Discrete Vulnerabilities to CDK2 Inhibition in Breast Cancer: Genetic Determinants & Therapeutic Opportunities

Erik S. Knudsen[1], Vishnu Kumarasamy[1], Adam P. Dommer[1], Jianxin Wang[1], Agnieszka K. Witkiewicz[1,2,3]

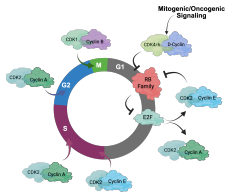
[1]Department of Molecular and Cellular Biology, [2]Department of Pathology, [3]Department of Medicine, Roswell Park Comprehensive Cancer Center, Elm and Carlton Street, Buffalo, NY, USA 14263.

Abstract

BACKGROUND: Cyclin-dependent kinase 2 (CDK2) is a critical effector of cell-cycle progression, including during late G1, S, and G2 phases. While CDK4/6 inhibitors have become a backbone in HR+/HER2- breast cancer therapy, resistance frequently emerges through activation of alternative CDK/Cyclin complexes. Notably, the CDK2/Cyclin E axis is frequently associated with resistance to CDK4/6 inhibitors and has driven the development of catalytic CDK2 inhibitors for breast cancer therapy. Here, we sought to: 1, delineate the mechanistic determinants of response to pharmacologic CDK2 inhibition in breast cancer models; 2, define biomarkers predictive of response; and 3, interrogate rational combination strategies to maximize therapeutic efficacy.

RESULTS: Using a panel of breast cancer models, it was found that CDK2 catalytic inhibitors induced a bimodal response with two fundamentally distinct phenotypic responses. A minority of cell lines underwent G1 arrest while most accumulated 4N DNA content consistent with a G2/M block. Co-expression of *p16INK4A* and high Cyclin E1 strongly correlated with the G1 arrest phenotype and marked sensitivity to CDK2 inhibition that was also observed *in vivo*. Models lacking this co-expression adopted the 4N arrest response and displayed upregulation of phospho-CDK1 (Y15) and Cyclin B1. Critically, this latter phenotype was observed in all HR+/HER2- models tested, including those with engineered resistance to CDK4/6 inhibitors. Using whole-genome CRISPR screens, loss of *CDK2* was identified as a top hit conferring resistance to catalytic inhibitors, underscoring the concept that inhibitor binding to CDK2 (rather than simple loss) is essential for the cytostatic effect. Further, deletion of *CDK2* reversed the G2/M arrest induced by the inhibitor and restored proliferation. A host of potential targets were identified that enhanced sensitivity to CDK2 inhibitors through CRISPR and drug screening approaches. These included CDK4/6 inhibitors that were broadly synergistic with CDK2 inhibitors irrespective of breast cancer subtype. The basis of this cooperation involved arrest in multiple phases of the cell cycle. Several additional combinatorial strategies have recently been defined that further credential CDK2 as a key therapeutic target moving forward. *Together, this work addresses the complexity of targeting CDK2 for the treatment of breast cancer and multiple opportunity areas for combinations.*

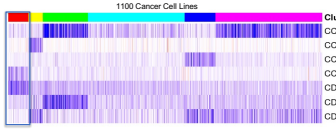
Rationale for Targeting CDK2



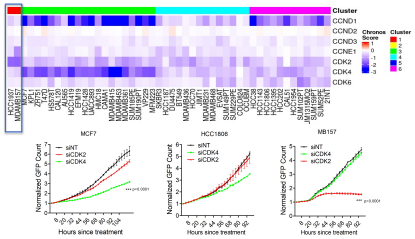
***CCNE1*-deregulated cancers:** Amplification of *CCNE1* or deregulation of Cyclin E expression is associated with poor prognosis and therapeutic resistance. In breast cancer this includes resistance to endocrine therapy and poor outcomes in TNBC where *CCNE1* amplification is more common.

CDK4/6 inhibitor resistance: Cyclin E and CDK2 activity are considered one of the key mechanisms through which HR+/HER2- breast cancers develop resistance to CDK4/6 inhibitors.

Differential Genetic Dependence on CDK2

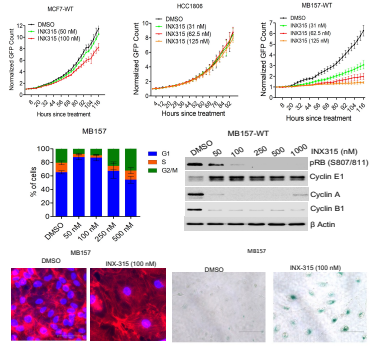


Unbiased genomic screens of 1,100 pan-cancer cell lines using Cancer DepMap data illustrate only a minority (~7%) of cancer cells are highly dependent on *CDK2* and *CCNE1*. This cluster is overrepresented for *CCNE1* amplification.



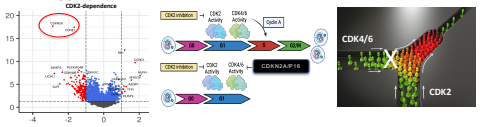
Select breast cancer cell models are exceptionally dependent on *CDK2*.

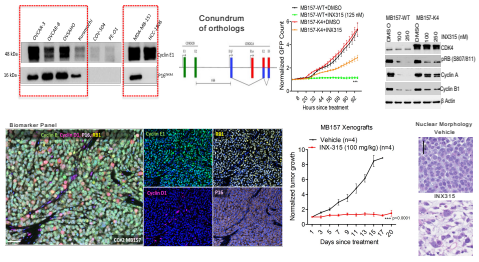
Exceptional Sensitivity to CDK2 Inhibitors



Select breast cancer cell models are exceptionally sensitive to CDK2 inhibitors. These cells arrest in the G1 phase of the cell cycle and undergo senescence-like arrest.

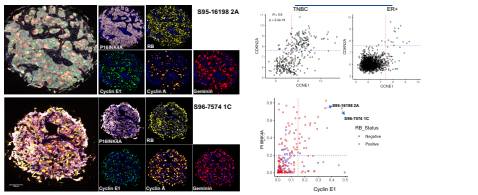
Determinants of Exceptional CDK2 Dependence





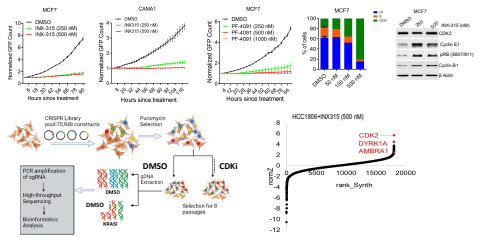
DepMap data indicate high expression of *CCNE1* and *CDKN2A* is associated with vulnerability to *CDK2* depletion. Functional studies support the critical role of suppressing CDK4/6 along with CDK2. High P16 contributes to CDK2 inhibitor monotherapy sensitivity.

Biomarkers in Breast Cancer

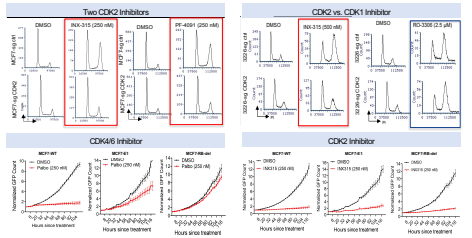


~10% of TNBC cases present as *CCNE1*, *CDKN2A*-high, indicative of sensitivity to CDK2i.

Common Response to CDK2 Inhibitors is CDK2-Dependent

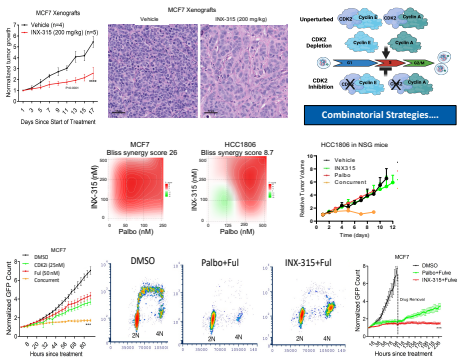


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CDK2 inhibitor G2/M arrest is CDK2-dependent.

Actionable *In Vivo* and Combinatorial Strategies



CDK2 inhibitor synergy is observed with CDK4/6 inhibitors and endocrine therapy.

Key Takeaways

- CDK2 inhibition is a promising approach for several cancer types
- CDK2 inhibitors were particularly effective in Cyclin E^{high}, P16^{high}-expressing breast cancer models
- Synergy is observed with CDK2 plus CDK4/6 inhibitors and CDK2 inhibitors plus endocrine therapy

Contact

Erik S. Knudsen and Agnieszka K. Witkiewicz
Roswell Park Comprehensive Cancer Center, Buffalo, NY
Email: erik.knudsen@roswellpark.org and agnieszka.witkiewicz@roswellpark.org
Website: https://www.roswellpark.org/research/labs/witkiewicz-knudsen-lab

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