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A large collection of integrated genomically characterized patient-derived xenografts highlighting the heterogeneity of triple-negative breast cancer

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Triple-negative breast cancer (TNBC) represents 10% of all breast cancers and is a very heterogeneous disease. Globally, women with TNBC have a poor prognosis, and the development of effective targeted therapies remains a real challenge. Patient-derived xenografts (PDX) are clinically relevant models that have emerged as important tools for the analysis of drug activity and predictive biomarker discovery. The purpose of this work was to analyze the molecular heterogeneity of a large panel of TNBC PDX ($n = 61$) in order to test targeted therapies and identify biomarkers of response. At the gene expression level, TNBC PDX represent all of the various TNBC subtypes identified by the Lehmann classification except for immunomodulatory subtype, which is underrepresented in PDX. NGS and copy number data showed a similar diversity of significantly mutated gene and somatic copy number alteration in PDX and the Cancer Genome Atlas TNBC patients. The genes most commonly altered were *TP53* and oncogenes and tumor suppressors of the PI3K/AKT/mTOR and MAPK pathways. PDX showed similar morphology and immunohistochemistry markers to those of the original tumors. Efficacy experiments with PI3K

Key words: triple-negative breast cancer, targeted therapies, patient-derived xenograft, integrated genomic analysis.

Abbreviations: BL1: Basal-Like 1; BL2: Basal-Like2; CGH: comparative genomic hybridization; CK: cytokeratin; ER: estrogen receptor; ERBB2: human epidermal growth factor receptor 2; HR: hormonal receptors; IHC: immunohistochemistry; IM: immunomodulatory; LAR: luminal androgen receptor; LST: large-scale state transitions; M: mesenchymal; METABRIC: Molecular Taxonomy of Breast Cancer International Consortium; MFS: metastasis-free survival; MSL: mesenchymal stem-like; NGS: next-generation sequencing; PDX: patient-derived xenografts; PR: progesterone receptor; RTV: relative tumor volume; SCNA: somatic copy number alteration; SMG: significantly mutated gene; TBP: Tata box binding protein; TCGA: The Cancer Genome Atlas; TGI: Tumor growth inhibition; TNBC: Triple-negative breast cancer; UNS: Unclassified

Additional Supporting Information may be found in the online version of this article.

*I.B. and E.M. contributed equally to this work

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and MAPK inhibitor monotherapy or combination therapy showed an antitumor activity in PDX carrying genomic mutations of *PIK3CA* and *NRAS* genes. TNBC PDX reproduce the molecular heterogeneity of TNBC patients. This large collection of PDX is a clinically relevant platform for drug testing, biomarker discovery and translational research.

What's new?

Triple-negative breast cancer (TNBC) is a highly heterogeneous disease in terms of molecular profile, histological features, clinical behavior, and drug response. Several clinical trials have been conducted for targeted therapies, but only in unselected TNBC patients, with disappointing results. This study shows that patient-derived xenografts (PDX) reproduce the molecular heterogeneity of TNBC. The presented genomic analysis identifies several interesting targetable drivers, particularly in the PI3K and MAPK pathways. PDX models provide a unique opportunity to test various treatments on individual tumors: already, two specific inhibitors (dual PI3K/mTOR and MEK inhibitor) and their combination are providing encouraging results.

Introduction

Triple-negative breast cancer (TNBC) represents approximately 10% of all breast cancers.¹ This disease is routinely defined by lack of expression of estrogen receptor (ER), progesterone receptor (PR) and no overexpression of human epidermal growth factor receptor 2 (ERBB2).² Globally, women with TNBC have a poor prognosis, particularly due to the absence of targeted therapy.³ TNBC is a highly heterogeneous disease in terms of histological features, clinical behavior, drug response and molecular profile.⁴ Various studies have addressed the heterogeneity of TNBC in terms of gene expression.^{5–7} Lehman *et al.* identified six classes of TNBC (BL1, basal-like 1; BL2, basal-like2; IM, immunomodulatory; M, mesenchymal; MSL, mesenchymal stem-like; and LAR, luminal androgen receptor) characterized by alterations of specific pathways.⁶ Chemotherapy is the main systemic treatment for TNBC, but fewer than 30% of patients with metastatic disease survive more than 5 years after the diagnosis and TNBC remains associated with a poor prognosis. The identification of molecular alterations in TNBC is essential in order to develop new biomarkers and to identify therapeutic targets for this type of breast cancer. In addition to *TP53* alterations, TNBC also displays heterogeneity in terms of somatic genomic alterations, as described in large cohorts of breast cancer (Molecular Taxonomy of Breast Cancer International Consortium [METABRIC] and The Cancer Genome Atlas [TCGA]).^{8,9} Nevertheless, some interesting targetable drivers have been described in terms of significantly mutated genes (SMG) or somatic copy number alteration (SCNA) analysis profiles, particularly genes of the PI3K and MAPK pathways. TNBC heterogeneity is not only confined to genomic features but also concerns morphologic and immunohistochemical characteristics.^{10,11} Several clinical trials have been conducted on targeted therapies in TNBC, but only in unselected TNBC patients, with disappointing results.

This marked heterogeneity of TNBC probably represents the main barrier for the implementation of personalized care and the development of new targeted therapies. There is a

need for preclinical models that faithfully represent this heterogeneity. Patient-derived xenografts (PDXs) models are robust preclinical models to test targeted therapies because they conserve each patient's molecular heterogeneity¹² and response to treatment.¹³ These models have emerged as an important translational research tool to identify new treatments and predictive biomarkers.^{14,15} PDX models provide the unique opportunity to test and compare different treatment and combinations of treatments on a patient's tumor without the limitations of clinical trials. We previously showed, in a small series of TNBC PDX, morphological and genomic fidelity between PDXs and matched patient's tumor and possibility to identify efficient treatment and predictive biomarkers.¹⁶

In the present study, we describe a large cohort of clinically annotated TNBC PDX with genomic, transcriptomic and immunohistochemical data in order to test targeted therapies adapted to specific tumor alterations.

Materials and Methods

Patients

Sixty-one PDXs were obtained from the engraftment of 150 early-stage triple-negative breast tumors or axillary lymph node metastases excised from patients between 2000 and 2017 with their informed consent.^{17,18} Forty of the 61 established TNBC PDXs were obtained from primary breast cancer, 19 were obtained from residual disease after neoadjuvant chemotherapy and 2 were obtained from primary axillary nodes (Supporting Information Tables S1 and S2).

Forty-four PDX from other subtypes of breast cancer observed at Institut Curie were also analyzed: 32 tumors with expression of hormonal receptors (HR; ER+ and/or PR+) and no amplification of HER2 (HER2–), five HR-positive (ER+ and/or PR+) tumors with amplification of HER2 (HER2+) and seven HR-negative (ER– and PR–) tumors with amplification of HER2 (HER2+). The characteristics of all tumors are detailed in Supporting Information Table S3.

Xenograft

Sixty-one triple-negative tumors and 44 HR+ and/or HER2+ specimens were obtained from BC patients with their informed consent. Tumor fragments were removed during surgery and grafted into the interscapular fat pad of female Swiss nude mice under anesthesia, as previously described.¹⁸ The experimental protocol and animal housing were in accordance with institutional guidelines as proposed by the French Ethics Committee (Agreement B75-05-18, France). Two models with specific genomic alterations were chosen for *in vivo* preclinical assays: HBCx92 mice (with *NRAS* mutation) were treated with selumetinib (50 mg/kg, bid) (MedChem Express®, Monmouth Junction, NJ), five times a week for 25 days. HBCx4B mice (with *PIK3CA* and *NF1* mutation) were treated (i) with PF-04691502 (MedChem Express®, 10 mg/kg, once daily), five times a week, for 25 days and (ii) with a combination of oral PF-04691502 (MedChem Express®, 10 mg/kg, once daily), five times a week and selumetinib (50 mg/kg, bid; MedChem Express®), five times a week for 25 days.

Tumor growth was evaluated by measuring two perpendicular tumor diameters with calipers twice a week. Individual tumor volumes were calculated: $V = a \times b^2/2$, where “a” is the largest diameter, “b” is the smallest diameter. For each tumor, volumes were expressed in relation to the initial volume as relative tumor volume (RTV). Tumor growth inhibition (TGI) of treated tumors vs. controls was calculated as the ratio of the mean RTV in the treated group to the mean RTV in the control group at the same time. Statistical significance of TGI was calculated by a paired Student’s *t* test by comparing tumor volumes in the treated and control groups.

Transcriptomic data analysis

Transcriptomic profiling of 61 TNBC PDX was performed by gene expression arrays. The concentration and integrity/purity of each RNA sample were measured using RNA 6000 LabChip kit (Agilent) and the Agilent 2100 bioanalyzer. GeneChip Human 1.1 ST arrays were hybridized according to Affymetrix recommendations using WT Expression Kit protocol (Life Technologies, Carlsbad, CA) and Affymetrix labeling and hybridization kits. Arrays were normalized according to the RMA normalization procedure using the Oligo package.¹⁹ No additional human–mouse cross-hybridization filtering were applied as our xenografts samples contains less than 5% of mouse cells (percentage determined using RT-PCR for quantification of transcripts of the ubiquitous expressed *TBP* gene with specific mouse and human primers pairs) and do not affect expression profiles provided by HuGene1.0 arrays.²⁰ The TNBC molecular subtypes of PDX were determined on the basis of gene expression data with TNBCtype software developed by Chen *et al.*²¹

Somatic mutation analysis

A total of 61 triple-negative PDXs were analyzed by targeted NGS of 95 genes, chosen among the most frequently mutated

genes in breast cancer (>1%) and including potential therapeutic targets (Supporting Information Table S4). NGS primers were specifically designed on human genome reference (<1% of the total read are common with mice). NGS was performed on an Illumina HiSeq2500 sequencer and the genomic variants were annotated with COSMIC and 1000 genome databases.²² Reads were aligned using BWA allowing up to 4% of mismatches with the reference. Only those reads with a mapping quality higher than 20 were used for variant calling, performed with GATK UnifiedGenotyper. Genomic alterations included single nucleotide variations of SMGs (i.e., base substitutions and short insertions/deletions).²³ Deleterious genomic alterations were defined as follows: (i) for oncogenes, only mutations driving to gain of function were considered (i.e., hotspots missense mutations, in-frame insertions/deletions/splicing described as oncogenic), (ii) for tumor suppressor genes (TSG), only mutations driving to loss of function were considered (i.e., biallelic truncating alterations (nonsense mutations, frameshift insertions/deletions/splicing) or monoallelic truncating alterations associated with heterozygous deletion detected by copy number analysis). Variants with low allelic frequency (<5%) or low coverage (<100×) were excluded from the analysis. No patient’s control (for constitutional analysis) was used as we reported only pathological variants, validated in literature or public database.

Genomic variants were biologically validated by comparison with COSMIC, TumorPortal and cBioportal databases (7, 15).

Somatic copy number alteration (SCNA) analysis

PDX were profiled using Affymetrix genomics array: 24 with SNP 6.0 and 37 with Cytoscan HD array. Genome-wide copy number analysis was conducted by means of Affymetrix SNP arrays, as previously described.^{24,25} SNP 6.0 or Cytoscan HD was processed with 500 and 250 ng of gDNA as starting material, respectively, as recommended by the supplier. Raw data were normalized with Genotyping console (SNP6.0 arrays) or Chromosome Analysis Suite (Cytoscan HD arrays). Focal amplification of oncogenes was defined by log ratio >1.58 (six copies per diploid genome) and maximum size <10 megabases. Biallelic inactivation of TSGs was defined by homozygous deletion or truncating mutation associated with heterozygous deletion. Copy number alterations were compared to cBioPortal data for TCGA breast cancer.^{26,27} All PDX copy numbers were represented by the circular binary segmentation algorithm²⁸ as implemented in the DNACopy package for R using a minimum width of 3, alpha less than 0.01 and up to 10,000 permutations. Downstream analysis of the sample population was performed with GISTIC2.0²⁹ based on default settings. Comparative genomic hybridization (CGH) explorer and CGHcall were used for visual representation of the results and figures.³⁰ The BRCAness signature was defined with large-scale state transitions (LST), defined as chromosomal break between adjacent regions of at least 10 Mb initially described by T. Popova with Gap methodology.³¹

Immunohistochemistry

Immunohistochemistry of PDX. Twenty-eight of the 61 TNBC PDXs were fixed in 10% neutral buffered formalin, paraffin embedded and hematoxylin–eosin stained. TNBC PDXs were included in TMA in duplicate. Two cores were picked from each tumor paraffin block using Tissue-Tek Quick-Ray System from Sakura and 6 × 10 matrix of 2 mm core recipient block; 4 μm TMA sections were adhered to Superfrost Plus slides (MICROM, Walldorf, Germany). Rabbit CK5 (Abcam, Cambridge, UK; ab52635, 1/400), CK8/18 (cloneE431-1, Invitrogen, Carlsbad, CA, 1/100), CK14 (Abcam, ab198167, 1/100), EGFR (Cell Signaling, #4267, 1/100) were used. Parallel-stained slides with preimmune rabbit IgG were used as negative controls. Incubation and revelation using the streptavidin–biotin–peroxidase complex method with DAB as substrate were performed in a Ventana Medical System (ROCHE, Tucson, AZ) DXT automat.

Immunohistochemistry of patients. Twenty-eight TN breast tumors corresponding to PDX were available for the comparison with PDX's tumors. Immunostaining was performed according to previously published protocols.³² Briefly, 4 μm tissue sections were prepared from a representative sample of the tumor. After rehydration and antigen retrieval in citrate buffer (10 mM, pH 6.1), tissue sections were stained for CK5 (BioSB, Santa Barbara, CA; BSB6602, 1/100), CK8/18 (BioSB, BSB5419, 1/400), CK14 (Novocastra, Buffalo Grove, IL, NCL-L-LL002, 1/100), EGFR (Invitrogen, 28–005, 1/200). Staining was revealed with the Vectastain Elite ABC peroxidase mouse Immunoglobulin G kit (Vector, Burlingame, CA) using diaminobenzidine (Dako A/S, Glostrup, Denmark) as chromogen.

Morphology and immunohistochemistry (IHC) of both patient and PDX tumors were compared by the same histologist (ML). The percentage of positive tumors was scored by the percentage of cells showing positive staining and staining intensity was scored on a scale from 0 to 3 (0 = no specific staining; + = weak; ++ = moderate; +++ = strong). IHC defined basal-like phenotype with positivity for basal cytokeratin (CK5 and/or CK14) and/or the presence of EGFR marker. Basal CK are absent and luminal cytokeratin (CK18) is positive in androgen or apocrine subtype.³³

Statistical analysis

Metastasis-free survival (MFS) was determined as the interval between diagnosis and detection of the first distant metastasis. Survival distribution was estimated with the Kaplan–Meier method. The proportion of transcriptomal TNBC subgroup was compared to a χ^2 test. The proportion of genomic alterations between PDX and TCGA was compared to a χ^2 test or Fisher's exact test, as appropriate.

Results

Clinicopathological characteristics of TNBC patients

The clinical and pathological characteristics of the patients corresponding to the established TNBC PDXs were analyzed

to determine whether they reflected the heterogeneity of TNBC. This cohort of 61 patients presented the usual characteristics of TNBC³⁴ (Supporting Information Tables S1 and S2). Mean age at diagnosis was 56 years. TNM staging mostly corresponded to T2 (59.4%), N0 (61.7%) with a small percentage of synchronous metastasis (10%). Fifty-seven percent of patients were treated by mastectomy and 79.7% by lymphadenectomy. This proportion can be explained by the extended period of PDX establishment (2000–2017). In 2000s, primary chemotherapy and node biopsy were performed less frequently. Histologically, 90% of tumors were invasive ductal carcinoma of no special type (NST). Six tumors were characterized by a rare histology: one micropapillary, one apocrine and four with metaplastic differentiation (squamous, fusiform or chondroid). One-third (32.7%) of tumors presented emboli and a majority had a high-SBR histological grade. The majority of patients received sequential chemotherapy (anthracycline then taxane) as adjuvant or neoadjuvant therapy. All patients who underwent tumorectomy also received adjuvant radiotherapy. Some patients treated by mastectomy received radiotherapy according to the Institut Curie guidelines. Five-year metastasis-free survival (MFS) was 61% in this TNBC cohort (data not shown).

Gene expression analysis and Lehmann classification

To analyze TNBC heterogeneity in terms of gene expression, we generated transcriptomic profiles by gene expression arrays and classified the PDX models among the various TNBC molecular subtypes, as defined by Lehmann *et al.*,⁶ using the TNBCtype tool.²¹ The distribution of the various subtypes in PDX was then compared to the TNBC classification based on the TCGA and METABRIC cohorts^{8,9} (Fig. 1). The frequencies of the various TNBC subtypes were similar except for IM and BL1 subtypes. IM subtype was underrepresented in this cohort of TNBC PDX: 3.5% vs. 18 and 20% in the TCGA and METABRIC cohorts, respectively ($p < 0.005$). BL1 subtype was overrepresented in this series of PDX: 31% vs. 17 and 19% in the TCGA and METABRIC cohorts, respectively ($p = 0.02$ and 0.05, respectively).

Histopathologic and immunophenotypic characterization

The morphology and IHC profile of 28 paired tumors were analyzed in order to compare the histology of PDX and patient tumors. Microscopic examination of hematoxylin and eosin (H&E) stained histological sections demonstrated that the original tumor characteristics were preserved in the PDX. Mouse xenografts displayed strong histological similarity with the clinical specimens including tumor cellularity, histopronostic grade (tubule and gland formation, nuclear pleomorphism and mitotic counts; Fig. 2). Xenograft tumors also retained the histopathological features of the original patient tumors in particular for rare subtypes such as metaplastic breast cancer (e.g., squamous cell carcinoma or metaplastic carcinoma with chondroid differentiation), indicating the same pattern of differentiation capacity (Fig. 3). Immunohistochemistry with

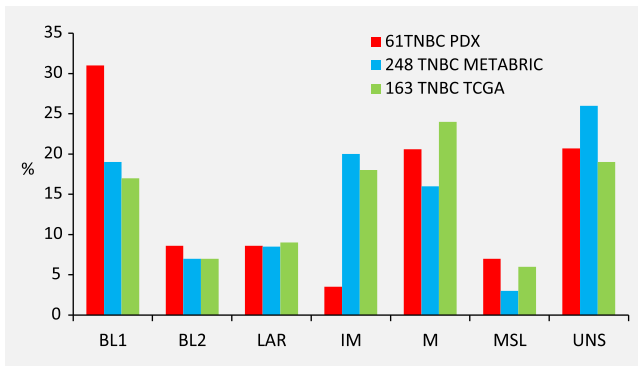


Figure 1. Repartition of the Lehmann subtype (%) in our 61 TNBC PDX and TNBC of TCGA and METABRIC cohort. Abbreviations: BL1, basal-like 1; BL2, basal-like2; IM, immunomodulatory; M, mesenchymal; MSL, mesenchymal stem like; LAR, luminal androgen receptor; UNS, unclassified.

epithelial markers (CK5, CK14 and CK8/18) and a specific biomarker (EGFR) was used to define a basal-like phenotype (Fig. 2 and Table S5). The majority of TNBC PDX (92%) presented a basal-like phenotype (EGFR and/or CK5 and/or CK14 positive). As expected, HBCx2, a LAR subtype tumor, expressed no basal cytokeratin but the luminal CK8/18.

Genomic alteration

The genomes of 61 TNBC PDX were analyzed by targeted NGS of 95 genes and CGH arrays. Data of SMGs and SCNAs are detailed in Figure 4. Details of the 65 SMGs are described in Table S6. As expected, the majority of TNBC PDX presented an alteration of the *TP53* gene (50.8%; 82.1% for the TCGA and 82.7% for the METABRIC cohort). Homozygous deletion of *CDKN2A* (9.8%) or *RBI* (4.9%) were the other most common cell cycle anomalies (in our targeted analysis). Receptor Tyrosine Kinase (RTK), PI3K and MAPK pathways were altered in 42.6, 40.9 and 32.7% of TNBC PDX, respectively. Some SMGs

or SCNAs with possible targeted therapies were present in this cohort. *PIK3CA* was the second most frequently altered gene in the PI3K pathway (18% including 10% of mutations and 8% of focal amplifications). Other effectors of this pathway were also altered: *AKT1* (4.9% of activating mutations and 4.9% of focal amplifications) and *PTEN* (11.5% of biallelic inactivations). This pathway was more frequently altered in BL2 (100%), LAR (80%) and MSL (75%) subtypes. Focal amplifications of *FGFR1* (8.1%), *FGFR4* (8.1%) and *IGF1R* (6.5%) were the three major oncogenes altered in the RTK pathway. Focal amplifications of *KRAS* (13.1%), *BRAF* (6.6%) and inactivating mutations of *NFI* (4.9%) were observed in the MAPK pathway. *BRCA1* (13.1%) and *BRCA2* (3.3%) mutations were the most common alterations in the DNA repair pathway. All 10 patients *BRCA1* or *BRCA2* mutated tumors presented a BRCAness signature as identified by CGH profiles. Comparison with TNBC tumors of the TCGA database (details in Supporting Information Table S7) showed a similar distribution of the major genomic alteration for SCNAs and SMGs. However, some RTK or MAPK pathway alterations were more frequent in PDX among which some are potentially actionable, such as *KRAS*, *BRAF* and *FGFR4* ($p < 0.05$) focal amplification (Fig. 5a and 5b). Focal amplification of *DDR2*, a collagen receptor kinase was also overrepresented (but not significantly) in PDX (16.4% vs. 6.7% in TCGA, $p = 0.06$). The global copy number profile of this cohort (Fig. 5c) highlights the classical characteristic of TNBC: 8q24 (*MYC*) focal amplification (>50% in our cohort), 1q and 10p gain, 8p and 5q losses. Eighty-eight percent of PDX models had an actionable alteration and 54% presented an association of at least two actionable alterations, particularly in the PI3K and MAPK pathways with possible targeted therapies combination (Supporting Information Table S8). SMG analysis of PDX included 44 ER+ and/or HER2+ PDX breast cancers, established by our group since 2003. The

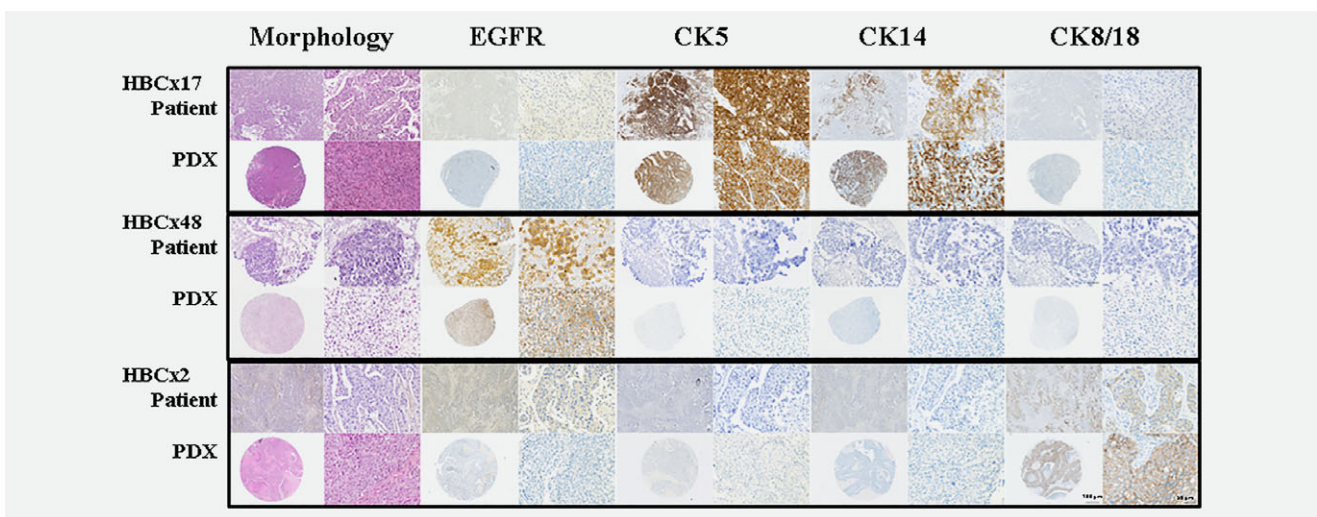


Figure 2. Comparison of morphology and IHC between patient and PDX.

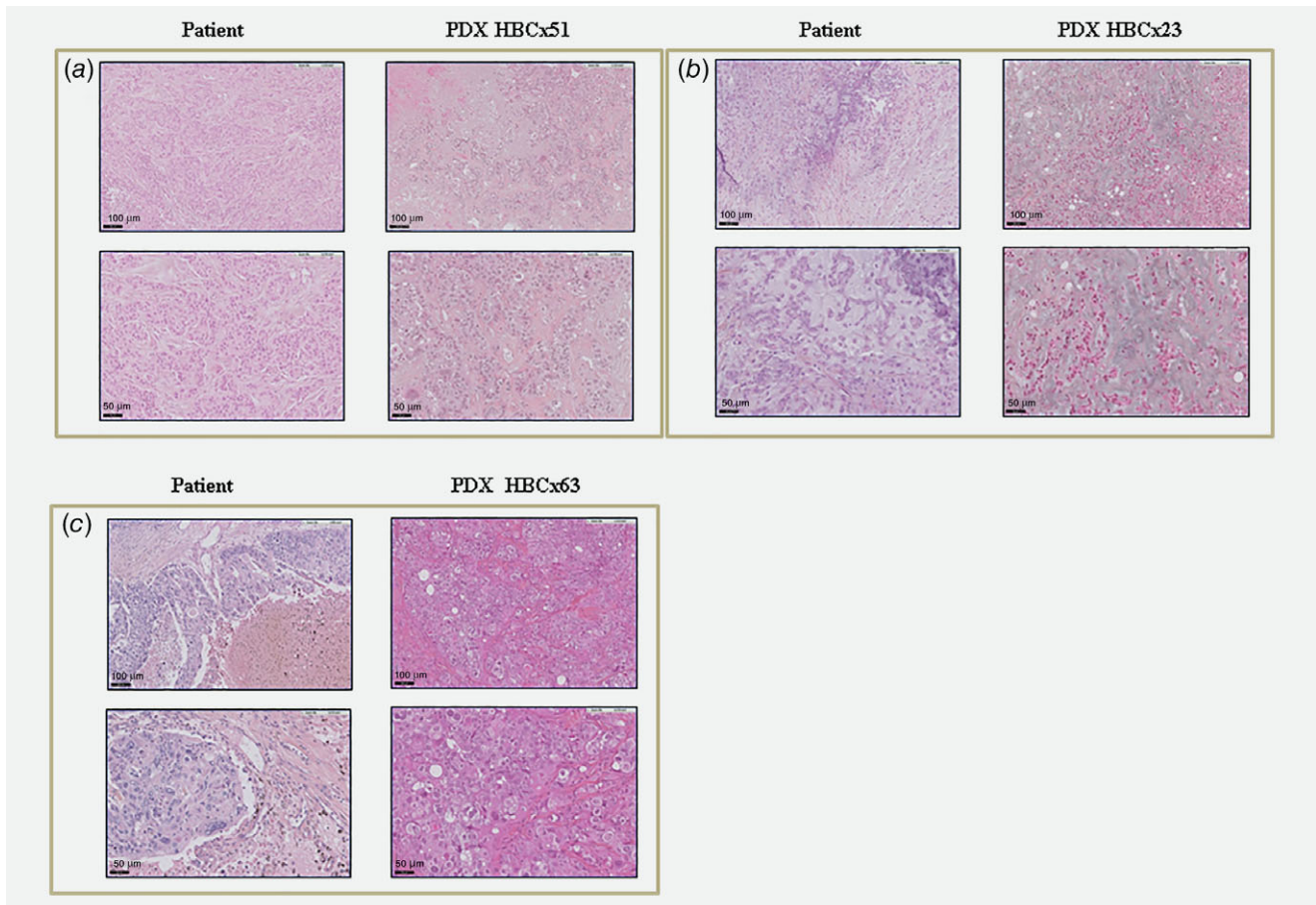


Figure 3. Morphological comparison between patient's tumor and PDX for (a) invasive carcinoma of no special type (NST) both patient's tumor and PDX have large nuclei and no evidence of glandular differentiation, and a high proliferative rate; (b) metaplastic carcinoma with mesenchymal differentiation: areas of chondroid and myxoid differentiation are observed in both patient tumor and PDX; (c) squamous cell carcinoma is characterized by cystic cavity lined by squamous epithelium. Typical squamous cells with keratinizing are observed in PDX tumor.

distribution of mutations in ER+ PDX highlighted four major mutated genes: *PIK3CA* (27%), *TP53* (24%), *MAP3K1* (11%) and *GATA3* (8%; Supporting Information Table S6). These predominant genes in ER+ breast cancer have been largely described in the literature.⁸

Testing of two targeted therapies, alone and in combination, in TNBC PDX

MAPK and PI3K pathways represented two major targetable pathways in this cohort. Consistent with this notion, we tested the antitumor activity of two promising inhibitors of these pathways: selumetinib (a MEK inhibitor) and PF-04691502 (a dual PI3KCA/mTOR inhibitor) in PDX with genomic alterations in PI3K and MAPK pathways. These two PDXs are resistant to chemotherapies commonly used in TNBC (anthracycline, taxane and platinum; Supporting Information Figure S1). Figure 6a shows the antitumor activity of selumetinib in the HBCx-92 PDX, harboring a *NRAS* hotspot mutation (p.Q61K). After 25 days of treatment, selumetinib (a MEK inhibitor) induced significant

TGI (85%). As some PDX presented multiple targetable alterations, PF-04691502 monotherapy was initially tested, followed by a combination of two targeted therapies in HBCx4B (*PIK3CA* hotspot mutation, p.E545K and *NF1* inactivating mutation, p.K2631 fs, associated with loss of heterozygosity) with PF-04691502 and selumetinib (Fig. 6b and 6c). Monotherapy induced a TGI of 70% without complete response. Combination therapy was significantly superior to monotherapy (TGI of 90%) and two mice obtained a complete response (Fig. 6d).

Discussion

We report the largest cohort of clinically annotated TNBC PDX, illustrating the genomic, transcriptomic and morphologic heterogeneity of TNBC. Several other authors have described genomic alterations in small cohorts of TNBC PDX, including description of morphological and genomic fidelity with the patient's tumor and possibility to identify efficient treatment and predictive biomarkers.^{12,16,35} The primary objective of transcriptomic classifications is to identify subtypes with biologic drivers in order to

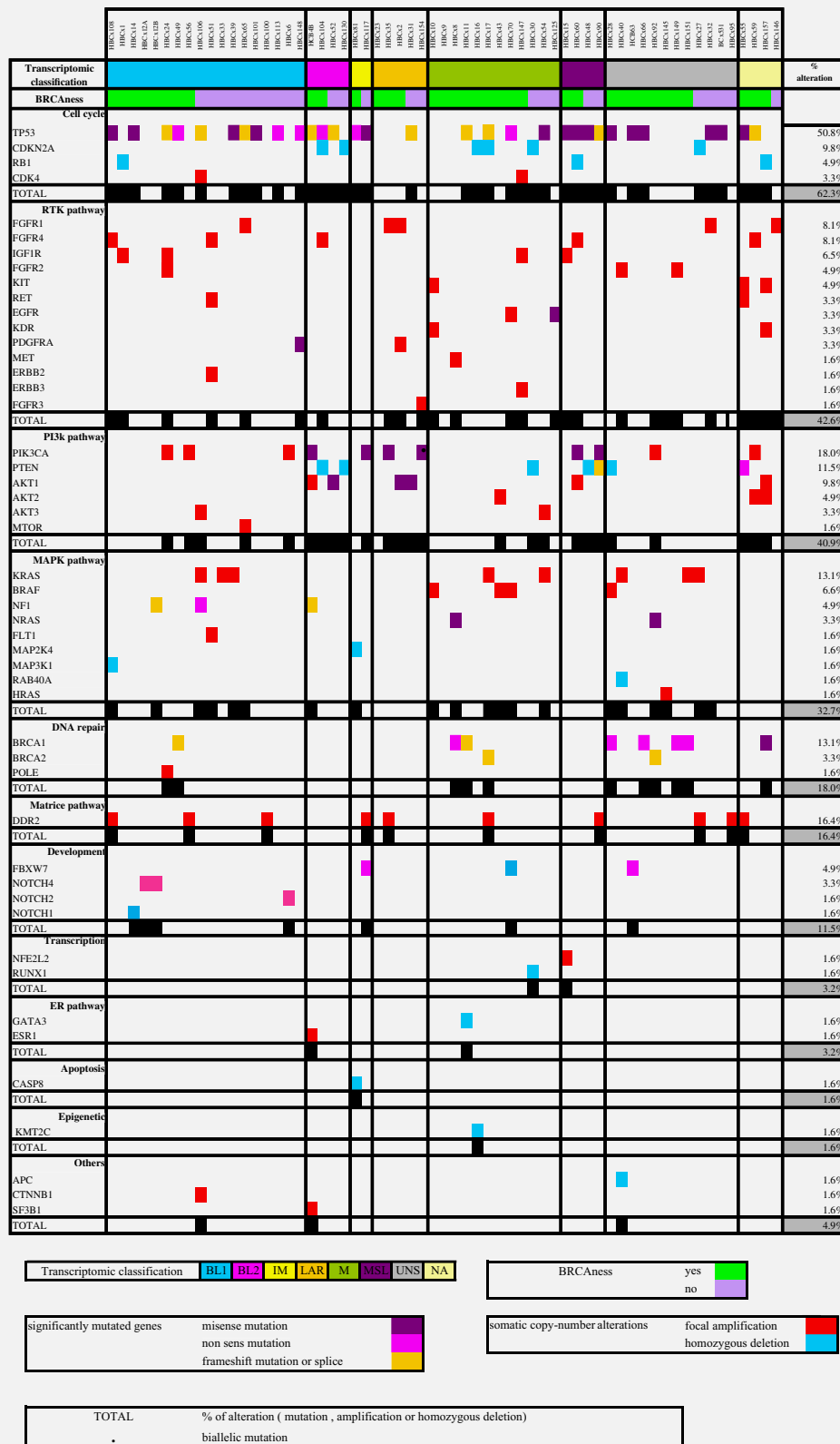


Figure 4. Details of SCNAs and SMGs of 61 TNBC PDX.

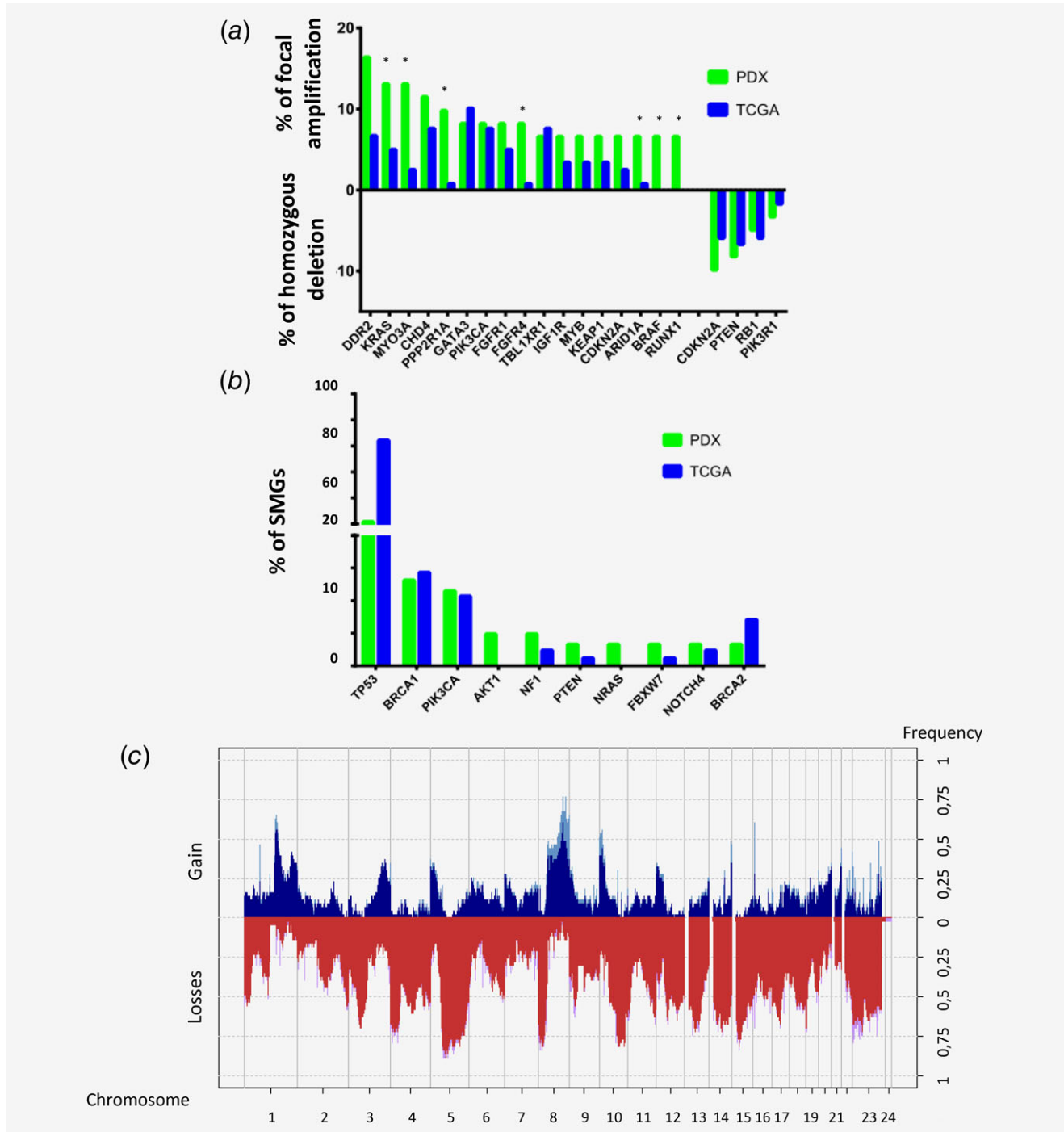


Figure 5. Comparison of SCNAs (a) and SMGs (b) for the most frequently altered genes between TN PDX and TCGA C: representation of global alteration of copies numbers of TN PDX.

guide personalized treatment. By comparing the distribution of TNBC PDX according to Lehmann's classification, with TNBC of TCGA⁸ and METABRIC data,⁹ the distribution of the various data sets was similar, except for IM (underrepresented in our cohort) and BL1 subtype (overrepresented in our cohort). As the IM subtype is characterized by expression of immunity genes,

most of which are expressed by immune cells, the low frequency of this subtype in PDX is only to be expected and can be explained by the loss of human stroma in PDX models. BL1 subtype is overrepresented in our PDX cohort, probably because the majority of IM subtypes were reclassified in the BL1 subgroup as described in Lehmann's second classification.³⁶ Transcriptomic

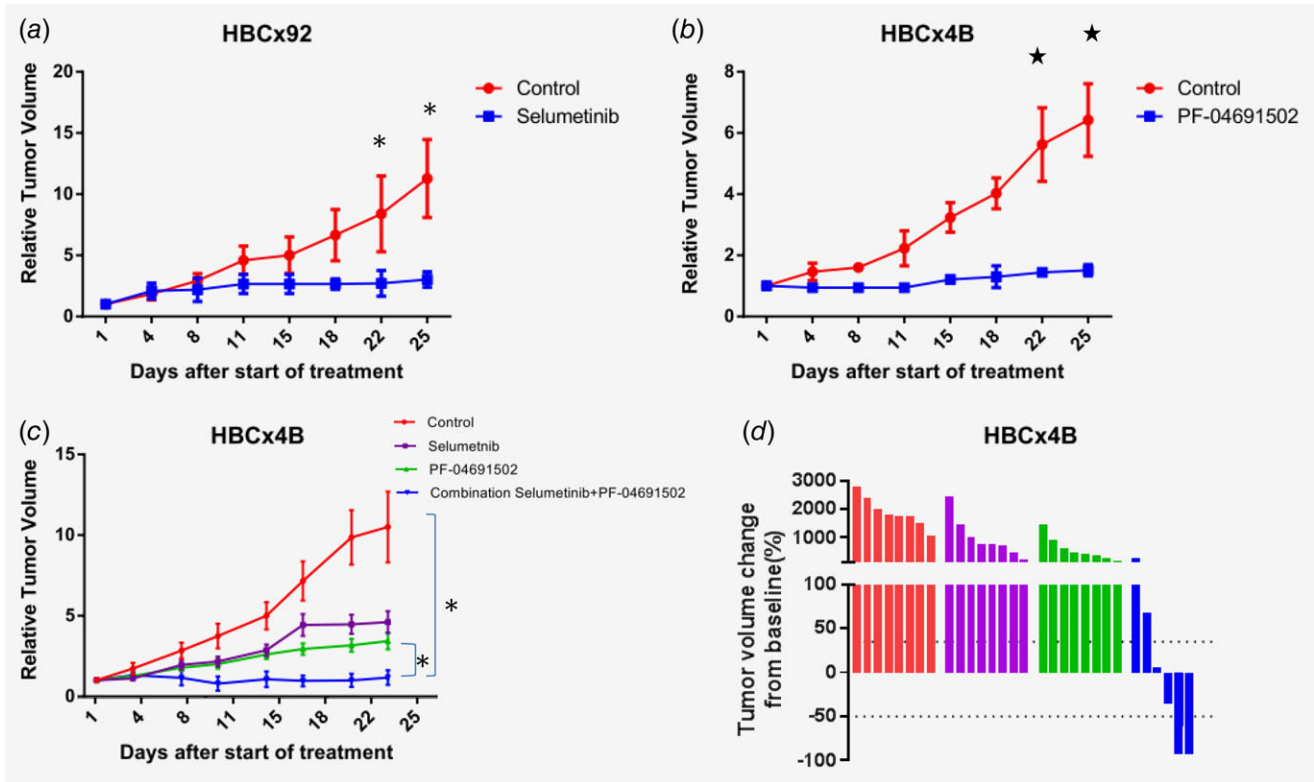


Figure 6. (a) Treatment of HBCx92 with selumetinib; (b) treatment of HBCx4B by PF-04691502; (c): treatment of HBCx4B with a combination of selumetinib+PF-0469150; (d) Waterfall plots representing the percent change in tumor for each mouse of HBCx4B treated with selumetinib, PF-04691502 and combination of selumetinib+PF-046915. Mean of RTV \pm SD. $n = 3$ mice per group (a and b) and $n = 8$ mice per group (c and d). *Significant difference, $p < 0.01$ (Mann–Whitney test), $p < 0.05$.

classifications are not used in clinical practice, but stress the importance of selection of the TNBC subtype before prescribing targeted therapy. However, except for the LAR and mesenchymal subtypes, it is difficult to demonstrate a specific correlation between transcriptomic subtype and response to targeted therapy.^{8,37}

In our study, the most frequently altered genes in TNBC are comparable to those described in the TCGA or METABRIC. Moreover, our presentation of global copy number alterations highlights the specific characteristics of TNBC: 8q24 (*MYC*) focal gain, 1q, 10p gain and 8p, 5q loss.⁸ Some transcriptomic subtypes in this cohort harbored specific somatic mutations already described in other studies^{6,37} LAR subtype corresponds to the luminal repertoire of genomic alterations: the most frequent mutations are *PIK3CA* and *AKT1* (40%, 2/5) and the less frequent mutations are *TP53* (20%). BL1 and BL2 present a high percentage of *TP53* mutations (55.5 and 75%, respectively).

As expected, the PI3K pathway was frequently altered in the present cohort: 40.9% of PDX presented at least one genomic alteration in this pathway. *PIK3CA* mutation and loss of *PTEN* were the most frequent alterations. This pathway was particularly altered in LAR, BL2 and MSL subtypes, as described in several other studies^{6,36} and inhibitors of this pathway could be useful in these particular subtypes. We have previously shown that everolimus induced a significant response

in 7 out of 15 TNBC PDX, irrespective of the *PIK3CA* mutational status.³⁸ In another study, seven TNBC PDX treated with rapamycin (mTOR inhibitor), showed a marked growth inhibition, supporting the value of this treatment in TNBC.³⁹ Few clinical data are available concerning the PI3K pathway inhibitor in TNBC⁴⁰ and various studies have tested specific PI3K inhibitors and have tried to identify biomarkers (SAFIR study, NCT02299999). Dual PI3K/mTOR inhibitor (PF-04691502) presents an advantage by ensuring more complete suppression of the pathway by inhibiting PI3K as well as mTORC1 and mTORC2 and theoretically abrogates possible negative feedback loops caused by inhibition of mTORC1 alone.⁴¹ In our study, we show that PF-04691502 inhibits tumor growth in our PDX with *PIK3CA* mutation, emphasizing the value of this type of specific PI3K pathway inhibition. The MAPK pathway presented rare but targetable alterations of oncogenic drivers, suggesting that targeting this pathway may be an option for TNBC tumors. Few published data are available concerning MEK inhibitors in TNBC. Trametinib was tested in eight PDX with a high range of MEK/ERK phosphorylation: the best response was observed in the PDX with the most highly activated MAPK pathway.³⁵ Interestingly, in a phase Ib trial in patients with solid tumors ($n = 31$) treated with gemcitabine and trametinib, the only complete response to therapy was

observed in a patient with TNBC.⁴² Selumetinib was tested in one model of breast cancer xenograft with regression of lung metastasis.⁴³ A MAPK inhibitor is currently under clinical investigation in TNBC with a specific targetable alteration of the MAPK pathway (selumetinib in the metastatic setting, SAFIR02, NCT02299999; or neoadjuvant setting, NCT02685657). Here, we showed that selumetinib markedly reduced tumor growth in our selected PDX with *NRAS* mutation confirming the value of this drug in selected tumors.

Our study provides evidence of the multiple alterations present in each tumor (54% presented at least two targetable alterations) which can have important clinical implications: (i) it is essential to identify patients likely to benefit from effective targeted therapies by means of genomic biomarkers (ii) two inhibitor monotherapies were tested in our study in chemoresistant PDX with specific alterations, resulting in significant growth inhibition but the combination of a dual PI3K/mTOR inhibitor and MEK inhibitor provided very encouraging results, with a better response than monotherapies and 30% of complete response.

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Conclusion

Our study shows that PDX reproduce the molecular heterogeneity of TNBC. This subtype is characterized by marked heterogeneity, explaining why TNBC “divides and rules.” The high percentage of tumors with concomitant targetable anomalies in our cohort emphasizes the various possibilities of combination therapy, particularly between PI3K, MAPK, cell cycle and DNA repair pathways, as recently described in TCGA.⁴⁴ These results are very encouraging to continue testing various targeted therapies and combination in our models in order to develop new treatments and combination in TNBC.

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