A platform of CDK4/6 inhibitor-resistant patient-derived breast cancer organoids illuminates mechanisms of resistance and therapeutic vulnerabilities

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Introduction

- CDK4/6 inhibitors (palbociclib, ribociclib, abemaciclib) + antiestrogens have revolutionized the treatment of metastatic ER+ breast cancer.
- However, tumors eventually acquire resistance. Patients with resistant cancers are left with limited treatment options.
- Mechanisms of resistance to CDK4/6i are quite heterogeneous. Potential resistance-conferring alterations include: *RB1, FAT1, PTEN, ARID1A, FGFR1/2, ERBB2, CCNE1/2, AURKA*, and *KRAS*.
- Models of CDK4/6i resistance are needed to capture the heterogeneity of resistance mechanisms and identify novel therapeutic strategies for CDK4/6i-resistant tumors
- Patient-derived organoids (PDOs) provide a rapid, robust and reliable platform that recapitulates intra-tumor heterogeneity, partially mimics the cancer microenvironment, and accurately predicts drug response.

Objective: To generate and characterize a platform of CDK4/6i-resistant breast cancer PDOs to serve as models for understanding acquired resistance to CDK4/6i + antiestrogens and identifying therapies to overcome resistance.

PDOs established from metastatic biopsies of ER+ breast cancers progressing on CDK4/6i + antiestrogens

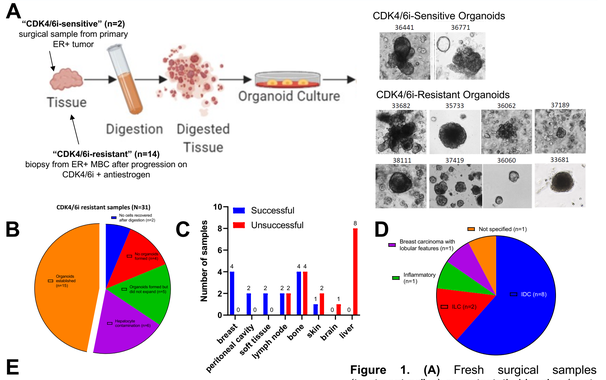

Figure 1. (A) Fresh surgical samples (treatment-naïve) or metastatic biopsies (post-progression on CDK4/6i) from ER+ breast cancers were digested and plated in Matrigel domes in defined organoid media. **(B)** Out of 31 metastatic biopsies from cancers that progressed on CDK4/6i, 15 PDOs were successfully established and passaged >4 times. **(C)** Biopsy sites from which PDOs were generated. **(D)** Histological subtypes represented in the CDK4/6i-resistant PDO collection. **(E)** Racial/ethnic background of established and unsuccessful PDOs.

Palbociclib fails to suppress proliferation and E2F/G2M signatures in CDK4/6i-resistant PDOs

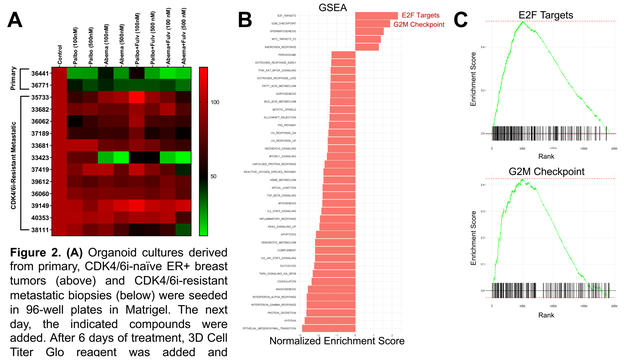

Figure 2. (A) Organoid cultures derived from primary, CDK4/6i-naïve ER+ breast tumors (above) and CDK4/6i-resistant metastatic biopsies (below) were seeded in 96-well plates in Matrigel. The next day, the indicated compounds were added. After 6 days of treatment, 3D Cell Titer Glo reagent was added and luminescence was quantified. The mean from 4 replicates per condition is plotted. **(B)** CDK4/6i-sensitive (n=2) and –resistant (n=6) PDOs were treated with 200 nM palbociclib in estrogen-deprived conditions. RNA was harvested and subjected to RNA-sequencing. Gene set enrichment analysis (GSEA) was performed on palbociclib-treated resistant vs. sensitive samples. **(C)** Cell cycle-associated pathways (E2F Target Hallmark signature and G2M Checkpoint Hallmark signature) were upregulated in palbociclib-treated CDK4/6i-resistant vs sensitive organoids.

Organoids from CDK4/6i-resistant tumors retain genomic alterations detected in patients

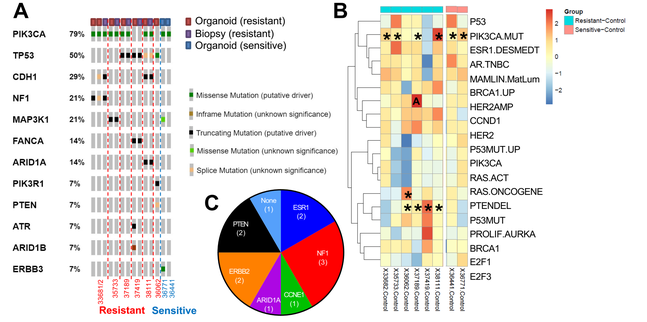

Figure 3. (A) DNA was extracted from CDK4/6i-sensitive and –resistant PDOs and subjected to targeted DNA-seq using the TEMPUS targeted capture NGS panel (684 cancer genes) and compared to the clinical NGS reports from matched biopsies. Putative cancer driver mutations (OncoKB) were analyzed using cBioportal.org. PDOs #33681 and #33682 were derived from the same patient. A clinical NGS report was not available for samples #36062, #36771, and #36441. **(B)** RNA-seq data from untreated CDK4/6i-sensitive and resistant PDOs was analyzed using single sample gene set enrichment analysis (GSEA) of 125 breast cancer-related signatures. Asterisks represent mutations of that gene in those organoids; "A" represents amplification. For example, the HER2-amp signature was highly upregulated in organoid #37189, which harbors HER2 amplification. **(C)** Clinical NGS reports were available for 12 matched patient biopsies for successful resistant PDOs. Frequency of alterations that have been associated with CDK4/6i or antiestrogen resistance is shown. DNA-seq analysis in PDOs is ongoing.

A subset of CDK4/6i-resistant PDOs retain sensitivity to PI3K pathway and/or G2/M inhibitors

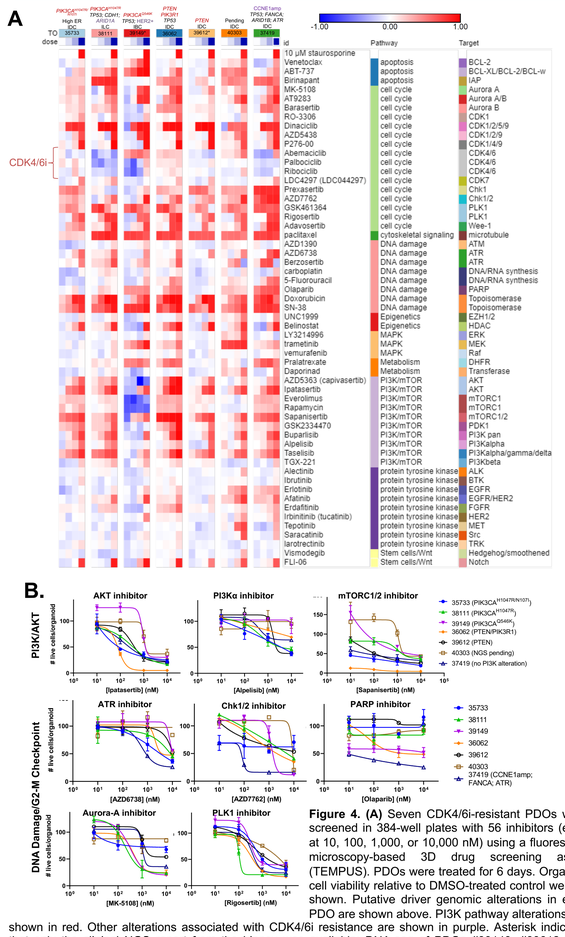

Figure 4. (A) Seven CDK4/6i-resistant PDOs were screened in 384-well plates with 56 inhibitors (each at 10, 100, 1,000, or 10,000 nM) using a fluorescent microscopy-based 3D drug screening assay (TEMPUS). PDOs were treated for 6 days. Organoid cell viability relative to DMSO-treated control wells is shown. Putative driver genomic alterations in each PDO are shown above. PI3K pathway alterations are shown in red. Other alterations associated with CDK4/6i resistance are shown in purple. Asterisk indicates that only the clinical NGS report from the biopsy was available; DNA-seq of PDOs #39149, #39612, and #40303 is in progress. **(B)** Dose-response curves of the organoid screen in (A) are shown.

CCNE1-amplified PDO is sensitive to CDK2/4/6 inhibitor

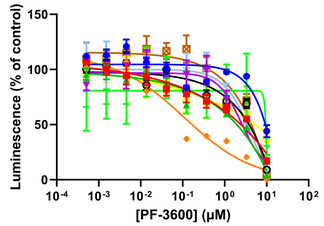

Figure 5. CDK4/6i-resistant PDOs (n=10) were treated with the indicated concentrations of the CDK2/4/6 inhibitor PF06873600 for six days. Cell viability of was measured using the 3D CellTiter-Glo reagent and normalized to untreated controls. Data points represent the mean of four replicates. IC50 values are shown in parentheses. PDO #37419 harbors a *CCNE1* (Cyclin E1) amplification.

Conclusions

- PDOs can be successfully established and cultured long-term from metastatic ER+ breast cancer biopsies.
- PDOs from patients progressing on CDK4/6i retain resistance in culture.
- Mutations in PDOs are concordant with clinical reports from biopsies and recapitulate alterations that have previously been associated with resistance to CDK4/6i and/or antiestrogens.
- CDK4/6i-resistant PDOs fail to suppress the E2F gene signature in palbociclib-treated organoids.
- CDK4/6i-resistant organoids are vulnerable to inhibitors of other cell cycle proteins and/or PI3K/AKT pathway inhibitors.
- **CDK4/6i-resistant PDOs represent a valuable model to understand and explore diverse mechanisms of drug resistance and therapeutic vulnerabilities.**

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