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A preclinical platform of breast cancer PDX and derived cellular models as a tool for pharmacological screening and functional studies

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Introduction

Despite considerable progress in understanding the biology and genetics of breast cancer, the development of effective therapies needs physiological and predictive preclinical models.
In this context, breast cancer (BC) patient-derived xenograft (PDX) models have become a standard tool as they reproduce the biology of tumors of origin, in term of histology, genotype and response to chemotherapy.

They have proven their relevance in the study of pathways leading to the development and progression of cancer, to the mechanisms linked to tumor resistance and to the identification of novel effective therapies. We present a preclinical platform of over 60 fully characterized BC PDX models and their *in vitro* cell derivatives for preclinical evaluation of new treatment modalities.

Our platform consists of a PDX collection of 41 TNBC, 8 ER+, 5 HER2+, 2 Luminal B models (ER+ HER2+) and 12 cellular models derived from these PDX, representing the variety of BC.

Materials & Methods

PDX model establishment and *in vivo* assays:
PDX models were obtained by transplantation of post-surgery tumor specimens either by grafting of tumor fragments in the interscapular region of nude mice or by injection of tumor cells into the fat pad of NOD-Scid mice. Molecular analyses were done included gene expression (RNASeq), copy number variation, whole exome sequencing and IHC markers staining. *In vivo* drug efficacy assays were performed with standards of care as single agent or in combinations. A/C 2/100 mpk IP qwk x2; PARPi (Olaparib 100 mpk PO qdx28; niraparib 50 mpk PO qdx28); Trastuzumab 10 mpk IP 2qwk x6; T-DM1 10 mpk q2wk x2; Fulvestrant 1 to 5 mg/dose qwk x8.
T400-OLA were obtained from T400 maintained under olaparib treatment until resistance.

Cellular model derivation and *in vitro* assay:
PDX-derived cell cultures were obtained from tumors explanted from mice and isolated by mechanical and enzymatic dissociation and maintained in culture at 37°C in a humidified atmosphere with 5% CO2 in complete growth medium.
Cells are maintained in culture in our XT Advanced medium (DMEM/F12 with 1% Penicillin-Streptomycin (10,000 U/mL), 2mM L-Glutamine, Insuline-Transferrin-Selenium 1X, Albumax II 0.4g/L and 8% FCS with or without Rho-associated kinase (ROCK) inhibitor, Y-27632 supplementation.
For ATP content measurement (CellTiter Glo® assay kit), cells were seeded in 96-well plates at a density of $1.25.10^3$ to 5.10^3 cells/well. Cells are incubated 48h at 37°C prior to the addition of T-DM1. Cell viability is assessed before drugs' addition (T0) and 5 days after test molecules addition.
For PARP inhibition assays, cells were seeded in 6-well plates at a density of 10^5 to 5.10^5 cells/well. Cells were incubated up to 3 days prior to addition of olaparib (10 µM) at D0. At D4, D11 and D18, the medium was renewed with olaparib renewal. At D0, D7, D14 and D21, cells were stained with crystal violet. Images of plates were acquired with a flatbed scanner before analysis. Quantification of the intensity/area was performed using ImageJ software and 'ColonyArea' Plugin (Camilo Guzmán et al. PLoS One. 2014 Mar 19;9(3)). Cell densities were compared to non-treated wells at each time points, with: TX : area value of treated well; NT: area value of non treated well and %AREA = 100xTX/NT (at D0, 7, 14, 21).

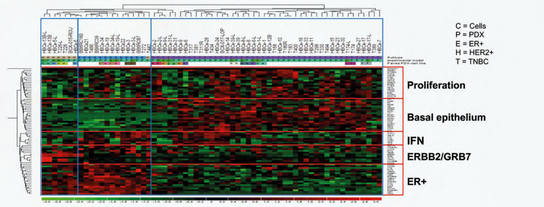
PDX engraftment on humanized mice:
Some of our BC PDX were engrafted on PBMC or CD34+ stem cells humanized mice.
For PBMC humanized mice, PDX were engrafted before injection of PBMC from 2 different donors (2.10^6 IP). For CD34+ humanized mice, 75.10^3 CD34+ cells were injected IV in NXG mice and 15 to 18 weeks later, PDX were engrafted only on mice presenting at least 25% of human cells in blood.
Tumor-Infiltrating Lymphocytes were purified from tumors using Tumor Dissociation Kit and CD45 (TIL) MicroBeads from Miltenyi. After antibodies staining, cells were acquired on a MACSQuant® Analyzer 10 Flow Cytometer and analyses were performed using FloLogic™ software.

Breast cancer-derived xenograft panel and derived cellular models

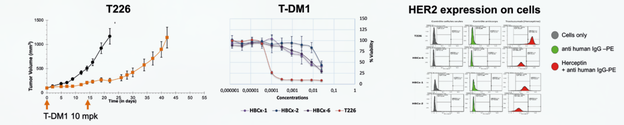
	Tumor patient Origin	Tumor patient sub type	PDX marker status	Key mutations	PARPi [1]	A/C [2]	Trastuzumab [3]	T-DM1	Fulvestrant	PDX-DC [4] available
HBCx-19	Peritoneal metastasis	ER+ / ILC	ER+	PIK3CA	NR	NR	/	/	NR	Yes
HBCx-3	Primary	ER+PR+HER2- / IDC	ER+	TP53; PTEN	NR	NR	NR	/	NR	Yes
T272	Primary	ER+PR+HER2- / IDC (IBC)	ER+	PIK3CA; ATM; PTEN	NR	R	/	/	R	No
BB6RC39	Primary	ER+PR+HER2+ / IDC	ER+PR+HER2-	BRCA2; ATM	R	/	/	/	R	No
HBCx-21	Primary	ER+PR+HER2- / DCIS	ER+PR+HER2-	ATM	NR	R	/	/	R	No
HBCx-22	Primary	ER+PR+HER2- / IDC	ER+PR+HER2-	PTEN; TP53; BRCA2; CDKN2A	R	R	/	/	R	No
HBCx-34	Primary	ER+PR+HER2- / IDC	ER+PR+HER2-	BRCA2;ATM	R	R	/	NR	R	No
T486	Primary	ER+PR+HER2+	ER+PR+HER2-	na	/	/	/	/	R	No
BB6RC87	Primary	ER+PR-HER2+ / IDC	ER+PR-HER2+		NR	/	/	/	NR	No
BCX-015-ROU	NA	ER+PR-HER2+ / IDC	ER+PR-HER2+	TP53; PIK3CA	/	/	NR	HR	NR	No
BB6RC160	Primary	ER+PR-HER2+ / IDC	HER2+	EGFR	/	/	/	/	NR	No
HBCx-13B	Axillary metastasis	HER2+ / IDC	HER2+	BRCA2; CDKN2A; TP53	NR	NR	R	R	/	No
HBCx-5	Primary	HER2+ / IDC	HER2+		NR	HR	NR	R	/	No
T226	Primary	HER2+ / IDC (IBC)	HER2+	TP53; ATM	NR	R	R	R	/	Yes
T442	Primary	HER2+	HER2+	TP53; PIK3CA; BRCA1; DNA-PK	/	/	R	R	/	No
BB6RC191	Primary	TNBC / IDC	TNBC	TP53	/	/	/	/	/	No
BB6RC52	Primary	TNBC / IDC	TNBC		/	/	/	/	/	No
BCX-017-LOP	NA	TNBC	TNBC	TP53; BRCA1; RB1	HR	HR	/	/	/	No
HBCx-1	Primary	TNBC / IDC	TNBC	TP53	NR	R	NR	/	/	Yes
HBCx-10	Primary	TNBC / IDC	TNBC	BRCA2; PTEN; RB1; TP53	HR	HR	/	NR	/	No
HBCx-11	Primary	TNBC / IDC	TNBC	TP53; BRCA1; MLL2;	R	R	NR	/	/	No
HBCx-12B	Axillary metastasis	TNBC / IDC	TNBC	TP53; NF1	NR	R	/	/	/	No
HBCx-14	Primary	TNBC / IDC	TNBC	TP53; HRAS; NOTCH1; RB1	HR	HR	/	/	/	No
HBCx-15	Primary	TNBC / IDC	TNBC	TP53	HR	HR	/	/	/	No
HBCx-16	Primary	TNBC / IDC	TNBC	TP53; PTEN	NR	NR	NR	/	/	No
HBCx-17	Primary	TNBC / IDC	TNBC	BRCA2; AKT1; CDKN2A; TP53	R	HR	/	/	/	Yes
HBCx-2	Axillary metastasis	TNBC / IDC	TNBC	TP53; RB1; AKT1	NR	NR	/	/	/	Yes
HBCx-23	Primary	TNBC / IDC	TNBC	TP53; CDKN2A	R	R	/	/	/	No
HBCx-24	Primary	TNBC / IDC	TNBC	TP53	NR	NR	/	/	/	No
HBCx-27	Primary	TNBC / IDC	TNBC	TP53; CDKN2A	NR	R	/	/	/	No
HBCx-28	Primary	TNBC / IDC	TNBC	TP53; BRCA1; PTEN	NR	R	/	/	/	No
HBCx-30	Primary	TNBC / IDC	TNBC	TP53; CDKN2A; PTEN	NR	NR	/	/	/	No
HBCx-31-L1	Primary	TNBC / IDC	TNBC	TP53; AKT1	/	/	/	/	/	Yes
HBCx-33	Primary	TNBC / IDC	TNBC	TP53	NR	HR	/	/	/	No
HBCx-39	Primary	TNBC / IDC	TNBC	TP53	NR	R	/	/	/	Yes
HBCx-6	Primary	TNBC / IDC	TNBC	NF1; RB1; TP53	HR	HR	/	/	/	Yes
HBCx-8	Primary	TNBC / IDC	TNBC	BRCA1; NRAS; TP53	NR	HR	/	/	/	Yes
HBCx-9	Primary	TNBC / IDC	TNBC	ATM; CDH1; TP53	NR	NR	NR	/	/	Yes
PDR01	Metastasis	TNBC	TNBC		/	/	/	/	/	No
PDR06	Recurrence	TNBC	TNBC	TP53	/	/	/	/	/	No
PDR07	Metastasis	TNBC	TNBC		/	/	/	/	/	No
T168	Primary	ER+PR+HER2- / IDC (IBC)	TNBC	TP53; BRCA1; DNA-PK	HR	HR	/	/	NR	No
T174	Primary	TNBC / IDC (IBC)	TNBC	TP53; PIK3CA; CDKN2A	NR	NR	/	/	/	Yes
T180R	Primary	TNBC / IDC	TNBC	TP53	NR	HR	/	/	/	No
T183	Primary	TNBC / IDC	TNBC	TP53	/	/	/	/	/	No
T298	Primary	TNBC / IDC	TNBC	PALB2; NOTCH1; PIK3CA	HR	HR	/	/	/	No
T317	Primary	TNBC	TNBC	PTEN; TP53; ATM	/	/	/	/	/	No
T330	Primary	TNBC / IDC	TNBC	TP53; BRCA1; NRAS	HR	HR	/	/	/	No
T381	Primary	TNBC	TNBC	TP53	NR	HR	/	/	/	No
T389	Primary	TNBC	TNBC	KRAS (G12C)	NR	NR	/	/	/	No
T392	Primary	TNBC	TNBC	TP53, BRAF, PIK3CA	/	/	/	/	/	No
T400	Primary	TNBC	TNBC	TP53; BRCA1	HR	HR	/	/	/	No
T412	Primary	TNBC	TNBC	TP53	/	/	/	/	/	No
T434	Primary	TNBC	TNBC	TP53	NR	/	/	/	/	No
T491	Primary	TNBC	TNBC		/	/	/	/	/	No
T494	Primary	TNBC	TNBC	PTEN; TP53; ATM	/	/	/	/	/	No

1. Olaparib or niraparib · 2. Adriamycin/Cyclophosphamide · 3. on SCID or SHO mice - no response on nude mice · 4. PDX-derived cells · 5. Tumor Growth Inhibition
NR Non Responder (TGI[5] < 58%) R Responder (90% > TGI[5] > 58%) HR High Responder (TGI[5] > 90%)

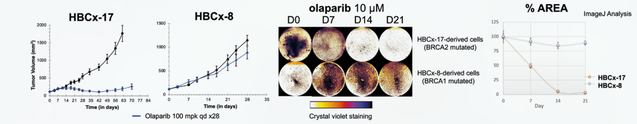
I Molecular profiling of breast cancer PDX and derived cellular models



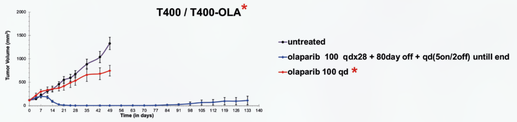
II T-DM1 response in HER2+ T226 PDX model and derived cellular model



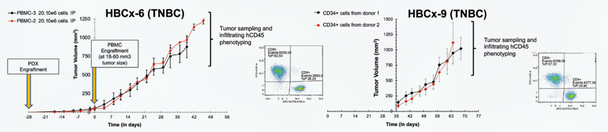
III Olaparib responses in BRCA1/2 mutated TNBC PDX and PDX-DC models



IV Obtention of a resistant model to olaparib from a very sensitive one



V Engraftment of TNBC PDX on humanized mice and TILs phenotyping



Results

1. Hierarchical clustering was performed by using a gene list that stratifies breast cancer samples according to their intrinsic molecular phenotype. This analysis showed that breast cancer PDX and PDX-DC are properly classified within the molecular subtypes corresponding to their histopathological features. Cellular models conserve gene expression feature of parental PDX (Fig I).
2. The HER2+ T226 model was the only cellular model sensitive to T-DM1 *in vitro*. This sensitivity was correlated with a high expression level of HER2, assessed by Herceptin/trastuzumab staining. *In vivo*, T226 responds to T-DM1, like all our HER2+ models (Fig. II).
3. Our TNBC PDX panel is very well characterized for the response to PARPi. *in vitro*, TNBC PDX-derived cells treated with olaparib presented a good correlation with *in vivo* sensitivity : cells derived from *in vivo* responder PDX model HBCx-17 presented an E50 (the day for which a 50% effect is observed - at day 6.62) (Fig. III).
4. To study olaparib resistance, mice bearing T400, a model very sensitive to olaparib, were treated with olaparib until obtention of growing tumors under treatment (Fig. IV). After re-engraftment, the acquired resistant status of T400-OLA was demonstrated.
5. TNBC PDX models (HBCx-6 and HBCx-9) are able to grow on humanized mice. The growing tumors are infiltrated by human cells, assessed by TILs purification and phenotyping.

Conclusion & Perspectives

Our PDX panel mostly reflects the molecular heterogeneity of breast cancer and reproduce accurately the molecular and drug response profile of human tumors. In addition to these PDX panel, we derived cellular models (PDX-DC) to offer a time- and cost-effective preclinical screening tool with good correlation with *in vivo* responses.

Engrafted on highly immunodeficient mice humanized with human PBMCs or CD34+ cells, these PDX models should facilitate bispecific T-Cell engager antibody testing or immune-checkpoint inhibitors evaluation.
This BC PDX panel and *in vitro* cell derivatives provide a powerful preclinical platform to improve our knowledge on BC biology and to rapidly evaluate response to new treatments and translate this knowledge to the clinic.