Discovery of first-in-class potent and selective oral degraders of KAT6A that demonstrate anti-cancer activity in pre-clinical models

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Background

- MYST proteins like KAT6A, KAT6B, and KAT7 are histone acetyltransferases that epigenetically regulate chromatin accessibility.[1-2]
- KAT6A expression is associated with cancer growth and is recurrently amplified in breast, lung, and other cancers.[1]
- KAT6A forms a tetrameric protein complex with BRPF1, ING5, and MEAF6 which enhances its regulation of cell cycle, estrogenic, and other oncogenic genes.[1-2]
- First-in-human clinical data with a dual KAT6A/B inhibitor demonstrated promising efficacy in heavily pre-treated patients with ER+/HER2- breast cancer and provided insight into on-target safety considerations like neutropenia.[3]
- MYST proteins have a synergistic relationship in hematopoietic cells, which may have safety implications for non-selective targeting of MYST proteins.[6]
- Non-enzymatic KAT6A dependencies have been reported in heme and ovarian malignancies.[4-5]
- We hypothesized a targeted protein degradation (TPD) approach would enable improved KAT6A selectivity and engage differential biology, with the potential to improve hematological safety and/or single agent anti-cancer activity for KAT6-targeted therapies.

Key Findings

- Identified first-in-class potent and selective KAT6A protein degraders with good cross species oral bioavailability.
- Selective KAT6A degraders have reduced activity in neutropenia-predictive pre-clinical assays, suggesting potential for a differentiated hematological safety profile from dual KAT6A/B inhibitors.
- Demonstrated proof-of-concept that selective KAT6A protein degradation is differentiated from dual KAT6A/B inhibition, leading to robust single agent activity *in vitro* and *in vivo*.
- Selective KAT6A degraders induced deep tumor regressions in ER+/HER2- breast cancer and KAT6A-amplified lung cancer xenografts at low oral doses.

Results

Table 1. Discovery of Potent KAT6A Degraders with Anti-Cancer Activity

Assay	KAT6A/Bi	PRT0A1	PRT0A2	PRT0A3	PRT0A4	PRT0A5
HeLa KAT6A HiBiT nM DC_{50}	NA	19.7	2.8	1.7	0.8	0.2
T47-D CTG nM Rel EC_{50} (E_{MAX})	0.5 (43%)	8.9 (77%)	1.4 (90%)	1.3 (85%)	1.0 (80%)	0.1 (85%)

KAT6A degradation potency at 24 h using a HeLa KAT6A HiBiT model and corresponding anti-proliferative activity in T47-D cell proliferation assays (CTG) are shown.

Figure 1. Oral KAT6A Degraders Have Excellent Global Selectivity and Potential for a Differentiated Hematological Safety Profile

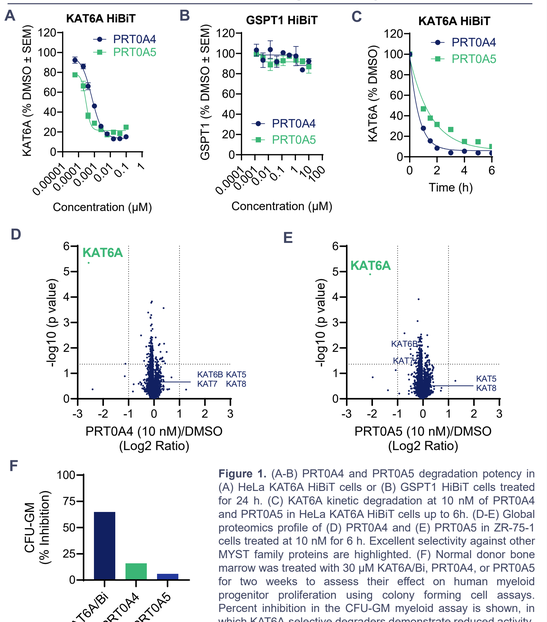

Figure 1. (A-B) PRT0A4 and PRT0A5 degradation potency in (A) HeLa KAT6A HiBiT cells or (B) GSPT1 HiBiT cells treated for 24 h. (C) KAT6A kinetic degradation at 10 nM of PRT0A4 and PRT0A5 in HeLa KAT6A HiBiT cells up to 6h. (D-E) Global proteomics profile of (D) PRT0A4 and (E) PRT0A5 in ZR-75-1 cells treated at 10 nM for 6 h. Excellent selectivity against other MYST family proteins are highlighted. (F) Normal donor bone marrow was treated with 30 μM KAT6A/Bi, PRT0A4, or PRT0A5 for two weeks to assess their effect on human myeloid progenitor proliferation using colony forming cell assays. Percent inhibition in the CFU-GM myeloid assay is shown, in which KAT6A-selective degraders demonstrate reduced activity.

Figure 2. KAT6A-Selective Degradation Drives Superior Anti-Cancer Activity To Dual KAT6A/B Inhibition in Breast and Lung Cancer Cell Lines

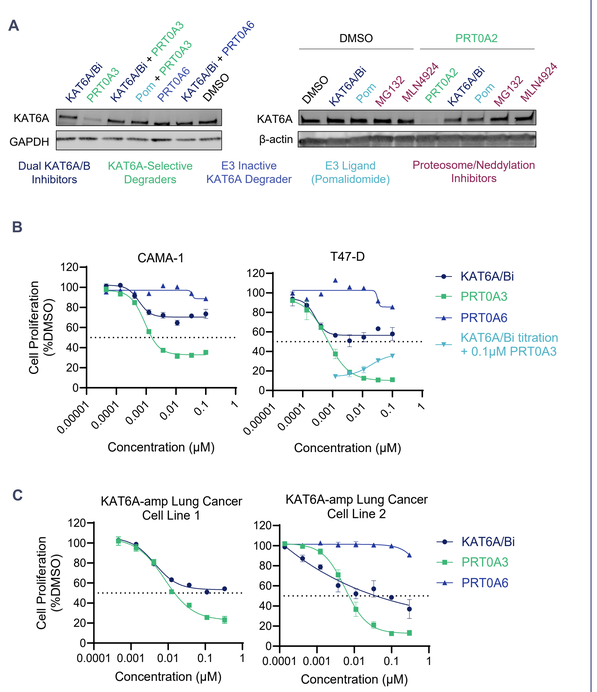

Figure 2. (A) Competition assays demonstrate target, E3-ligase, and proteosome dependent degradation. Cancer cells were treated for 0.5-2 h (0.1-1 μM KAT6A/Bi, MG132, or MLN4924; >5 μM Pomalidomide) before treating with KAT6A-selective degraders (0.1 μM) for 6 h. (B) Anti-proliferative activity (CTG) of a dual KAT6A/B inhibitor, KAT6A-selective degrader, and E3-inactive KAT6A-selective degrader (PRT0A6) in ER+ CAMA-1 and T47-D breast cancer cells. In T47-D cells, the deeper maximum response driven by KAT6A degradation is reversible by dose-dependent competition with a dual KAT6A/B inhibitor. (C) Anti-proliferative activity (CTG) of the indicated compounds in two KAT6A-amplified lung cancer cell lines, highlighting a deeper maximum response with a KAT6A-selective degrader.

Figure 3. KAT6A-Selective Degraders Have Robust Activity in KAT6A-Amplified Cancer Cells

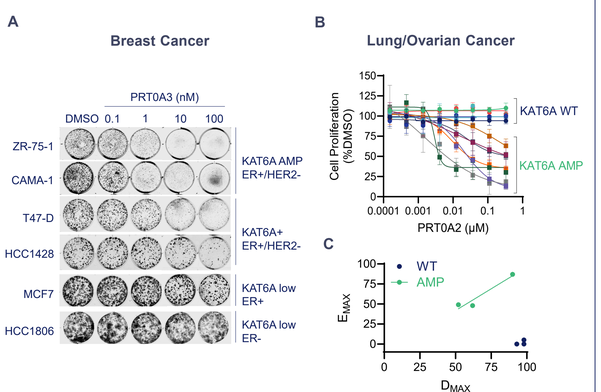

Figure 3. (A) Sensitivity of a panel of breast cancer cell lines treated with KAT6A-selective degrader PRT0A3 for >2 weeks and evaluated by clonogenic assay. Status of KAT6A and ER are annotated. (B) A panel of KAT6A WT and KAT6A-amplified lung and ovarian cancer cell lines were evaluated for their sensitivity to KAT6A-selective degrader PRT0A2 in CTG assays for ≥7 days. (C) Comparison of E_{MAX} and D_{MAX} for 3 KAT6A WT and AMP lung cancer cell lines with varying anti-proliferative activity from panel B are shown.

Figure 4. KAT6A-Selective Degradation Has a Differentiated Mechanistic Profile to Dual KAT6A/B Inhibition

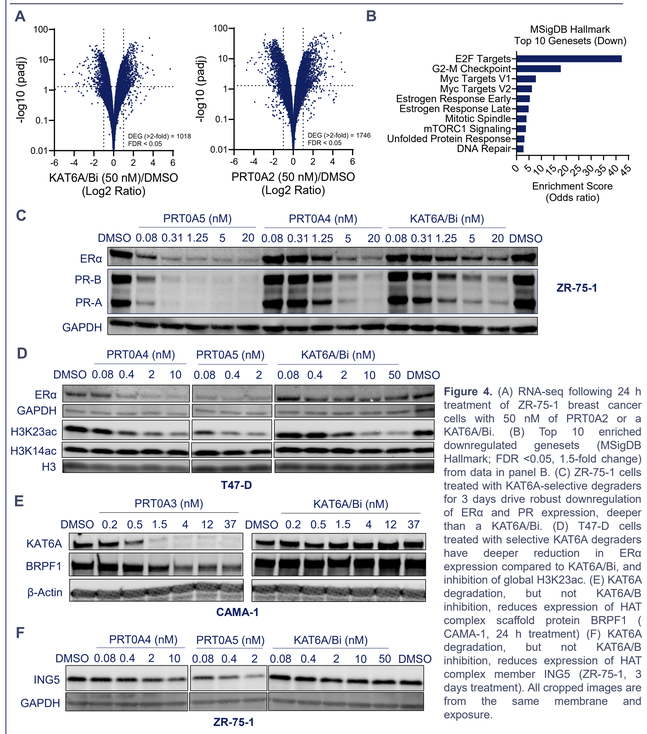

Figure 4. (A) RNA-seq following 24 h treatment of ZR-75-1 breast cancer cells with 50 nM of PRT0A2 or a KAT6A/Bi. (B) Top 10 enriched downregulated genesets (MSigDB Hallmark; FDR <0.05, 1.5-fold change) from data in panel B. (C) ZR-75-1 cells treated with KAT6A-selective degraders for 3 days drive robust downregulation of ERα and PR expression, deeper than a KAT6A/Bi. (D) T47-D cells treated with selective KAT6A degraders have deeper reduction in ERα expression compared to KAT6A/Bi, and inhibition of global H3K23ac. (E) KAT6A degradation, but not KAT6A/B inhibition, reduces expression of HAT complex scaffold protein BRPF1 (CAMA-1, 24 h treatment) (F) KAT6A degradation, but not KAT6A/B inhibition, reduces expression of HAT complex member ING5 (ZR-75-1, 3 days treatment). All cropped images are from the same membrane and exposure.

Figure 5. KAT6A-Selective Degraders Improve Response Compared to and In Combination with SoC Therapies While Retaining Activity in Therapy Resistant Cancer Cells *In Vitro*

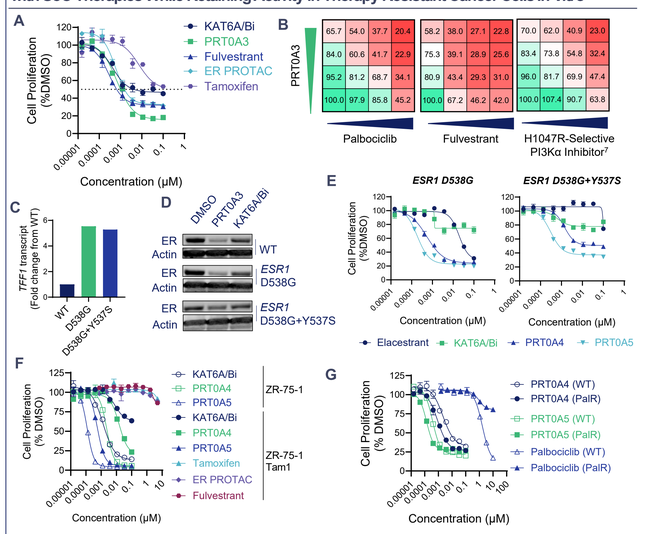

Figure 5. (A) Anti-proliferation activity of endocrine/ER-targeted agents, dual KAT6A/Bi, and KAT6A-selective degrader are compared in a T47-D CTG assay. (B) T47-D cells (*PIK3CA* H1047R) were treated with a KAT6A-selective degrader in combination with Palbociclib, Fulvestrant, or a H1047R-selective PI3Kα inhibitor[7] and cell viability is shown (CTG). (C) Functional validation of constructed *ESR1* mutant T47-D cell lines, which have increased constitutive transcript of *TFF1*. (D) KAT6A-selective degradation reduces ERα protein more than a dual KAT6A/Bi (10 nM, 24 h) in parental and *ESR1* mutant T47-D cells. (E-G) KAT6A-selective degraders retain anti-proliferative activity in (E) *ESR1* mutant T47-D, (F) Tamoxifen-resistant ZR-75-1 (ZR-75-1 Tam1), and (G) Palbociclib-resistant (PalR) T47-D cells, tested against relevant endocrine/ER-targeted agents.

Figure 6. KAT6A-Selective Degraders Demonstrate Robust Target Engagement *In Vivo*

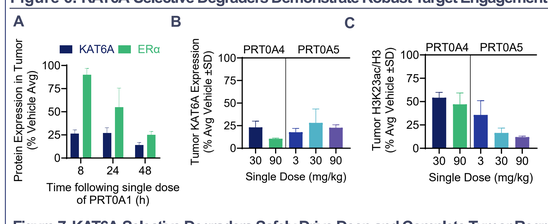

Figure 6. (A) Tumor PD from a ZR-75-1 CDX after a single dose of PRT0A1. KAT6A is degraded and ERα expression is reduced over time. (B-C) Tumor PD from a ZR-75-1 CDX 8 h following single oral doses of PRT0A4 or PRT0A5. (B) KAT6A is robustly degraded and (C) H3K23ac levels are shown.

Figure 7. KAT6A-Selective Degraders Safely Drive Deep and Complete Tumor Regressions in Breast and Lung Cancer Xenografts, Superior to a Dual KAT6A/B Inhibitor

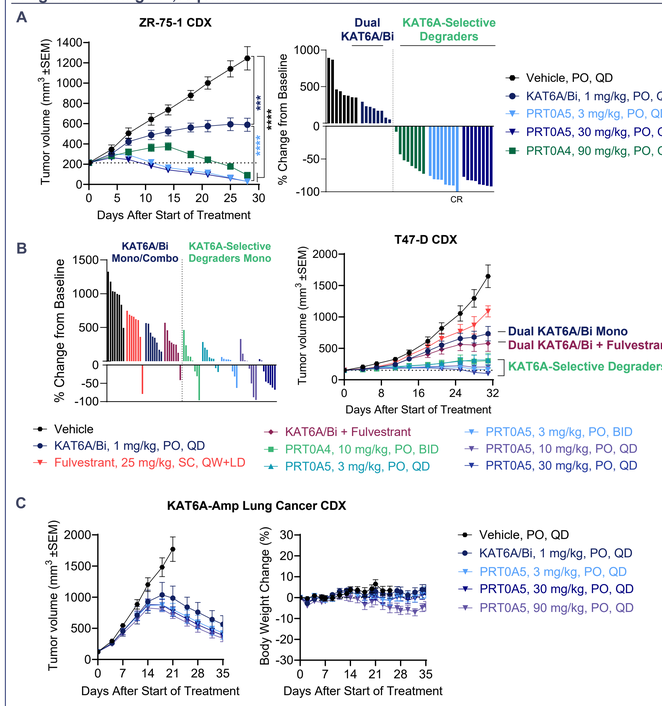

Figure 7. (A) Oral KAT6A-selective degraders safely achieved deep and complete tumor regressions at low oral doses in an orthotopic ZR-75-1 xenograft model, superior to a dual KAT6A/B inhibitor. (B) Oral KAT6A-selective degraders induced significant tumor growth inhibition and tumor regressions in T47-D xenografts as a monotherapy, outperforming combination of a KAT6A/Bi with Fulvestrant. (C) Oral KAT6A-selective degraders induced delayed regression of tumors from a KAT6A-amplified lung cancer xenograft and were well-tolerated. ***P<0.0005, **** P <0.0001.

Conclusions

- Identified what is believed to be first-in-class sub-nanomolar, selective, and readily orally bioavailable KAT6A degraders.
- Early pre-clinical markers suggest that achieving selective degradation of KAT6A over other MYST proteins via TPD could yield a differentiated hematological safety profile, which warrants further investigation.
- Selective KAT6A protein degradation drives significantly deeper anti-cancer responses compared to non-selective dual KAT6A/B inhibitors, supported by a deeper and differentiated mechanistic effect.
- Selective KAT6A degraders display robust anti-cancer activity in KAT6A-amplified breast, lung, and ovarian cancer cells.
- Observed deeper anti-cancer activity of selective KAT6A degraders in breast cancer cells compared to SoC therapy, sustained activity in *ESR1* and *PIK3CA* mutated, endocrine therapy, and CDK4/6i-resistant cells as well as robust combination benefit with SoC and potential next generation breast cancer therapies.
- Demonstrated KAT6A-selective degraders have robust *in vivo* target engagement and deep tumor regressions in breast and lung cancer xenografts as a monotherapy at low oral daily doses.

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