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An Organoid Model System to Study Resistance Mechanisms, Predictive Biomarkers, and New Strategies to Overcome Therapeutic Resistance in Early-Stage Triple-Negative Breast Cancer

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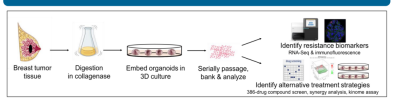
The I-SPY Trials

BACKGROUND

- Therapy resistance: significant challenge in the treatment of breast cancer.
- Organoids: promising technology used for growing breast cancer cells, but the extent to which it can model treatment resistance is largely unknown.
- This research: using patient-derived organoid cultures in the context of computational analyses of large molecular and clinical datasets (I-SPY2) to study resistance mechanisms, biomarkers, and alternative treatment strategies in early-stage triple-negative breast cancer (TNBC).

METHODS & DATA



- An organoid biobank, enriched for inflammatory breast cancer (IBC), was established from organoid cultures derived from breast tumor samples, digested to the organoid level using collagenase, and grown in three dimensional cultures using a basement membrane extract and a fully-defined organoid medium (1).
- Next, previously analyzed I-SPY2 gene expression and protein biomarkers associated with resistance (identified in pre-treatment patient tumors) were explored to determine if they were present in organoids propagated from breast cancer post-treatment residual disease (2,3).
  - Bulk RNA sequencing data of 11 TNBC organoids were normalized and merged with the TCGA dataset (4) to enable analysis of (TN)BC subtypes (5-7) and I-SPY2 gene expression biomarkers in a larger context.
  - Immunofluorescence analysis of protein biomarkers (from I-SPY2 RPPA analysis) was performed, using breast cancer cell lines as controls.
- A high-throughput 386 anti-cancer drug screen was performed (with and without cisplatin) in a tumor organoid modeling resistance to cisplatin. The most promising compounds were selected for subsequent synergy analysis.
- High-throughput kinase activity-mapping assays (HT-KAM, or 'kinome assay') in this organoid model are in progress, with the goal of identifying (druggable) kinase mediators of cisplatin sensitivity and resistance (8).

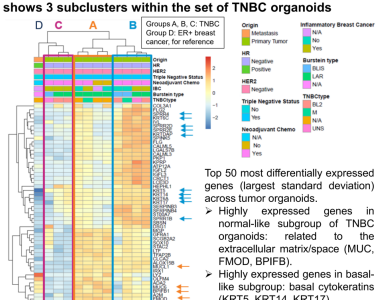
Table 1: TNBC organoids are characterized predominantly by either normal-like/luminal androgen receptor or basal-like features

Single cell analyses are ongoing to confirm preliminary findings that the normal-like subgroup contains a heterogeneous mixture of cell types.

Tumor organoid	Grade	Triple Negative	Neoadjuvant Chemo	Inflammatory BC	PAM50 subtype	Burstein subtype	TNBCtype
	Clinical characteristics				RNA-Seq based subtyping		
TORG16 P4	N/A	No	N/A	N/A	Her2	N/A	N/A
TORG27 P7	3	Yes	No	N/A	Normal	LAR	N/A
TORG43 P3	3	Yes	Yes	No	Normal	LAR	N/A
TORG46 P5	3	Yes	No	No	Normal	LAR	N/A
TORG47 P5	3	Yes	Yes	Yes	Normal	LAR	M
TORG48 P3	3	Yes	Yes	Yes	Normal	LAR	UNS
TORG49 P2	2	Yes	Yes	No	Basal	BLIS	M
TORG51 P4	1	Yes	Yes	No	Basal	BLIS	BL2
TORG53 P2	3	Yes	Yes	Yes	Basal	LAR	BL2
TORG39 P8	N/A	Yes	Yes	Yes	Basal	BLIS	UNS
TORG40 P5	N/A	Yes	Yes	Yes	Basal	LAR	BL2
TORG40 P7	N/A	Yes	Yes	Yes	Basal	LAR	BL2

RESULTS

Figure 1: Bulk RNA-Seq analysis and GSEA of 11 samples shows 3 subclusters within the set of TNBC organoids



Top 50 most differentially expressed genes (largest standard deviation) across tumor organoids.

- Highly expressed genes in normal-like subgroup of TNBC organoids: related to the extracellular matrix/space (MUC, FMOD, BPIFB).
- Highly expressed genes in basal-like subgroup: basal cytokeratins (KRT5, KRT14, KRT17).

IBC cases present in all 3 subsets

Hallmark gene sets present in ≥2 comparisons*	Group A (TORG 27, 43, 46, 47)	Group B (TORG 48, 49, 51, 53)	Group C (TORG 39, 40P5, 40P7)
	• KRAS signaling DOWN • Androgen response (in comparison A vs. B)	• Wnt Beta Catenin signaling • MYC targets V2 • Allograft rejection • MYC targets V1 • P53 pathway • Inflammatory response • TNFa signaling via NFKB • KRAS signaling DOWN + • KRAS signaling UP • DNA Repair	• E2F targets • G2M checkpoint • MYC targets V1 • Mitotic Spindle

*Comparisons: group A organoids vs all other organoids, group B vs others, group C vs others, group A vs B, A vs C, B vs C
Bold: unique to this subgroup
Categories: DNA damage, immune, proliferation, signaling

Table 2: RPPA biomarker analysis by I-SPY2 investigators highlights potential markers of resistance to veliparib-carboplatin (VC) (3)

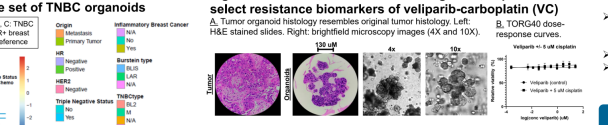
These biomarkers were individually significantly associated with non-pCR (nominal p-value <0.05).

RESULTS

Figure 2: TORG40, a TNBC and IBC organoid, expresses select resistance biomarkers of veliparib-carboplatin (VC)

A. Tumor organoid histology resembles original tumor histology. Left: H&E stained slides. Right: bright-field microscopy images (4X and 10X).

B. TORG40 dose-response curves.



C. Gene expression biomarkers related to (non-) response to VC: low PARPi_orig score in TORG40.

D. Immunofluorescence analysis of RPPA biomarkers. Blue = DAPI, green = EpCAM, red = pMEK1/2 (top) / pFOXO1 (bottom).

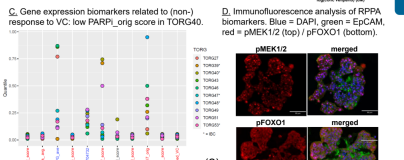
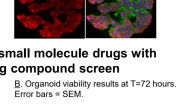


Figure 3: Top synergistic/additive small molecule drugs with cisplatin in TORG40 in the 386-drug compound screen

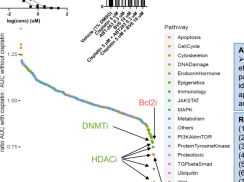
A. Organoid cell viability in response to BV6 and ABT-263 +/- cisplatin, showing synergy/additivity of cisplatin with these compounds (results at T=72 hours)

B. Organoid viability results at T=72 hours. Error bars = SEM.



C. Ranking of 386 compounds based on their synergy/additivity with cisplatin in TORG40. Ratio of AUCs between condition with cisplatin and without cisplatin was used to rank the compounds and select top hits of therapeutic interest based on largest reduction in AUC with the addition of cisplatin (indicated with arrows).

Drugs that appeared most synergistic/additive with cisplatin were related to epigenetics (HDAC-, DNMT inhibitors) and apoptosis (IAP-, Bcl2 inhibitors).



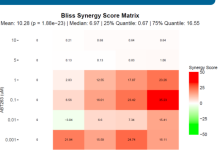
CONCLUSIONS

- Therapeutic resistance in residual disease tumor organoid cultures can be matched to I-SPY2 resistance biomarkers and signatures.
- Tumor organoid cultures modeling drug resistance states are a useful complement to existing research models of breast cancer and can be used for compound testing.
- We demonstrate the ability to model IBC and subtypes of TNBC.
- A pipeline is being developed to propagate residual tumors from patients enrolled in I-SPY2 into organoid cultures to create a broader platform for preclinical drug testing informed by tumor biology.

FUTURE DIRECTIONS

Figure 4: Preliminary Bliss Synergy and Kinome Assay Data

A. Preliminary Bliss Synergy Score analysis of cisplatin and ABT-263 (analyzed using Synergyfinder package in R). Future results could help select promising and clinically relevant dose combinations to guide future research, including organoid-based mechanistic studies.



B. Preliminary data from a pilot kinome assay (8) comparing cisplatin-treated vs. untreated TORG40 organoids highlight new potentially druggable kinases to overcome resistance to cisplatin, including FGFR3, NLK and MAPK9.

Long-term goal:

- leverage organoid model system to enable faster, more successful translational studies and find new treatment options for resistant disease.

Advocate perspective

- Using new in-vitro combined with in-silico tools to overcome therapy resistance can avoid unnecessary adverse effects and have the potential to select the most effective drugs for patients. The authors have developed a novel patient-derived organoid culture model system to study why some patients' tumors resist a particular drug therapy, identify new biomarkers of resistance, and screen for potentially more efficacious treatments. This personalized approach could provide a more successful outcome for individual patients with various TNBC subtypes and IBC. In addition, establishing a biorepository of organoids can support future drug discovery.

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