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MT1621 for thymidine kinase 2 (TK2) deficiency: Mechanism of action is via mitochondrial DNA incorporation

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Introduction

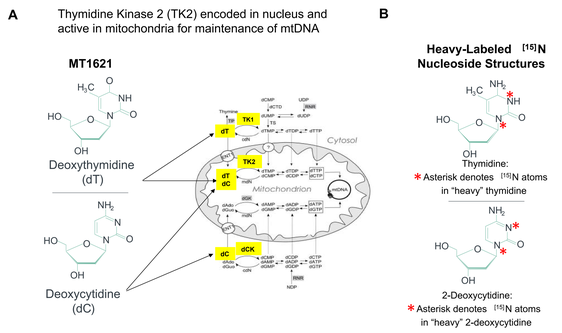
Thymidine kinase 2 (TK2) deficiency[1]:

- Is an ultra-rare autosomal recessive mitochondrial DNA depletion syndrome[2]
- Presents with severe progressive myopathy across all ages
- Leads to significant morbidity (loss of ambulation, need for ventilatory support)
- Has a mortality rate of ~50%[1,3]
- Has no currently approved treatments
- Is caused by a critical TK2 enzyme deficiency, leading to deficiency in the mitochondrial DNA (mtDNA) replication pathway
  - Mutations in the *TK2* gene result in impaired phosphorylation of deoxycytidine (dC) and deoxythymidine (dT), leading to depletion of the deoxynucleotriphosphate (dNTP) pool, which is required for mtDNA replication
  - mtDNA copy number depletion leads to insufficient synthesis of mitochondrial respiratory chain complexes

MT1621: Pharmacologic mechanism of action

- MT1621, an oral, fixed-dose combination of dC and dT, is being developed as substrate enhancement therapy for TK2 deficiency (**Figure 1A**)
- MT1621 increases the available pool of dNTPs for mtDNA replication by increasing dC and dT substrates for:
  - Mitochondrial TK2, maximizing residual mitochondrial salvage activity
  - Cytosolic TK1 and deoxycytidine kinase (dCK) in cytosolic salvage pathways
- Results from a TK2-/- mouse model support a mechanism whereby oral administration of dC and dT increases mtDNA copy number to prolong survival and delay disease progression[4,5]
- Although the metabolic pathways for dC/dT metabolism are well understood, physiological concentrations of substrate and product are difficult to model
  - Balanced nucleoside administration is important, as unbalanced nucleoside pools can lead to loss of fidelity in mtDNA replication

Figure 1. Mechanism of action of MT1621 (dC/dT) in mtDNA replication



Objectives

- To characterize the molecular mechanism of pyrimidine nucleoside treatment in TK2 deficiency using a TK2 knock-out mouse model (TK2-/-) to assess incorporation of isotopically labeled dC and dT into mtDNA

Methods

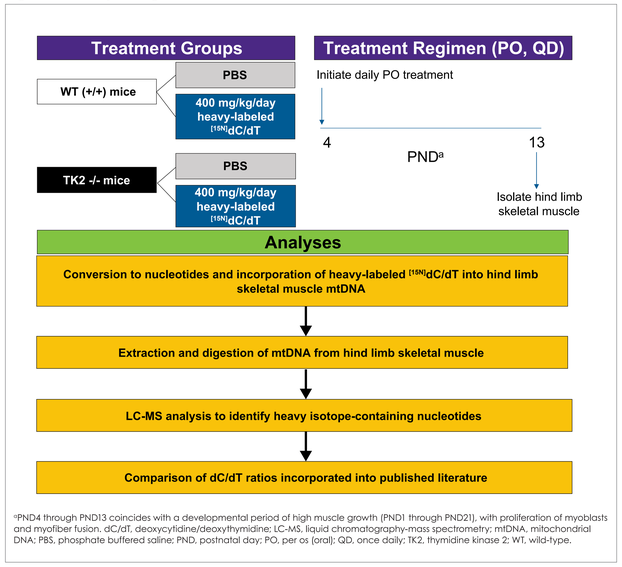
Mouse model of TK2 deficiency[6,7]

- All experiments were conducted in compliance with the Generalitat de Catalunya for the Care and Use of Laboratory Animals
- The in vivo protocol was approved by the Ethics Committee for Animal Experimentation of the Vall d'Hebron Research Institute, and the study was approved by the Universities' Institutional Animal Care and Use Committees (IACUC)
- TK2-/- knockout mice (C57BL/6 genetic background) have been previously characterized and described[5-7]
- Mice were housed in a controlled environment with a 12-hour light/dark cycle and ad libitum access to water and standard rodent chow

Study design

- Wild-type (WT) (N=3 or 6/group) or TK2-/- (N=2 or 4/group) mice were orally administered a once-daily dose of vehicle (phosphate-buffered saline; PBS) or 400 mg/kg/day isotopically labeled heavy [15N]-dC and [15N]-dT from postnatal day 4 (PND4) to PND13 (**Figure 1B, Figure 2**). All animals in each litter were dosed until they were genotyped
  - Dose timing coincided with a developmental period characterized by active proliferation of myoblasts and myofiber fusion (PND1 through PND21) when salvage pathways are highly expressed due to cell division
- Skeletal muscle from both hind limbs was collected at PND13 and frozen for mtDNA isolation and analysis
- mtDNA was extracted (Abcam kit ab65321); mtDNA was then enzymatically digested and analyzed by targeted liquid chromatography-mass spectrometry (LC-MS; QExactive Plus Mass Spectrometer, Thermo Scientific, Waltham, MA, USA) with an Ultimate 3000 nano system
- Nucleoside AUC data were processed in Skyline software (MacCoss Lab, University of Washington, Seattle, WA, USA) to quantify the heavy and light isotope ratio of dC and dT

Figure 2. Study design

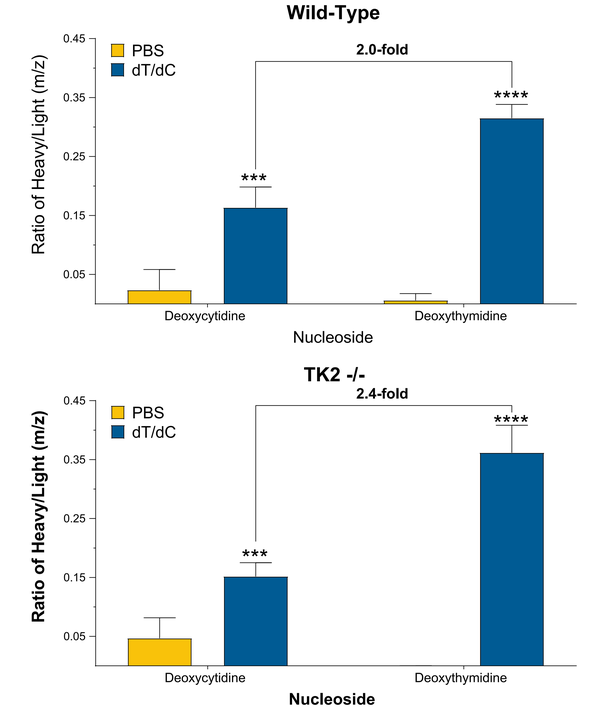


[a]PND4 through PND13 coincides with a developmental period of high muscle growth (PND1 through PND21), with proliferation of myoblasts and myofiber fusion. dC/dT, deoxycytidine/deoxythymidine; LC-MS, liquid chromatography-mass spectrometry; mtDNA, mitochondrial DNA; PBS, phosphate buffered saline; PND, postnatal day; PO, per os (oral); QD, once daily; TK2, thymidine kinase 2; WT, wild-type.

Results

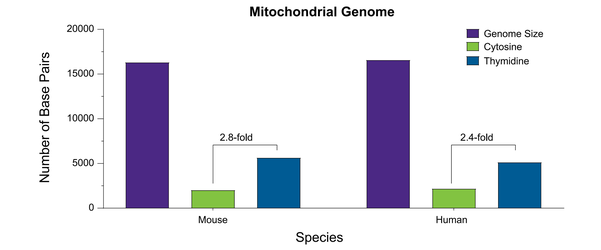
- Nucleosides administered to mice are incorporated into mtDNA in vivo (**Figure 1**)
  - mtDNA incorporation of isotopically labeled dC and dT in WT and TK2-/- mice given dT/dC was substantially increased over PBS control mice (**Figure 3**)
- Relative dT incorporation was approximately 2-fold that of dC in both WT and TK2-/- mice (**Figure 3**)
  - These ratios are consistent with literature-derived base composition in the mouse mitochondrial genome (**Figure 4, Table 1**)[8]
- Base composition of mtDNA is comparable in mouse and human (**Table 1**)[8]
  - mtDNA contains about 16,000 base pairs, with a predictable dT:dC ratio of approximately 2.8-fold in mice and 2.4-fold in humans
  - In contrast, the nuclear genome contains ~3 million base pairs, where incorporation ratios are poorly defined

Figure 3. Relative incorporation of dC and dT in skeletal muscle of wild-type and TK2 -/- mice



***P<0.001; ****P<0.0001. dT/dC, deoxythymidine/deoxycytidine; PBS, phosphate buffered saline; TK2, thymidine kinase 2.

Figure 4. Literature-derived predictions for mitochondrial genome composition



Species comparison data are from Toren et al 2016.[8]

Table 1. Base composition of mouse and human mitochondrial genome

Species	Genome size (base pairs)	dC (base pairs)	dT (base pairs)	Ratio (dT/dC)
Mouse	16,299	2013	5629	2.8
Human	16,569	2169	5124	2.4

Species comparison data are from Toren et al 2016.[8]
dC, deoxycytidine; dT, deoxythymidine.

Conclusions

- After oral administration of dC/dT to WT and TK2-/- mice:
  - dC and dT were incorporated into skeletal muscle mtDNA in both WT and TK2-/- mice
  - mtDNA incorporation was comparable to the expected ratios of dT to dC in the mouse mitochondrial genome
- These data support mtDNA incorporation as the mechanism of action for MT1621 substrate enhancement therapy in TK2-deficient mice
- The similarity of the base composition in the mitochondrial genomes of both mouse and human supports the relevance of these findings as a mechanism of action for human pathways in MT1621 clinical studies[9]
- These data support a mechanism of action for MT1621 via a TK2-independent phosphorylation pathway for pyrimidine nucleoside incorporation of mtDNA

References

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Disclosure

LST: Employee, stock owner, Modis Therapeutics, a wholly owned subsidiary of Zogenix, Inc.; **DK, GH:** Paid consultant, Modis Therapeutics, a wholly owned subsidiary of Zogenix, Inc. **AK:** No disclosures; **RM, YC, CB-B, VHIR, and The Biomedical Network Research Centre on Rare Diseases (CIBERER)** have filed patent applications covering potential use of deoxynucleoside treatment for POLG deficiency and other mtDNA replication defects in humans. VHIR and CIBERER have licensed pending patent applications related to these technologies to MODIS Therapeutics, Inc., and VHIR and CIBERER may be eligible to receive payments related to the development and commercialization of the technologies. Any potential licensing fees earned will be paid to VHIR and CIBERER and are shared with all inventors mentioned above through VHIR and CIBERER policies on distribution and objectivity in research. YC serves as a paid consultant to MODIS Therapeutics, Inc., a wholly owned subsidiary of Zogenix, Inc.

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