Targeted protein degradation reveals a cryptic NUDT5–PPAT axis governing nucleoside analog drug efficacy

Nicholas C.K. Valerie*[,1], Seher Alam[2], Massimiliano Gaetani[3,4,5], Gabriel Tidestav[6], Femke M. Hormann[7], Emma Rose Scaletti[8], Maria J. Pires[2], Johan Boström[2], Ann-Sofie Jemth[7], Julia Eklund[9], Fredrik Klingegård[9], Rémi Caraballo[9], Dante Rotilli[10], Siebe van den Elzen[1,11], Eri van Berkum[2], Olov Wallner[7], Jonas Malmström[6], Ulf Martens[12], Bo Lundgren[12], Pål Stenmark[8], Sean G. Rudd[7], Per I. Arvidsson[11,13], and Mikael Altun*[,1,11]

[1]SciLifeLab, Division of Clinical Physiology, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital; [2]Division of Clinical Physiology, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital; [3]Chemical Proteomics, Division of Chemistry I, Department of Medical Biochemistry and Biophysics, Karolinska Institutet; [4]SciLifeLab, Chemical Proteomics Unit; [5]Chemical Proteomics, Swedish National Infrastructure for Biological Mass Spectrometry (BioMS); [6]SciLifeLab, Department of Oncology-Pathology, Karolinska Institutet; [7]Recipharm OT Chemistry AB, Uppsala, Sweden; [8]Department of Biochemistry and Biophysics, Stockholm University; [9]SciLifeLab, Drug Discovery and Development Platform, Department of Biochemistry and Biophysics, Stockholm University; [10]Department of Science, "Roma Tre" University; INBB - Biostructures and Biosystems National Institute, Rome, Italy; [11]SciLifeLab, Drug Discovery and Development Platform; [12]SciLifeLab, Biochemical and Cellular Assay Facility, Department of Biochemistry and Biophysics, Stockholm University; [13]Department of Medical Biochemistry and Biophysics, Karolinska Institutet

Nucleoside analog (NA) chemotherapies exploit the nucleotide requirements of proliferating cancer cells, yet the molecular factors determining NA efficacy remain poorly defined. Genome-wide loss-of-function screens recently identified the Nudix hydrolase NUDT5 as a potential enabler of thiopurine efficacy[1]. Here, we utilize targeted protein degradation (TPD) to identify a non-enzymatic scaffolding function of NUDT5 that governs cellular sensitivity to NA drugs[2, 3]. From a series of inhibitor-derived degraders, we identify DDD2 as a potent, selective, and VHL-dependent NUDT5 PROTAC using a cell-based TPD platform that couples a modular degradation reporter with E3 ligase engagement assays. DDD2 or RNAi targeting NUDT5, but not potent inhibitors, confer resistance to most NA drugs proportionally with its depletion. Mechanistically, NUDT5 depletion locks purine metabolism into *de novo* synthesis (DNPB) *via* loss of a functional interaction with phosphoribosyl amidotransferase (PPAT), the committal step of the pathway. Persistent DNPB then misallocates phosphoribosyl pyrophosphate (PRPP) to constrain salvage pathway flux. Accordingly, NUDT5-PPAT complex formation is driven by all salvageable nucleotide precursors and NA drugs, implying this node broadly regulates nucleotide metabolism. Our findings present a versatile framework for mechanistic evaluation of TPD molecules in cells and establish the NUDT5-PPAT axis as a key determinant of global NA drug responses.

References

1. Doench, J.G. et al. Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nature Biotechnology* **34**, 184-191 (2016).
2. Alam, S. et al. A cell-based degrader assessment platform facilitates discovery of functional NUDT5 PROTACs. *bioRxiv*, 2025.2006.2013.659494 (2025).
3. Valerie, N.C.K. et al. NUDT5 regulates the global efficacy of nucleoside analog drugs by coordinating purine synthesis and PRPP allocation. *bioRxiv*, 2025.2011.2020.689348 (2025).