Development of small molecule NUDT22 inhibitors for uses in cancer

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1 Introduction

NUDT22 is a so far unstudied member of the highly diverse **NUDIX protein superfamily**.

We previously identified a unique **hydrolase activity of NUDT22 towards UDP-glucose** in a family-wide biochemical substrate screen resulting in the **production of uridine mono-phosphate (UMP) and glucose 1-phosphate (G-1-P).**

We furthermore solved the first co-crystal structure of NUDT22 and its substrate UDP-glucose [1].

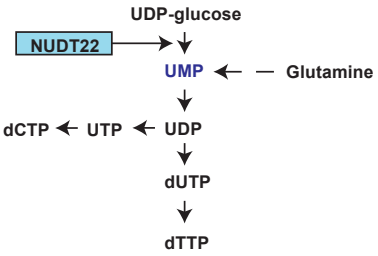

Figure 1. NUDT22 is involved in a novel pyrimdinge salvage pathway

NUDT22 is an interesting new anti-cancer target

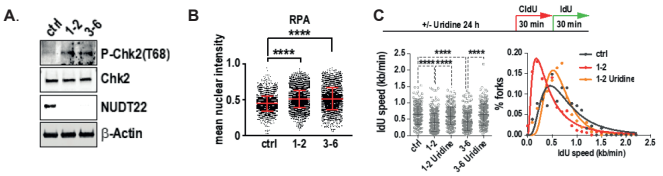

Figure 2. **A.** Cell cycle checkpoint activation in *NUDT22* depleted cells. **B.** Quantification of nucllear intensity of DNA replication stress marker RPA-32 upon *NUDT22* depletion. **C.** Changes replication fork speed upon *NUDT22* depletion as determined by DNA fiber assay.

Our mechanistic studies reveal increased replication stress in *NUDT22* deficient U-2 OS cells which can be rescued by uridine supplementation (***Fig. 2***). We therefore propose the discovery of a novel NUDT22-mediated Glutamine-independent pyrimidine salvage pathway (***Fig.1***).

2 *NUDT22* gene alterations in breast cancer tissues

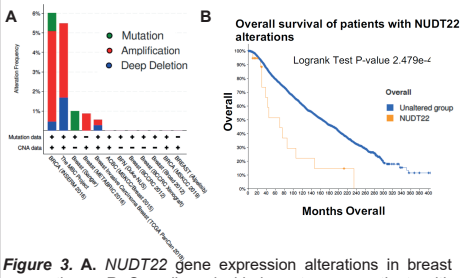

Figure 3. **A.** *NUDT22* gene expression alterations in breast cancer tissue. **B.** Overall survival in breast cancer patients with or without *NUDT22* gene alterations.

Here we analysed the TCGA database based on *NUDT22* expression levels in breast cancer tissues indicating the presence of *NUDT22* gene amplifications (***Fig. 3A***). Furthermore, overall survival is decreased in breast cancer patients with *NUDT22* alterations (***Fig. 3B***).

3 Aims and Methods

Development of first-in-class NUDT22 inhibitors by computer-aided drug design (CADD).

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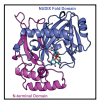
Experimental evaluation

(Enzymatc Activity Assay on recombinant NUDT22 & Target engagement - Differential Scanning Fluorimetry/ Cellular Thermal Shift Assay)

4 *In silico* screening identifies Compound 1 as first-in-class NUDT22 inhibitor

In silico docking of **NCI/DTP compound library** (200,000 molecules) and



Enzymatic Activity Assay IC50 →

Compound A

IC50 > 650 µM

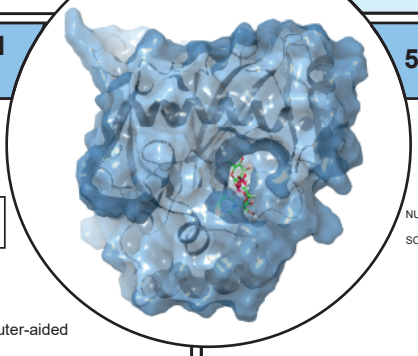


Figure 4. Scheme of the identification of Compound A as weak NUDT22i by computer-aided drug design and experimental evaluation.

Here we proof the suitability of computer-aided drug design (CADD) to identify Compound A as first-in-class NUDT22 inhibitor. We performed an *in silico* docking screen of the co-crystal structure of NUDT22 and UDP-glucose [1] on the National Cancer Institute/Development Therapeutics Program (NCI/DTP) compound library on the followed by the evaluation of the top-40 compounds based on their potential to inhibit NUDT22 (***Fig. 4***).

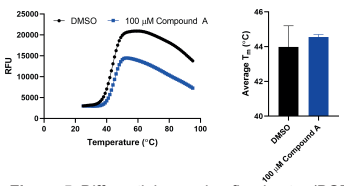


Figure 5. Differential scanning fluorimetry (DSF) was used to identify a shift in ΔTm upon Compound A binding.

We used Differential Scanning Fluorimetry (DSF) to determine whether Compound 1 stabilises recombinant NUDT22 protein. Preliminary data suggest a shift in the specific melting temperature (ΔTm) of NUDT22 of 0.5ºC upon treatment with 100 µM Compound 1 and, therefore target engagement (***Fig. 5***).

5 Chemical optimisation of Inhibitor 1 results in stronger target engagement

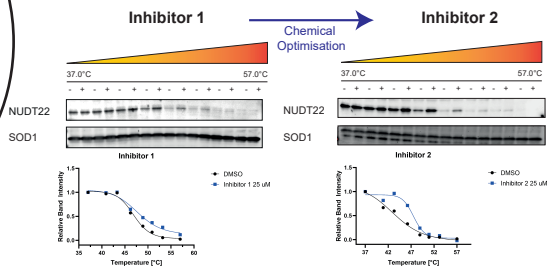


Figure 6. Cellular thermal shift assay (CETSA) was used to determine target engagement of a chemical optimised NUDT22i compared to the original Hit.

A second virtual screen of an in-house compound library identified Inhibitor 1 as potential NUDT22i. Chemical optimisation of Inhibitor 1 caused an increase in cellular target engagement from ΔTm of 0.9°C to 3.5°C as determined in a cellular thermal shift assay (CETSA) in U-2 OS cell lysate (***Fig. 6***).

6 Conclusions

- We identified Compound A as first-in-class small molecule NUDT22 inhibitor by using computer-aided drug design (CADD).
- Chemical optimisation of Inhibitor 1 results in an increase in NUDT22 stabilisation and, therefore, target engagement.

References

[1] M. Carter et al., "Human NUDT22 Is a UDP-Glucose/Galactose Hydrolase Exhibiting a Unique Structural Fold," Structure, vol. 26, no. 2, pp. 295-+, Feb 2018, doi: 10.1016/j.str.2018.01.004.

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