Single cell analysis enables tracking the evolution of resistance to CDK4/6 inhibitors in ER+ breast cancer

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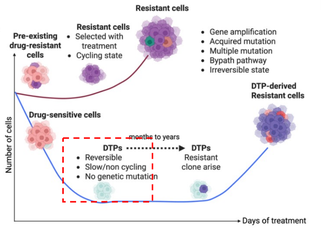
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Introduction

The combination of CDK4/6 inhibitors (CDK4/6i) with endocrine therapy prolongs survival of patients with ER+/HER2- metastatic breast cancer.

However, nearly all patients with advanced disease treated with this combination eventually progress, often with multiple resistance mechanisms.



Drug tolerant persister (DTP) cells represent a reservoir of surviving cells from which drug-resistant clones eventually emerge[1,2]. We hypothesize that targeting the DTP state, which emerges under selective pressure from CDK4/6i, could prevent the emergence of drug resistance.

Characterization of drug tolerant peristers under Palbociclib and estrogen deprivation pressure

DTP cell cycle arrest is reversible

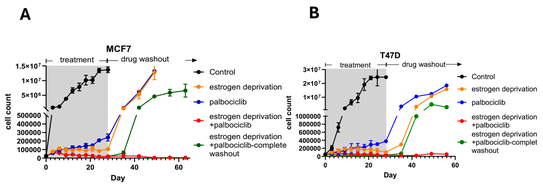
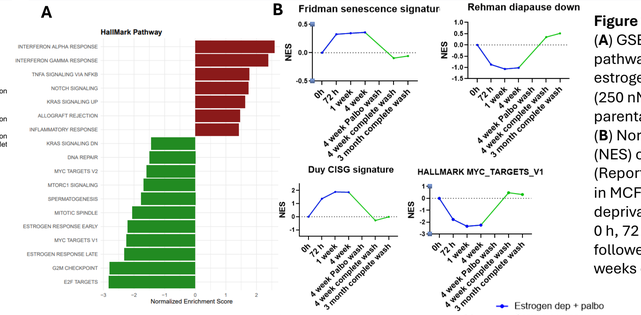


Figure 1.
(**A, B**) Growth of (**A**) MCF7 and (**B**) T47D cells treated with palbociclib (250nM), estrogen deprivation, or both for 4 weeks followed by treatment withdrawal (washout) or maintenance of the CDK4/6i and estrogen-free media. The total number of cells/well were measured using the Coulter Counter and normalized to day 0. Data represent the mean + SEM (n=3).

The DTP state is associated with transcriptomic reprogramming



CISG ; chemotherapy-induced stress genes[5,6]

Figure 2.
(**A**) GSEA analysis of Hallmark pathways in MCF7 cells treated with estrogen deprivation and palbociclib (250 nM) for 4 weeks compared to parental cells.
(**B**) Normalized enrichment score (NES) of **known DTP signatures** (Reported in HER2+ breast cancer[6]) in MCF7 cells treated with estrogen deprivation + palbociclib(250nM) for 0 h, 72 h, 1 week, or 4 weeks, followed by complete washout for 4 weeks or 3 months.

DTP signatures were similarly enriched in patients with neoadjuvant palbociclib treatment

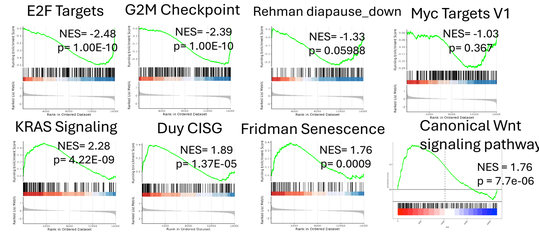


Figure 3. GSEA plots of microarray data from ER+/HER2- tumors (n=5) from patients in the NeoPalAna study. Patients were treated with neoadjuvant palbociclib and anastrozole (5 cycles) and compared to pre-treatment biopsies.

scRNA-seq with cell barcoding to track the heterogeneity of drug-tolerant clones

Wnt signaling is enriched in both MCF7 and T47D DTP subclusters

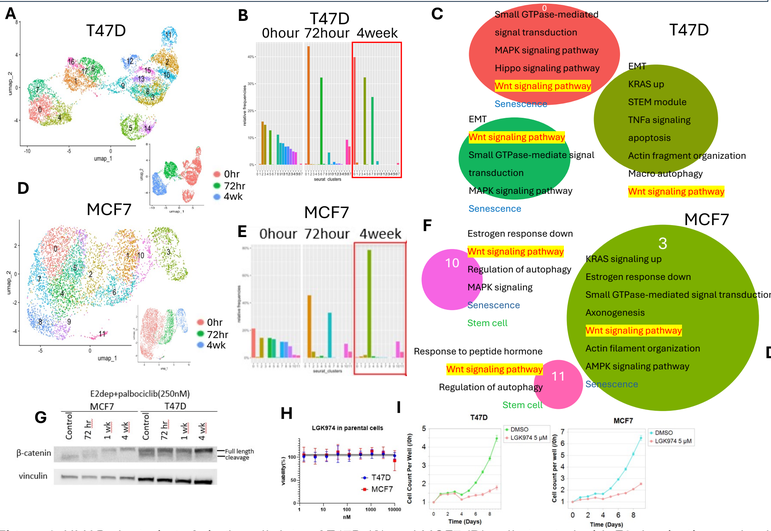


Figure 4. UMAP clustering of single cell data of T47D (**A**) and MCF7 (**D**) cells treated with E2 deprivation and palbociclib (250 nM) for 0 h, 72 h, or 4 wks. (**B, E**) Cluster distribution was annotated at each time point. (**C,F**) Summary of GSEA (Hallmark, GO, C2, KEGG) in 3 main T47D (**C**) and MCF7 DTP clusters (**F**). (**G**) Western blotting of β-catenin. (**H**) Cell viability assay in parental T47D and MCF7 cells treated with the PORCN inhibitor LGK974 for 72 h. (I) Incucyte cell count of T47D and MCF7 cells treated with E2dep+palbo for 4 wks, followed by treatment with DMSO or 5 μM LGK974 for 9 days.

LARRY barcode tracking to find expanding clones

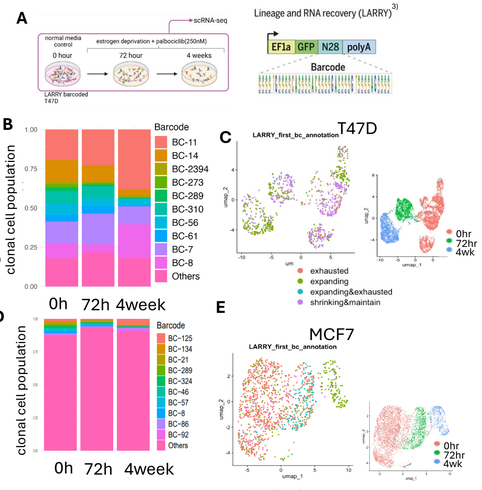
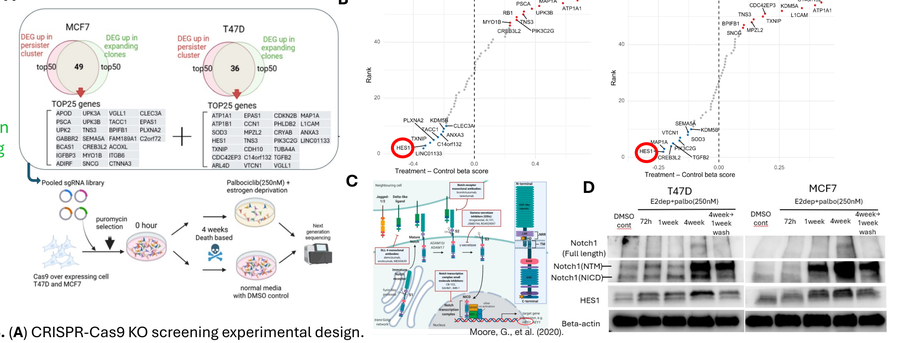


Figure 5. (**A**)Experimental design. (**B,D**) Fraction of each barcoded clone following treatment with estrogen deprivation plus palbociclib for the indicated timepoints in T47D and MCF7. (**C, E**) UMAP colored by change patterns of clonal population dominance in T47D (**C**) and MCF7 (**E**). Expanding clones: ratio of 4 weeks/0 h >1; Exhausted: clonal population >0 at 0h but undetectable at 4 weeks; Expanding & exhausted: clonal population >0 at 72 h but undetectable at 0 h or 4 weeks; Shrinking & maintain: ratio of 4 week/0 h ≤1. 101 and 15 clones expanded at 4 weeks, while 376 and 27 clones were eliminated during treatment of MCF7 and T47D cells, respectively.

Targeted CRISPR/Cas9 dropout screen identifies HES1 as essential for DTP survival

DEGs up in Expanding clones at 4weeks



Moore, G., et al. (2020).

Figure 6. (**A**) CRISPR-Cas9 KO screening experimental design. The top 25 differentially expressed genes (DEGs) that were both upregulated in DTP cells (by scRNA-Seq) and upregulated in expanding clones at 4 weeks (barcoding analysis) in MCF7 and T47D cells were selected for a pooled sgRNA library. sgRNAs that were enriched or depleted during 4 weeks of treatment will be identified by next gen sequencing. (**B**) Rank plot of normalized beta score. (**C,D**)Notch pathway schema and Western Blot for Notch1 and HES1.

Conclusions

- Cells that survive long-term treatment with CDK4/6i plus estrogen deprivation exhibit characteristics of DTPs, such as: Reversible sensitivity to CDK4/6i + estrogen deprivation, cell cycle arrest, transcriptional programs associated with DTPs
- Residual tumors following neoadjuvant treatment with CDK4/6i + estrogen suppression similarly show enrichment of gene signatures associated with DTPs
- scRNA-seq analysis revealed genes/pathways that are upregulated in the DTP state; the Wnt pathway could be targeted to reduce survival of drug-tolerant cells and prevent the emergence of drug resistance
- Tracking LARRY barcodes enabled identification of subclones that expand during long-term CDK4/6i treatment.
- Targeted CRISPR/Cas9 dropout screen in DTPs revealed HES1 as essential for DTP survival.

References

1. Sharma SV, *et al. Cell*. 2010;141(1):69-80.
2. Oren Y, *et al. Nature*. 2021;596(7873):576-82.
3. Weinreb C, *et al. Science*. 2020;367(6479):eaaw3381.
4. Ma CX, *et al. Clin Cancer Res*. 2017; 23 (15): 4055–4065
5. Duy C, et al. Cancer Discov. 2021;11:1542–61.
6. Chang, CA, et al. 2022. Cancer Discovery 12 (4): 1022–45.

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