Discovery of a novel KAT6A/B inhibitor with anti-tumor activity coupled with novel response biomarkers

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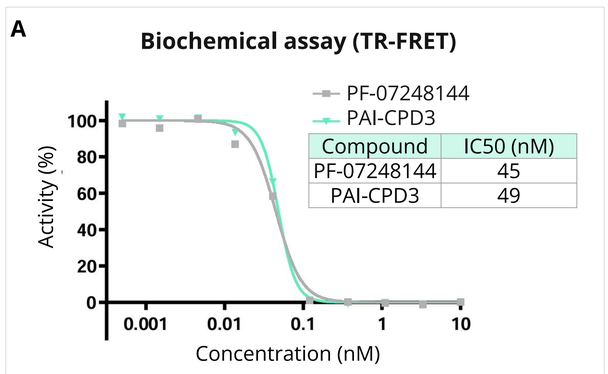
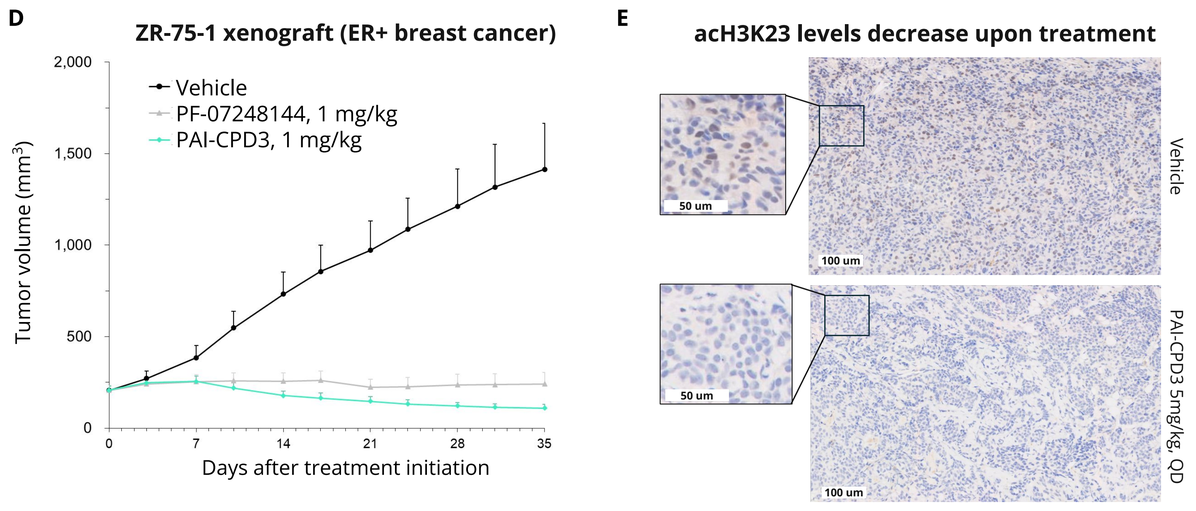
Introduction

- Lysine histone acetyltransferase 6 (KAT6A/B) is a MYST family member of histone acetyltransferases (HATs), known to regulate gene expression by acetylation of both histone and non-histone substrates.
- KAT6A/B is a promising cancer drug target, specifically in estrogen receptor positive (ER+) breast cancer, but anti-cancer activity in other cancer indications has also been shown, suggesting a potential benefit for wider patient populations[1].
- We discovered a novel, highly potent KAT6A/B inhibitor, showing anti tumor activity both *in-vitro* and in xenograft models, as well as improved selectivity compared to the benchmark compound PF-07248144.
- Combining drug sensitivity with basal and dynamic proteomics data, Protai's AIMS™ platform[2] discovered a novel biomarker for sensitive cells outside ER+ breast cell lines.

PAI-CPD3 shows high potency *in-vitro* and *in-vivo*



(A) PAI-CPD3 demonstrates high inhibitory potency in a time-resolved (TR)-FRET biochemical assay, equivalent to PF-07248144.
(B) Western blot showing reduction in acetylated H3K23 upon treatment of ER+ T47D breast cancer cell line with the indicated compounds.
(C) PAI-CPD3 demonstrates growth inhibition of ER+ breast cancer cell line ZR-75-1, treated for 10 days, equivalent to PF-07248144.
(D) Female BALB/c nude mice were treated with KAT6 inhibitors (1 mg/kg QD, 5 weeks). PAI-CPD3 shows substantial tumor growth inhibition (N=5), outperforming PF-07248144 in a similar treatment regimen. Body weight change relative to the weight on day 0, shows no significant weight loss (not shown).
(E) Acetylated H3K23 IHC staining 35 days after treatment initiation, 8 hours post final dosage.



AIMS™ informs PAI-CPD3 favorable selectivity profile

Biochemical HAT enzymatic activity shows selectivity advantage to PAI-CPD3

Biochemical assays comparing PAI-CPD3 to PF-07248144 show equivalent potency in KAT6A/B inhibition (10 dose IC50, 3-fold serial dilution starting at 30µM) (**A**).
Both compounds also show equivalent selectivity over other HATs, with a slight advantage of PAI-CPD3 in KAT8 and KAT5 (single dose, duplicate, 10µM) (**B**).

KAT6A over KAT6B selectivity

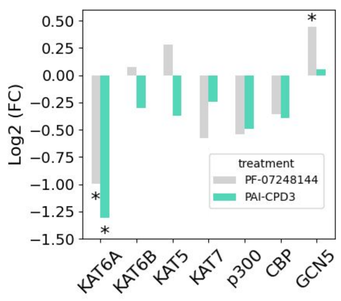
A

Compound	KAT6A IC50 (nM)	KAT6B IC50 (nM)
PF-07248144	2.37	19.38
PAI-CPD3	1.46	24

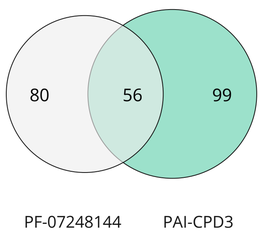
B HATs enzymatic inhibition panel

Protein dynamics show similar post-treatment biology

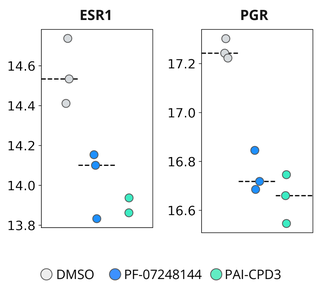
A Individual HAT alterations upon treatment



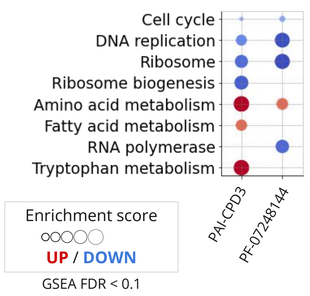
B Number of proteins downregulated upon KAT6i
(log_2 fold change < -0.5, p-value < 0.05)



C Compounds affect ESR1 and PGR to a similar extent



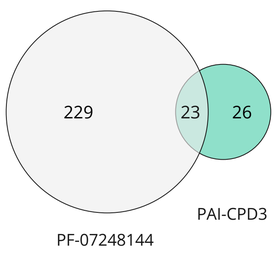
D Compounds are similar on pathway level



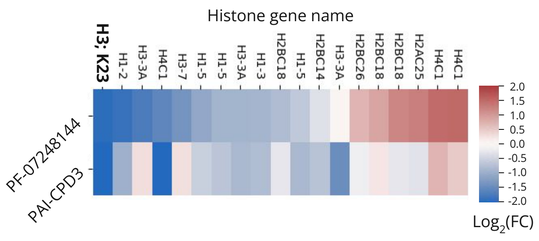
Protein dynamics compound comparison was carried out on ZR-75-1 cell lines at 0.1uM for 24h. Proteomic analysis of downregulated features showed significant inhibition of KAT6A and minimal inhibition of other HATs (**A**); and overall similar dynamics of both compounds in the number of proteins downregulated upon treatment (**B**), downregulation of ESR1 and PGR (**C**), and significantly altered pathways (**D**).

Acetylation dynamics reveals potential cleaner profile of PAI-CPD3

A Number of acetyl-sites downregulated upon KAT6i
(log_2 fold change < -0.5, p-value < 0.05)



B More histone acetyl sites changing in PF-07248144 compared to PAI compounds

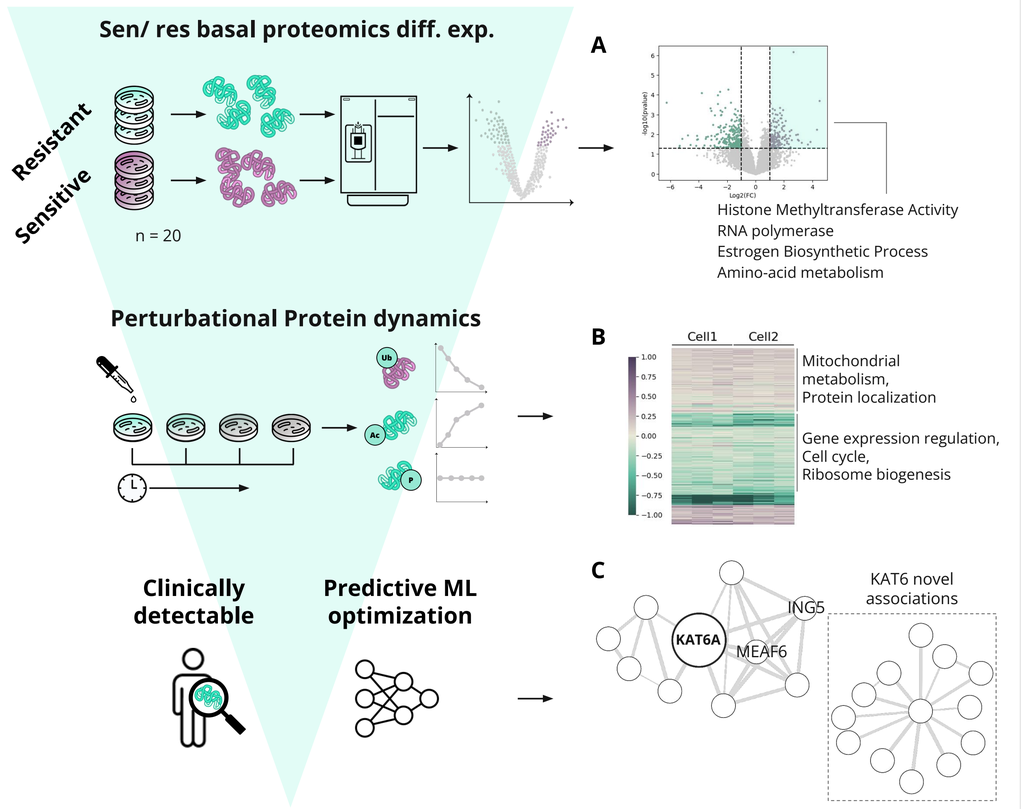


Acetylation dynamics compound comparison was carried out on ZR-75-1 cell line at 0.1uM for 2h. Acetyl-dynamics shows potential selectivity advantage of PAI-CPD3, evident in the total number of regulated acetyl sites (**A**) and specifically in histone sites, higher in PF-07248144 (**B**). The KAT6A substrate H3K23 is marked on the left.

Proteomic biomarker identifies sensitivity beyond ER+ breast cancer

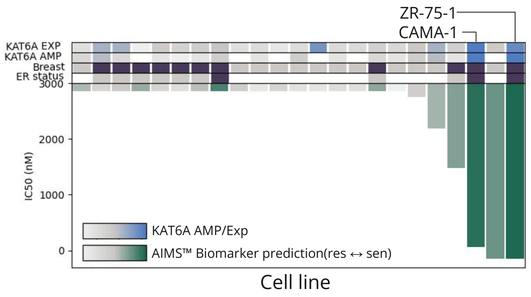
AIMS™ biomarker discovery workflow

Predictive biomarker discovery for KAT6i treatment is composed of several data layers.
Differential analysis of both basal-state proteomics (**A**) and on-treatment dynamics (**B**) of KAT6i sensitive and resistant cell lines (both in and outside breast cancer) highlights several candidates for KAT6 predictive biomarker, both KAT6A-related and novel KAT6A associations (**C**, KAT6A PPI network).



AIMS™ predicts sensitivity in cell lines regardless of ER+ status

Applying the selected biomarkers to internal proteomic datasets coupled with KAT6i IC50 values (barplot) demonstrates that biomarker candidates predict KAT6 sensitivity beyond ER status and KAT6A copy number amplification/protein expression.



Summary

- PAI-CPD3, a novel KAT6A/B inhibitor, exhibits potency and *in-vivo* efficacy in ER+ breast cancer.
- PAI-CPD3 demonstrates selective acetylation impact, both on histones and non histone sites.
- KAT6A/B directed treatment represents opportunity for ER+ breast cancer patients as well as other tumor types, supported by a proteomic biomarker.

References

1. *Mukohara, et al. Nature Medicine (2024)*
2. *Alchanati et al. AACR (2024)*