Tertiary lymphoid structure (TLS)-related gene expression and outcomes in HER2-Positive breast cancer in the Real-World Database

Saranya Chumsri[1], Sachin Kumar Deshmukh[2], Thiti Susiriwatananont[1], Panuch Eiamprapaporn[1], Yi Liu[3], Sharon Wu[2], Joanne Xiu[2], Nadine Norton[4], Shipra Gandhi, Evanthia Roussos Torres, Maryam B. Lustberg, Edith A. Perez[1], Aziza Nassar[5], George W. Sledge[2], E. Aubrey Thompson[3], Keith L. Knutson[6].

[1]Jacoby Center for Breast Health, Mayo Clinic, FL; [2]Caris Life Sciences, Phoenix, Arizona; [3]Department of Health and Human Services, Mayo Clinic, FL; [4]Department of Cancer Biology, Mayo Clinic, FL; [5]Laboratory Medicine and Pathology, Mayo Clinic, FL; [6]Department of Immunology, Mayo Clinic, FL, USA

BACKGROUND

- Tertiary lymphoid structures (TLS), have been associated with improved outcomes across various cancer types.
- limited data exist on the prognostic relevance of TLS in HER2+ BC
- In our prior work, we demonstrated that the presence of functional TLS, defined by lymphoid aggregates with elevated expression of BCL6 or IL21R, correlated with improved outcomes in the NCCTG N9831 trial.
- In this study, we evaluated TLS-related gene and outcomes in the real-world database.

METHODS

- 1344 HER2+ (HR-HER2+: 560; HR+HER2+: 784) BC samples were analyzed via NGS (592-gene panel, NextSeq; WES/WTS, NovaSeq; Caris Life Sciences, Phoenix, AZ).
- Above (high) or below (low) the 50th percentile expression levels of TLS-related genes (*IFNG, ICOSLG, CXCL13, CXCR3, BCL6, IL21R, ICOS, PDCD1, CXCR5, CXCL9, TBX21, CD38, CXCL10, CXCL11, IL21, CD200*) were used to calculate overall survival (OS).
- *IL21R*-high (H) and -low (L) tumors were classified by RNA expression above or below the 50th percentile.
- Immune cell fractions were calculated by deconvolution of WTS: Quantiseq.
- Real-world overall survival (OS) was obtained from insurance claims and calculated from tissue collection to last contact using Kaplan-Meier estimates.
- Statistical significance was assessed using chi-square and Mann-Whitney U tests with multiple comparison adjustments (q < 0.05).

RESULTS

Table 1: Overall survival by RNA expression of TLS gene in HR-HER2+ and HR+HER2+ BC

TLS Gene	HR-HER2+						HR+HER2+					
	HR	95% CI	High	Low	Δ (month)	p-value	HR	95% CI	High	Low	Δ (month)	p-value
IL21R	0.77	0.61-0.97	41.7 m (N=280)	35.2 m (N=280)	6.5	**0.02**	0.77	0.62-0.93	50.5 m (N=390)	43.6 m (N = 390)	6.87	**0.009**
CXCL9	0.73	0.58-0.92	41.9 m (N=280)	35.2 m (N=280)	6.7	0.007	0.83	0.68 - 1	50 m (N=390)	46.3 m (N = 390)	3.58	0.08
CD38	0.79	0.63-0.99	41.71 m (N=280)	35.4 m (N=280)	6.2	0.04	0.94	0.77 - 1.1	47.6 m (N=390)	47.8 m (N = 390)	-0.19	0.5
CXCL10	0.69	0.55-0.87	46.5 m (N=280)	33 m (N=280)	13.4	0.002	0.95	0.77 - 1.2	43.8 m (N=390)	49 m (N = 390)	-5.1	0.62
CXCR3	0.88	0.7-1.1	39.7 m (N=280)	36.1 m (N=280)	3.6	0.28	0.74	0.6-0.9	53 m (N=390)	42.3 m (N = 390)	10.6	0.003
PDCD1	0.84	0.66-1	39.7 m (N=280)	36.6 m (N=280)	3	0.14	0.77	0.63-0.94	53.3 m (N=390)	43.5 m (N = 390)	9.7	0.01
TBX21	0.84	0.66-1	38 m (N=280)	36.6 m (N=280)	1.4	0.14	0.81	0.66-0.99	49.5 m (N=390)	45.2 m (N = 390)	4.3	0.03
CD200	0.85	0.68-1	41.7 m (N=280)	35.5 m (N=280)	6.2	0.18	0.73	0.66-0.1	49.6 m (N=390)	45.4 m (N=390)	4.1	0.04

Lowest Hazard Ratio — Highest Hazard Ratio; Shortest ΔSurvival — Longest ΔSurvival

In HR-HER2+ BC, high expression of *IL21R, CXCL9, CD38*, and *CXCL10* were associated with improved OS. In HR+HER2+ BC, high expression of *CXCR3, PDCD1, TBX21, CD200* and *IL21R,* were associated with improved OS. Higher expression of *IL21R* was significantly associated with OS in both HR-HER2+ or HR+HER2+ BC.

Table 2: Patients' demographic information of IL21R high vs low HR-HER2+ and HR+HER2+

		HR-HER2+		HR+HER2+	
		IL21R low	IL21R high	IL21R low	IL21R high
Count		280	280	393	391
Median age [range]		58 [23 - >89]	58 [23 - >89]	58 [23 - >89]	59 [20 - >89]
Race	White	67% (134/200)	72.73% (144/198)	70.26% (215/306)	75.83% (229/302)
	Black	25% (50/200)	18.69% (37/198)	21.24% (65/306)	14.9% (45/302)
	Asian/Pacific Islander	3.5% (7/200)	3.03% (6/198)	3.92% (12/306)	4.3% (13/302)
	Other	4.5% (9/200)	5.56% (11/198)	4.58% (14/306)	4.97% (15/302)
Ethnicity	Not Hispanic or Latino	86.79% (184/212)	84.76% (178/210)	86.02% (277/322)	86.24% (282/327)
	Hispanic or Latino	13.21% (28/212)	15.24% (32/210)	13.98% (45/322)	13.76% (45/327)
Tumor site	Primary	28.57% (80/280)	47.14% (132/280)	34.35% (135/393)	46.29% (181/391)
	Metastatic	71.43% (200/280)	52.86% (148/280)	65.65% (258/393)	53.71% (210/391)

Race and ethnicity data is self-reported

Figure 1. Immune cell infiltration

	HR-HER2+		HR+HER2+	
	IL21R low	*IL21R* high	*L21R* low	*IL21R* high
B cell	2.96	4.42 *	4.28	5.63 *
DC	2.57	2.51	2.42	2.55
Mϕ M1	2.63	3.92 *	2.15	3.03 *
Mϕ M2	3.28	4.05 *	4.43	5.69 *
Neutrophil	3.53	3.57	2.68	2.75
NK cell	2.58	2.82 *	2.47	2.74 *
T cell CD8+	0	0.89 *	0	0.7 *
Tregs	1.41	2.91 *	1.51	2.82 *

Low Median% — High Median%

In both HR-HER2+ and HR+HER2+, *IL21R* high groups had higher infiltration of B cells, M1 MΦ, M2 MΦ, NK cells, CD8+ T cell, Tregs. *q<0.05

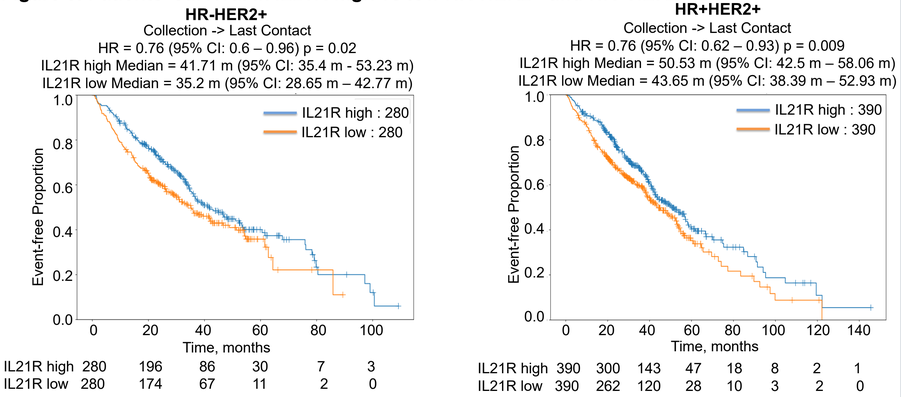
Figure 2. MHC Class I and II gene expression

		HR-HER2+		HR+HER2+	
		IL21R low	*IL21R* high	*IL21R* low	*IL21R* high
MHC I	*HLA-A*	98.99	215.38	104.43	192.52
	HLA-B	108.88	254.29	117.42	229.23
	HLA-C	117.70	217.07	125.45	198.62
	HLA-E	62.78	126.33	59.43	114.07
MHC II	*HLA-DQA1*	18.21	52.11	16.51	52.95
	HLA-DOA	4.73	13.09	4.65	12.61
	HLA-DPA1	96.35	242.67	96.53	229.01
	HLA-DMA	24.06	55.52	23.84	51.66

Low TPM (Median) — High TPM (Median)

In both HR-HER2+ and HR+HER2+, *IL21R* high group had higher expression levels of MHC class I genes (*HLA-A, HLA-B, HLA-C, HLA-E*, FC: 1.6-2.3), MHC class II genes (*HLA-DQA1, HLA-DOA, HLA-DPA1, HLA-DMA*, FC: 2.2-3.2). **All q<0.05.**

Figure 3. Patients' survival in *IL21R* high vs low HR-HER2+ and HR+HER2+ BC



In both HR-HER2+ and HR+HER2+, *IL21R* high had better survival compared to *IL21R* low.

CONCLUSIONS

- Functional TLS identify an immune-active tumor phenotype associated with improved outcomes in HER2+ breast cancer across both a clinical trial and a real-world cohort.
- IL21R emerged as a promising prognostic biomarker in both HR-/HER2+ and HR+/HER2+ subgroups, supporting its potential role in immune-based risk stratification for HER2+ breast cancer.