# The Cyclin D1-CDK6 Complex Drives Resistance to the CDK4-Selective Inhibitor Atirmociclib in Estrogen Receptor-Positive Breast Cancer

Poster #18


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Reference

## BACKGROUND/SIGNIFICANCE

The cyclin D1-CDK4 complex drives G1-S progression in estrogen receptor-positive breast cancer (ER+ BC), accounting for the success of dual CDK4/6 inhibitors combined with hormonal therapy in this BC subtype. CDK4-selective inhibitors, including the index compound atirmociclib, are being developed to mitigate the hematologic toxicity associated with the CDK6 inhibitory activity of CDK4/6 inhibitors. Despite the promising results from the recent clinical trials of atirmociclib, acquired resistance to CDK4-selective inhibitors is still expected to universally occur. To address the mechanism of resistance, we developed ER+ BC cancer models resistant to atirmociclib using the MCF7 and T47D cell lines and identified the cyclin D1-CDK6 complex as a mechanism of resistance that overcomes atirmociclib-induced cell cycle arrest.

## RESULTS

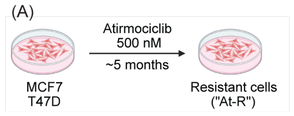


**Figure 1. Emergence of acquired resistance to atirmociclib in ER+ metastatic breast cancer cells.**
**(A)** Schematic of generation of atirmociclib-resistant (At-R) cells in MCF7 and T47D. **(B)** DMSO or atirmociclib (At) 500 nM was treated for 1 day to parental (Par) and atirmociclib-resistant (At-R) cells, followed by cell cycle analysis by flow cytometry with EdU/DAPI staining. Mean+SD, n=3 (MCF7), n=2 (T47D). **(C)** IC50 value measured by CyQuant assay after 1-week treatment of atirmociclib in parental and At-R cells n=1. **(D)** Increased CDK6 protein levels in At-R cells. Bands in different lanes on the same blot were cropped to position next to each other.

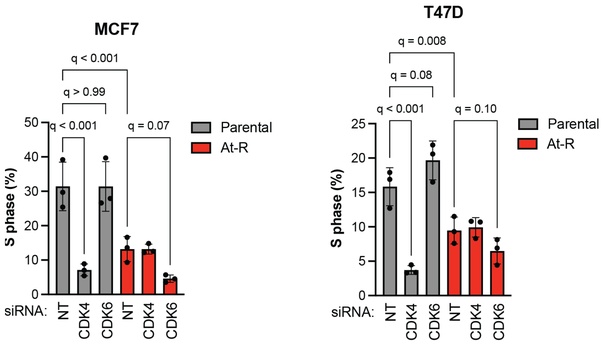


**Figure 2. CDK6 supports G1-S progression in ER+ BC cells with acquired resistance to atirmociclib.**
Cell cycle analysis by flow cytometry with EdU/DAPI staining after 48 hours of siRNA transfection. Mean±SD, N=3. One-way ordinary ANOVA with Holm-Sidak multiple testing correction. The adj.P-value of select sets of comparison is denoted on the graph.

## RESULTS

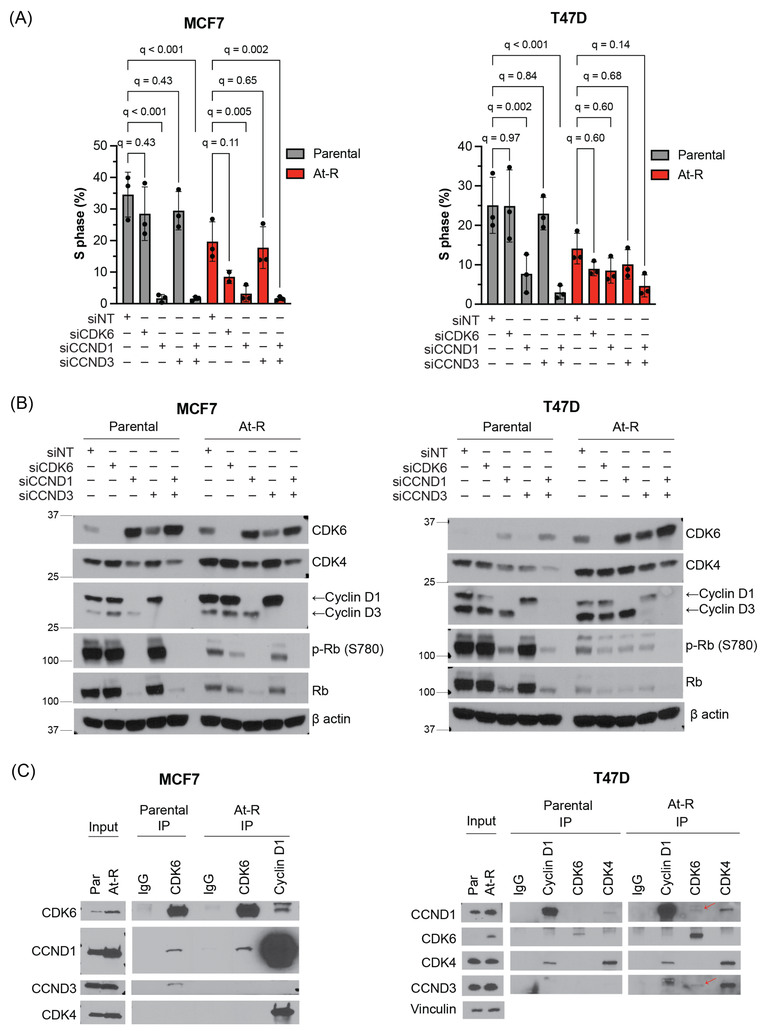


**Figure 3. The cyclin D1-CDK6 complex is necessary for G1-S progression in ER+ BC cells with acquired resistance to atirmociclib.**
**(A)** Cell cycle analysis by flow cytometry with EdU/DAPI staining after 48 hours of siRNA transfection. Mean±SD, N=3. One-way ordinary ANOVA followed by Holm-Sidak multiple testing correction. **(B)** Western blot analysis of the same samples from (A). **(C)** Immunoprecipitation (IP) analysis from the whole-cell lysates. Bands in different lanes on the same blot were cropped to position next to each other.

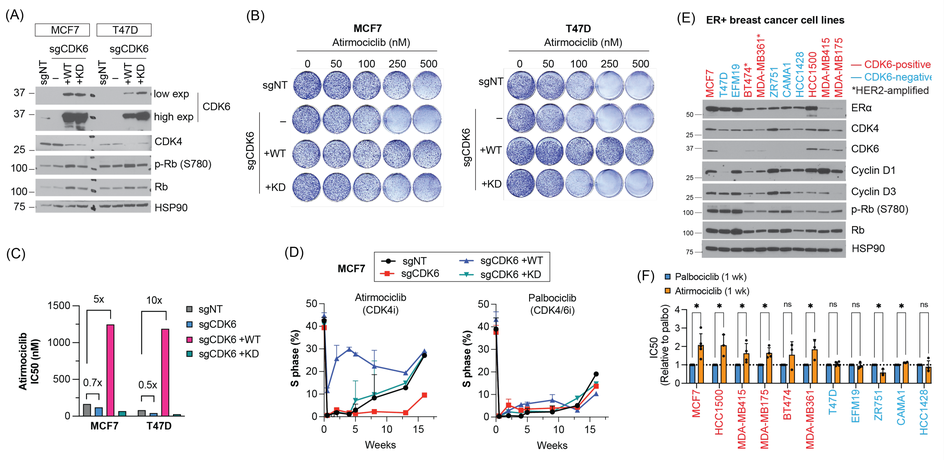


**Figure 4. CDK6 kinase function determines the efficacy of atirmociclib and its loss delays the emergence of acquired resistance to atirmociclib in ER+ BC cells.**
**(A)** Western blot analysis of CDK6 deletion by CRISPR-Cas9 and stable complementation of either CDK6 wild-type or kinase-dead form. **(B)** Clonogenic assay after 2 weeks of atirmociclib treatment as denoted. **(C)** IC50 values of atirmociclib measured by CyQuant assay after 1 week treatment. Mean, N=2 **(D)** 500 nM of atirmociclib or palbociclib was treated for 16 weeks and cell cycle phases were periodically measured with EdU/DAPI staining. (E) Select set of ER+ breast cancer cell lines categorized by CDK6 expression status. (F) Comparison of IC50 values measured by CyQuant assay. Mean±SD, N=3. Lognormal t-test. *: P<0.05

## RESULTS

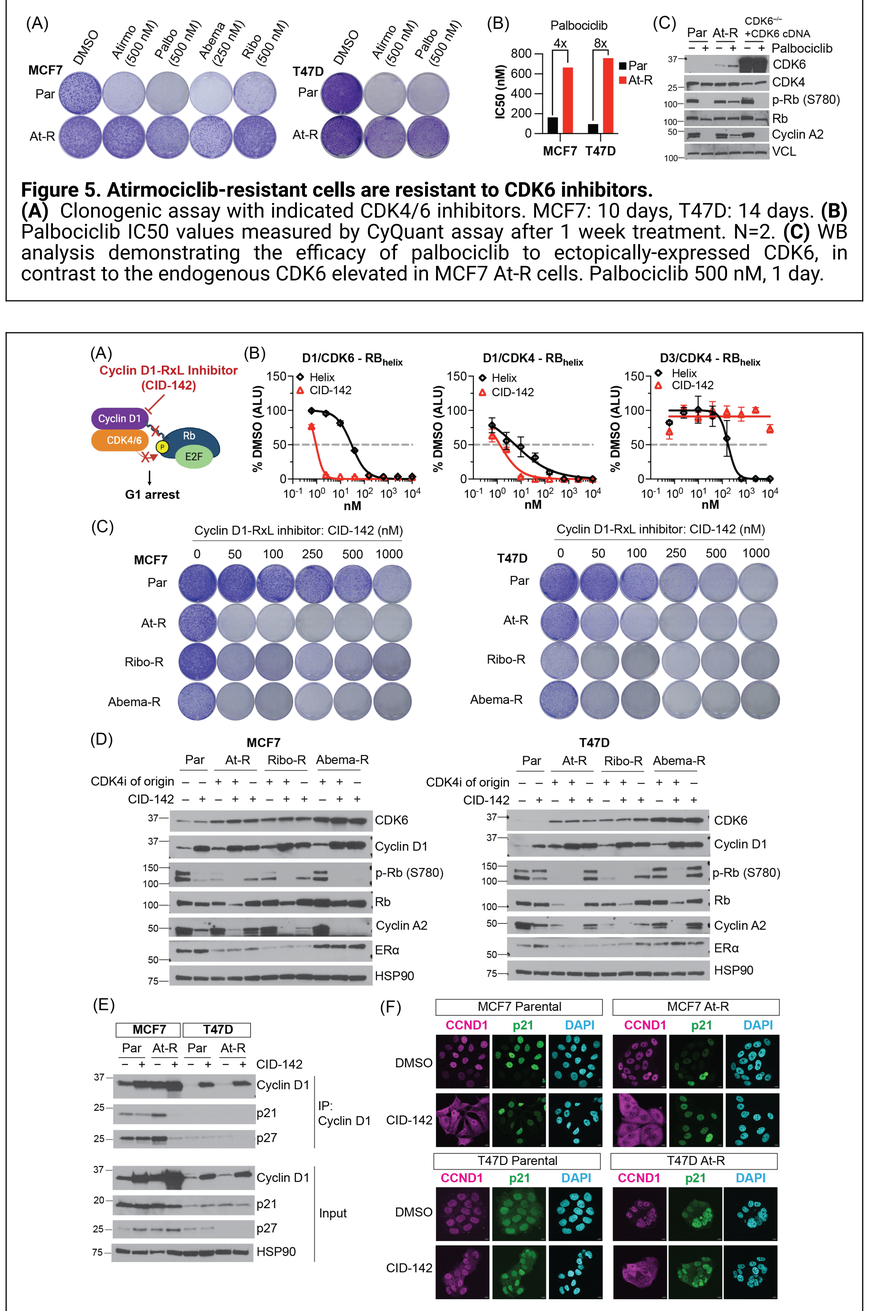


**Figure 5. Atirmociclib-resistant cells are resistant to CDK6 inhibitors.**
**(A)** Clonogenic assay with indicated CDK4/6 inhibitors. MCF7: 10 days, T47D: 14 days. **(B)** Palbociclib IC50 values measured by CyQuant assay after 1 week treatment. N=2. **(C)** WB analysis demonstrating the efficacy of palbociclib to ectopically-expressed CDK6, in contrast to the endogenous CDK6 elevated in MCF7 At-R cells. Palbociclib 500 nM, 1 day.

**Figure 6. Cyclin D1-RxL inhibitor blocks the growth of atirmociclib-resistant cells.**
**(A)** Depiction of CID-142, cyclin D1-RxL inhibitor. **(B)** AlphaLISA using a GST-tagged 20 amino acid from Rb described by Topacio et al. and biotinylated D1/D3 complexed with CDK4/6. Find details from the reference with QR code in the title. **(C)** Clonogenic assay after 2 weeks of CID-142 treatment as indicated. **(D)** WB of several CDK4 inhibitor-resistant cells treated with CID-142. **(E)** Cyclin D1 and CIP/KIP proteins co-IP analysis with CID-142. **(F)** Immunofluorescence analysis of cyclin D1 and p21 with CID-142, showing the nuclear exclusion of cyclin D1. Scale bar: 10 μm. **(D, E, F)** CID-142: 100 nM, 1 day

## CONCLUSION

1. The cyclin D1-CDK6 complex is necessary for acquired resistance and sufficient for intrinsic resistance to atirmociclib in ER+ BC cells.
2. CDK6 inhibitors yield limited effects to curb the growth of the CDK6-driven atirmociclib-resistant cells.
3. The cyclin D1-RxL inhibitor effectively inhibits the growth of atirmociclib-resistant ER+ BC cells and is currently being tested in HR+ patient-derived xenograft models with acquired resistance to CDK4/6 inhibitors.

## ACKNOWLEDGMENT


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