PX578: A First-in-Class POLG Activator to Increase mtDNA for Treatment of Mitochondrial DNA Depletion Syndromes

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Abstract

Mitochondrial DNA depletion syndromes (MDDS) are severe primary mitochondrial disorders (PMDs) caused by marked reductions in mitochondrial DNA (mtDNA) copy number, leading to impaired oxidative phosphorylation and multi-organ dysfunction. Mutations in DNA polymerase γ (*POLG*), the sole polymerase for mtDNA replication, are most prevalent, with over 300 variants identified. These conditions result in profound morbidity and early mortality, and this genetic diversity underscores the urgent need for mutation-agnostic therapies. Clinically, MDDS manifests heterogeneously, but neurological symptoms such as seizures, neuropathy and ataxia predominate, often accompanied by hepatic and muscular involvement.

PX578 represents a first-in-class, orally bioavailable, brain-penetrant small-molecule activator of Polγ designed to restore mtDNA replication and mitochondrial function independent of MDDS patient genotype. PX578 binds an allosteric site at the interface between the catalytic subunit POLGA and the proximal POLGB subunit, enhancing enzyme activity and processivity. In patient-derived fibroblasts and POLG mutant neural stem cells, PX578 drives mtDNA synthesis, increases mtDNA copy number, and improves mitochondrial function. Preclinical studies in two MDDS mouse models demonstrate robust efficacy and favorable pharmacology, supporting its potential as a disease-modifying therapy.

PX578 is currently in Phase 1 trials in healthy volunteers, with MDDS patient studies planned for 2026. By directly addressing the fundamental defect of mtDNA depletion, PX578 represents a novel, mechanism-based therapeutic strategy for a broad spectrum of MDDS. This approach represents a transformative advance in mitochondrial medicine with the potential to reverse or stop disease progression.

Methods

POLγ Activation Assay: Increase in fluorescence (response) after a 2-hour polymerase reaction relative to untreated using a 20-nt primer pre-hybridized to a single-stranded DNA (ssDNA) template.

mtDNA Depletion and Recovery Experiments: Fibroblasts were treated with 50 ng/mL ethidium bromide (EtBr) for 7 days(T7) After EtBr removal, cells were cultured in low serum to induce quiescence for 3 days (T7 +3), then PX578 or 0.01% DMSO vehicle was added for 14 days followed by mtDNA quantification via RT-qPCR.

Seahorse Extracellular Flux: Oxygen consumption rate (OCR) was measured using the XF Cell Mito stress test kit. Neural stem cells (NSCs) homozygous for the G848S POLγ mutation were treated with PX578 or 0.01% DMSO vehicle for 10 days.

TK2 KO Mouse: TK2-KO mice were administered a daily oral dose of either PX578 (100 mg/kg in Esbilac milk) or vehicle beginning on Day 4 of life. In a separate cohort of mice, under identical experimental conditions, liver mtDNA copy number was determined by qPCR on Day 15 of life.

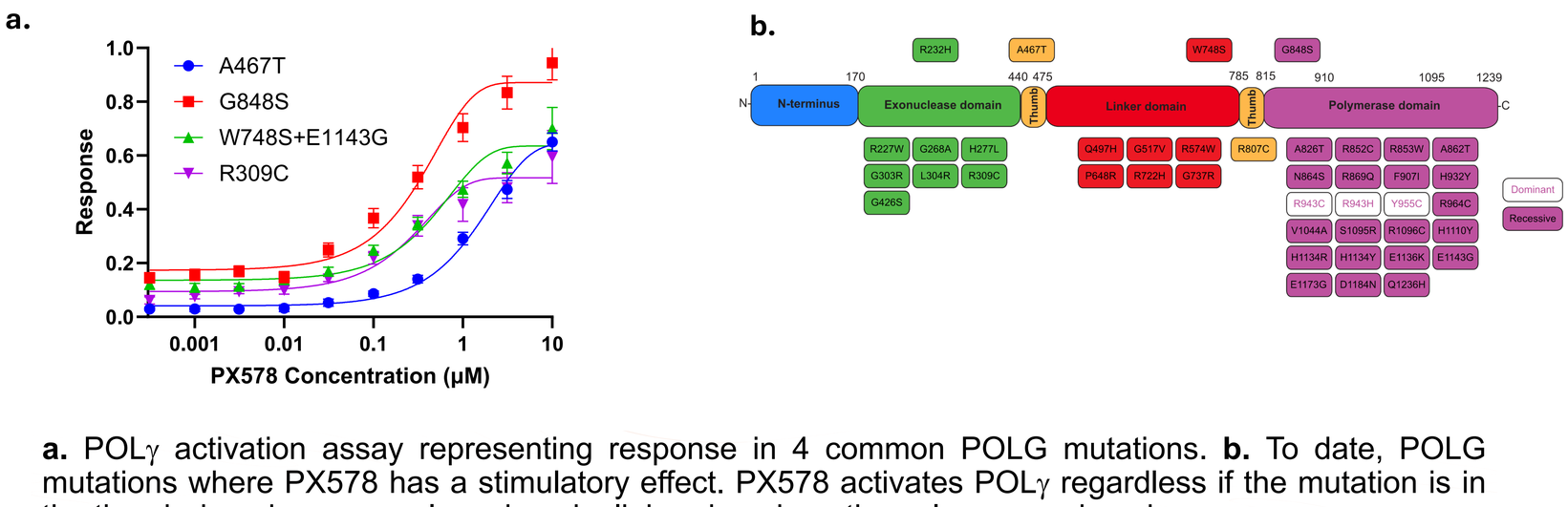
DGUOK KO Mouse: Eight-week-old mice were treated with vehicle or PX578 (100 mg/kg) by daily oral gavage for 28 days. Samples were collected on Day 29, and plasma transaminase levels and liver mtDNA copy number were measured.

Conclusions

- PX578 is a first-in-class, orally-administered, brain-penetrant small molecule activator of mtDNA POLγ - sole DNA polymerase responsible for mtDNA replication
- PX578 activates both mutant and wild-type POLγ, including the three most common mutations: A467T, G848S, and W748S
- PX578 binds to POLγ leading to increased mtDNA synthesis and improved cellular energetics, which is at the core of metabolic dysfunction in PMDs
- PX578 elicits beneficial pharmacology in animal models where mtDNA is below a defined physiological range

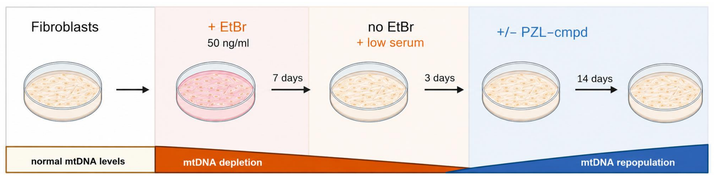
Results

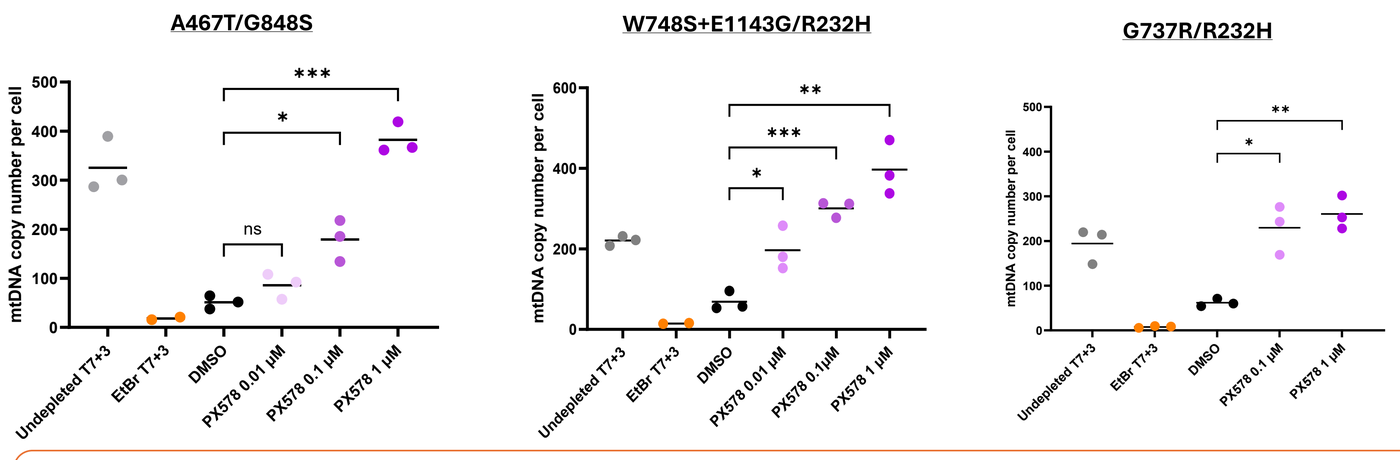
PX578 Activates Mutant POLγ



a. POLγ activation assay representing response in 4 common POLG mutations. **b.** To date, POLG mutations where PX578 has a stimulatory effect. PX578 activates POLγ regardless if the mutation is in the thumb domain, exoneucleus domain, linker domain or the polymerase domain.

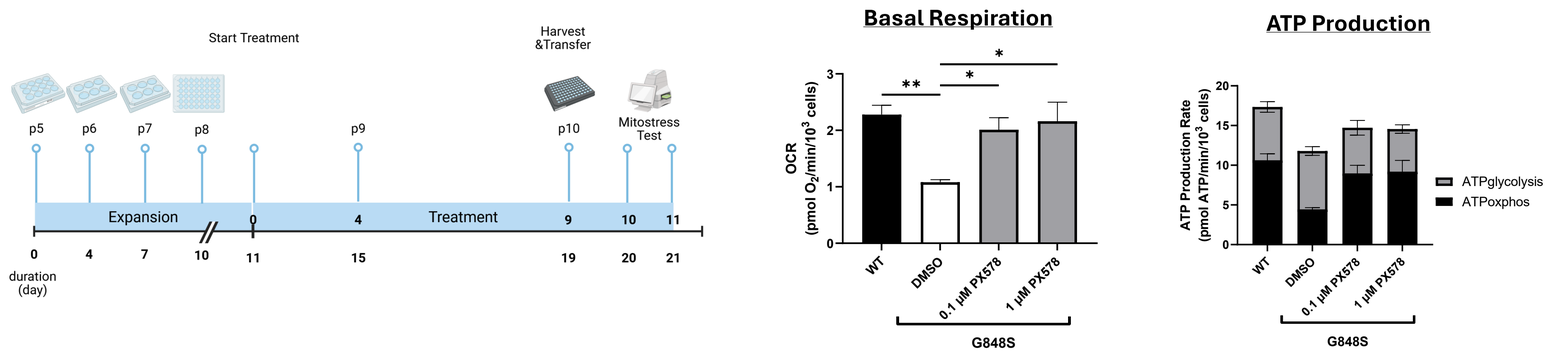
PX578 Accelerates mtDNA Recovery in POLG Patient Fibroblasts





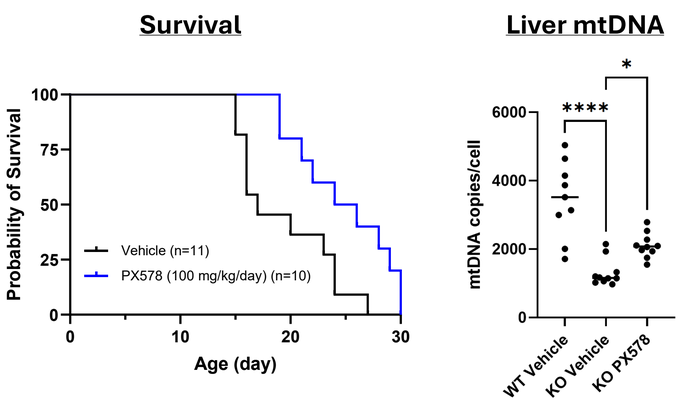
EtBr-depleted quiescent patient fibroblasts carrying POLG mutations were treated with either DMSO or 0.01, 0.1 and 1 uM PX578 for 14 days
T7+3: 7-day depletion in proliferation phase and 3-day induction of quiescence phase without EtBr.

PX578 Enhances Cellular Respiration in Neural Stem Cells



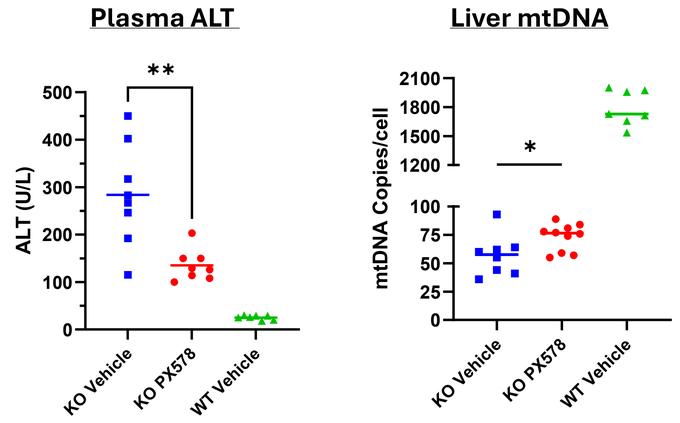
Human neural stem cells homozygous for the G848S POLG mutation were cultured with 0, 0.1 and 1.0 μM of PX578 for 10 days prior to Seahorse assay.

PX578 Increases Survival and mtDNA in TK2-KO Mice



Mice given daily oral dose of PX578 (100 mg/kg) or vehicle starting at day 4 of life. Liver mtDNA copy number assessed on day 15 of life.

PX578 Lowers ALT and Increases mtDNA in DGUOK-KO Mice



8-week-old mice given daily oral dose of PX578 (100 mg/kg) or vehicle for 29 days. Plasma alanine aminotransferase (ALT) and liver mtDNA assessed on day 29.