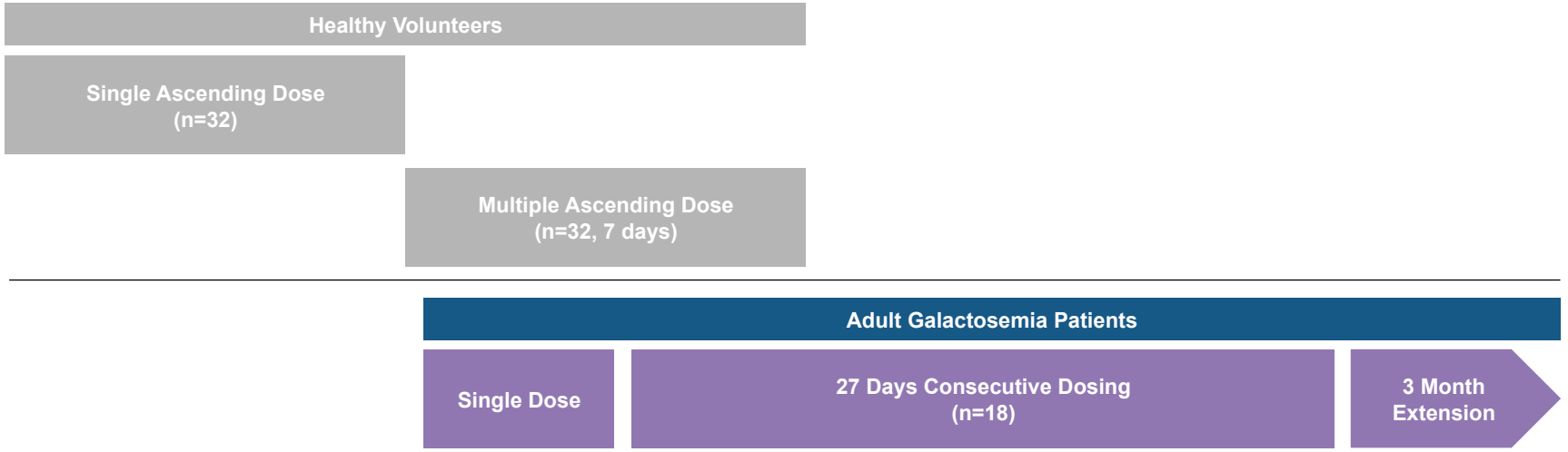
# Galactosemia Phase 1/2 Registrational Study (ACTION-Galactosemia)

Multi-Center Placebo-Controlled Study in Healthy Volunteers & Adult Galactosemia Patients




Galactosemia Endpoints:

- Safety
- Pharmacokinetics/ Pharmacodynamics
- **Efficacy Biomarker - Galactitol**

*Data from the adult Galactosemia patient portion of the trial expected in **4Q 2019***



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# Study Endpoints

**Primary**

- Overall safety and adverse events (AEs)
- Safety will be assessed by the following:
  - AEs
  - Clinical safety laboratory tests (hematology, chemistry, urinalysis)
  - Physical examinations
  - Vital signs
  - Electrocardiograms (ECGs)

**Secondary**

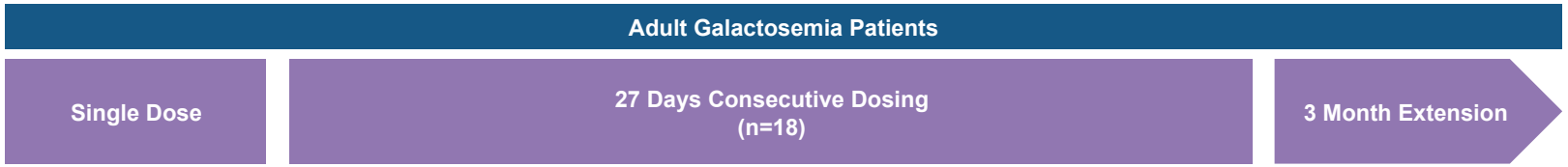
- PK parameters in healthy subjects and subjects with CG
- Biomarker assessment in Galactosemia patients:
  - Galactitol in urine & blood
  - Galactose and other metabolites (galactonate, Gal1P) in urine & blood

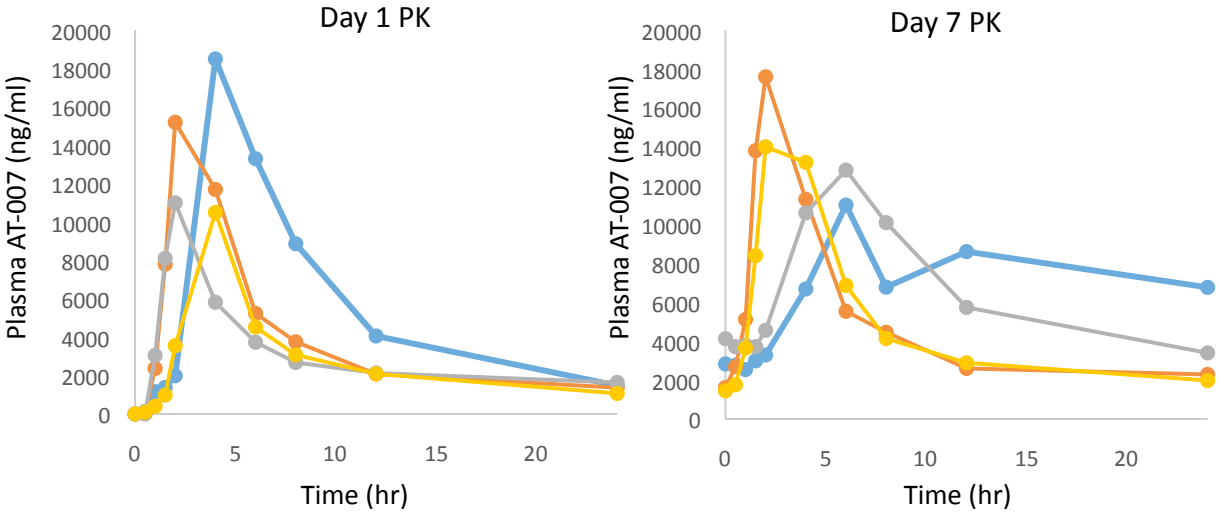
**Exploratory**

- Major metabolites of AT-007 (if any) in the urine of healthy subjects and subjects with CG
- AT-007 levels in the CSF of healthy subjects
- MR Spectroscopy of the brain in a subset of subjects with CG (for galactitol quantitation in the brain)

# Galactosemia Phase 1/2 Registrational Study (ACTION-Galactosemia)

Multi-Center Placebo-Controlled Study in Healthy Volunteers & Adult Galactosemia Patients




**Pharmacokinetics**

AT-007 PK in patients with Classic Galactosemia is similar to that in healthy volunteers

**Safety**

One cohort of patients (4 subjects on active drug 5mg/kg) has been dosed to date

No drug-related adverse events have been reported



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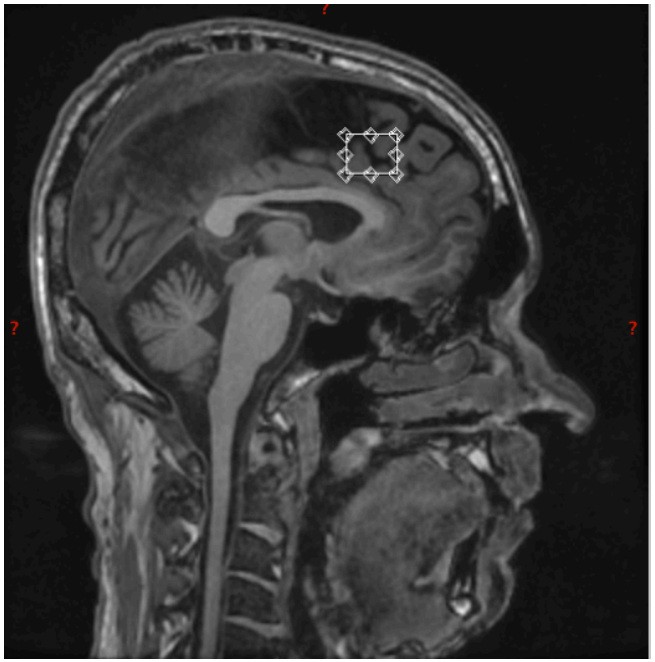
# Baseline Characteristics of Pts with Classic Galactosemia enrolled to date

- Elevated urine galactitol, all patients
- Brain accumulation of galactitol, all patients
- EKG conduction abnormalities, most patients
- Anxiety and depression, most patients
- Relevant cognitive deficits, most patients
- History of seizures, most patients





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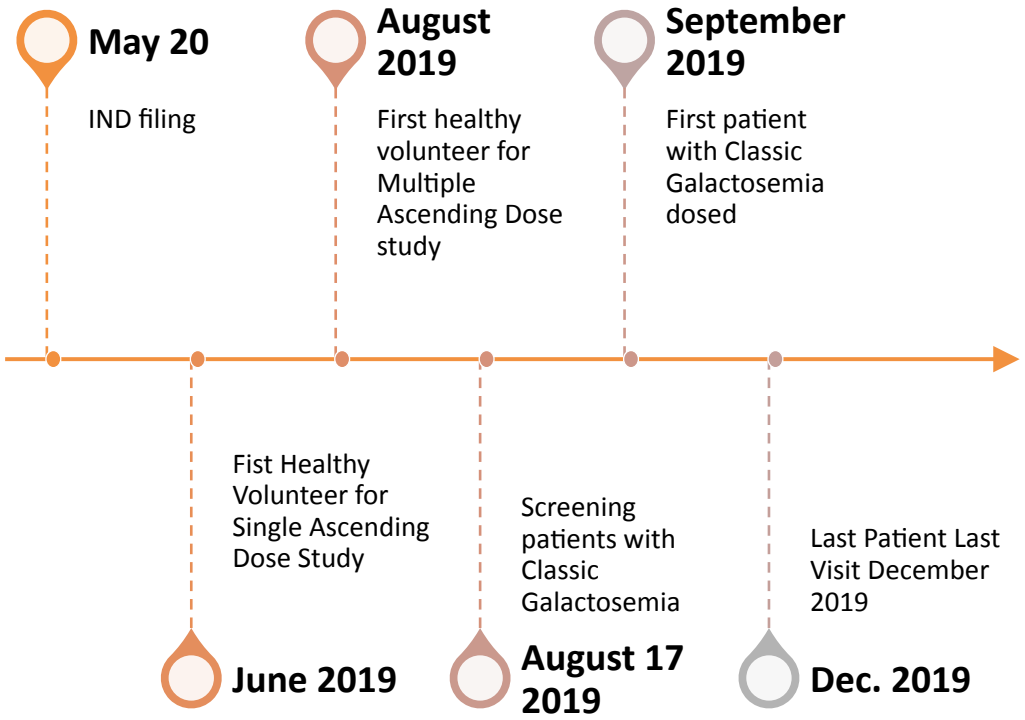
# Timelines

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# Summary & Conclusions

- **Treatment with AT-007 in a classic Galactosemia disease model of GALT null rats corrects the following sequelae:**
  - Biochemical characteristics of Classic Galactosemia
  - Phenotypical characteristics of Classic Galactosemia
  - Behavioral characteristics of Classic Galactosemia
- **A Clinical study in Healthy Volunteers and in adult patients with Classic Galactosemia is currently underway**
  - AT007 is well tolerated with no drug-related adverse events to date
  - Baseline characteristics of patients with Classic Galactosemia further confirm the severity of the disease in this population

# Questions and Answers

All

# Thank you

# Thank you

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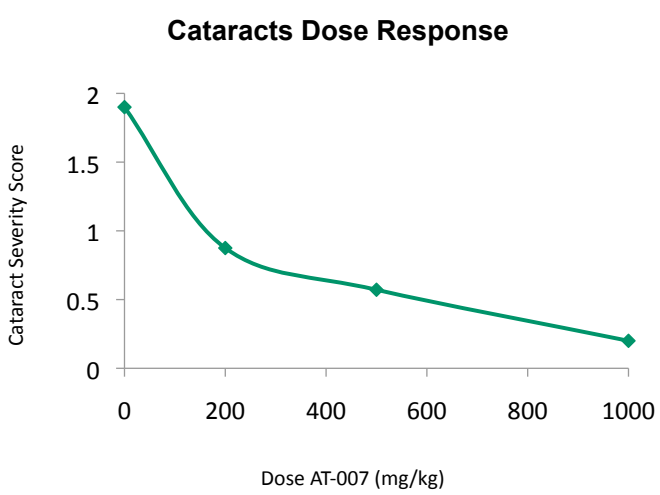
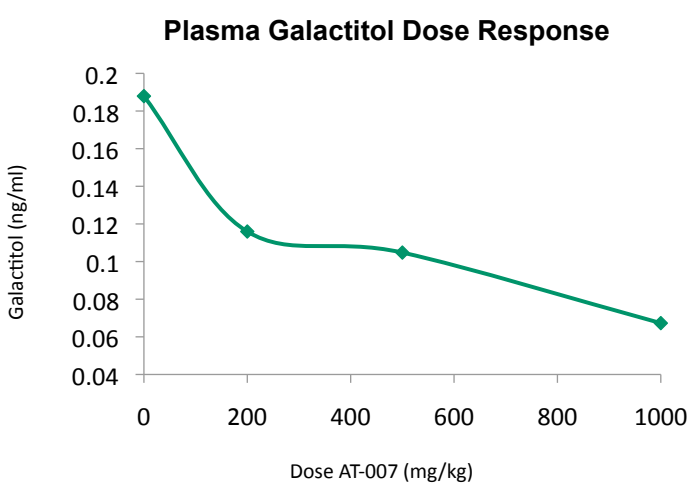
# AT-007 Dose Response

**Greater doses of AT-007 reduced galactitol levels and the severity of cataracts**




**No cataracts in WT or AT-007 treated GALT null rats, but visible cataracts in all GALT null placebo rats at Neonatal Day 22**



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