Nucleoside Bypass Therapy for Thymidine Kinase 2 Deficiency (TK2d)

MitoAction Expert Series
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Michio Hirano, MD
Lucy G. Moses Professor
Principal Investigator, North American Mitochondrial Disease Consortium (NAMDC)
H. Houston Merritt Neuromuscular Research Center
Neuromuscular Medicine Division
Columbia University Irving Medical Center

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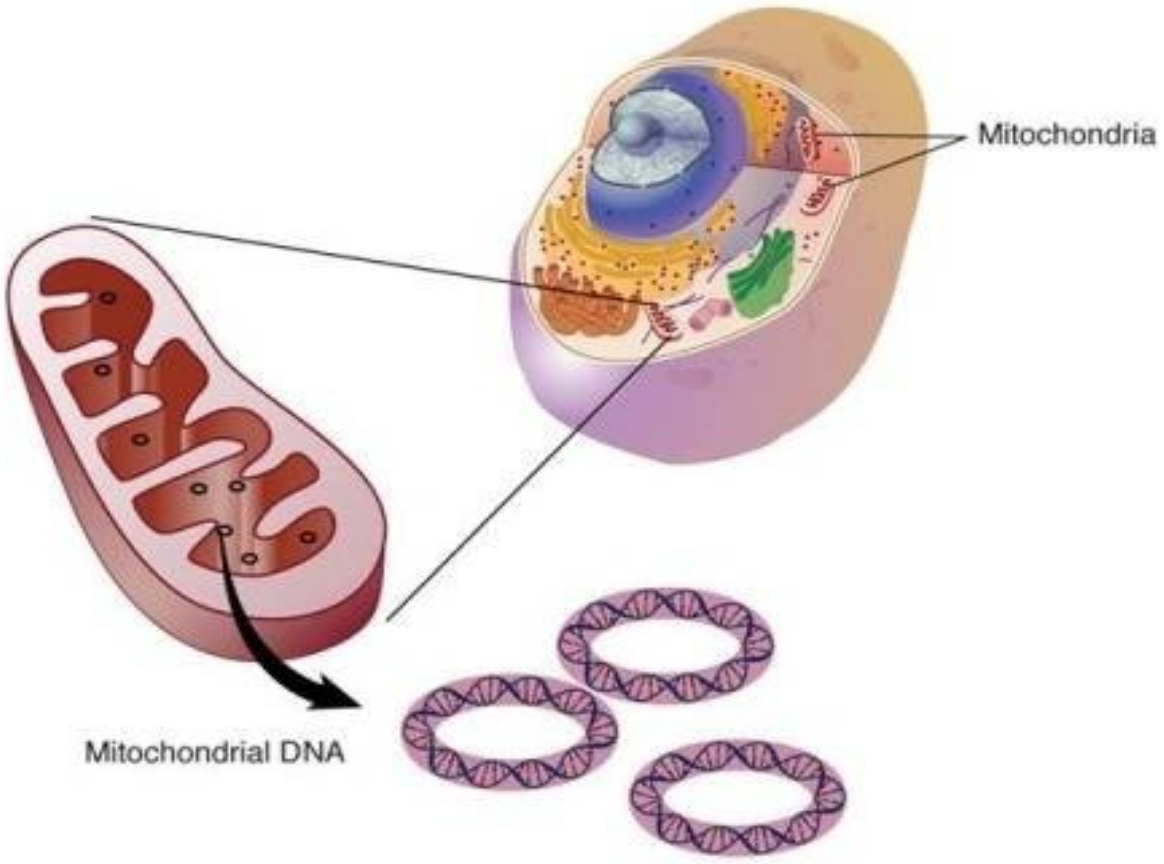
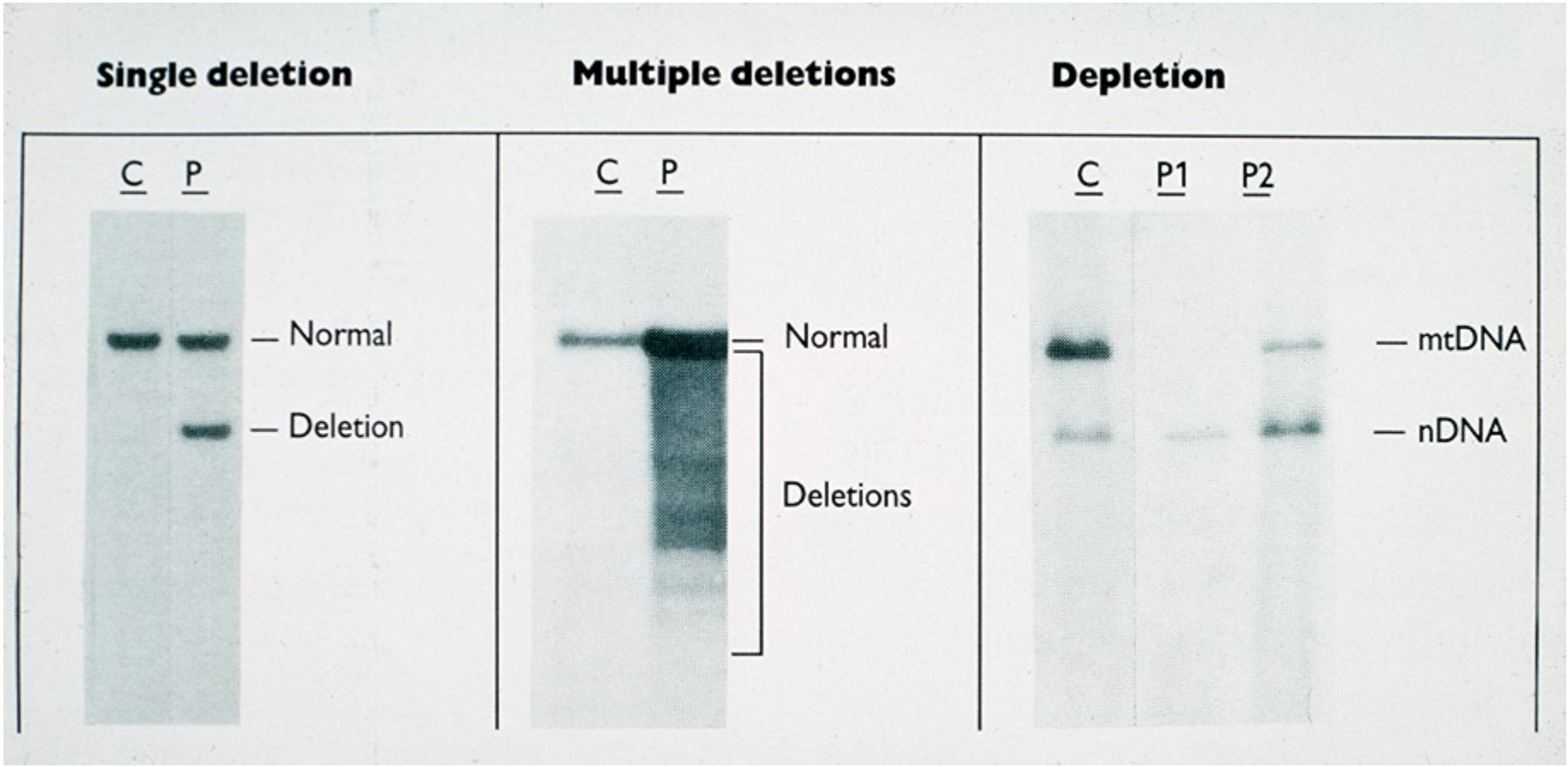
Disorders of mtDNA maintenance

Clinically heterogeneous group of mitochondrial disorders characterized by **mtDNA depletion, multiple deletions, or both** in affected tissues.



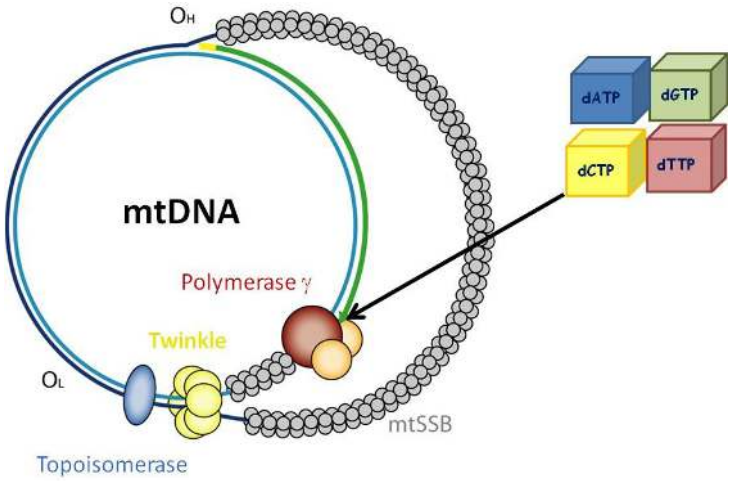
Defects of mtDNA Maintenance



Autosomal dominant or recessive progressive external ophthalmoplegia

Mitochondrial DNA depletion syndrome
-myopathy
-hepatopathy
-hepatocerebral disease
-Alpers syndrome
-Navaho neurohepatopathy

Mutations in 34 nuclear genes cause mtDNA depletion, multiple deletions, or both



- **mtDNA replication**
  - *-POLG1*
  - *-POLG2*
  - *-TWNK*
  - *-SSBP1*
  - *-MGME1*
  - *-DNA2*
  - *-RNaseHI*
  - *-TFAM*
  - *-TOP3A*
  - *-LIG3*

- **Nucleoside/nucleotide metabolism**
  - *-TYMP*
  - *-ANT1*
  - ***-TK2***
  - *-DGUOK*
  - *-RRM2B*
  - *-RRM1*

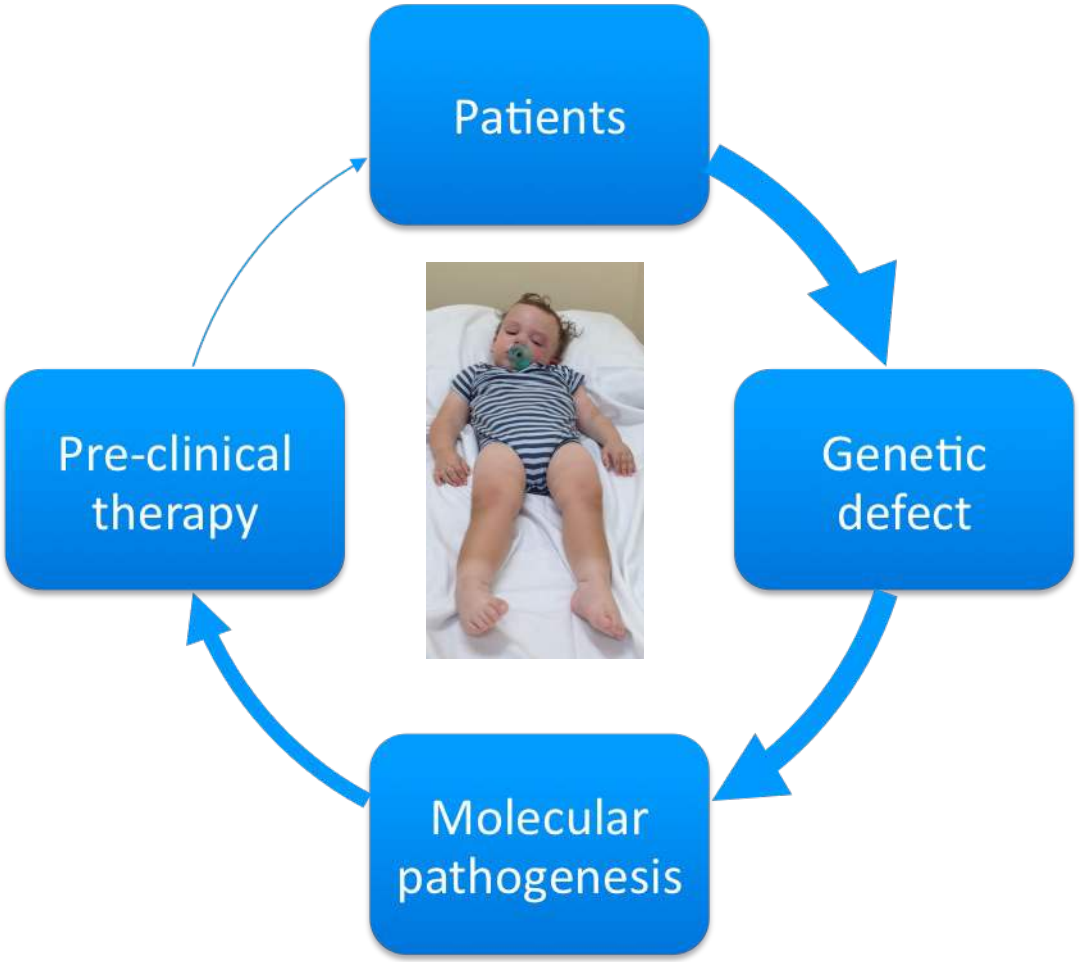
- **Mitochondrial dynamics**
  - *-OPA1*
  - *-MFN2*
  - *-SPG7*
  - *-AFG3L2*
  - *-MICOS13*
  - *-GFER*

- **Membrane channels**
  - *-SLC25A4*
  - *-MPV17*
  - *-SLC25A1*
  - *-SLC25A10*

- **Other Function/ Mechanism unknown**
  - *-SUCLA2*
  - *-SUCLG1*
  - *-GFER*
  - *-AGK*
  - *-FBXL4*
  - *-ABAT*

Lopez-Gómez et al. Neuromusc Disord 2022

TK2 deficiency:
From bedside to bench and back



What is thymidine kinase 2 deficiency (TK2d)?

TK2 Disease and Therapy Overview

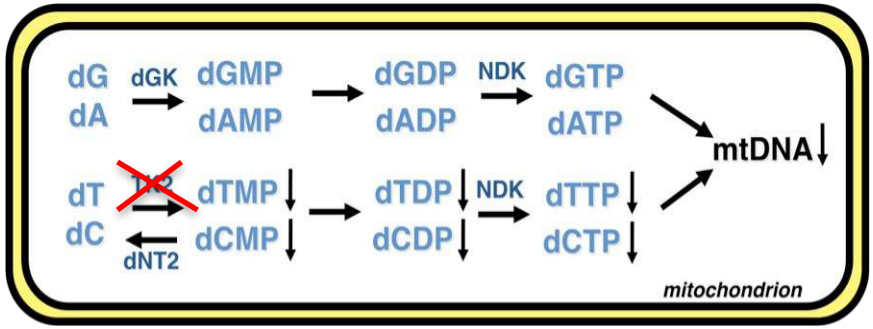
Mutant mitochondrial thymidine kinase in mitochondrial DNA depletion myopathy

Ann Saada[1*], Avraham Shaag[1*], Hanna Mandel[2], Yoram Nevo[3], Staffan Eriksson[4] & Orly Elpeleg[1]

nature genetics • volume 29 • november 2001

Table 2 • The activity of thymidine kinase 2 in muscle mitochondria

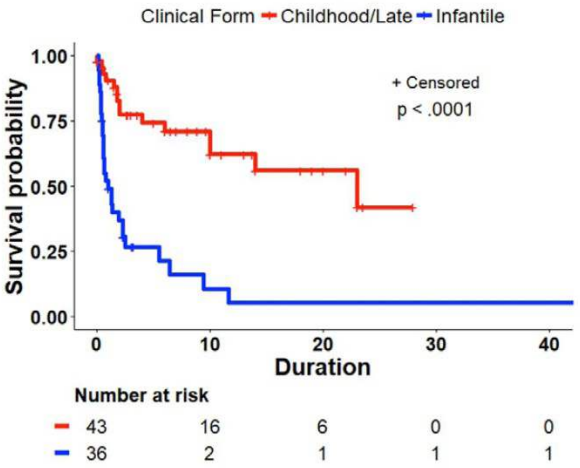
Sample	Substrate	
	[^{3}H]dThd	[^{3}H]dCyt
patient 1	3.42±0.38	1.80±0.06
patient 2	1.03±0.31	0.71±0.59
patient 4	1.76±0.42	0.68±0.08
controls (range)	7.55±1.45 (5.40–10.51)	5.65±1.89 (4.55–7.59)



- Early onset: from birth to 30 months
- Progressive weakness of skeletal and respiratory muscles
- Elevated CK and lactic acid
- Mean age of death: 2.6 years

Disease Spectrum of 92 TK2-deficient Patients

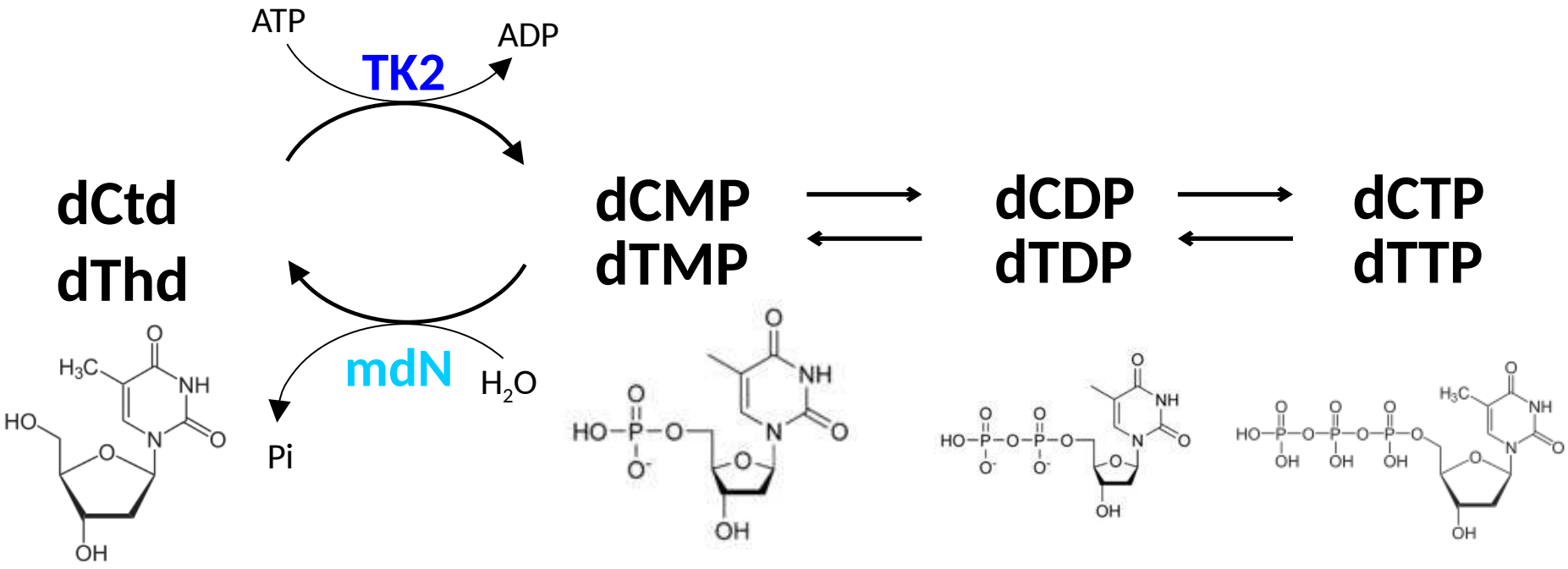
	Infantile-onset myopathy 39 (42.4%)	**Childhood-onset myopathy** 37 (40.2%)	**Late-onset myopathy** 16 **(**17.4%)
Onset	≤12 months	>1-<12 years-old	≥12 years-old
Symptoms	Diffuse muscle weakness, early respiratory failure	Proximal muscle weakness, areflexia	Muscle weakness
EMG	Myogenic +/- neuropathic pattern	Myogenic +/- neuopathic pattern	Myogenic pattern
CK	↑↑↑	↑↑↑	normal-↑↑
mtDNA depletion	+++	+++	+/-
mtDNA deletions	–	–	+++
Other signs & symptoms	seizures 7, encephalopathy 5, cognitive dysfunction 3, ptosis 4, facial diplegia 3, dysphagia 3, multiple bone fractures 2, nephropathy 1, rigid spine 1, coma episodes 1, cardiomyopathy 1, bi-ventricular hypertrophy 1, arrhythmia 1 and esophageal atresia 1	facial diplegia 11, ptosis 9, PEO 3, hearing loss 2, cognitive decline 1, encephalopathy 1, prolonged QT 1, arrhythmia 1, multiple bone fractures 1, renal tubulopathy 1, and gynecomastia 1	ptosis 9, PEO 8, dysphagia 6, respiratory insufficiency 5, dysarthria 3, cardiomyopathy 2, gynecomastia 1, Neuropathy 1, Hearing loss 1



Garone et al. J Med Genet 2018

What does thymidine kinase 2 (TK2) do?

TK2 catalyzes the first phosphorylation of pyrimidine deoxynucleosides in mitochondria



How can we study TK2d in the laboratory?

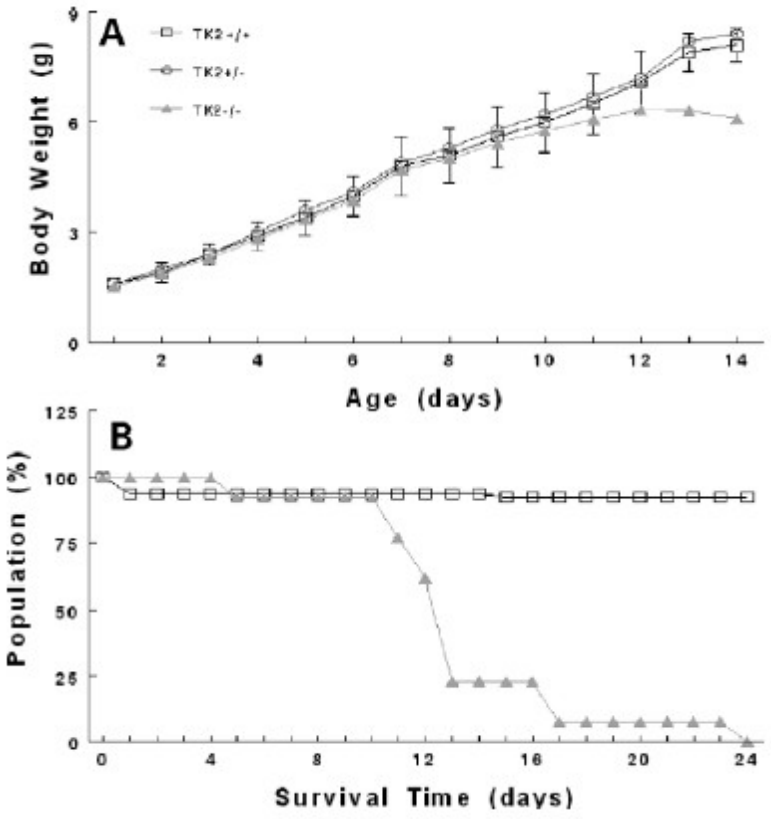
Cell models

Animal models

Thymidine kinase 2 (H126N) knockin mice show the essential role of balanced deoxynucleotide pools for mitochondrial DNA maintenance

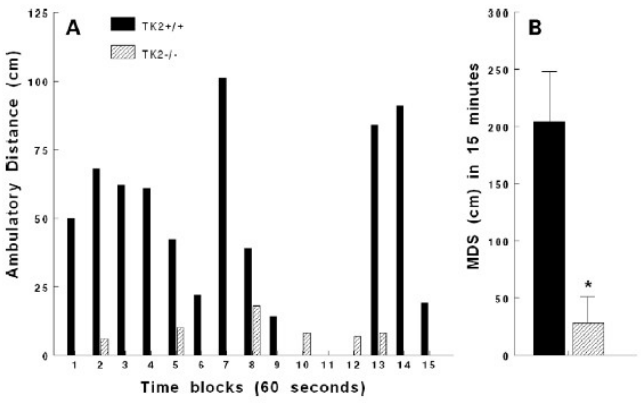
Hasan O. Akman[1,†], Beatriz Dorado[1,†], Luis C. López[1], Ángeles García-Cazorla[1,2], Maya R. Vilà[1,3], Lauren M. Tanabe[4], William T. Dauer[1,4], Eduardo Bonilla[1,5], Kurenai Tanji[5] and Michio Hirano[1,*]

Human Molecular Genetics, 2008, Vol. 17, No. 16



After post-natal day 10:

- Reduced spontaneous movements
- Generalized coarse tremor
- Severely impaired gait



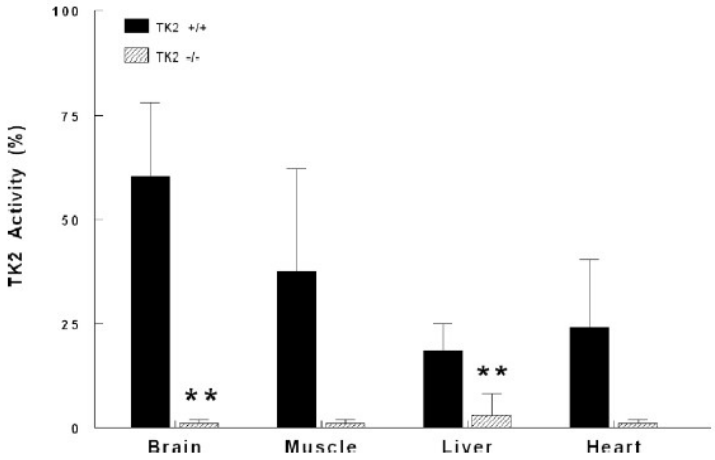
Why should we study cell and animal models?

1. To understand pathomechanisms of diseases

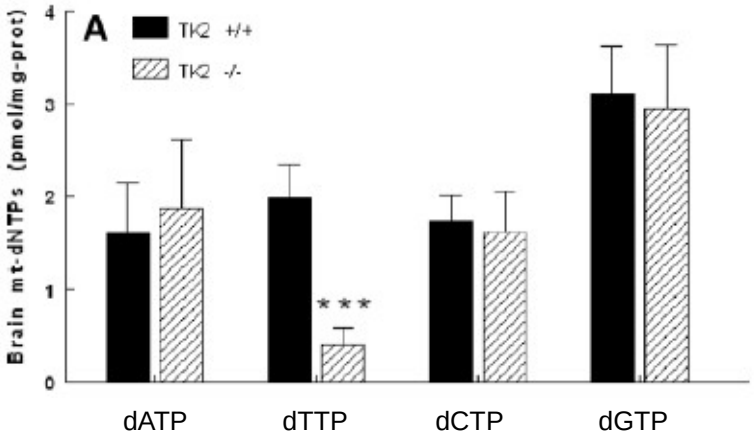
2. To identify and assess therapies for diseases

Pathogenesis of Tk2 deficiency

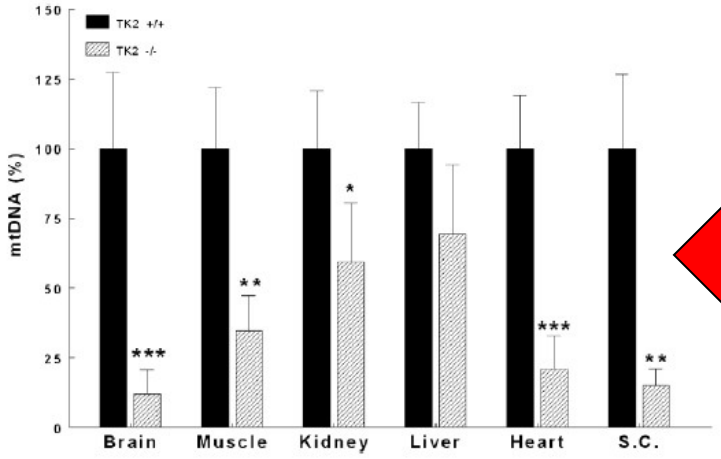
Tk2 activity

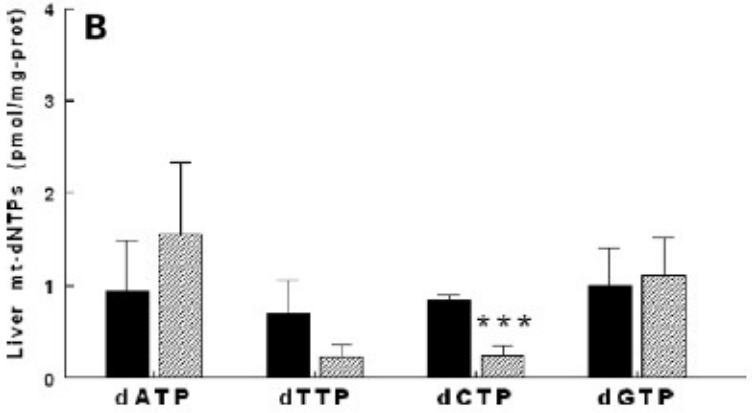


dNTP pool

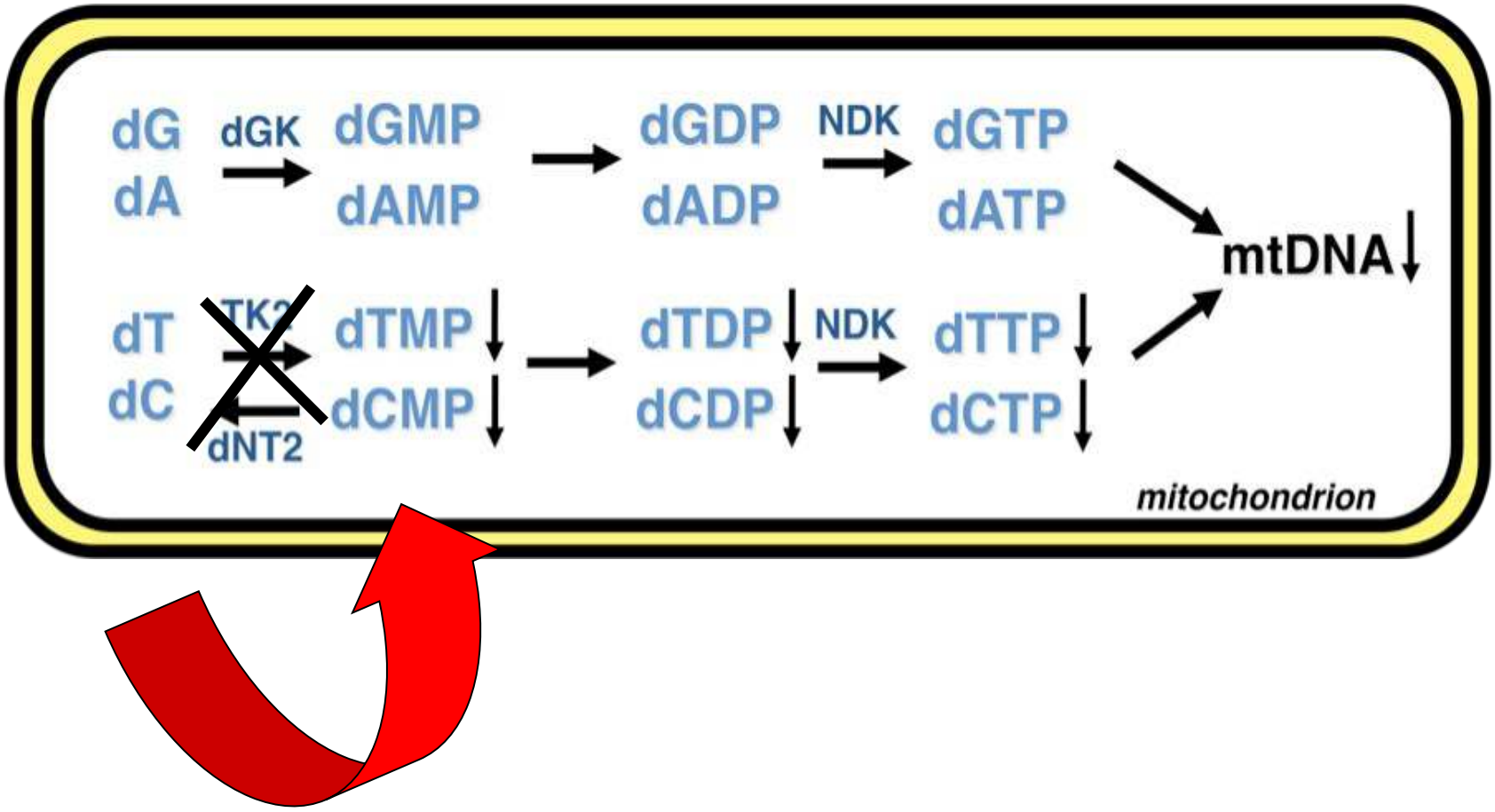


mtDNA copy number





Deoxypyrimidine monophosphate therapy

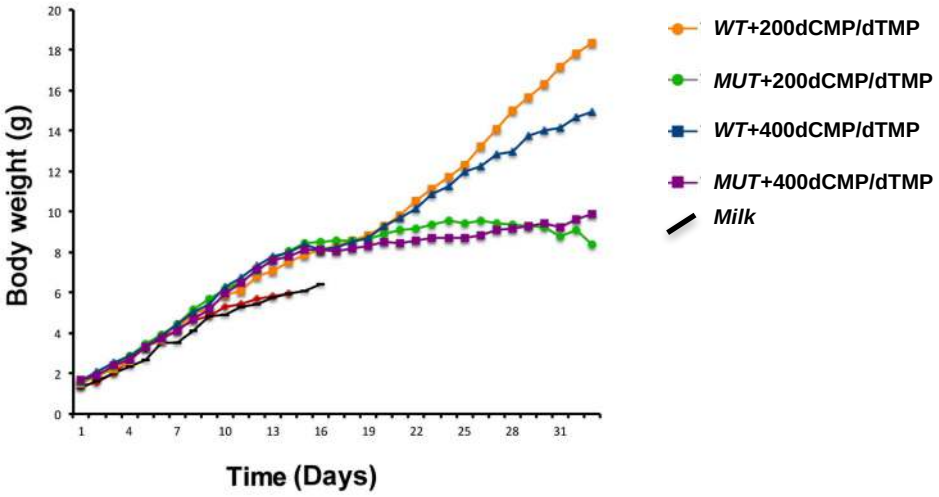


Deoxypyrimidine monophosphate bypass therapy for thymidine kinase 2 deficiency

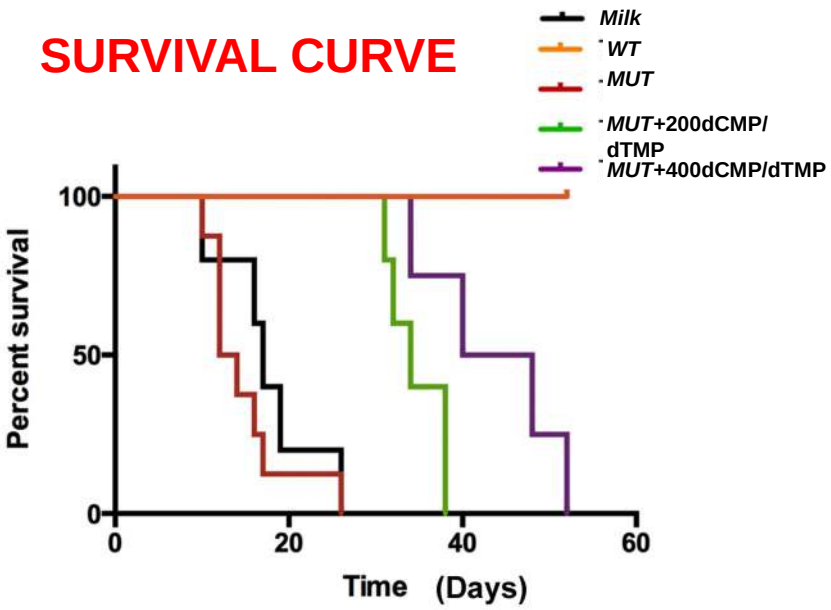
Caterina Garone[1,2], Beatriz Garcia-Diaz[1], Valentina Emmanuele[1,3], Luis C Lopez[4], Saba Tadesse[1], Hasan O Akman[1], Kurenai Tanji[5], Catarina M Quinzii[1] & Michio Hirano[1,*]

EMBO Mol Med 2014;6:1016-27

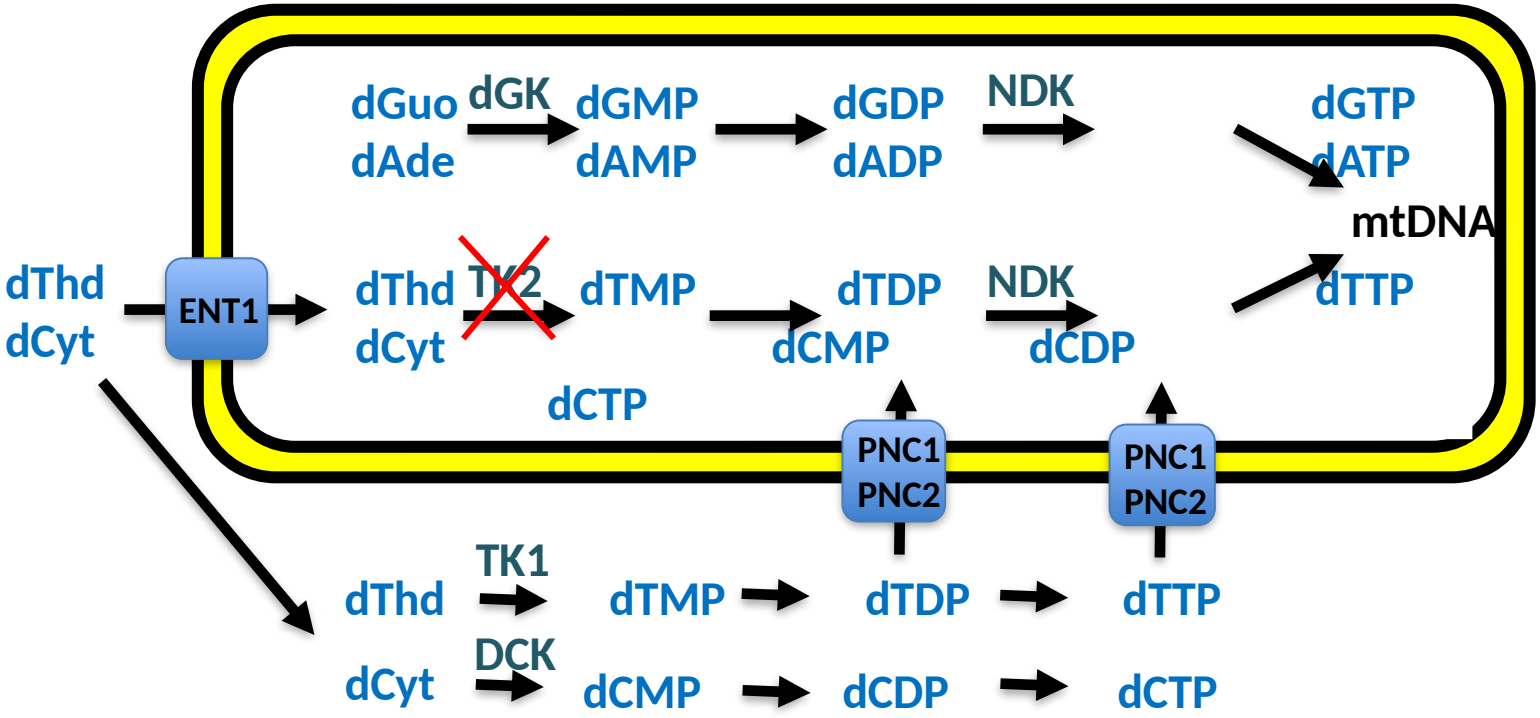
GROWTH CURVE



SURVIVAL CURVE



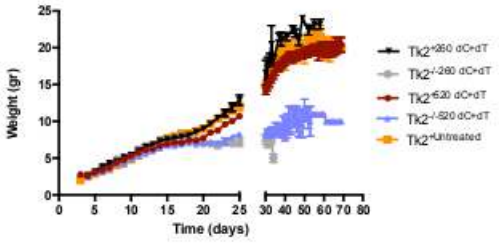
Deoxynucleoside "molecular bypass" therapy



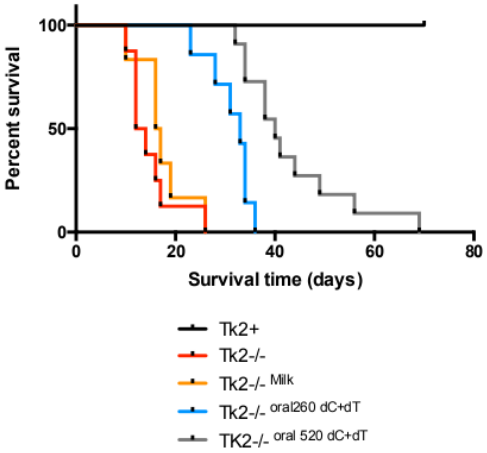
Pharmacological treatment

Oral dC+dT treatment

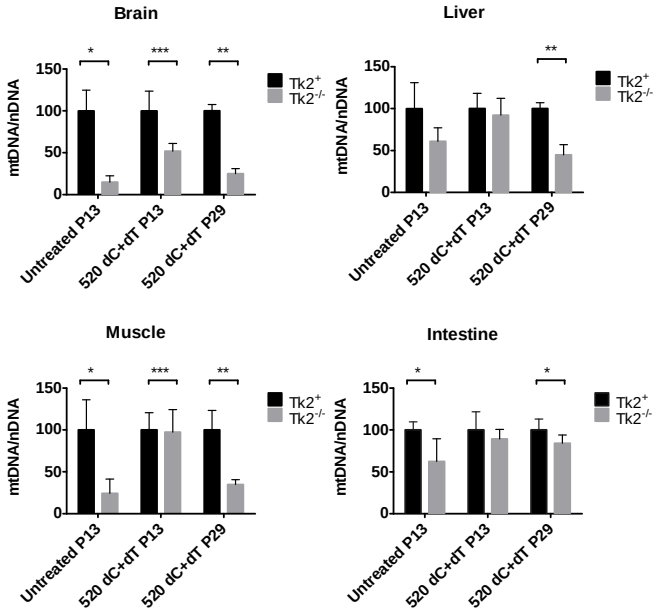
Delays disease onset



Prolongs lifespan



Rescue mtDNA copy number at P13
Only partial rescue at P29
Less efficient in brain



Patient A.E.

Normal early development: sitting at 6-7 months, cruising at 12 months

At 12 months: trouble holding up head

At 15 months: unable to cruise or sit

Admitted to a hospital for severe weakness.

- CK 1,290-2,098 (NL 24-295)
- Venous lactate 3.9 mM (NL 0.5-2.2).
- Muscle biopsy: severe mtDNA depletion
- *TK2* mutations: p. Lys50IlefsX99 and p.Thr108Met.

A.E.

Age 19 months

- Severe quadriparesis (1-2/5 strength)
- Placed on mechanical ventilation 24 h/day
- Gastrostomy

Age 21 months

- Treatment initiated via emergency IND
- dTMP+dCMP 100mg/kg/day for one month then 200mg/kg/day for 3 years

A.E.

2015:

dT+dC 260 mg/kg/day then 400mg/kg/day

After 2 months, he was able to stand for 5 minutes with support of a person and after 4 months, used biceps to lift forearms off the bed and index finger to press computer keys

A.E.

9 years-old:

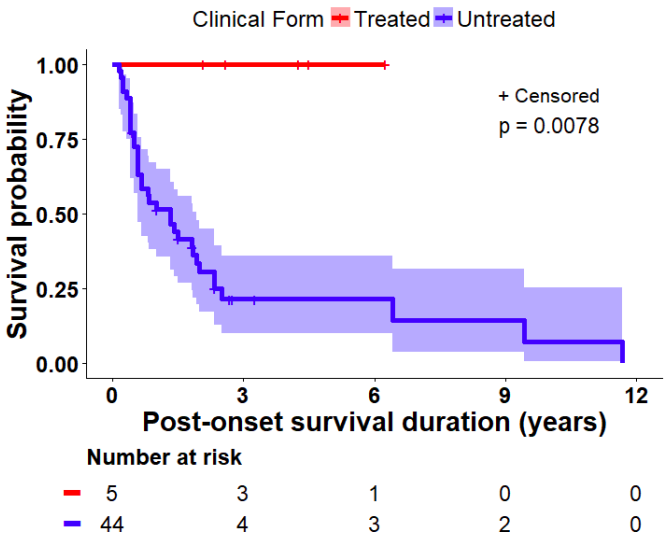
- gained weight from 10.4 to 28.4 kg;
- lifted arms and legs from bed; gained ability to hold and manipulate small objects, use an iPad and television remote;
- said words and spells name;
- sat with mild support of a person or in wheelchair 2-3 hours;
- stood in stander up to 90 minutes
- dT+dC 400mg/kg/day for >4 years: still uses ventilator and feeding tube, but…

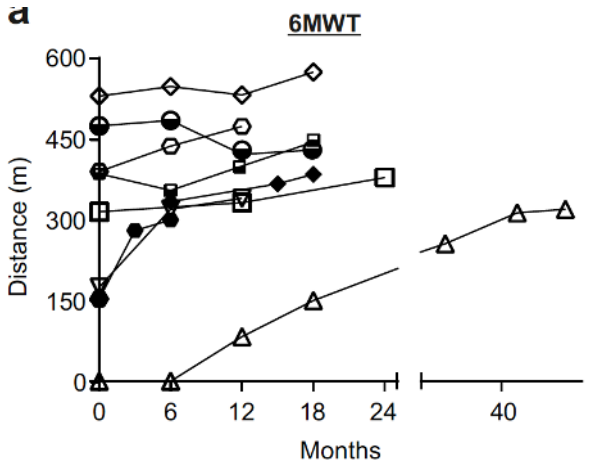
Deoxynucleoside Therapy for Thymidine Kinase 2–Deficient Myopathy

Cristina Domínguez-González, MD,[1,2,3] Marcos Madruga-Garrido, MD,[4] Fabiola Mavillard, PhD,[5,6] Caterina Garone, MD, PhD,[7] Francisco Javier Aguirre-Rodríguez, MD,[8] M. Alice Donati, MD,[9] Karin Kleinsteuber, MD,[10] Itxaso Martí, MD,[11] Elena Martín-Hernández, MD,[2,3,12] Juan P. Morealejo-Aycinena, MD,[13] Francina Munell, MD,[14] Andrés Nascimento, MD,[3,15] Susana G. Kalko, PhD,[3,15] M. Dolores Sardina, MD,[16] Concepcion Álvarez del Vayo, MD,[6,17] Olga Serrano, MD,[18] Yuelin Long, BS,[19] Yuqi Tu, BS,[19] Bruce Levin, PhD,[19] John L. P. Thompson, PhD,[19] Kristen Engelstad, MS,[20] Jasim Uddin, BS,[20] Javier Torres-Torronteras, PhD,[3,21] Cecilia Jimenez-Mallebrera, PhD,[3,15] Ramon Martí, PhD,[3,21] Carmen Paradas, MD, PhD,[5,6] and Michio Hirano, MD[20]

Ann Neurol 2019;86:293-303

16 TK2-deficient patients treated with deoxynucleos(t)ides under compassionate use protocols for at least one year prior to September 1, 2017.





TK2d treatment experience at
Columbia University Irving Medical Center (CUIMC)
2012-19

- The first treated US patient started dTMP+dCMP in November, 2012 under an emergency IND
- 2012-2019: 22 additional patients were enrolled in a US investigator-initiated expanded access program
- FDA recommended: 1) stop enrollment of new patients into the research expanded access (EA) protocol and 2) initiation of new treatment-naïve patients into a new industry-sponsored study or a Sponsor-Investigator (SI) research study to obtain industry-standard data required for drug registration.

MT1621 Clinical Program

Study	Description	N	Status
TK2 DEFICIENCY STUDIES			
	Untreated Patient Dataset (literature)	~130	Ongoing
MT1621-101	Ph 2 RETROspective study	38	Completed
MT1621-102	Ph 2 Prospective, open-label continuation study	47*	Ongoing
MT1621-107	Ph 2 Retrospective chart review study to collect vital status of untreated and treated patients not participating in Zogenix sponsored trial	~ 45 untx ~20 tx	Ongoing
CLINICAL PHARMACOLOGY STUDIES			
MT1621-103	PK and food effect (healthy volunteers)	14	Completed
MT1621-105	PK and food effect (healthy volunteers)	14	Completed
MT1621-106	Renal Impairment Study	32	Dosing complete

AWCT

*includes 35 pts who were in Study 101+ 12 addl pts;
AWCT = adequate well-controlled trial

ZOGENIX

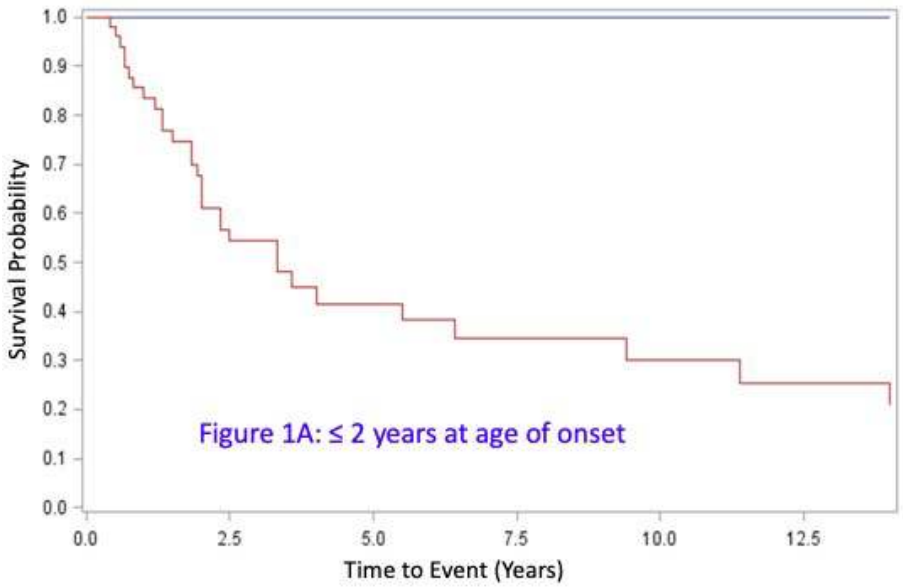
Retrospective analysis of compassionate use deoxynucleos(t)ide therapy for TK2d

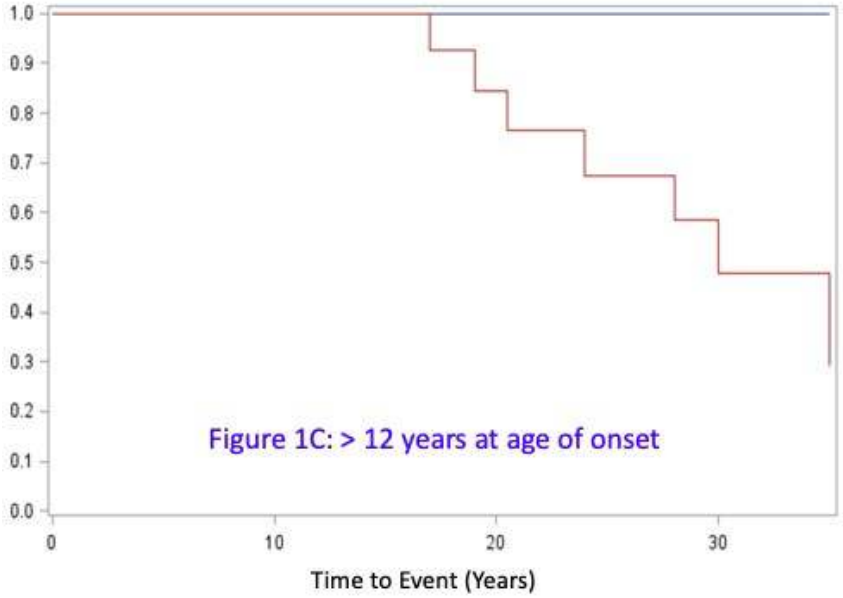
- **38 patients with TK2 deficiency have been treated with deoxynucleos(t)e therapy under an expanded access program through December 31, 2018.**
- **Median treatment 71 weeks (range 92 days-7 years)**

Subject Demographics in Study MT-1621-101	
n	38 subjects
Age at Onset	
≤ 2 years	15 (40%)
2-12 years	14 (37%)
>12 years	9 (24%)
Median Age of Onset (Q1,Q3)	2.5 (1.4,11.7) years
Male	21 (55%)
Female	17 (45%)
Baseline Status	
Ambulatory	16 (42%)
Ventilator Support	19 (50%)
Feeding Tube	8 (21%)

Direct Adjusted Survival Curves Modeled from Treated and Historical Untreated Data

(Assuming Treatment from Time of Onset, Red=Untreated, Blue=Treated)

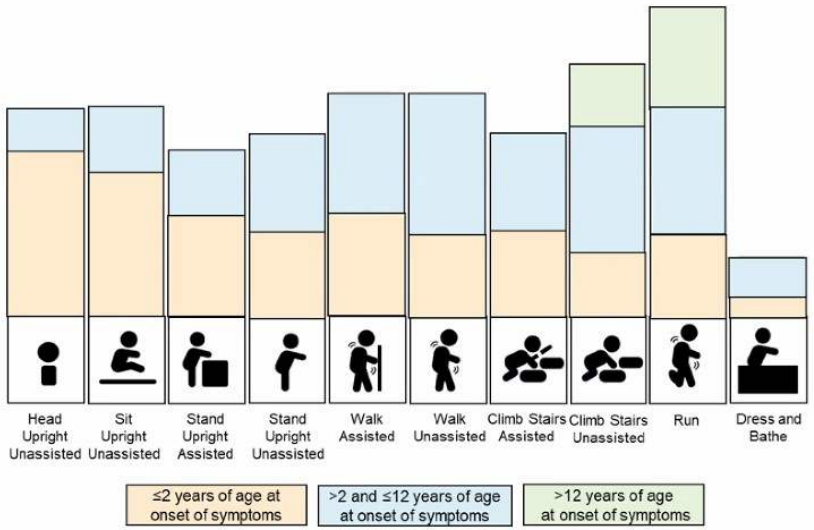




p=0.0006

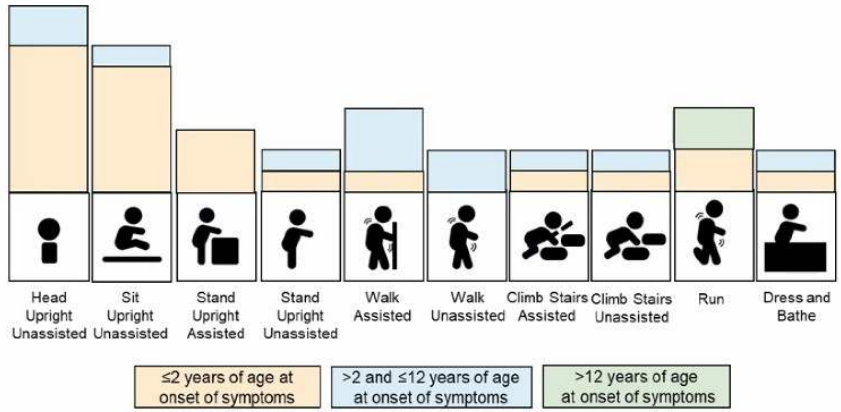
Study MT1621-101 Efficacy on Major Motor Milestones

25 of 38 patients (66%) lost motor milestones



Post Treatment *Regain* of Motor Milestones

17 of 25 patients (68%) regained motor milestones



ZOGENIX

Thymidine + Deoxycytidine (dT+dC)

- A Potential Treatment for TK2d

16 month-old boy

- Onset at age 10 months
- Lost ability to sit, stand, and walk

Age 26 months
after 10 months of dT+dC therapy

Adult Spanish TK2d patient

- 38 years-old woman
- Onset: age 5 years-old.
- Diagnosis: age 35 years-old
- Homozygous TK2 p.T108M mutation. mtDNA copy number in muscle: 25%
- Symptoms:
  - Fatigue and exercise intolerance
  - Facial, axial, and proximal limb weakness. No dysphagia.
  - Respiratory muscle weakness: Orthopnea. At age 35 yo: Vital capacity 53% began using BiPAP.
- At age 35 yo, began chemical grade deoxynucleosides 200mg/kg/day. Developed diarrhea.

Adult Spanish TK2d patient

- Response to treatment

PRE-TREATMENT

AFTER 3 MONTHS OF TREATMENT

Adult Spanish TK2d patient: Response to Treatment

PRE-TREATMENT

AFTER 6 MONTHS OF TREATMENT

TK2 Deficiency Team Acknowledgements

Special thanks to the patients and their families

Columbia University

Laboratory Team

Carlos Lopez-Gomez, PhD
Caterina Garone, MD
Orhan Akman, PhD
Maria Sanchez-Quintero, PhD
Beatriz Garcia-Diaz, PhD
Valentina Emmanuele, MD, PhD
Saba Tadesse, BS
Rebecca Levy, MD, PhD
Henley Hewan, MD
Catarina M Quinzii, MD
Kurenai Tanji, MD, PhD
Salvatore DiMauro, MD
Michio Hirano, MD

Guangping Gao, PhD
Elisa Konofagou, PhD
Carlos Sierra, PhD
Rebecca Levy, MD

Clinical Team

Kristin Engelstad, MS, GC
Caterina Garone, MD
Emanuele Barca, MD, PhD
Andres Berardo, MD, PhD
Robert Fryer, MD, PhD
Shufang Li, MD
Valentina Emmanuele, MD
Saba Tadesse, BS
Rachel Salazar, PT
Donnielle Rome-Martin, OT
Jasim Uddin, MS
Xiomara Rosales, MD
Yuelin Long, BS
Yuqi Tu, BS
Bruce Levin, PhD
Seamus Thompson, PhD
Salvatore DiMauro, MD
Michio Hirano, MD

Modis/Zogenix/UCB Therapeutics

Susan Van Meter
Cal Lucas
Stacey Harte
Richard Kim, MD
Carl Chiang, MD
Paul Hoskins,
Deric Esquivel
Christy Pifer
Karen Lai
Tracy Bortoli
Lindsay Fischer
Terri Sampo
Dennis Kim
Bradley Galer, MD
Arnold Gammaitoni
Joanne Quan, MD
Andrea Schatz
Peter Barber
Daniel DiPietro
Linda Thanos
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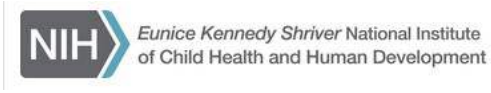

TK2 Deficiency Team Acknowledgements

Special thanks to the patients and their families

International Collaborators

Madrid, Spain
Christina Dominguez, MD; Elena Martín-Hernández, MD; Olga Serrano, MD

Sevilla, Spain
Carmen Paradas, MD; Marcos Madruga-Garrido, MD; Fabiola Mavillard, PhD; Concepcion Álvarez del Vayo, MD; Susana G. Kalko, PhD; Cecilia Jimenez-Mallebrera, PhD

Barcelona, Spain
Andrés Osorio Nascimento, MD; Francina Munell, MD; J. Torres-Torronteras; Ramon Marti, PhD; Francisco J. Aguirre-Rodriguez, MD

Badajoz, Spain
M. Dolore Sardina, MD

San Sebastian, Spain
Itxaso Martí, MD

Bologna, Italy
Caterina Garone, MD

Florence, Italy
Maria Alice Donati, MD

Naharia, Israel
Hanna Mandel, MD; Tzipora Falik Zaccai, MD

Tel Aviv, Israel
Mira Ginsberg, MD

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Galit Tal, MD

Santiago, Chile
Karin Kleinsteuber, MD

Guatemala City, Guatemala
Juan P. Morealejo-Aycinena, MD

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