Preclinical Evaluation of NUDT15 Genotype-Guided Thiopurine Dose Individualization using CRISPR-Cas9 Mouse Model


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Introduction of Thiopurines

6MP

• Thiopurines (i.e., 6MP, 6TG) are widely used therapeutic agents in cancers (e.g., acute lymphoblastic leukemia) and autoimmune diseases (e.g., inflammatory bowel diseases)

6TG

• Myelosuppression is the main side effect

• Dose titration is done based on WBC but challenging
Inherited Polymorphism in **NUDT15** is a Novel Genetic Determinant of Thiopurine Toxicity

- Inherited polymorphisms in *TPMT* are well known to be associated with thiopurine toxicity
- *TPMT*-genotype guided thiopurine dosing algorithm is a prototype of pharmacogenetics-driven precision medicine

**GWAS in pediatric ALL cohort (N=657)**

Relling et al, *Clin Pharmacol Ther* 2013
Yang et al., *J Clin Oncol* 2015
**Objective**

Establish *Nudt15* knockout mouse model to evaluate the effectiveness of thiopurine dosing individualization *in vivo*
Nudt15 Knockout in Mouse Using CRISPR-Cas9

**Exon 1**

*Nudt15* 1 2 3 4 5

5′ · · · GCTATGGCCGC · · · 60 nt · · · GCATCTCGTGCCTGCTCCTTTCTGGGGAAAGGAA · · · 3′

3′ · · · CGATACGGGGCG · · · 60 nt · · · CGTAGGAGCGACGCAGGAAGACCCCTTTCTT · · · 5′

**Start codon**

**sgRNA target site**

**PAM**

**Nudt15**−/−
c.78_79insT

WT Nudt15 Knockout in Mouse Using CRISPR-Cas9

![Western blot analysis](attachment:image.png)

Control  WT  c.78_79insT

**β-actin**

**WT Nudt15**
Thiopurine Toxicity was Mitigated by Reducing MP Dose

Daily intraperitoneal administration with 6MP (20, 5, or 1 mg/kg)

Toxicity evaluation
- Body weight loss
- Survival
- CBC (bone marrow suppression)

Dosage (mg/kg)

Nudt15+/+ (n = 15)  
Nudt15-/- (n = 16)

BW Reduction (%)

Survival Rate

Day

KO 20mg/kg  
WT 5mg/kg  
KO 20mg/kg

Day

KO 5mg/kg  
WT 1mg/kg  
KO 20mg/kg  
WT 5mg/kg  
KO 1mg/kg  
WT 1mg/kg
KO mice experienced more severe leukopenia than WT mice at the same dosage.

Hematological toxicity was mitigated by MP dose reduction from 20 mg/kg to 1 mg/kg in both genotypes.

Cytotoxicity in KO mice exposed to 1 mg/kg was comparable to that of WT mice receiving 20 mg/kg.
MP Dose Reduction Effectively Normalized DNA-TG Accumulation *in vivo*

**Mouse Bone Marrow at Day 5**

- Daily i.p. injection with 6MP (40, 20, 5, or 1mg/kg)

**Peripheral Blood in Patients**

- Children with ALL in Japan/Singapore (N=95)
- DNA-TG levels during maintenance therapy
  - Unique cases: N=95
  - WBC Samples: N=153
Reduced MP Dosage Efficiently Suppressed Leukemia Burden in KO Mice

**Blast% in peripheral blood**

- **WT**
  - 0 mg/kg
  - 1 mg/kg
  - 20 mg/kg

- **KO**
  - 0 mg/kg
  - 1 mg/kg

**Leukemia-free survival**

- **WT**
  - 20 mg/kg
  - 1 mg/kg

- **KO**
  - 0 mg/kg
  - 1 mg/kg

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**Experimental Details**

- **Bone marrow cells** Retroviral transduction
  - BCR-ABL1,GFP
  - Nudt15+/+
  - (n = 10 ~ 14)

Daily intraperitoneal administration with 6MP (20 or 1 mg/kg)

Leukemia Burden
- Blast%
- Leukemia-free survival
A *Nudt15* knockout mouse model was established by CRISPR/Cas9 genome editing.

Across MP dosages, *Nudt15* knockout mice experienced severe leukopenia, rapid weight loss and earlier toxic death compared to wildtype mice.

*Nudt15* knockout mice showed excessive accumulation of a thiopurine active metabolite (i.e., DNA-TG) in a MP dosage-dependent fashion, as a plausible cause of increased toxicity.

MP dose reduction effectively normalized systemic exposure to DNA-TG in *Nudt15* knockout mice and largely eliminated *Nudt15* deficiency-mediated toxicity without compromising anti-leukemic efficacy *in vivo*. 