Development and characterization of patient-derived xenografts of HR+/HER2- breast cancer following progression on CDK4/6 inhibitor-based therapy

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Abstract

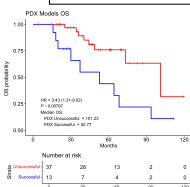
A limitation in the field of research around HR+/HER2- breast cancer is the lack of models that represent meaningful features of the disease. On the NCT04526587 clinical study, standard of care tumor tissues were collected to develop patient-derived xenografts (PDX) from patients that had progressed on CDK4/6 inhibitor-based therapy. As of September 2025, a total of 12 models have been developed. In general, there was a greater success in establishing models from solid tissue biopsies relative to liquid collections, such as ascites or effusion. The models expressed variable levels of the estrogen receptor and were molecularly characterized by whole-exome sequencing and gene expression analyses. The PDX models were highly represented by *ESR1* mutations as well as *PIK3CA* and other common genetic lesions in metastatic HR+/HER2- breast cancer. All models fell into the Luminal B or HER2 subtypes of breast cancer based on absolute assignment of breast cancer intrinsic subtypes. The subtype of each model remained relatively static with passaging in NSG mice supplemented with estrogen pellets. Assessment of therapeutic sensitivity illustrated a general resistance to CDK4/6 inhibitors relative to established xenograft models (e.g. MCF7 xenografts). However, combination treatment with CDK4/6 and CDK2 inhibitors was highly effective in halting proliferation. Combined treatment resulted in potent arrest and generated *in vivo* gene expression changes indicative of therapy-induced senescence. In select cases, PDX tumors failed to proliferate following the cessation of treatment, supporting the potency of this therapeutic regimen. Pilot studies have used cell culture of select PDX models to define unique mechanisms of resistance and vulnerabilities. Together, this work is providing new resources and insights upon which to combat therapeutic resistance in metastatic HR+/HER2- breast cancer.

Demographics and Clinical Characteristics

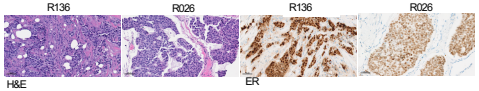
Patient Characteristics	Successful PDX Models (n=12)	Unsuccessful PDX Models (n=38)
CDK4/6i therapy at progression (%)		
Palbociclib	8 (66.67)	29 (76.32)
Ribociclib	2 (16.67)	7 (18.42)
Abemaciclib	2 (16.67)	2 (5.26)
First-line	9 (75.00)	33 (86.84)
Months PFS (median)	16.37	24.97
Endocrine therapy at progression (%)		
Aromatase inhibitor	11 (91.67)	30 (78.95)
Fulvestrant	1 (8.33)	8 (21.05)
Race (%)		
African American	3 (25.00)	4 (10.53)
Hispanic of Latino	1 (8.33)	0 (0.00)
Caucasian American	8 (66.67)	34 (89.47)
Menopause status		
Premenopausal	6 (50.00)	7 (18.42)
Menopausal	6 (50.00)	30 (78.95)
Male	0 (0.00)	1 (2.63)
Histologic diagnosis (%)		
Invasive ductal carcinoma	6 (50.00)	28 (73.68)
Invasive lobular carcinoma	3 (25.00)	6 (15.79)
Other	3 (25.00)	3 (7.89)
Unknown	0 (0.00)	1 (2.63)
Metastatic status at presentation (%)		
De novo	4 (33.33)	18 (47.37)
Recurrent	8 (66.67)	20 (52.63)
Metastatic sites (%)		
Non-visceral	8 (66.67)	20 (52.63)
Visceral	4 (33.33)	18 (47.37)

Overall Survival and Established Models



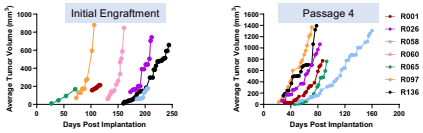


Model	Somatic Mutations	Germline Mutations	Pre-CDK Biopsy	PDX Source Biopsy	
R001	ESR1, PIK3CA, ATM, TP53, PTEN, KMT1A	Negative	breast	ER/PR+ HER2-	liver	ER/PR/HER2 -
R026	ESR1, ERBB2	Not tested	lung	ER/PR+ HER2-	bone/soft tissue	PR- ER/HER2 +
R058	BRCA2, APC, ESR1, TP53	Negative	lymph node	ER/PR+ HER2-	fluid	ER/PR + HER2-
R060	ESR1	Not tested	NA	no biopsy done	liver	ER/PR + HER2-
R065	SMMT3A, ESR1, PIK3CA, PTEN, TBX3, TP53	HOXB13	breast	ER/PR+ HER2-	lymph node	ER/PR/HER2 -
R097	PIK3CA, CDH1, CCND1, ARID1A , FGF19, FGF3, FGF4	Negative	bone	ER/PR+ HER2-	fluid	non-diagnostic fluid
R136	ESR1, PIK3CA, FGFR1, NSD3, TP53, CCND1, CDKN2A, CDKN2B, FGF19, FGF4, MTAP	Negative	breast	ER/PR+ HER2-	breast	ER/PR + HER2-
R274	PIK3CA, TP53	Negative	breast	ER/PR+ HER2-	liver	ER/PR/HER2 -
R290	PTEN, BRCA2, PIK3CA, TP53	Negative	breast	ER/PR+ HER2-	liver	ER/PR/HER2 -
R119	Not Tested	Negative	pleura	ER/PR+ HER2-	liver	ER/PR + HER2-
R243	PIK3CA, MAP3K1, RB1, TP53	Negative	bone	ER/PR+ HER2-	bone	ER/PR/HER2 -
R261	GATA3, STAT3, TP53	Negative	bone	ER+ PR/HER2-	liver	ER/PR/HER2 -



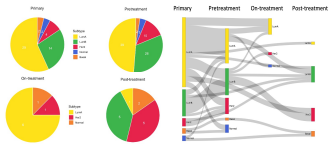
Overall survival, mutational status, and tumor histology from patient biopsies used to create PDX models.

Growth Characteristics



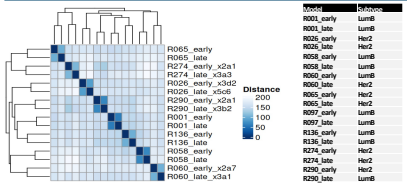
PDX tumor growth accelerated over time with subsequent passages.

Clinical Progression of Molecular Subtypes



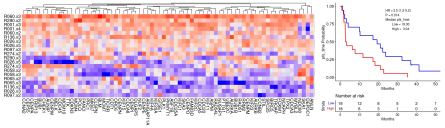
Clinical sample molecular subtype transitions from mostly LumA to LumB/Her2 post-therapy.

PDX Subtypes



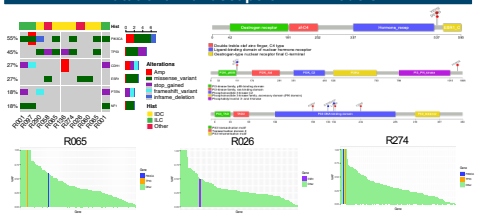
PDX subtype is predominantly conserved through subsequent passages.

Heterogeneity in Gene Expression Associated with PFS

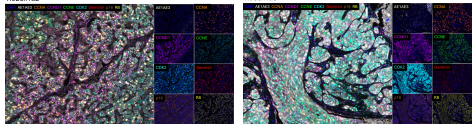


RB signature gene expression dichotomization associates with PFS.

Mutational Landscape of PDX Models

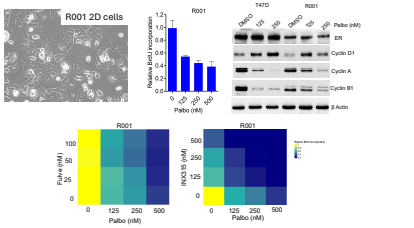


Multispectral Immunofluorescence

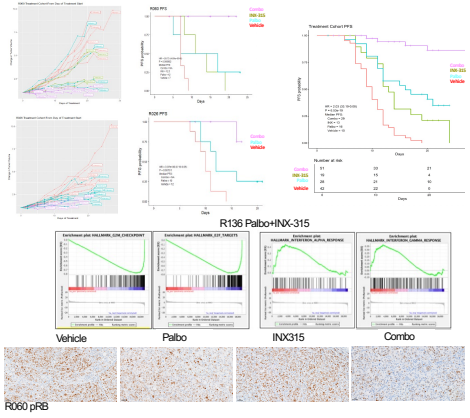


Cell cycle biomarker panel staining of PDXs can inform therapeutic response.

Cell Lines to interrogate combination therapies



Low dose CDK2 inhibitor rescues resistance *in vivo*



CDK2 plus CDK4/6 inhibitor treatment limits PDX tumor growth and improves PFS.

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