Chemokines as predictive biomarkers for immune checkpoint inhibitor (ICI) efficacy in triple negative breast cancer (TNBC)

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BACKGROUND

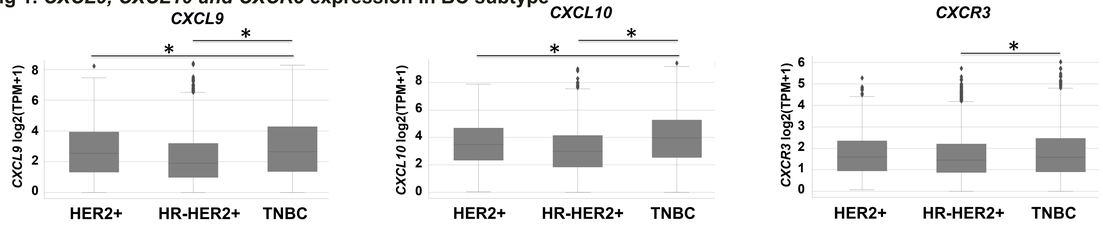
- TNBC, although an aggressive breast cancer (BC) subtype, is highly immunogenic and the only BC subtype where the ICI pembrolizumab is approved.
- However, predictive biomarkers for pembrolizumab benefit are limited.
- The chemokines *CXCL9* and *CXCL10* attract CD8 T cells into the tumor microenvironment (TME) and are associated with chemotherapy benefit, but little is known about their role in predicting pembrolizumab benefit in TNBC. We investigated the association of *CXCL9, CXCL10* and their cognate receptor *CXCR3* with TME and ICI efficacy.

METHODS

- 3,038 TNBC samples were analyzed via NGS (592-gene panel, NextSeq; WES/WTS, NovaSeq; Caris Life Sciences, Phoenix, AZ).
- Tumor mutational burden (TMB) totaled somatic mutations per tumor (high > 10 mt/MB).
- *CXCL9/CXCL10/CXCR3*-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

Fig 1. *CXCL9, CXCL10 and CXCR3* expression in BC subtype



TNBC expressed higher levels of *CXCL9* and *CXCL10* (median (TPM): 2.6 and 3.9) compared to N = 1,082 HER2+ (2.5 and 3.5, p< 0.05) and N = 4,918 HR+HER2- (1.9 and 2.9, p<0.05) BC. CXCR3 expression was higher in TNBC compared to HR+HER2- (1.5 vs 1.4,q < 0.05), but no difference when compared to HER2+ (1.5 vs 16, p = 0.96) BC. *p<0.05

Table 1: TNBC patients' sample demographic information

		CXCL9		*CXCL10*		*CXCR3*	
		Low	High	Low	High	Low	High
Count (N)		1469	1469	1469	1469	1469	1469
Median age [range]		61 (22 - >89)	61 (22 - >89)	61 (22 - >89)	60 (22 - >89)	61 (22 - >89)	61 (22 - >89)
Race	White	59.66% (670/1123)	62.13% (694/1117)	60.76% (689/1134)	61.03% (675/1106)	57.74% (645/1117)	64.02% (719/1123)
	Black	29.83% (335/1123)	27.93% (312/1117)	29.28% (332/1134)	28.48% (315/1106)	31.96% (357/1117)	25.82% (290/1123)
	Asian/Pacific Islander	3.92% (44/1123)	4.21% (47/1117)	3.88% (44/1134)	4.25% (47/1106)	3.49% (39/1117)	4.63% (52/1123)
	Other	6.59% (74/1123)	5.73% (64/1117)	6.08% (69/1134)	6.24% (69/1106)	6.8% (76/1117)	5.52% (62/1123)
Ethnicity	Not Hispanic or Latino	83.24% (884/1062)	82.88% (881/1063)	83.66% (896/1071)	82.45% (869/1054)	83.09% (870/1047)	83.02% (895/1078)
	Hispanic or Latino	16.76% (178/1062)	17.12% (182/1063)	16.34% (175/1071)	17.55% (185/1054)	16.91% (177/1047)	16.98% (183/1078)
Tumor site	Primary	45.41% (667/1469)	53.3% (783/1469)	44.38% (652/1469)	54.32% (798/1469)	47.99% (705/1469)	50.71% (745/1469)
	Metastatic	54.59% (802/1469)	46.7%(686/1469)	55.62% (817/1469)	46.68%(671/1469)	52.01% (764/1469)	49.29%(724/1469)

Race/ethnicity data is self-reported

Table 2: TNBC patients' survival pembrolizumab to last contact

Gene	HR	95% CI	High (month)	Low (month)	Δ (month)	p-value
CXCL9	0.65	0.5 - 0.84	26.48 (N = 363)	15.69 (N = 189)	10.79	0.001
CXCL10	0.74	0.57 - 0.95	25.95 (N = 341)	20.56 (N = 211)	5.39	0.02
CXCR3	0.68	0.52 - 0.88	32.6 (N = 318)	18.32 (N = 234)	14.27	0.003

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

CXCL9/CXCL10/CXCR3-H TNBC had higher median OS post pembrolizumab [CXCL9-H vs -L: 26.5 vs 15.7 months (mo), HR: 0.65 (95% CI 0.5-0.84); *CXCL10-H* vs -L: 26.0 vs 20.6 mo, HR 0.74 (0.57-0.95); *CXCR3-H* vs -L: 32.6 vs 18.3 mo, HR 0.68 (0.52-0.88), all p < 0.05]

Fig 2. Immune gene expression

	CXCL9 Low	*CXCL9* High	*CXCL10* Low	*CXCL10* High	*CXCR3* Low	*CXCR3* High
CD274	2.63	6.89	0.65	1.98	2.52	7.22
PDCD1	0.24	0.89	0.27	0.83	0.22	0.99
PDCD1LG2	0.88	2.23	0.87	2.31	0.86	2.36
CTLA4	0.72	3.15	0.79	3.03	0.68	3.41
LAG3	2.10	6.56	1.97	6.87	2.00	6.81
HAVCR2	12.74	26.57	11.77	28.02	11.77	28.28
FOXP3	1.49	4.27	1.51	4.14	1.38	4.57
IDO1	0.88	5.93	0.90	6.26	0.93	5.59
TNFSF14	0.29	0.60	0.29	0.59	0.26	0.65
TIGIT	0.49	2.27	0.56	2.15	0.47	2.50
BTLA	0.67	2.88	0.77	2.57	0.59	3.13
CEACAM1	22.34	28.27	20.62	29.48	18.89	31.58
CD47	43.23	67.56	39.27	73.44	41.42	69.50

Low TPM (Median) — High TPM (Median)

CXCL9-H, CXCL10-H and CXCR3-H had higher expression of immune-checkpoint genes. All q<0.05.

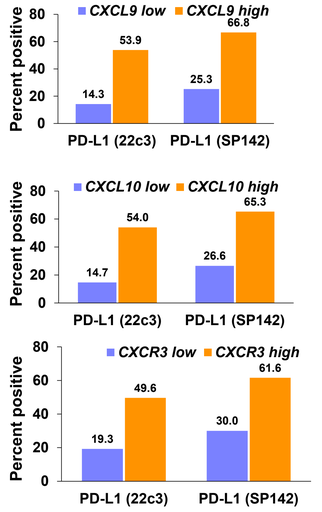
Fig 3. Immune cell infiltration

	CXCL9 Low	*CXCL9* High	*CXCL10* Low	*CXCL10* High	*CXCR3* Low	*CXCR3* High
B cell	3.51	4.39 *	3.62	4.26 *	3.36	4.59 *
MΦ M1	2.4	3.79 *	2.19	3.99 *	2.47	3.71 *
MΦ M2	2.63	3.37 *	2.77	3.21 *	2.46	3.52 *
Neutrophil	4.54	4.15 *	4.39	4.21 *	4.34	4.28 *
NK cell	2.9	2.83	2.89	2.86	2.77	2.95
DC	2.87	3.04	2.8	3.13	2.83	3.06
T cell CD8+	0	1.22 *	0	1.04 *	0	1.27 *
Tregs	0.99	2.53 *	1.13	2.37 *	0.99	2.6 *

Low Median% — High Median%

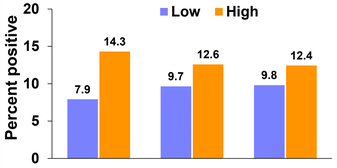
CXCL9-H, CXCL10-H and *CXCR3-H* elevated B cells, M1, M2 MΦ and CD8 T cells, Tregs infiltration, but not neutrophils. *q<0.05.

Fig 4. PD-L1 positivity



CXCL9-H, CXCL10-H and CXCR3-H had higher PD-L1 positivity, all q<0.05

Fig 5. TMB-high analysis



CXCL9-H, CXCL10-H and CXCR3-H had TMB-high, all q<0.05

CONCLUSIONS

High *CXCL9/CXCL10/CXCR3* expression is associated with longer survival in patients with TNBC post pembrolizumab and characterized by an immune-enriched TME. Further investigation is needed to evaluate this chemokine axis in TNBC and its potential as a therapeutic target to enhance ICI efficacy.