Short or long-term treatment with CDK4/6 inhibitors in patients with ER+ breast cancer: Characterization and comparative analysis of resistance in seventeen XPDX models

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ABSTRACT

Background: Mechanisms of resistance to CDK 4/6 inhibitors (CDK4/6i) have been well studied and several alterations identified including RB loss and altered expression of related genes such as CCNE1, E2F4 and CDK6. However, whether duration of clinical treatment might elicit specific mechanism(s) of CDK4/6i resistance is unclear. To better understand if duration of clinical treatment correlates with unique resistance mechanisms, we established, characterized, and compared a panel of ER+ breast XPDX models from patients who benefitted then progressed on a CDK4/6i. Patients were separated into two groups by time to progression (TTP): those who responded up to twelve months (RES12) and patients with clinical response greater than one year (RES13+).

Methods: Seventeen breast cancer XPDX models were analyzed, including eight previously described (7xRES12; 1xRES13+: SABCS2021: T Hernandez et al). Nine new models were established from seven patients: six from fluid samples with three designated as ductal (ST3105B, ST3105C, STM001B) and three lobular carcinoma (STM182, STM229, STM229B); two from lymph node biopsies (ST5676, STM127) and one from a liver core biopsy (ST4887B), all reported as ductal carcinoma. STM182 was classified as RES12 and the remaining eight as RES13+. These models were passaged and challenged with CDK4/6i to confirm resistance. Receptor expression was determined by IHC and genomic analyses including WES and RNAseq, were performed to identify mechanisms of resistance. For in vivo studies, CDK4/6i were dosed PO once daily at 50 mg/kg; endpoints included tumor volume (TV) and time from treatment initiation (TTI) with %T/C values and tumor regression reported at study completion; a %T/C of ≤20 versus control was considered sensitive. Tumor regression (%T/C<0) versus Day 0 TV was also reported.

Results: Clinical TTP for RES12 (n=8) was four to twelve months and RES13+ (n=9) from thirteen to forty-two months. All models retained ER expression in evaluated passages with similar histology compared with archival clinical samples. Sequencing identified several variants including RB1 truncations or deletions and increased gene expression in CCND1, CCNE1 and the PIK3CA/AKT pathway. Interestingly, 5/8 RES12 models reported ESR1 mutations or fusions versus 1/9 RES13+ and PIK3CA mutations were reported in 1/8 RES12 versus 5/9 RES13+. Several RES13+ models also reported variants and increased amplification in the RICTOR/TORC2 pathway versus RES12.

Conclusion: We have established, characterized, and compared a panel of seventeen breast XPDX models from fourteen female patients representing early or late acquired resistance to CDK4/6i therapy and identified potential differences in each set. These models and resulting data are useful in developing novel therapies for CDK4/6i-resistant patients.

MATERIALS AND METHODS

XPDX Model Establishment: Tissue samples were implanted into female athymic nude mice supplemented with estradiol and resulting tumors (designated P0) were serially passaged until model stabilization. Models were confirmed human using qPCR to GADPH and receptor expression (ER/PR/HER2) determined. At P2, hormone dependency was determined and models further developed using intact female athymic nudes in the presence or absence of exogenous estradiol. FFPE samples were collected at each passage and confirmed as similar to patient tissue using histological analysis.

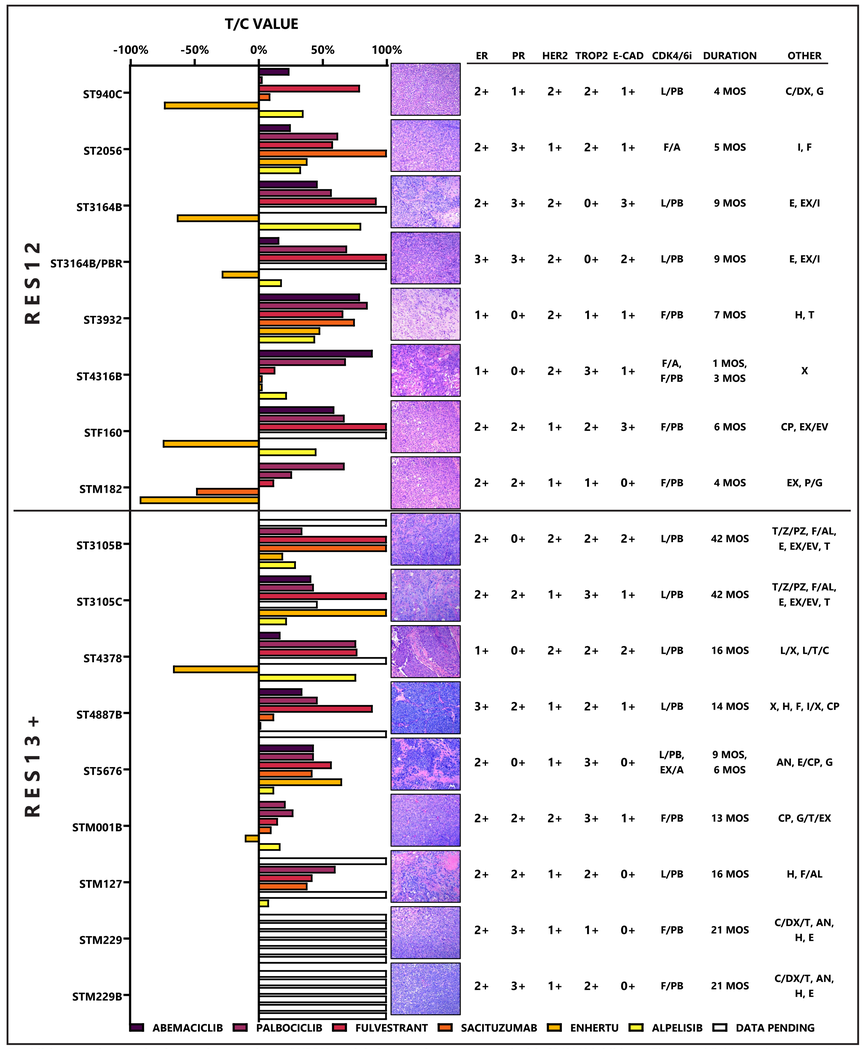
Immunohistochemistry (IHC): For protein expression, slides were cut from FFPE tissue blocks from patient or model samples and stained with hematoxylin and eosin (H&E) or the following commercial antibodies: ERα: Estrogen Receptor (SP1) α-rabbit mAb; PR: Progesterone Receptor (SP42) α-rabbit mAb; HER2: HER2/Neu (SP3) α-rabbit mAb; E-cadherin: (ab15148) α-rabbit mAb; Trop2: Trophoblast antigen 2 (EP431) α-rabbit mAb, using the Leica Bond III platform. Target expression was scored (0^+-3^+) by a board-certified pathologist at Precision Pathology Services (San Antonio, TX) using standardized protocols.

DNA/RNA Sequencing: Using an Illumina-based platform, DNA or RNA extracted from established models was subjected to WES at 100x depth or RNA sequenced at 50M. Secondary and tertiary analysis was performed by the XenoSTART Bioinformatics Group.

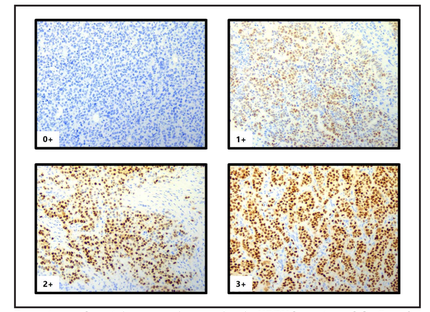
Test Agents: Pharmaceutical-grade fulvestrant, chemotherapy, antibody treatments, and research-grade small molecule agents were formulated according to internal protocols and utilized in all efficacy studies. Treatment regimens including route, dose, and schedule are available upon request.

In Vivo Efficacy Studies: All studies were IACUC approved. Tumor fragments from each model were harvested from host animals (P4+) and implanted subcutaneously (SC) into immune-deficient mice. Study animals were matched by tumor volume (TV) into control and treatment groups and dosing initiated at a mean tumor volume of 200-300mm^3. Data including tumor dimensions and animal weights was collected using a digital caliper and scale; tumor dimensions were converted to volume using the formula:
TV (mm^3) = width^2 (mm) x length (mm) x 0.52.
Each study was completed once mean control tumor volume reached designated tumor volume or completion date endpoint. %T/C values were calculated comparing ΔTV between treatment (T) and control (C) groups at study completion; a %T/C ≤20% versus control was considered sensitive. Tumor regression (%T/C <0%) is calculated versus Day 0 tumor volume for that treatment group.

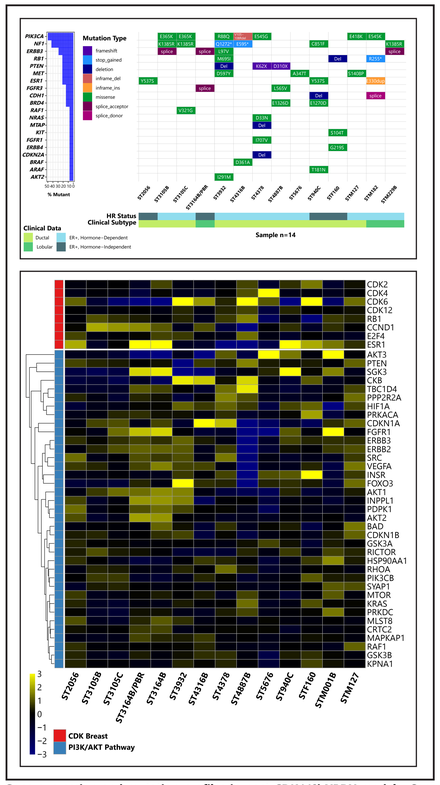
RESULTS



Establishment, characterization, and comparison of XPDX models from patients pretreated with CDK4/6 inhibitors: Tissue and fluid samples collected from patients post-treatment with CDK4/6i were implanted into female athymic nude mice supplemented with estradiol. Resulting models were notated with patient clinical histories and characterized by H&E, WES, RNA$_{Seq}$ and IHC and relevant drug sensitivities evaluated in vivo. [A=Abemaciclib, AL=Alpelisib, AN=Anastrozole, C=Cyclophosphamide, CP=Capecitabine, DX=Doxorubicin, E=Eribulin, EV=Everolimus, EX=Exemestane, F=Fulvestrant, G=Gemcitabine, H=Tamoxifen, I=Investigational, L=Letrozole, P=Platinum, PB=Palbociclib, PZ=Pertuzumab, T=Taxane, X=Radiation, Z=Trastuzumab]



Receptor and protein expression scoring in XPDX breast models: Samples from XPDX breast models were collected at tumor volume endpoint (P4+), embedded in paraffin, and stained using antibodies for ERα (c-term), PR, and HER2 receptors and Trop2 and E-cadherin proteins. Each stain was assigned a score of 0+, 1+, 2+, or 3+using signal intensity and proportion with each score represented above.



Gene expression and mutation profiles in post CDK4/6i XPDX models: Gene expression is log2 scaled with yellow indicating higher and blue lower relative gene expression; data is calculated from stranded RNAseq with gene expression normalized using trimmed mean of M-values (TMM) and gene expression values centered by gene to show relative gene expression between models. Mutation profiles of key genes are listed and varients colored according to mutation type. Exome sequencing of the XPDX models to a depth of >100X was followed by variant calling using MuTect and Varscan2 and annotation using VEP. CNV calling was done with CNVkit.

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Establishment and characterization of a panel of breast XPDX models representing innate or acquired resistance to trastuzumab deruxtecan (T-DXd)

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Abstract ID: 3855

ABSTRACT

Background: Trastuzumab deruxtecan (T-DXd) is an ADC consisting of an anti-HER2 antibody linked to a novel topoisomerase I inhibitor payload approved for T-DM1-refractory, HER2+ breast cancer (BC) and metastatic, recurrent HER2-low BC. While this therapy is effective in some patients, resistance often develops. To aid in developing new therapies for T-DXd-resistant BC and better understand potential resistance mechanisms, we established a panel of six breast XPDX models representing innate or acquired resistance to T-DXd. These models, designated ST4565C, ST4565D, STM148B, STM148C, STM148D, and ST4480B/EHR were developed and characterized for receptor expression, genomics, and drug sensitivities toward chemotherapies and targeted agents including T-DXd, T-DM1, and margetuximab.

Methods: ST4565C/D was established from a patient with ER+/HER2+, metastatic BC who was T-DXd treatment naïve. ST4565C was collected post chemo- and HER2-targeted therapies; ST4565D was collected following eribulin/margetuximab. STM148 models (B/C/D) were established from a patient with ER-/HER2+ metastatic BC following trastuzumab, T-DM1, and eleven months of T-DXd. ST4480B/EHR was established by chronic in vivo T-DXd dosing to the parent ST4480B ER+/HER2+ model until resistance developed. Resulting models were passaged and receptor expression confirmed by IHC and genomic analyses, including WES and RNAseq, were performed to further characterize models. In vivo, models were tested with agents including: trastuzumab, T-DM1, and T-DXd; endpoints included tumor volume (TV) and time from treatment initiation (TTI) with %T/C values and tumor regression reported at study completion; a %T/C of ≤ 20 versus control was considered sensitive. Tumor regression (%T/C=<0) versus Day 0 TV was also reported.

Results: Models retained receptor expression and similar histology comparable with archival clinical or model samples. In vivo, all ST4565 and STM148 models were refractory to trastuzumab, T-DM1, and T-DXd up to 10 mg/kg weekly with an average %T/C of 75%. ST4480B/EHR showed similar resistance to trastuzumab and T-DM1 at 10 mg/kg, and T-DXd at 3 mg/kg; T-DXd tested at 3 mg/kg in the ST4480B parent model resulted in tumor regressions. Sequencing of ST4565C/D identified a novel fusion, MTAP/CDKN2A/B deletions and elevated expression of FGFR1 and CCND1. In STM148 models an AKAP8L-NOTCH3 fusion and PIK3CA and CCNE1 mutations were reported. ST4480B/EHR reported PIK3CA^{E545K} and an ESR1-CCDC170 fusion identical to the parent model, but differential expression was noted in several genes in the RICTOR/TORC2 pathway.

Conclusion: We established and characterized six breast XPDX models representing innate or acquired resistance to T-DXd. These models are valuable tools in understanding resistance mechanisms and in developing novel therapies for T-DXd-resistant patients.

MATERIALS AND METHODS

XPDX Model Establishment: Tissue samples were implanted into female athymic nude mice supplemented with estradiol and resulting tumors (designated P0) were serially passaged until model stabilization. Models were confirmed human using qPCR to GADPH and receptor expression (ER/PR/HER2) determined. At P2, hormone dependency was determined and models further developed using intact female athymic nudes in the presence or absence of exogenous estradiol. FFPE samples were collected at each passage and confirmed as similar to patient tissue using histological analysis.

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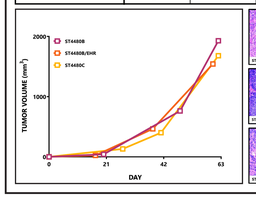
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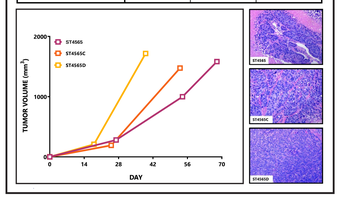
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RESULTS

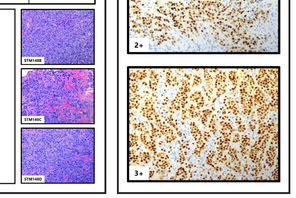
CLINICAL TREATMENT HISTORY			
THERAPY LINE	ST4480B	ST4480B/EHR	ST4480C
3RD PRIOR	T/PZ/Z	T/PZ/Z	T/PZ/Z
2ND PRIOR	TD	TD	TD
1ST PRIOR	CP/Z/TC	CP/Z/TC	CP/Z/TC
MODEL VARIANTS	SAMPLE COLLECTED		
ESR1:CCDC170 FUSION	X	X	X
PIK3CA^{E545K}	X	X	X
PIK3CA^{G1007R}	--	--	X
TP53^{E192*}	X	X	X
MODEL IHC	INTENSITY		
ER	2+	2+	2+
PR	2+	2+	3+
HER2	2+	2+	2+
TROP2	2+	2+	2+
E-CAD	2+	1+	3+

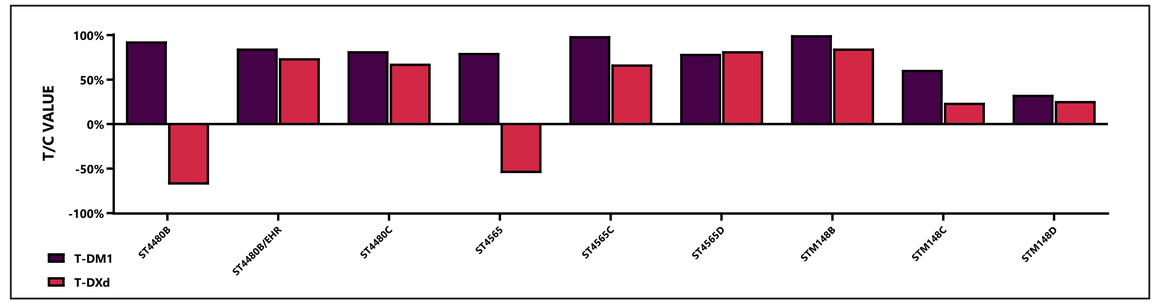


CLINICAL TREATMENT HISTORY			
THERAPY LINE	ST4565	ST4565C	ST4565D
3RD PRIOR	--	FU/DX/C	T/Z/PZ
2ND PRIOR	--	T/Z/PZ	TD/AN
1ST PRIOR	--	TD/AN	E/MG
VARIANTS	SAMPLE COLLECTED		
ERBB2^{IHH}	X	X	X
NRXN3-KDM6A fusion	X	X	X
IFT43-KDM5C fusion	X	X	X
MTAP/CDKN2A/CDKN2B loss	--	X	X
MODEL IHC	INTENSITY		
ER	2+	3+	2+
PR	3+	2+	0+
HER2	2+	1+	0+
TROP2	0+	2+	1+
E-CAD	2+	2+	1+

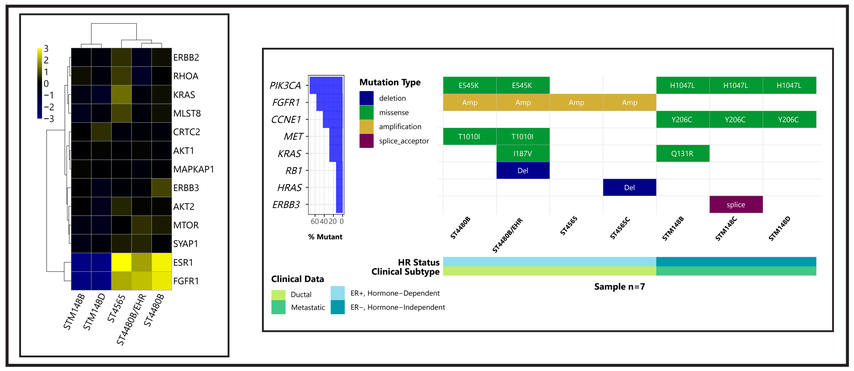


CLINICAL TREATMENT HISTORY			
THERAPY LINE	STM148B	STM148C	STM148D
3RD PRIOR	E/ZA	E/ZA	E/ZA
2ND PRIOR	L/ZA	L/ZA	L/ZA
1ST PRIOR	IX	IX	IX
VARIANTS	SAMPLE COLLECTED		
AKAP8-NOTCH fusion	X	X	X
CCNE1^{Y206C}	X	X	X
PIK3CA^{H1047L}	X	X	X
MLLT10^{F893L}	X	X	X
MODEL IHC	INTENSITY		
ER	0+	0+	0+
PR	2+	2+	1+
HER2	3+	2+	2+
TROP2	3+	3+	3+
E-CAD	1+	1+	2+





Establishment, characterization, and comparison of XPDX models from patients pretreated with T-DM1, T-DXd or conditioned in vivo: Tissue and fluid samples collected from patients pre- or post-treatment with T-DM1 or T-DXd were implanted into female athymic nude mice supplemented with estradiol. Resulting models were notated with patient clinical histories and characterized by H&E, WES, RNA$_{seq}$ and IHC and relevant drug sensitivities evaluated in vivo. [AN=Anastrozole, C=Cyclophosphamide, CP=Capecitabine, DX=Doxorubicin, E=Eribulin, FU=5-FU, IX=Ixabepilone, L=Letrozole, MG=Margetuximab, PZ=Pertuzumab, T=Taxane, TC=Tucatinib, TD=T-DM1, Z=Trastuzumab, ZA=T-DXd]. For receptor and protein staining, samples from XPDX breast models were collected at tumor volume endpoint (P4+), embedded in paraffin, and stained using antibodies for ERα (c-term), PR, and HER2 receptors and Trop2 and E-cadherin proteins. Each stain was assigned a score of 0+, 1+, 2+, or 3+using signal intensity and pro-portion with each score represented above.



Gene expression and mutation profiles in T-DM1/T-DXd XPDX models: Gene expression is log2 scaled with yellow indicating higher and blue lower relative gene expression; data is calculated from stranded RNAseq with gene expression nor-malized using trimmed mean of M-values (TMM) and gene expression values centered by gene to show relative gene expression between models. CRTC2 is upregulated in later stage model STM148D vs STM148B, and this had been linked to cancer progression through ID1 activation. Trastuzumab-resistant ST4480B (ST4480B/EHR) shows decreased expression of ESR1 and FGFR1, both known to be involved in HER2 signaling. Mutation profiles of key genes are listed and variants colored according to mutation type. Exome sequencing of the XPDX models to a depth of >100X was followed by variant calling using MuTect and Varscan2 and annotation using VEP. CNV calling was done with CNVkit. STM148B (H1047R) and ST4480B (E545K) have common PIK3CA gain-of-function driver mutations. The FGFR1 amplification observed is a common mechanism of trastuzumab resistance. The MET T1010I is a germline variant that predisposes individuals to metastatic breast cancer.

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