**MT1621 for thymidine kinase 2 (TK2) deficiency:**

**Mechanism of action is via mitochondrial DNA incorporation**

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

**Introduction**

**Thymidine kinase 2 (TK2) deficiency:**

- Is an ultra-rare autosomal recessive mitochondrial DNA depletion syndrome
- Presents with severe progressive neurodegeneration
- Leads to mitochondrial dysfunction and subsequent organ failure
- Is caused by critical TK2 enzyme deficiency, leading to deficiency in the mitochondrial DNA (mtDNA) replication pathway

**Methods**

**Mouse model of TK2 deficiency:**

- All experiments were conducted in compliance with the Generalitat de Catalunya for the Care and Use of Laboratory Animals.
- In 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 Instituto de Salud Carlos III, Madrid, Spain.
- Results from a TK2-/- mouse model support a mechanism whereby oral administration of dC and dT into mtDNA enhances dNTP availability for mtDNA replication.

**Results**

- Despite dC and dT, deoxycytidine/deoxythymidine; LC-MS, liquid chromatography-mass spectrometry; mtDNA, mitochondrial DNA.
- These results are consistent with literature-derived base composition in the mouse mitochondrial genome.

**Conclusions**

- These data support dNTP incorporation as the mechanism of action for MT1621 substrate enhancement therapy in TK2 deficient mice.
- The ability of dNTP incorporation to maintain mitochondrial DNA in both mouse and human subjects supports the relevance of these findings as a mechanism of action for human pathogenic mtDNA in TK2-/- clinical studies.

**Disclosure**

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**Table 1. Base composition of mouse and human mitochondrial genome**

<table>
<thead>
<tr>
<th>Species</th>
<th>Base composition</th>
<th>A (base pairs)</th>
<th>C (base pairs)</th>
<th>G (base pairs)</th>
<th>T (base pairs)</th>
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<tbody>
<tr>
<td>Mouse</td>
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<td>Human</td>
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**Figure 1A**

- Thymidine Kinase 2 (TK2) encoded in nucleus and active in mitochondria of muscle

**Figure 1B**

- Conversion to nucleotides and incorporation of heavy-labeled dC/dT into mitochondrial DNA.

**Figure 2**

- Treatment groups

**Figure 3**

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

**Figure 4**

- Literature-derived predictions for mitochondrial genome composition.