Discovery of NKT5097: a first-in-class, highly potent and selective, orally bioavailable CDK2/4 dual degrader for cancer therapy

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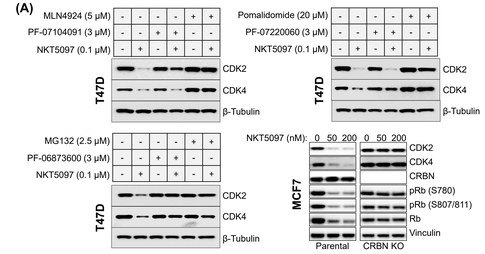
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Background

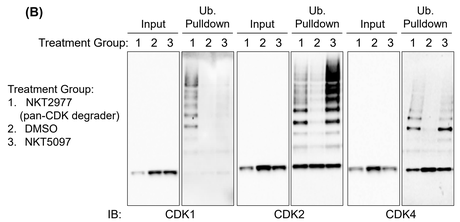
- CDK4 is the pivotal driver of HR+HER2- breast cancer and CDK4/6 inhibitors in combination with endocrine therapy have revolutionized the treatment landscape. However, several challenges remain with currently approved CDK4/6 inhibitors, including severe hematotoxicity associated with CDK6 inhibition and the development of resistance, often mediated by CDK2/cyclin E pathway activation. Therefore, simultaneously and selectively targeting CDK4 and CDK2 represents a promising therapeutic strategy for HR+HER2- breast cancer.
- The combination of CDK2 and CDK4 inhibitors has shown promising activity but was limited by overlapping toxicities in patients.
- The high homology between CDK1 and CDK2, as well as between CDK4 and CDK6, poses a significant challenge for the discovery of selective CDK2/4 dual inhibitors.
- We employed the targeted protein degradation approach and have discovered NKT5097, a first-in-class, selective, and orally bioavailable CDK2/4 dual degrader. Here, we report the preclinical characterization of NKT5097 across a panel of human cancer cell lines and animal tumor models. NKT5097 potently and selectively degrades CDK2 and CDK4, leading to specific G1 cell cycle arrest and significant anti-proliferation effect in CDK2-dependent, or CDK4-dependent, or CDK2/4 co-dependent tumor cell lines. Furthermore, the anti-tumor efficacy and potential off-target effects of NKT5097 were evaluated and benchmarked against CDK2- or CDK4-specific inhibitors currently in clinical development.

Results

NKT5097 Induced CDK2/4 Degradation through Recruiting CDK2/4 to Cereblon and the Ubiquitin-Proteasome System



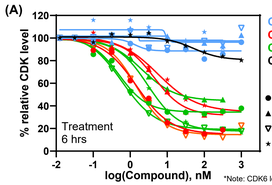
(A) CDK2/4 degradation requires binding of NKT5097 to both CDK2/4 and cereblon; and is mediated via the ubiquitin-proteasome system. NKT5097 failed to degrade CDK2/4 when proteasome activity was inhibited (MLN4924 or MG132), cereblon interaction was blocked (excess pomalidomide), or cereblon was knocked out. CDK2 degradation was blocked by CDK2/4/6 inhibitor PF-06873600 and CDK2-selective inhibitor PF-07104091, but not by CDK4 inhibitor PF-07220060. Conversely, CDK4 degradation was blocked by PF-06873600 and PF-07220060, but not PF-07104091.



(B) NKT5097 treatment induced ubiquitination of CDK2 & CDK4, but not CDK1. T47D cells were treated for 3 hrs with either DMSO, NKT5097 (200 nM) or NKT2977 (200nM; a pan-CDK degrader). Poly-ubiquitinated proteins were pulled-down from cell lysates using TUBEs (Tandem Ubiquitin Binding Entities, LifeSensors). Proteins were then eluted from the TUBE-conjugated beads and probed for CDK1, CDK2, and CDK4 by immunoblot. CDK6 was not detectable in the T47D cell lysates.

Results

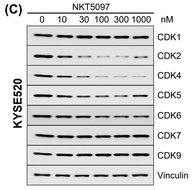
NKT5097 Preferably Degrades CDK2 and CDK4 with Minimal Off-Target Degradation



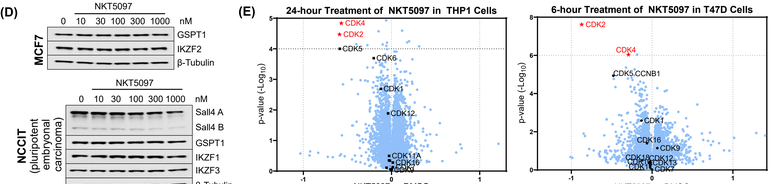
(B)

Cancer Cell Line	CDK2 DC_{50} (nM)	CDK2 D_{max} (%)	CDK4 DC_{50} (nM)	CDK4 D_{max} (%)
CAMA1	0.97	98%	1.34	68%
T47D	0.49	97%	0.44	75%
MCF7	1.42	75%	2.24	66%
ZR-75-1	1.77	87%	4.48	63%
SKBR3	1.03	94%	1.06	69%
A2780	0.71	97%	0.53	82%
KYSE520	3.2	75%	4.62	88%

*Note: CDK6 levels are below detection in T47D, MCF7 and A2780 cells.

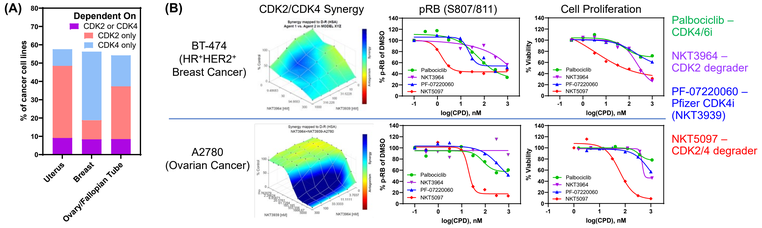


(A-B) NKT5097 potently degrade CDK2 and CDK4 in various cancer cells. Cancer cells were treated for 6 hrs (A) or 24 hrs (B). Total cellular CDK levels were measured using HTRF total CDK cellular kits (Cisbio/Revvity) following manufacturer's protocol. **(C) NKT5097 selectively degrades CDK2 and CDK4, while sparing CDK1/6/7/9.** Cells were treated with NKT5097 for 24 hrs and subject to immunoblotting analysis.



(D) NKT5097 has minimal effects on thalidomide neosubstrates. Immunoblotting analysis of cells treated with NKT5097 for 24 hrs. **(E) Global proteomic profiling demonstrates that NKT5097 selectively degrades CDK2 and CDK4.** T47D cells were treated with DMSO or 200 nM NKT5097 (n=4 each) for 6 hours. THP1 cells were treated with DMSO or 500 nM NKT5097 (n=3 each) for 24 hours. Lysates were treated with the TMT plex kit, which was followed by liquid chromatography-tandem mass spectrometry–based proteomics analysis. The volcano plots indicate protein ranking per abundance ratio (log_2 fold change) for NKT5097 over DMSO and the statistical p value in the format of $-Log_{10}$.

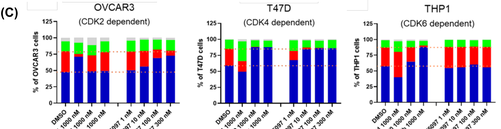
NKT5097 Has the Potential to Target a Broader Patient Population



(A) Distribution of CDK2 and CDK4 dependency across gynecologic and breast cancer cell lines. DepMap uses CRISPR knockout screens to evaluate gene function across cancer cell lines, with a dependency score ≤ −1 defining dependency.
(B) NKT5097 demonstrates potent anti-cancer activity in CDK2/4 Co-dependent cells.

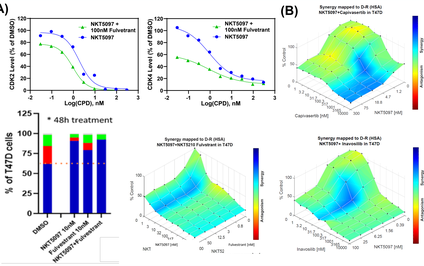
NKT5097 Inhibits Rb Phosphorylation and Arrests G1-S Progression in CDK2- or CDK4-dependent Cancer Cells

(A-B) NKT5097 inhibited Rb phosphorylation in CDK2- or CDK4-dependent human cancer cells. Cancer cells were treated for 24 hrs. (A) Immunoblot was performed to assess protein levels. (B) Total cellular phospho-Rb levels were measured using HTRF total phospho-Rb cellular kits (Cisbio/Revvity) following manufacturer's protocol.



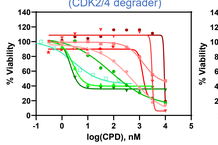
(C) NKT5097 blocked G1-to-S cell cycle progression in CDK2- or CDK4-dependent cancer cells. Cells were treated DMSO or compounds at the indicated concentrations for 48 hours. DNA content was assayed by flow cytometry to determine the relative distribution of cells in each phase of the cell cycle.

NKT5097 Combined with Fulvestrant, Capivasertib, or Inavolisib Enhances Cellular Response

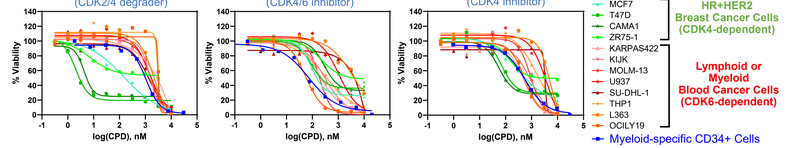


(A) Combination of NKT5097 with Fulvestrant (endocrine therapy) demonstrates enhanced multiple cellular effects in HR+HER2- T47D breast cancer cells.
(B) Combination of NKT5097 with Capivasertib (AKT inhibitor) or Inavolisib (PIK3CA inhibitor) shows synergistic effects on cell proliferation inhibition.

NKT5097 Demonstrates Superior Potency and CDK2/CDK4 Selectivity Compared with Clinical CDK2 or CDK4 Inhibitors

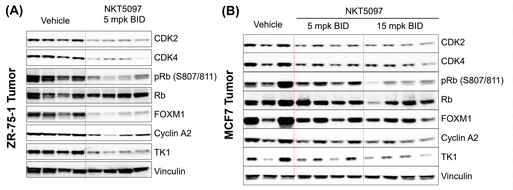


(A) NKT5097 exhibits high potency in CCNE1-amplified CDK2-dependent cancer cells and great selectivity against CDK2(4)-independent tumor cells.



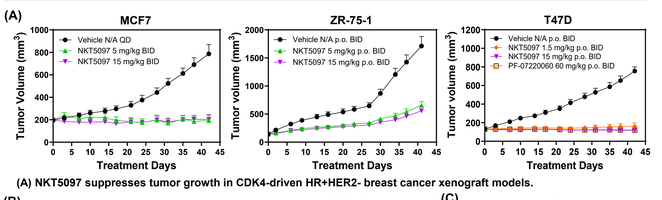
(B) NKT5097 has low risk of hematotoxicity due to its superior CDK4 vs CDK6 selectivity. Myeloid toxicity assay was conducted following the protocol from Mahalingaiah 2018. (A-B) Cancer cells were treated for 7 days and cell proliferation was measured using the Cell-Titer-Glo assay (Promega).

NKT5097 Demonstrates Robust Pharmacodynamic Effects in vivo

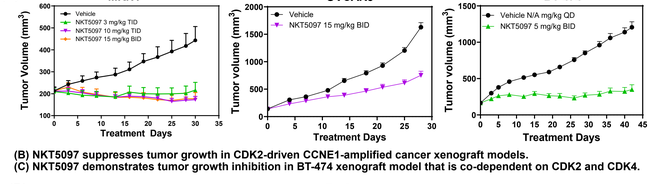


(A-B) NKT5097 treatment in HR+HER2– breast cancer xenografts resulted in degradation of CDK2 and CDK4, inhibition of Rb phosphorylation, and subsequent modulation of downstream effectors, including cyclin A2, FOXM1, and TK1. Mice were treated with NKT5097 for 3 days and tumors were collected 6 hrs post last dose

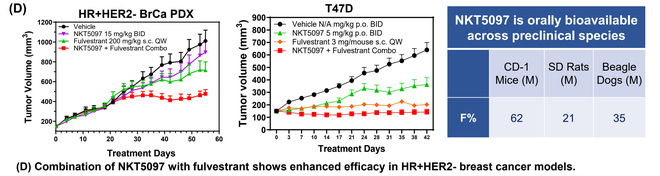
NKT5097 Demonstrates in vivo Efficacy



(A) NKT5097 suppresses tumor growth in CDK4-driven HR+HER2- breast cancer xenograft models.



(B) NKT5097 suppresses tumor growth in CDK2-driven CCNE1-amplified cancer xenograft models.
(C) NKT5097 demonstrates tumor growth inhibition in BT-474 xenograft model that is co-dependent on CDK2 and CDK4.



(D) Combination of NKT5097 with fulvestrant shows enhanced efficacy in HR+HER2- breast cancer models.

NKT5097 is orally bioavailable across preclinical species

	CD-1 Mice (M)	SD Rats (M)	Beagle Dogs (M)
F%	62	21	35

Summary

- The findings reported here establish NKT5097 as a potent, selective and orally bioavailable dual degrader of CDK2 and CDK4. NKT5097 offers distinct advantages over the combined use of CDK2 and CDK4 inhibitors, including more comprehensive pathway inhibition and a lower risk of overlapping toxicity, owing to its high potency against CDK2/4 and strong selectivity over CDK1/6. Therefore, as the first-in-class CDK2/4 dual degrader, NKT5097 presents a unique opportunity to serve as a backbone therapy for HR+HER2- breast cancer and other cancers with CDK2 and/or CDK4 dysregulation.
- NKT5097 is currently being investigated in a phase I clinical study (NCT07029399). Preliminary PK data from the first cohort indicated favorable systemic exposure consistent with human PK projections. In addition, initial PD data from the first cohort showed deep reduction of TKa level in HR+HER2- breast cancer patients previously treated with CDK4/6 inhibitors. (For further detail, refer to the NiKang press release.)

Reference

1. Jhaveri K et al. Expert Rev Anticancer Ther 2021; 21(10): 1105-24
2. Mahalingaiah PK, Curr Protoc Toxicol 2018;76(1):e45