TBCRC061: Immune biomarkers of standard-of-care chemoimmunotherapy response and resistance in real-world patient population

Justin Balko, PharmD, PhD[1,2], Paula Gonzalez-Ericsson, MD[1], Brandie Taylor, PhD[1], Andres Ocampo, BS[1], Joseph Serrenho, BS[1], Susan Opalenik, PhD[1], Violeta Sanchez, BS[1], Melinda Sanders, MD[2], Valentina Hoyos, MD[3], Mothaffar Rimawi, MD[3], Yara Abdou, MD[4],Sara Nunnery, MD[5] Rita Nanda, MD[6], Frederick Howard, MD[6], Jesus Anampa-Mesias, MD[7], Laura Kennedy[1]

[1]Medicine, Vanderbilt University Medical Center, Nashville, TN, [2]Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN, [3]Medicine, Baylor College of Medicine, Houston, TX, [4]Medicine, Lineberger Comprehensive Cancer Center, Chapel Hill, NC, [5]Medicine, Tennessee Oncology, Nashville, TN, [6]Medicine, University of Chicago, Chicago, IL, [7]Medicine, Montefiore Medical Center, Bronx, NY..

Background

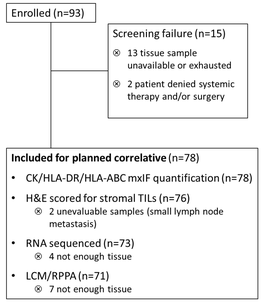
- Currently, only a small fraction (<10%) of patients with breast cancer receiving immune checkpoint inhibitors (ICI) as standard of care (SOC) achieve clinical benefit.
- Immunotherapy carries serious and long-lasting immune-related adverse events, highlighting the need for effective biomarkers for patient selection.
- Most biomarkers evaluated thus far, including PD-L1, stromal tumor-infiltrating lymphocytes (sTILs), TMB and most gene expression profiles, are generally prognostic or predictive of chemotherapy response, and lack immunotherapy benefit specificity
- Some biomarkers, like DetermaIO, tumor-specific MHC-I, and MHC-II have been identified as potential predictive biomarkers for immunotherapy-specific response across multiple tumor types and trials.
- We present the results from the early-stage cohort of TBCRC061, a non-interventional correlative trial that leveraged archival tissues from patients treated with SOC ICI to validate these biomarkers.

Methods

- Patients with breast cancer receiving standard of care immunotherapy were identified and consented across 5 sites – UNC, BCM, VUMC, University of Chicago, and Montefiore. The neoadjuvant cohort completed accrual in 2025 and is be presented here.
- Pre-treatment formalin-fixed and paraffin embedded archival samples were collected. Relevant patient demographics and response to treatment were extracted from the medical record.
- Transcriptomic profiling by RNA sequencing and pathologic assessments for MHC-I (immunofluorescence; IF), MHC-II (IF), and sTILs (H&E) , including TIME estimation (Grousso et al, JCI) was performed.
- We estimated predictive capacity for pCR or RCB0-1 across biomarkers, including multiple pre-constructed gene expression signatures. Since a chemotherapy-alone control cohort is not available due to contemporary use of KEYNOTE-522 in all eligible TNBC patients, RNA sequencing data from CALGB40603 were used as a comparator arm for transcriptomic studies.

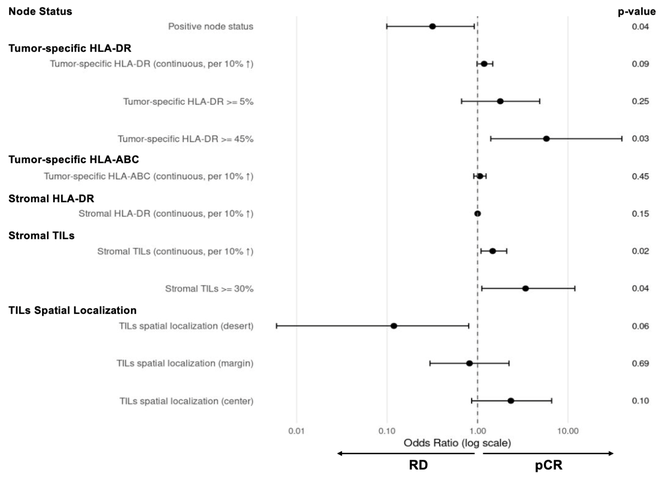
1. Patient population and CONSORT diagram

	All patients (n=78)	Completed treatment regimen (n=67)
Age at diagnosis (median)	51	51
Hormone receptor status		
ER and PR negative	75 (96%)	64 (96%)
ER negative, PR low	1 (1%)	1 (1%)
ER low, PR negative	2 (3%)	2 (3%)
Stage at diagnosis		
IA (T1cN0)	5 (6%)	4 (6%)
IIA	29 (37%)	25 (37%)
IIB	21 (27%)	19 (28%)
IIIA	18 (23%)	14 (21%)
IIIB	3 (4%)	3 (5%)
IIIC	2 (3%)	2 (3%)
Node status		
positive	50 (64%)	43 (64%)
negative	28 (36%)	24 (36%)
Completed treatment regimen		
yes	67 (86%)	67 (100%)
no	11 (14%)	
RCB score		
0	48 (61%)	39 (58%)
I	10 (13%)	9 (14%)
II	13 (17%)	12 (18%)
III	7 (9%)	7 (10%)
Tumor-specific HLA-DR		
≥5%	46 (59%)	38 (57%)
≥45%	15 (19%)	14 (21%)



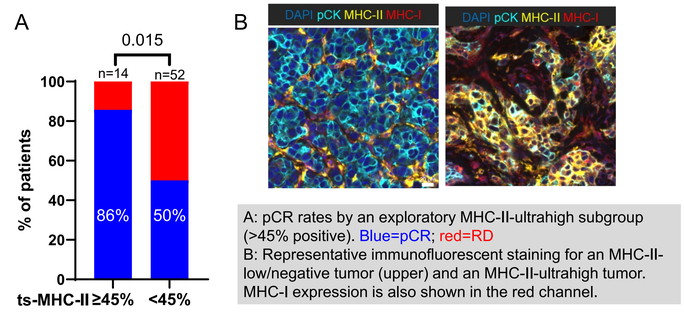
Results

2. Univariate analysis of tissue-based features



Odds ratio for achieving a pCR for patients that completed neoadjuvant regimen with pembrolizumab (n=66).

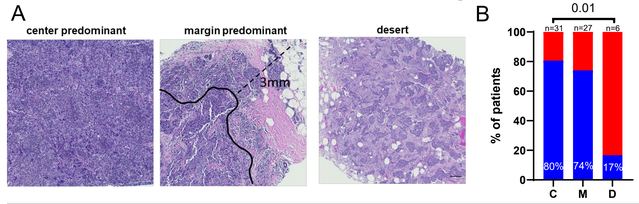
3. MHC-II ultra-high expressers are associated with exceptional pCR rates



A: pCR rates by an exploratory MHC-II-ultrahigh subgroup (>45% positive). Blue=pCR; red=RD
B: Representative immunofluorescent staining for an MHC-II-low/negative tumor (upper) and an MHC-II-ultrahigh tumor. MHC-I expression is also shown in the red channel.

Results (cont.)

4. The 'immune desert' phenotype identifies a group of low-TILs patients resistant to the KEYNOTE-522 regimen



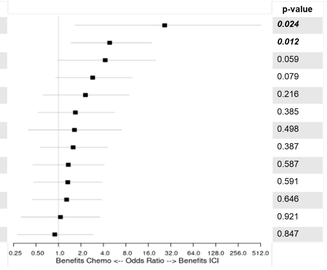
A: Examples of TILs-based tumor-immune microenvironment (TIME) phenotypes. B: pCR rates by TIME categories. blue=pCR; red=RD

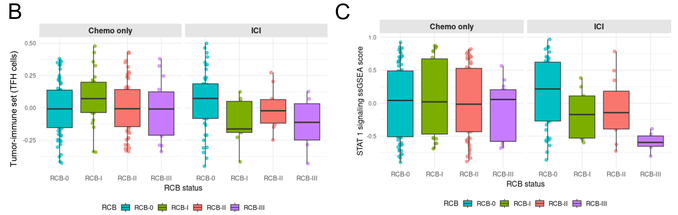
5. Exploratory analysis of RNA signatures associated with ICI benefit

A

Response (RCB0 only) ~ treatment * pathway (chemo as reference point)

Gene signature	Source	OR	95% CI	p-value
Tumor-immune set (TFH cells)	PMID: 24138885	26.51	(1.66, 520.11)	*0.024*
STAT1 signaling	I-SPY2	4.85	(1.48, 17.69)	*0.012*
Chemokine12	I-SPY2	4.23	(0.98, 20.07)	0.059
IFN signature	I-SPY2	2.87	(0.91, 9.77)	0.079
Tcell-Bcell	I-SPY2	2.3	(0.62, 8.89)	0.216
ICS5 signature	I-SPY2	1.69	(0.53, 5.68)	0.385
CD4 memory activated T cell	PMID: 25822800	1.64	(0.4, 6.99)	0.498
Dendritic cells	I-SPY2	1.57	(0.57, 4.51)	0.387
B cells	I-SPY2	1.35	(0.45, 4.11)	0.587
Mast cells	I-SPY2	1.33	(0.47, 3.83)	0.591
CD8 cluster	PMID: 24916698	1.29	(0.44, 3.82)	0.646
Bcell-Tcell	PMID: 31730857	1.06	(0.32, 3.61)	0.921
IGG cluster	PMID: 21214954	0.89	(0.28, 2.88)	0.847





A: Exploratory gene set analysis for preferential benefit of the addition of immunotherapy using non-bevacizumab arms of CALGB40603 (chemo alone) as a comparator. B: Stratification of ssGSEA T_{FH} scores by RCB status and C: STAT1 scores

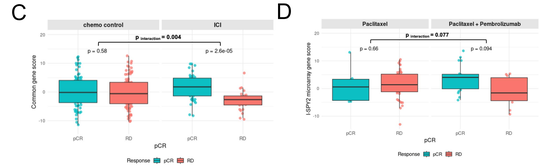
Results (cont.)

6. Combined logistic regression and bootstrapped elastic net regression selects predictive genes and validates the GLM output finding seven common genes



A: Schema for bootstrapped elastic net regression (BENR). B: Genes commonly selected between typical logistic regression and BENR approaches. C: Distribution of gene score across pCR and RD outcomes in CALGB40603 (chemo control) and TBCRC061. D: Validation in ISPY2 pembro4 and control arms.

Genes extracted by both logistic and elastic net regression	
IFNg driven immune activation and antigen presentation	CD274, IDO1, WARS1, CMTR1
Presence of tertiary lymphoid structures/T-follicular helper cells	CXCL13, MYO7A
Cytotoxic function	SLAMF7



Conclusions

- Multiple tumor microenvironmental features are associated with benefit from pembrolizumab in early TNBC, but their validation in randomized trials with chemotherapy-alone control arms are required to uncouple the chemotherapy-predictive effects (e.g. sTILs).
- Many immune-based gene expression signatures demonstrate this phenotype (generally predictive of pCR but trend toward slightly higher benefit with ICI).
- Immune-desert tumors may require alternative therapy strategies, as they are highly resistant to the KN522 regimen.
- Using feature selection from multiple approaches, subsets of genes may be identified that offer specificity to ICI benefit.

Acknowledgements

We would like to acknowledge the Breast Cancer Research Foundation, Susan G. Komen, and TBCRC for supporting this work. We would also like to acknowledge the patients for agreeing to make their tissue and clinical records available for research use for this study..

This presentation is the intellectual property of the author/presenter. Please contact them at justin.balko@vumc.org for permission to reprint and/or distribute.