Activity of CDK9 PROTAC (THAL-SNS-032) in HER2 positive breast cancer

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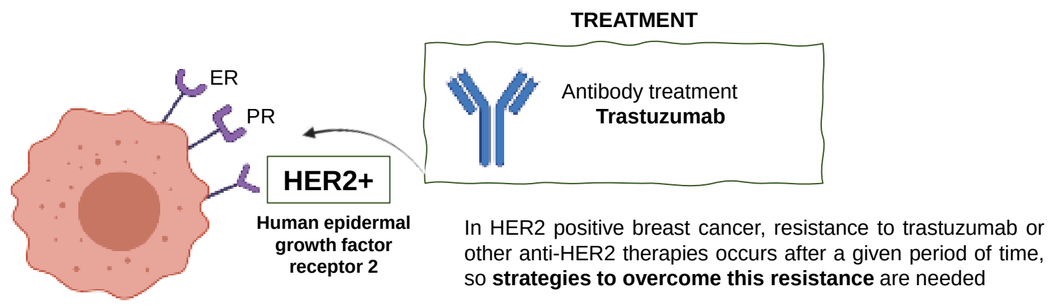
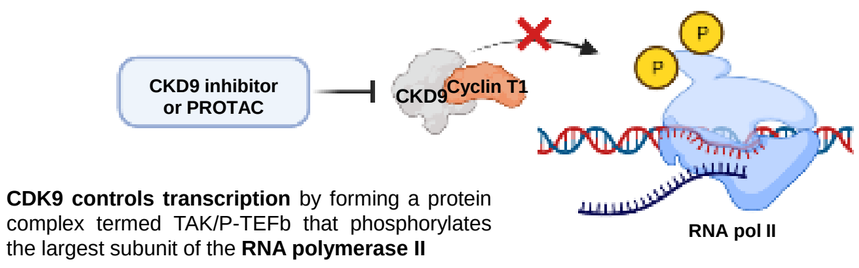
ABSTRACT

Cyclin dependent kinases (CDKs) are a broad family of proteins involved in cell cycle and tran-scriptional regulation. Transcriptional CDKs have been linked to cancer as they control expression of several oncogenic genes. In this article, we explore the antitumoral activity of a novel prote-olysis targeting chimera (PROTAC) compound against CDK9. Breast cancer cell lines from dif-ferent subtypes were used including BT474-derived cellular models, representative of adaptive resistance to different anti-HER2 therapies. Transcriptomic mapping of CDKs in breast cancer demonstrated that expression of CDK9 predicted detrimental outcome in basal-like tumors and, particularly, in the luminal B subtype with HER2+ expression. The novel CDK9 PROTAC, THAL-SNS-032 displayed a profound inhibitory activity in MCF7, T47D, and BT474 cells, with less effect in SKBR3, HCC1569, HCC1954, MDA-MB-231, HS578T and BT549 cells. The three cell lines with HER2 overexpression and no presence of ER, SKBR3, HCC1569 and HCC1954, displayed EC50 three times higher compared to ER positive and dual ER/HER2 positive cell lines. BT474-derived trastuzumab re-sistance cell lines, displayed a particular sensitivity to THAL-SNS-032. Western blot analyses showed that THAL-SNS-032 caused a decrease in CDK9 levels in BT474, BT474-RH, and BT474-TDM1R cells, and a significant increase in apoptosis compared with the kinase inhibitor SNS-032, with no major changes in cell cycle phases. In summary, the potent and efficient antitumoral properties of the CDK9 PROTAC THAL-SNS-032 opens the possibility of using this type of compounds in breast cancer only if specifically delivered to tumoral cells, particularly in ER/HER2 positive and HER2 resistant tumors.

BACKGROUND





CDK9 as potential new target in HER2+

RESULTS

In silico analysis of CDKs in breast cancer

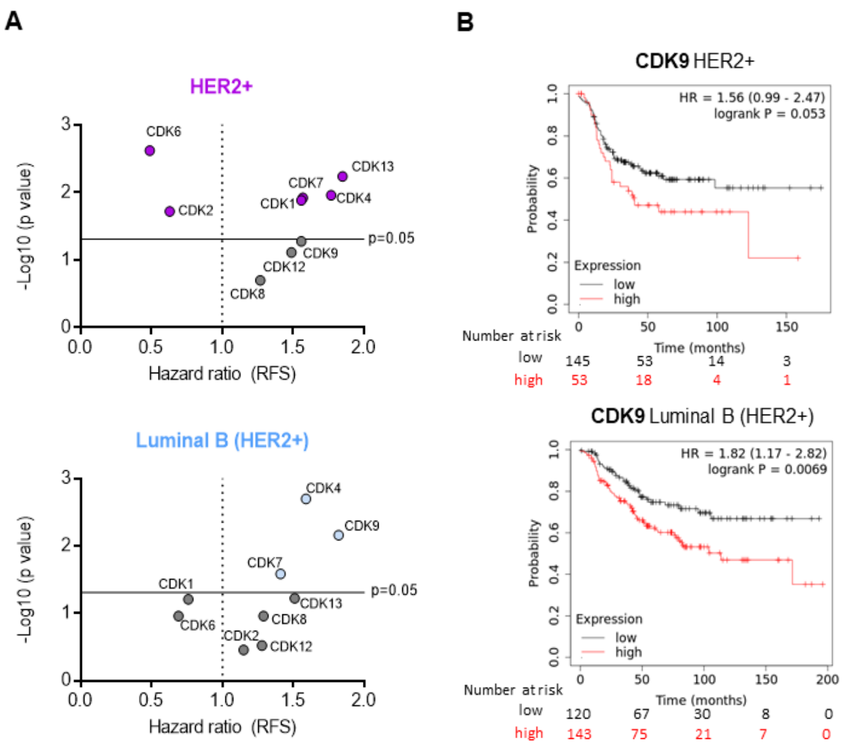


Figure 1. Correlation between CDKs expression level and clinical outcome in breast cancer. A. Graphs displaying HR values extracted from Kaplan–Meier survival plots of the association between CDKs individually expressed and patient prognosis, including relapse-free survival (RFS), for HER2+ (n=198) and Luminal B with Status HER2+ (n=263) **B**. Kaplan–Meier survival plots of the association between CDK9 mean expression levels and patient prognosis, including relapse-free survival (RFS) (n=1764) for all breast subtypes: HER2+ (n=198) and Luminal B with Status HER2+ (n=263).

THAL-SNS-032 is active in ER/HER2 positive cell lines

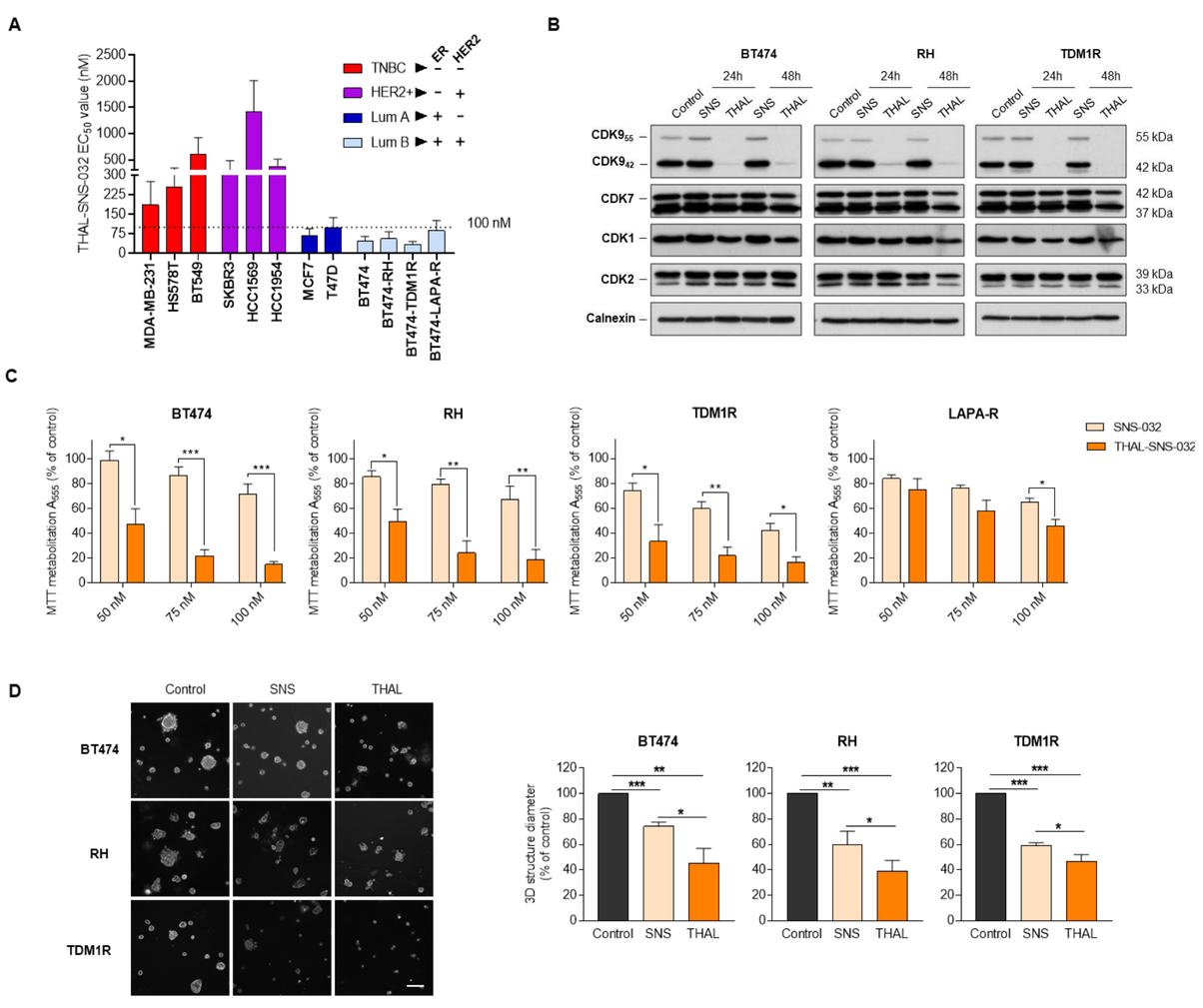


Figure 2. Evaluation of antitumoral activities of SNS-032 and THAL-SNS-032 in breast cancer lines. A. THAL-SNS-032 EC50 obtained by MTT proliferation assays in breast cancer cell lines after 72 h of treatment. **B.** Western blot showing expression levels of CDK9, CDK7, CDK1 and CDK2 in BT474, RH, and TDM1R cells treated with SNS-032 and THAL-SNS-032 at the times indicated at 50 nM. Calnexin was used as a loading control. **C.** Cell viability evaluated by MTT assays of BT474, BT474-RH, BT474-TDM1R, and BT474-LAPA-R cells treated with SNS-032 and THAL-SNS-032 at the doses indicated (72 h). **D**. SNS-032 or THAL-SNS-032 were applied for 72 h to BT474, RH, and TDM1R cells seeded in a Matrigel matrix (100 nM). Invasion capacity was assessed by measuring sphere diameters of invading 3D structures, and results are presented as percentage referred to untreated cells. Scale bar = 100 µm.

Effect of THAL-SNS-032 on the cell cycle and cell death

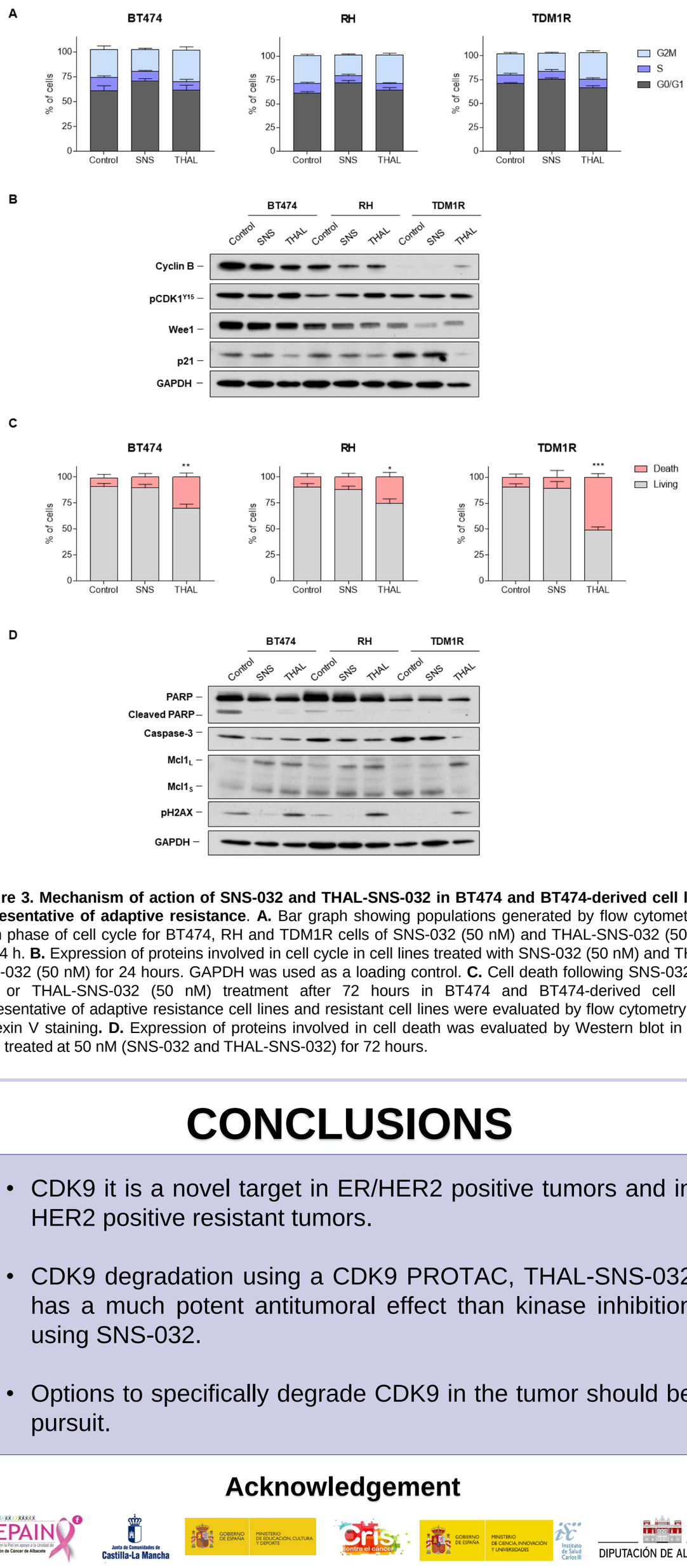


Figure 3. Mechanism of action of SNS-032 and THAL-SNS-032 in BT474 and BT474-derived cell lines representative of adaptive resistance. **A.** Bar graph showing populations generated by flow cytometry in each phase of cell cycle for BT474, RH and TDM1R cells of SNS-032 (50 nM) and THAL-SNS-032 (50 nM) for 24 h. **B.** Expression of proteins involved in cell cycle in cell lines treated with SNS-032 (50 nM) and THAL-SNS-032 (50 nM) for 24 hours. GAPDH was used as a loading control. **C.** Cell death following SNS-032 (50 nM) or THAL-SNS-032 (50 nM) treatment after 72 hours in BT474 and BT474-derived cell lines representative of adaptive resistance cell lines and resistant cell lines were evaluated by flow cytometry with annexin V staining**. D.** Expression of proteins involved in cell death was evaluated by Western blot in cells lines treated at 50 nM (SNS-032 and THAL-SNS-032) for 72 hours.

CONCLUSIONS

- CDK9 it is a novel target in ER/HER2 positive tumors and in HER2 positive resistant tumors.
- CDK9 degradation using a CDK9 PROTAC, THAL-SNS-032 has a much potent antitumoral effect than kinase inhibition using SNS-032.
- Options to specifically degrade CDK9 in the tumor should be pursuit.

Acknowledgement