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

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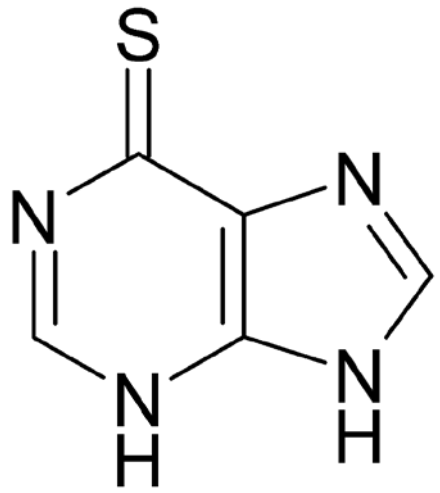
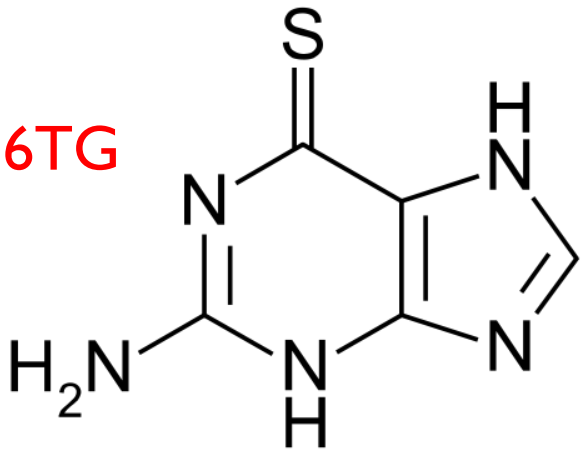
Introduction of Thiopurines

6MP



6TG

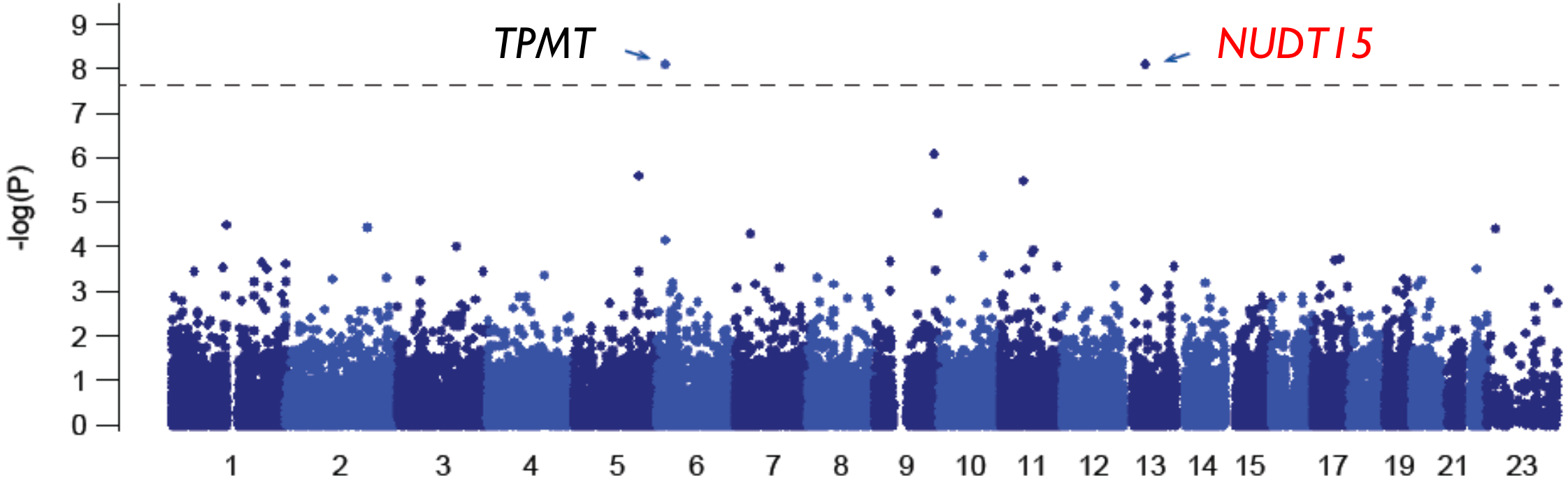


- Thiopurines (i.e., 6MP, 6TG) are widely used therapeutic agents in cancers (e.g., acute lymphoblastic leukemia) and autoimmune diseases (e.g., inflammatory bowel diseases)
- Myelosuppression is the main side effect
- Dose titration is done based on WBC but challenging

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)

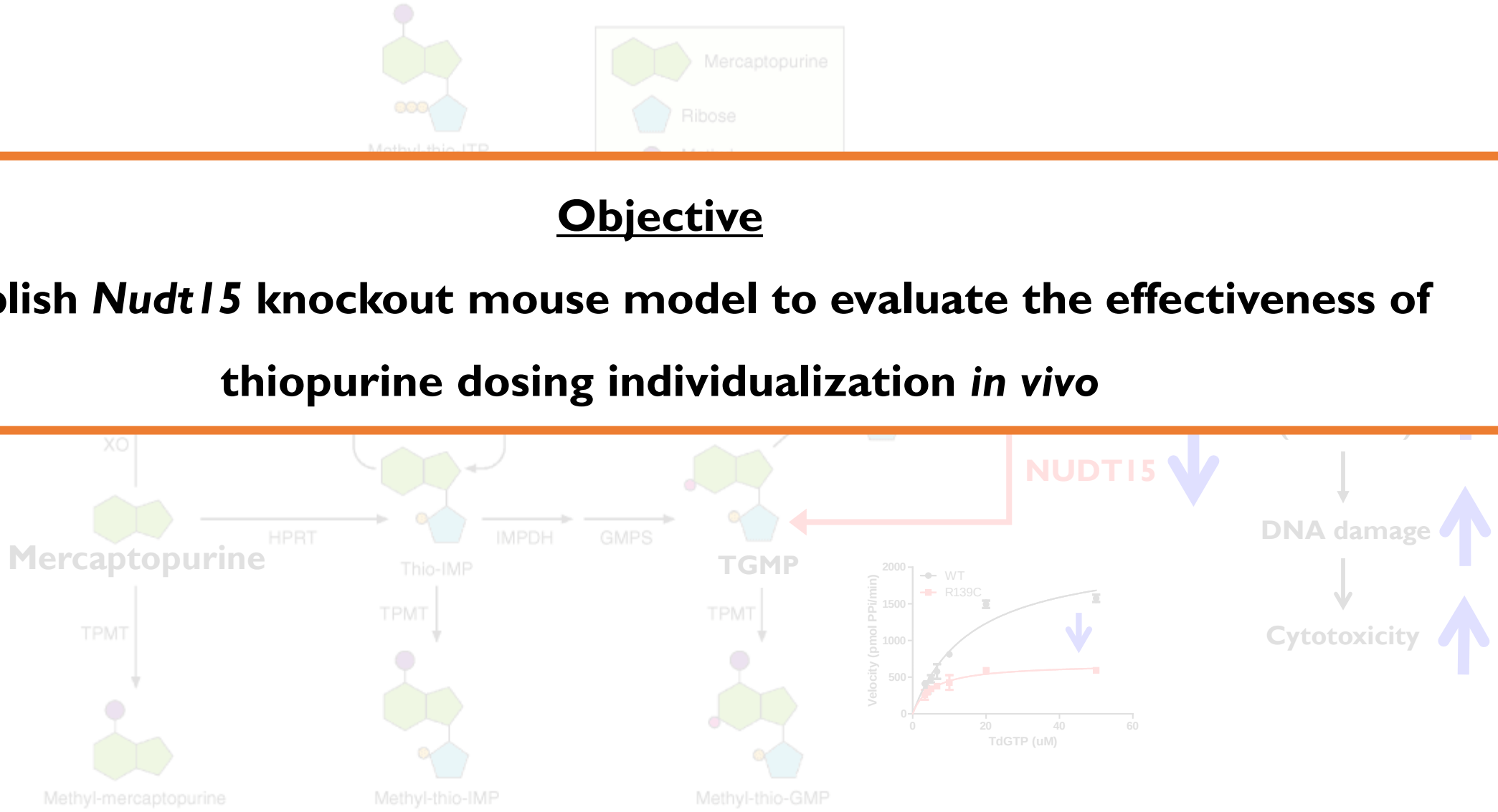


Relling et al, ***J Natl Cancer Inst*** 1999
Relling et al, ***Clin Pharmacol Ther*** 2013
Yang et al., ***J Clin Oncol*** 2015

NUDT15 and Thiopurine Metabolism

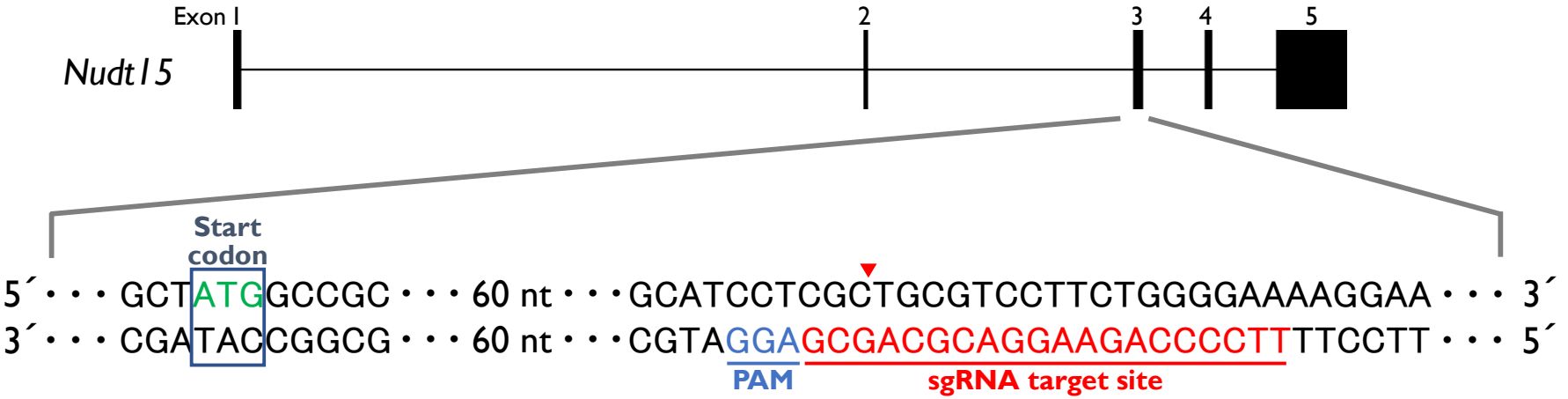
Objective

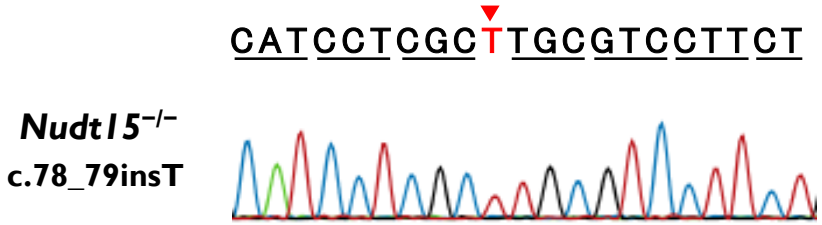
Establish *Nudt15* knockout mouse model to evaluate the effectiveness of thiopurine dosing individualization *in vivo*

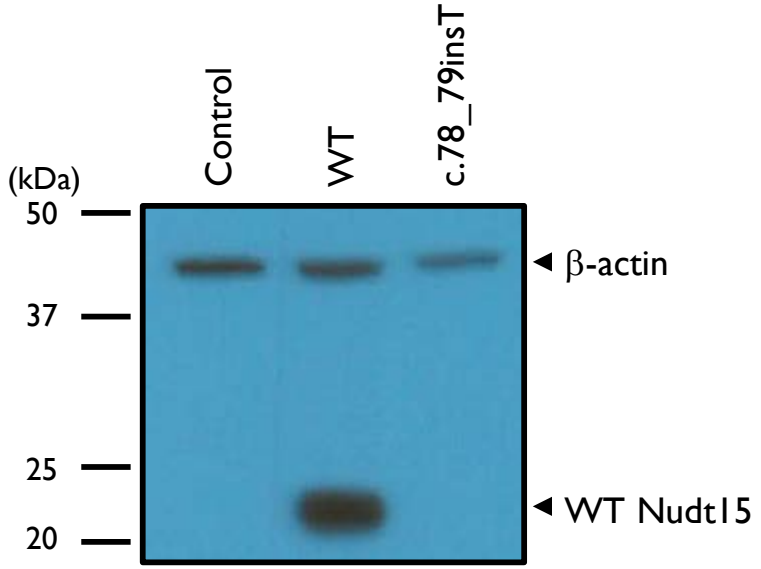


Moriyama et al., ***Nat Genet*** 2016

Nudt15 Knockout in Mouse Using CRISPR-Cas9

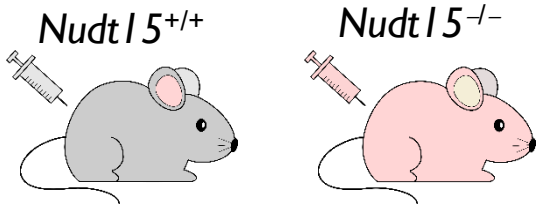






Thiopurine Toxicity was Mitigated by Reducing MP Dose

Mice



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

Toxicity evaluation

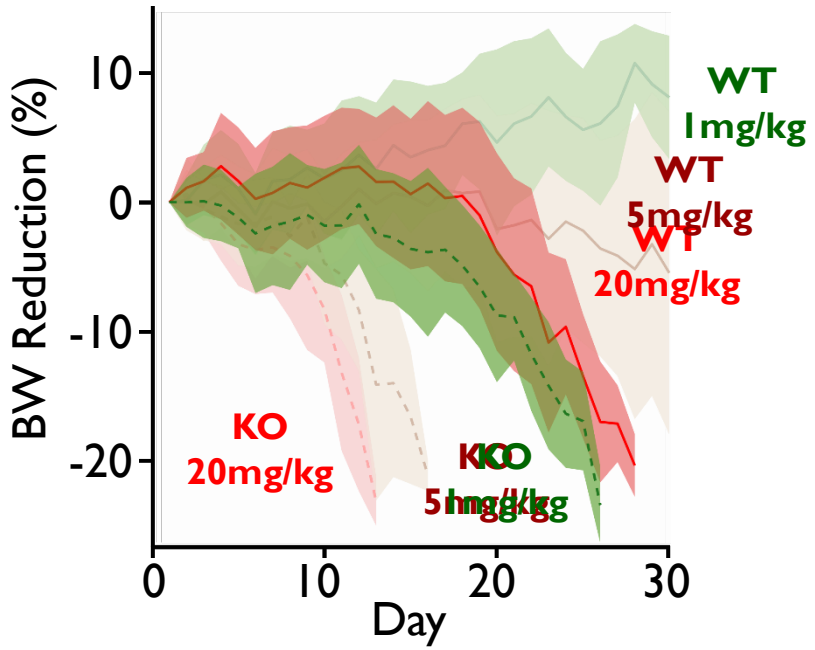
- Body weight loss
- Survival
- CBC (bone marrow suppression)

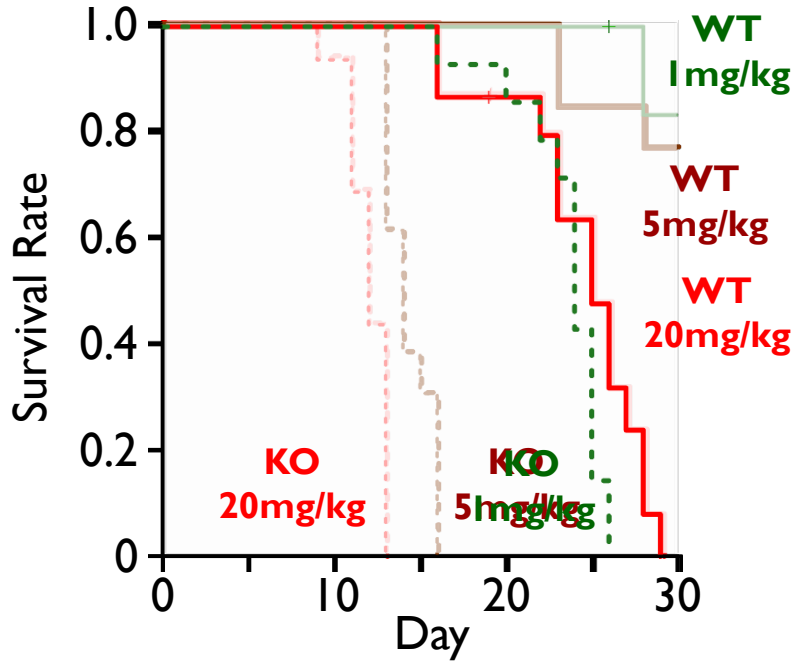
Dosage (mg/kg)

20

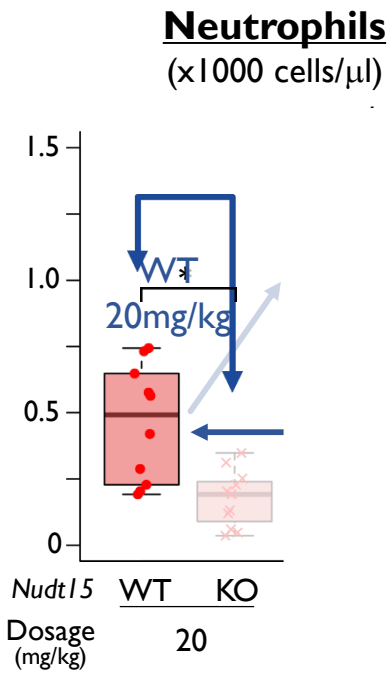
Nudt15$^{+/+}$ (*n* = 15)

Nudt15$^{-/-}$ (*n* = 16)



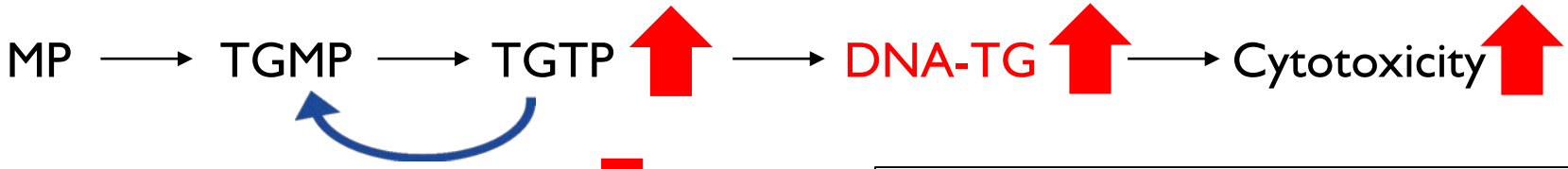


Nudt15 KO Mice Experienced Severe Hematological Toxicity

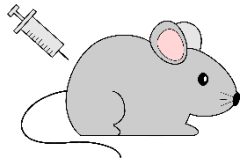


- KO mice experienced more severe leukopenia than WT mice at the same dosage
- Hematological toxicity was mitigated by MP dose reduction from 20 mg/kg to 1 mg/kg in both genotypes
- Cytotoxicity in KO mice exposed to 1mg/kg was comparable to that of WT mice receiving 20 mg/kg

MP Dose Reduction Effectively Normalized DNA-TG Accumulation *in vivo*



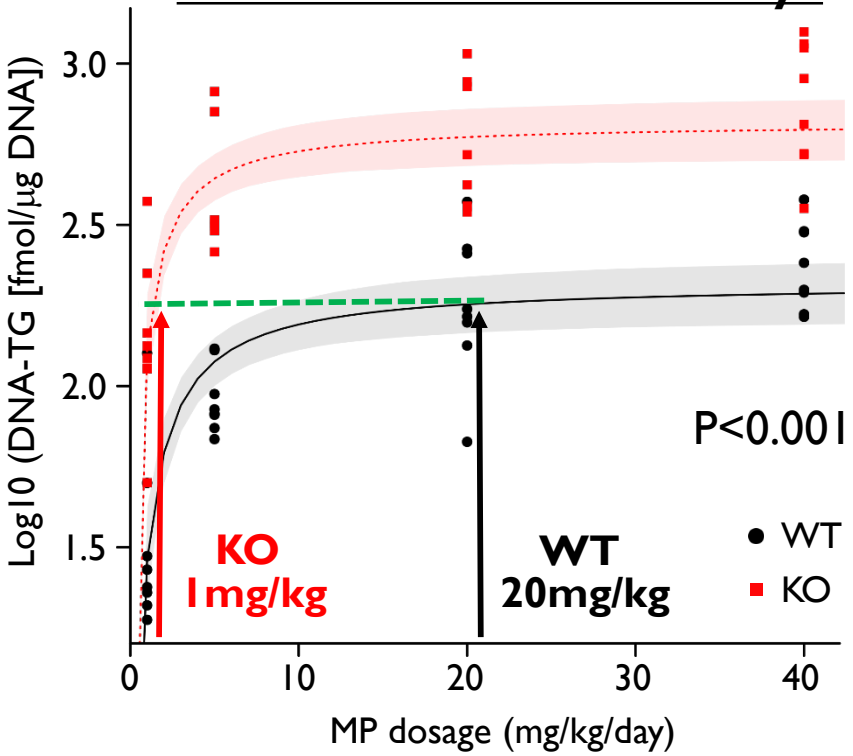
Nudt15
WT or **KO**



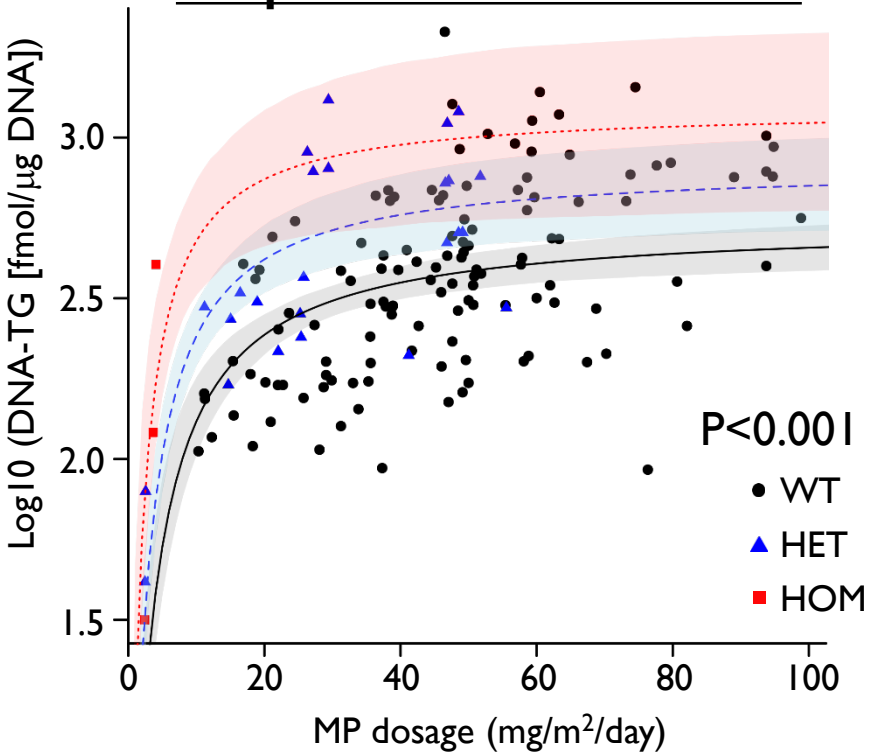
Daily i.p. injection with 6MP
(40, 20, 5, or 1mg/kg)

- ❑ Children with ALL in Japan/Singapore (N=95)
- ❑ DNATG levels during maintenance therapy
  - Unique cases: N=95
  - WBC Samples: N=153

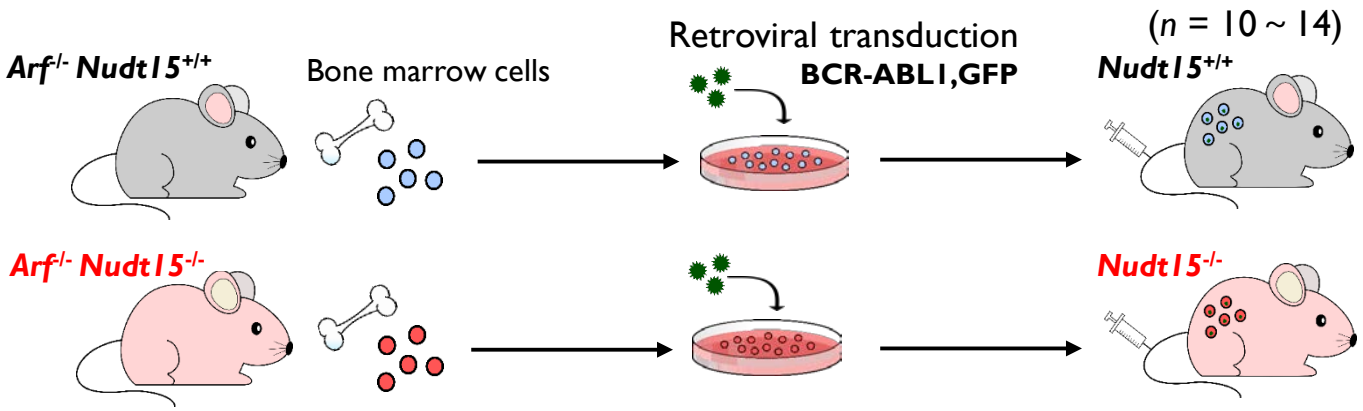
Mouse Bone Marrow at Day5



Peripheral Blood in Patients



Reduced MP Dosage Efficiently Suppressed Leukemia Burden in KO Mice



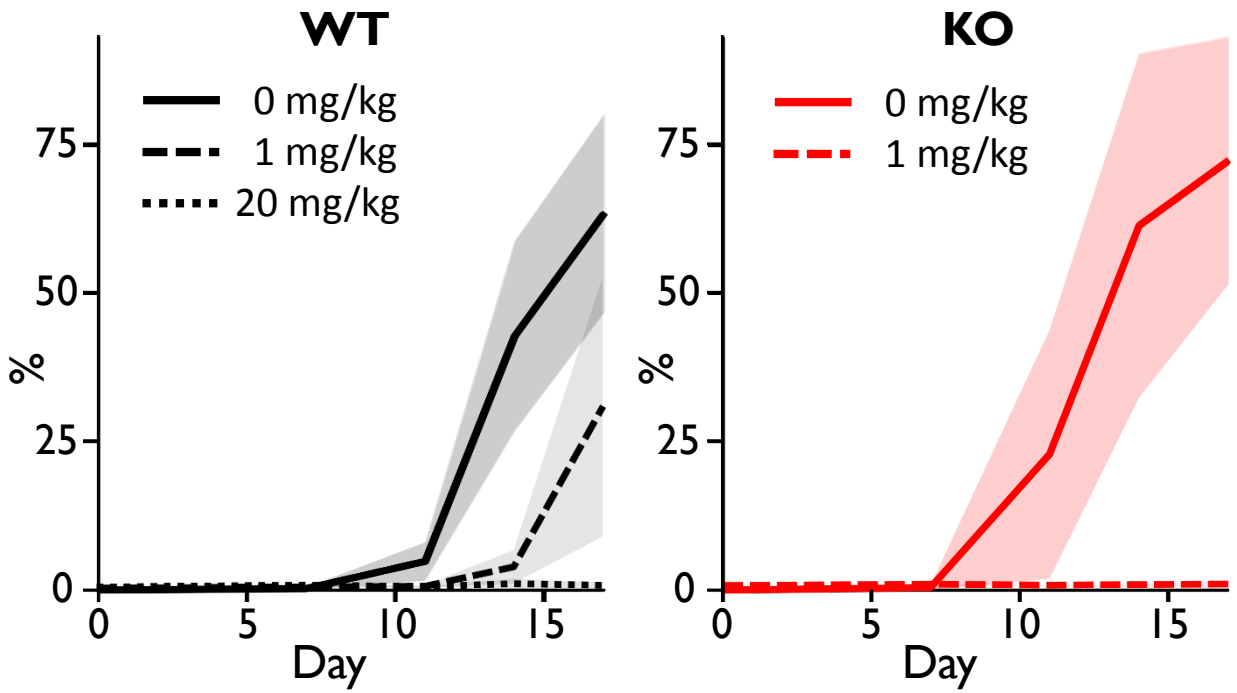
Daily intraperitoneal administration with 6MP (20 or 1 mg/kg)

Leukemia Burden

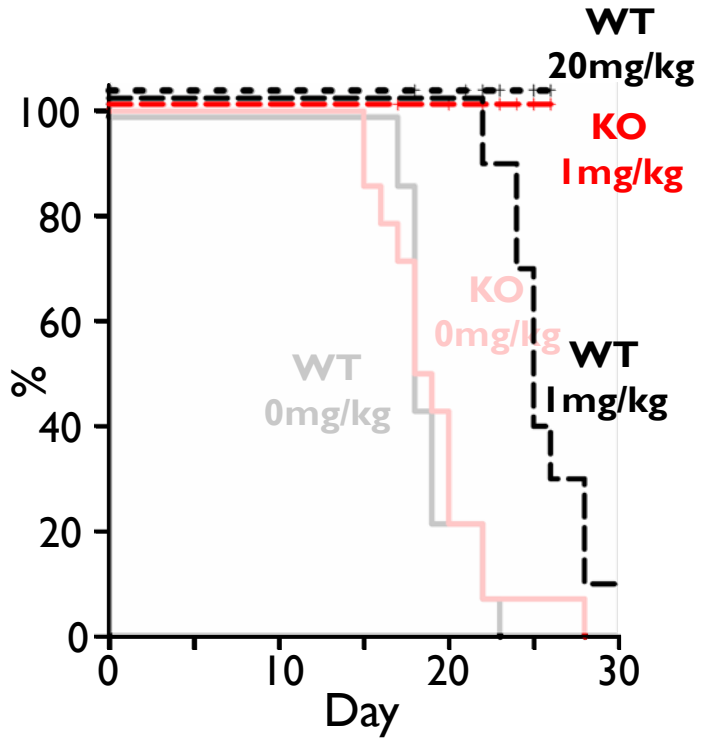
Blast%

Leukemia-free survival

Blast% in peripheral blood



Leukemia-free survival



Summary

- 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*.