SAN ANTONIO BREAST CANCER SYMPOSIUM®

UT Health San Antonio Mays Cancer Center | AACR American Association for Cancer Research®

P1-05-23

An observational cohort study of Multiplex8+: a spatially informed assay that uses multiplexed RNA-FISH guided laser capture microdissection followed by total RNA-sequencing

Evan D. Paul[1,2,*], Barbora Huraiová[1,2], Natália Matyašovská[1,2,3], Daniela Gábrišová[1,2], Soňa Gubová[1,2], Tomáš Ondris[1,2], Michal Gala[1,2], Liliane Barroso[1,2], Helena Ignačáková[1,2], Natália Valková[1,2], Fresia Pareja[4], Jakob N. Kather[5-7], Pavol Čekan[1,2]

[1] MultiplexDX, s.r.o., Comenius University Science Park, Bratislava, Slovakia.; [2] MultiplexDX, Inc., Rockville, MD, USA.; [3] Institute of Clinical Biochemistry and Diagnostics, University Hospital, Faculty of Medicine in Hradec Kralove, Charles University, Hradec Kralove, Czech Republic; [4] Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA. [5] Else Kroener Fresenius Center for Digital Health, Technical University Dresden, Dresden, Germany.; [6] Department of Medicine I, University Hospital Dresden, Dresden, Germany.; [7] Medical Oncology, National Center for Tumor Diseases (NCT), University Hospital Heidelberg, Heidelberg, Germany. * Presenting author.

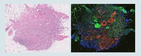
Background

- On a retrospective cohort of 1,082 FFPE breast tissues, we previously developed and validated mFISHseq, a novel, spatially informed tool that integrates multiplexed RNA fluorescent in situ hybridization (FISH) of the four main breast cancer biomarkers (*ESR1/PGR/ERBB2/MKI67*), which are used to guide laser capture microdissection (LCM) followed by RNA-sequencing[1-4].
- Here, we applied a Research Use Only (RUO) version of this test called Multiplex8+ to an observational cohort of 53 patients, where we assessed intrinsic molecular and TNBC subtypes, prognostic risk, and the expression of 40 genes and 28 gene signatures related to cancer hallmark pathways and treatment response.
- **Objectives**: We compared Multiplex8+ to immunohistochemistry and associated our list of genes and gene signatures with response to a variety of treatments, including chemotherapies and novel targeted therapies like CDK4/6 inhibitors and antibody drug conjugates (ADCs).

Methods

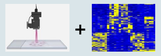
The Multiplex8+ assay[1]

H&E and Multiplexed RNA-FISH



ACD RNAScope Multiplex Fluorescent V2 Assay
- Estrogen (*ESR1*)
- Progesterone (*PGR*)
- Her2 (*ERBB2*)
- Ki67 (*MKI67*)

Laser capture microdissection (LCM) and RNA-SEQ



- H&E + Biomarker-guided capture of ROIs
- Takara SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian
- NovaSeq 6000 –100M reads/sample (2 x 100 PE)

Patient Report

- 4 key biomarkers
- Molecular/TNBC subtype
- Prognostic risk
- Genes and gene signatures
- Recommendations

Genes / gene signatures

Drug type / pathway	Genes	Gene signatures
Prognosis	1	3
Proliferation	2	2
Luminal	2	4
Her2	3	3
Chemotherapy	9	8
Immunotherapy	3	5
CDK4/6 inhibitors	5	0
PI3K inhibitors	0	1
DNA damage and repair	2	1
Angiogenesis / Hypoxia	1	1
Antibody drug-conjugates	20	0
Total	40	28

Overview of RUO cohort

Characteristic	HR+/HER2−	HR+/HER2+	HR−/HER2+	HR−/HER2−	Total
No. of patients	10	9	3	30	53*
Average age (years)	51	49	54	45	47
Tumor size (T)					
pT1	3	3	2	12	20 (38%)
pT2	4	1	0	5	10 (19%)
pT3 + pT4	1	2	0	3	6 (11%)
Unavailable	2	3	1	10	17 (32%)
Node status (N)					
Negative	3	1	1	14	19 (36%)
Positive	4	4	0	5	13 (25%)
Unavailable	3	4	2	11	21 (40%)
Grade (G)					
G1	1	1	0	0	2 (4%)
G2	2	5	0	8	15 (28%)
G3	5	1	3	18	27 (51%)
Unavailable	2	2	0	4	9 (17%)

* One patient did not have clinicopathological information

References: 1. Paul, E.D. et al. medRxiv 2023.12.05.23299341; doi: https://doi.org/10.1101/2023.12.05.23299341 (2023). **2**. Paul, E.D. et al. Annals of Oncology (2024) 9 (suppl_4): 1-34. 10.1016/esmoop/esmoop103010. **3**. Loeffler, C.M.L. et al. J Clin Oncol 42, 2024 (suppl 16; abstr 3069). **4**. Pareja F. et al. Annals of Oncology (2024) 35 (suppl_2): S309-S348. 10.1016/annonc/annonc1577.

Results – IHC concordance and predictive efficacy

1 Multiplex8+ has 90.5% concordance with IHC across ER (47/55=85%), PR (51/55=93%), HER2 (53/55=96%), and Ki67 (29/34=85%) biomarkers.

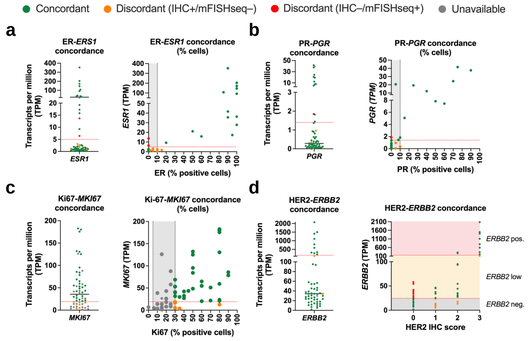


Figure 1. Graphs depict concordance between immunohistochemistry (IHC) and Multiplex8+ RNA-SEQ results for (**a**) estrogen receptor (ER-*ESR1*), (**b**) progesterone receptor (PR-*PGR*), (**c**) Ki67 marker of proliferation (Ki67-*MKI67*), and (**d**) HER2 receptor (HER2-*ERBB2*). The dotted red line in each figure denotes the TPM threshold used to call positive or negative biomarker expression. Samples with Unavailable lack pathology information.

2 Multiplex8+ has high predictive accuracy, especially for targeted therapies, where 91% (10/11) of the predictions were correct as supported by patient follow-up data regarding clinical response. By combining ADC-processing biomarkers, Multiplex8+ may stratify patients into ADC-treatment responsive subgroups.

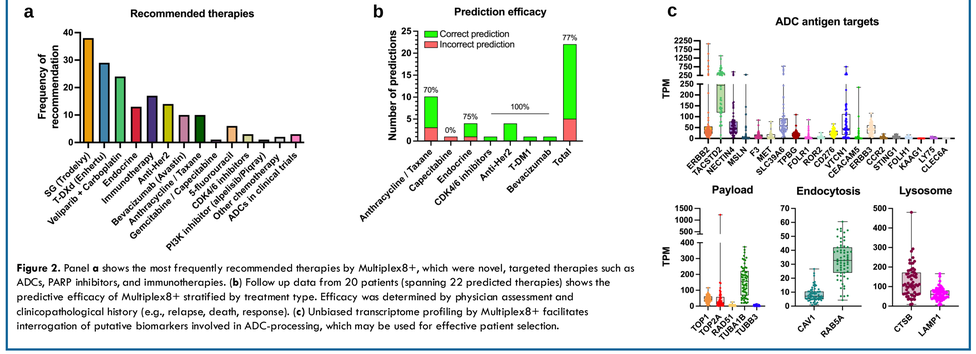


Figure 2. Panel **a** shows the most frequently recommended therapies by Multiplex8+, which were novel, targeted therapies such as ADCs, PARP inhibitors, and immunotherapies. (**b**) Follow up data from 20 patients (spanning 22 predicted therapies) shows the predictive efficacy of Multiplex8+ stratified by treatment type. Efficacy was determined by physician assessment and clinicopathological history (e.g., relapse, death, response). (**c**) Unbiased transcriptome profiling by Multiplex8+ facilitates interrogation of putative biomarkers involved in ADC-processing, which may be used for effective patient selection.

Results – Patient vignettes

3 Multiplex8+ provides unique insights into tumor biology and predicts response to multiple lines of therapy.

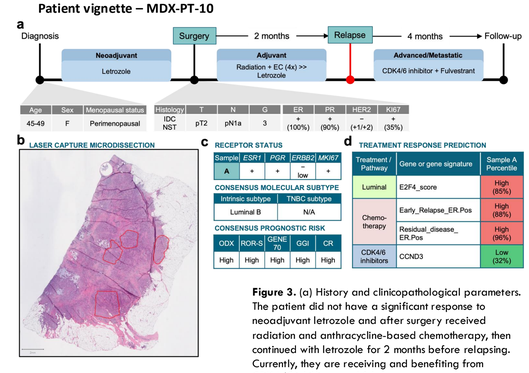


Figure 3. (a) History and clinicopathological parameters. The patient did not have a significant response to neoadjuvant letrozole and after surgery received radiation and anthracycline-based chemotherapy, then continued with letrozole for 2 months before relapsing. Currently, they are receiving and benefiting from CDK4/6 inhibitors and fulvestrant treatment. (**b**) Multiplex8+ conducted on the surgical resection (areas for LCM shown in red annotations). (**c**) Multiplex8+ confirmed previous IHC results and showed high risk of recurrence by all risk classifiers, which was clinically confirmed by the patient's rapid relapse. (**d**) The treatment predictive gene signatures show that although the hormone receptor expression is high, the patient is likely resistant to aromatase inhibitors and sensitive to CDK4/6 inhibitors, which is supported by low expression of CCND3, a marker of resistance to CDK4/6 inhibitors. The lack of response to epirubicin / cyclophosphamide chemotherapy was also predicted from high Early_Relapse_ER.Pos and Residual_disease_ER.Pos signatures.

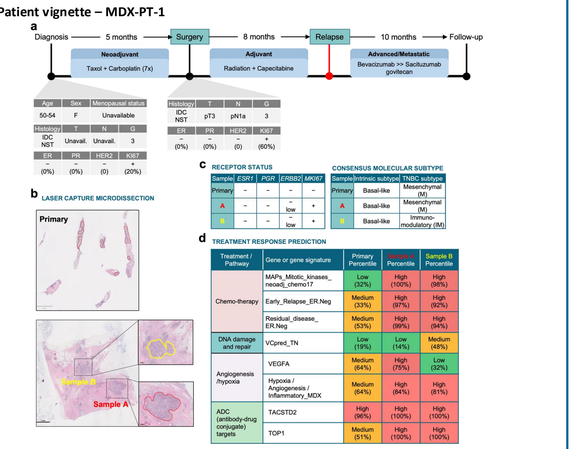


Figure 4. (**a**) History and clinicopathological parameters. (**b**) Multiplex8+ performed on initial biopsy and the resected tumor. The histopathology revealed two distinct regions of the resected tumor that were characterized either with or without infiltrating lymphocytes. (**c**) Multiplex8+ results for key biomarkers and intrinsic molecular / TNBC subtypes. Note the two regions were classified as different TNBC subtypes. (**d**) Treatment predictive signatures predicted response to bevacizumab (Avastin) and high expression for TACSTD2 and TOP1 (i.e., antigen and payload targets for sacituzumab govitecan). This provided two viable treatments in the first and second lines for metastatic TNBC. This evidence concurred with follow up data documenting a durable clinical response following bevacizumab. Interestingly, analysis of the primary tumor also provides biological rationale for the lack of response to neoadjuvant taxane/carboplatin, which would be predicted given the M TNBC subtype and low signature scores for taxanes (MAPs_Mitotic_kinases_neoadj_chemo17) and carboplatin (VCpred_TN).

Summary and Conclusions

- We demonstrated the feasibility of implementing Multiplex8+ in a real-world setting and illustrated its clinical potential in predicting response to a variety of treatments.
- Multiplex8+ showed high concordance with IHC (91%), mirroring the 93% accuracy observed in our large-scale retrospective cohort of 1,082 breast tissues[1].
- By interrogating the expression of targets related to ADC antigens, cytotoxic payloads, endocytosis, lysosome function, and resistance, we illustrate a hypothetical framework that could be used in a future prospective validation for identifying patients responsive to ADCs.

Funding and contact

Funding: MultiplexDX and the European Union's Horizon 2020 research and innovation programme under an EIC Accelerator grant (agreement No 946693).

Scan here to learn more and download the poster

Copies of this poster obtained through Quick Response (QR) Code are for personal use only and may not be reproduced without permission from SABCS® and the author of this poster.

Contacts: paul@multiplexdx.com
pavol@multiplexdx.com