Mitochonic Acid 5: ITP Application

Title: Mitochonic Acid 5 (MA-5): Lifespan and Healthspan Extension in Genetically Heterogeneous Mice
Applicants: Rapamycin News

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2. Rationale and Background

Mechanism: Mitochonic Acid 5 (MA-5), an indole-3-acetic acid derivative, targets mitochondrial bioenergetics and cellular senescence pathways. The proposed intervention utilizes either the S-enantiomer or a 50/50 racemic mixture. MA-5 operates via a pleiotropic mechanism:

1. It stabilizes mitochondrial cristae, facilitating ATP production even in the presence of severe Complex I defects.
2. It acts as an allosteric activator of nicotinamide phosphoribosyltransferase (NAMPT), functioning as a direct NAD+ booster independent of NAD+ precursors.
3. It activates the DNA-dependent protein kinase (DNA-PK) pathway in the absence of DNA damage signals. This activation results in significantly increased protein levels of SIRT1, as well as SIRT2-7, without requiring an upregulation in mRNA expression.

Relevance to Longevity: Mitochondrial dysfunction and NAD+ depletion are primary hallmarks of aging. Current NAD+ boosting strategies (e.g., NMN, NR) rely on precursor availability and are rate-limited by NAMPT activity, which declines with age. By directly allosterically activating NAMPT, MA-5 circumvents this bottleneck. Furthermore, the simultaneous stabilization of cristae and elevation of SIRT1-7 protein networks targets the intersection of metabolic decline and epigenetic instability. This mechanism mimics aspects of caloric restriction and pharmacological preconditioning without the requirement for metabolic stress.

Previous Evidence: The primary efficacy data for MA-5 derives from the Ndufs4 knockout mouse, a widely used model for Leigh Syndrome (a severe pediatric mitochondrial disease characterized by Complex I deficiency). In this model, MA-5 significantly prolonged lifespan and delayed the onset of neurological deficits (1). While Ndufs4 knockout mice do not represent a standard wild-type aging model, the prevention of ATP depletion and the rescue of mitochondrial survival pathways provide a strong rationale for testing healthspan and lifespan extension in genetically heterogeneous (UM-HET3) mice experiencing age-related mitochondrial dysfunction. Additional data is required to confirm whether these disease-rescue mechanics translate to wild-type longevity.

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3. Activity, Dosage, Bioavailability, and Toxicity

Pharmacokinetics: MA-5 is orally bioavailable and has been successfully administered systemically in murine models. For the ITP, stability in standard mouse chow (e.g., LabDiet 5LG6) at room temperature must be verified. Indole derivatives generally exhibit sufficient thermal stability to survive standard pelleting processes, though microencapsulation may be required to prevent degradation during long-term storage.
Toxicity: Previous in vivo studies indicate that MA-5 is well-tolerated at therapeutic doses. The proposed dose of 10 mg/kg/day is expected to be well below the threshold for acute toxicity. However, long-term toxicological data in UM-HET3 mice over a full lifespan is currently a knowledge gap and must be monitored.
Chemical Structure: MA-5 is a synthetic indole derivative. Its core structure is based on 4-(2,4-difluorophenyl)-2-(1H-indol-3-yl)-4-oxobutanoic acid. The chemical formula is C18H13F2NO3.

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4. Suggested Treatment Protocol

Route: Dietary administration (standard ITP protocol).
Dosage Calculation: The proposed clinical dose for testing is 10 mg/kg/day.
Assuming an average UM-HET3 mouse weighs 30 g (0.03 kg) and consumes approximately 3.5 g of food per day:

- Target Dose (10 mg/kg/day) x Body Weight (0.03 kg) = 0.3 mg/day/mouse

To achieve this daily intake via diet:

- 0.3 mg / 0.0035 kg (daily food intake) = 85.7 mg of MA-5 per kg of chow

Target dietary concentration: **85.7 ppm**.
Start Age: 4 months of age (lifelong administration).
Biomarkers: Efficacy of target engagement should be verified at 6 and 12 months using:

- Western blot for SIRT1 and SIRT3 protein levels in peripheral blood mononuclear cells and hepatic tissue.
- Quantification of intracellular NAD+ and NADH ratios in skeletal muscle.
- NAMPT activity assays.

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5. Cost of a Life-long Intervention Study

Supply: Currently available via Sigma-Aldrich.
Budget Calculation:
Assuming a standard single-site ITP cohort of 100 mice (50 male, 50 female) treated for up to 3 years:

- Total compound per day: 0.3 mg x 100 mice = 30 mg/day
- Total compound required: 30 mg/day x 365 days x 3 years = 32,850 mg (approx. 32.85

grams).

Based on the current Sigma catalog price of 15.80 USD per mg:

- 32,850 mg x 15.80 USD = 519,030 USD

Constraint & Practical Solution: A chemical cost exceeding 500,000 USD for a single site is fundamentally prohibitive for the standard ITP budget. Proceeding with this application strictly requires abandoning the Sigma retail source. A custom synthesis contract with a laboratory (e.g., WuXi AppTec, MedChemExpress bulk division) must be established to secure 50 to 100 grams of MA-5 at a wholesale synthesis cost (typically 5,000 to 15,000 USD total) to make the trial financially viable.

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6. Animal Safety Information

While acute toxicity is low, the mechanism of action presents specific theoretical risks for chronic administration:

1. **Chronic DNA-PK Activation:** Activating DNA-PK independent of DNA damage over a lifespan could theoretically dysregulate normal DNA repair fidelity or interfere with V(D)J recombination in maturing lymphocytes.
2. **Monitoring Protocol:** Animals must be routinely monitored for unexpected weight loss, signs of immunosuppression (opportunistic infections), and standard ITP necropsy protocols to assess any potential acceleration in tumorigenesis.

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7. Statement of Understanding

"I understand all information presented in the proposal can be freely shared with members of the ITP Steering Committee and the sponsors of the ITP at the National Institute on Aging. I understand the ITP intends to submit the results of all ITP-supported studies for publication in the peer-reviewed literature, regardless if they produce data showing positive or negative effects on lifespan and healthspan."

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8. References

1. Suzuki, T., et al. (2015). "Mitochonic acid 5 binds mitochondria and ameliorates renal tubular and cardiac myocyte damage." Journal of the American Society of Nephrology, 27(7), 1925-1932.
2. Matsuhashi, T., et al. (2017). "MA-5 facilitates mitochondrial ATP production and survival of patient fibroblasts." Tohoku Journal of Experimental Medicine, 242(4), 269-278.
3. Lei, X., et al. (2022). "Mitochonic acid 5 activates the DNA-PK-SIRT1 pathway to protect against mitochondrial dysfunction." Cell Death & Disease, 13(1), 1-15.
