

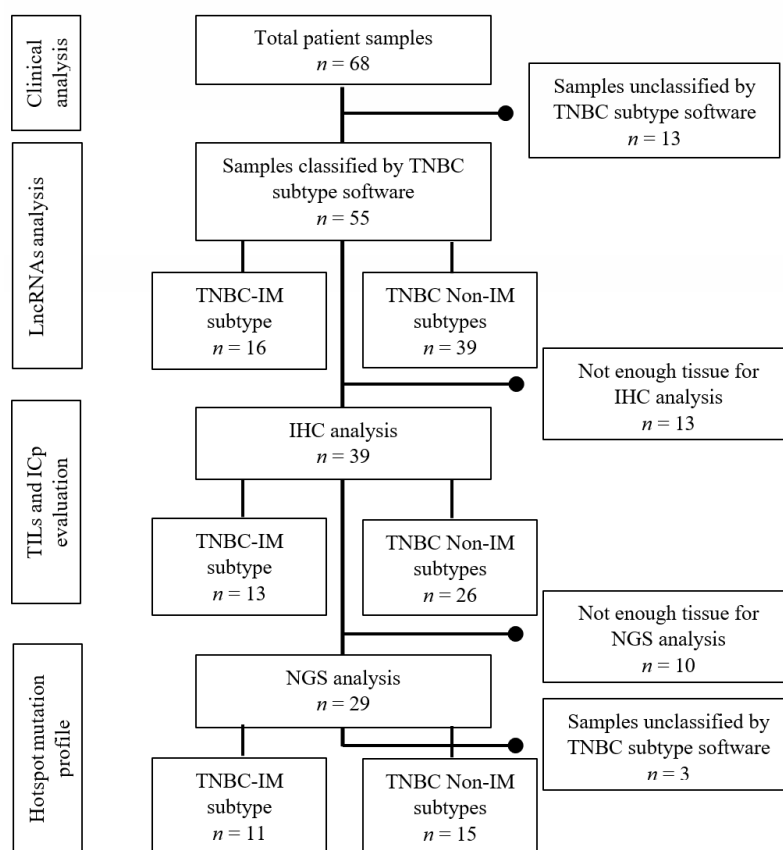
## Supplementary Materials

# Immune Milieu and Genomic Alterations Set the Triple-Negative Breast Cancer Immunomodulatory Subtype Tumor Behavior

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**Figure S1.** Distributions of the Vanderbilt subtypes in Mexican women with triple-negative breast cancer (TNBC) ( $n = 68$ ): basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), luminal androgen receptor (LAR), mesenchymal (M), and mesenchymal stem-like (MSL). Another 11 samples could not be identified by this software with a significant confidence level.



**Figure S2.** Strobe flow diagram. TNBC: Triple negative breast cancer; IM: immunomodulatory; IHC: immunohistochemistry; NGS: next generation sequencing; lncRNAs: long non-coding RNAs (lncRNAs); TILs: Tumor infiltrating lymphocytes; ICp: immune checkpoints.

**Table S1.** Gene content of cancer panel.

Genes
<i>AKT1</i> (3), <i>ALK</i> (21–25), <i>BRAF</i> (11, 15), <i>CDK4</i> (2), <i>CDKN2A</i> (1 *, 2, 3), <i>CTNNB1</i> (3), <i>DDR2</i> (18), <i>DICER1</i> (24, 25), <i>EGFR</i> (18–21), <i>ERBB2</i> (8, 17, 20), <i>ERBB4</i> (10, 12), <i>FBXW7</i> (8–12), <i>FGFR1</i> (13, 15), <i>FGFR2</i> (7, 12, 14), <i>FGFR3</i> (7, 9, 14, 16), <i>FOXL2</i> (1 *), <i>GNAI1</i> (4, 5), <i>GNAQ</i> (4, 5), <i>GNAS</i> (8), <i>H3F3A</i> (2 *), <i>H3F3B</i> (2 *), <i>HIST1H3B</i> (1), <i>HRAS</i> (2–4), <i>IDH1</i> (4), <i>IDH2</i> (4), <i>KIT</i> (8–11, 13, 17, 18), <i>KRAS</i> (2–4), <i>MAP2K1</i> (2, 3), <i>MET</i> (2, 14–20), <i>MYOD1</i> (1), <i>NRAS</i> (2–4), <i>PDGFRA</i> (12, 14, 18), <i>PIK3CA</i> (2 *, 3, 6 *, 8, 10, 21), <i>PTPN11</i> (3), <i>RAC1</i> (3), <i>RAF1</i> (7, 10, 12, 13 *, 14 *, 15 *), <i>RET</i> (11, 13, 15, 16), <i>ROS1</i> (38 *, 41 *), <i>SF3B1</i> (15–17), <i>SMAD4</i> (8–12), <i>TERT</i> (promoter *, 1 *, 8 *, 9 *, 13 *), <i>TP53</i> (full coding region)

Exon number in parentheses, \* Hotspots only.

**Table S2.** Type of recurrence sites of stage III patients ( $n = 57$ ).

Recurrence	%( $n/n$ )
No	29.8 (17/57)
Yes	70.2 (40/57)
CNS recurrence	43.9 (25/57)
Metastases Other Sites no CNS	
Visceral	29.8 (17/57)
No Visceral	5.3 (3/57)
Both	14.0 (8/57)
Metastases to Lung	36.8 (21/57)
Metastases to Liver	19.3 (11/57)
Metastases to Bone	21.1 (12/57)

CNS: Central Nervous System.

**Table S3.** Differential gene expression analysis coding and long non-coding regions.

Gene ID	LOGFC	AVEEXPR	t-Test	p Value	ADJ. p Value	B
<i>FDCSP</i>	2.943361527	6.850946853	3.955305312	0.000208904	0.01170946	0.515853226
<i>IGKC</i>	2.24664711	7.356507915	3.954025916	0.000209787	0.01173637	0.511982038
<i>IGHM</i>	2.231013275	6.286047314	3.847805474	0.000297036	0.01551561	0.193058658
<i>IGKC</i>	2.123936034	8.079436342	3.918282083	0.000235952	0.012891516	0.404112859
<i>IGHA1</i>	2.103705005	5.952013419	4.292874769	$6.72 \times 10^{-5}$	0.005139488	1.56053933
<i>IGKC</i>	2.069324519	8.551306661	4.440568321	$4.04 \times 10^{-5}$	0.003517919	2.031150831
<i>IGHG1</i>	2.013379674	7.688921093	4.268084854	$7.31 \times 10^{-5}$	0.00546486	1.482316947
<i>IGHV4-31</i>	1.982978135	8.208467165	4.08699288	0.000134883	0.008346984	0.917983833
<i>IGLV1-44</i>	1.981639885	3.70740469	4.957223829	$6.45 \times 10^{-6}$	0.00092192	3.732406847
<i>IGHG1</i>	1.96910446	9.086445794	4.905015932	$7.79 \times 10^{-6}$	0.001037077	3.55701903
<i>IGKC</i>	1.938970572	6.809754967	4.620531925	$2.15 \times 10^{-5}$	0.002295999	2.614586371
<i>CXCL13</i>	1.818226308	5.593121881	6.145128678	$7.61 \times 10^{-8}$	$7.92 \times 10^{-5}$	7.871888858
<i>IGLL5</i>	1.785391259	10.22665725	4.951568066	$6.59 \times 10^{-6}$	0.000931851	3.71337264
<i>IGKV<math>\equiv</math></i>	1.745843704	7.592397044	4.980519724	$5.93 \times 10^{-6}$	0.000860145	3.810894316
<i>CXCL9</i>	1.731316043	7.966204912	4.261334825	$7.48 \times 10^{-5}$	0.005535427	1.461057195
<i>IGKC</i>	1.695100885	6.158572658	4.898921853	$7.97 \times 10^{-6}$	0.001050526	3.536593105
<i>IGKC</i>	1.653521857	10.01740769	3.907324131	0.000244584	0.013275411	0.3711542
<i>IGKV3D-15</i>	1.649032155	8.347893953	4.799719601	$1.14 \times 10^{-5}$	0.001381514	3.205503434
<i>TRAJ17</i>	1.645074727	5.680195043	5.019889742	$5.14 \times 10^{-6}$	0.000764423	3.943849061
<i>IGHG1</i>	1.578146078	6.779645809	4.255736544	$7.63 \times 10^{-5}$	0.00558278	1.443437827
<i>IGLV3-25</i>	1.567399741	8.841218986	3.807293988	0.000338748	0.017111742	0.072741306
<i>CXCL10</i>	1.549755728	8.615580185	4.207080894	$9.00 \times 10^{-5}$	0.006305284	1.290800526
<i>IGK</i>	1.533307511	9.65561248	4.459600572	$3.78 \times 10^{-5}$	0.003358301	2.092347901
<i>GBP4</i>	1.467080145	7.179058901	4.890195916	$8.22 \times 10^{-6}$	0.001069571	3.507363001
<i>IGKC</i>	1.462262791	9.112632218	4.133286272	0.000115473	0.007430553	1.061031265
<i>YME1L1</i>	1.454346779	6.020678752	6.511737909	$1.86 \times 10^{-8}$	$4.52 \times 10^{-5}$	9.187052419
<i>CD38</i>	1.45006825	6.098312657	5.997847749	$1.34 \times 10^{-7}$	$9.73 \times 10^{-5}$	7.346951261
<i>IRF4</i>	1.442298063	6.352035066	5.180562042	$2.86 \times 10^{-6}$	0.000491682	4.490319118
<i>FCRL5</i>	1.434365642	5.104564812	4.892925942	$8.14 \times 10^{-6}$	0.00106385	3.516505848
<i>IGHM</i>	1.408334848	7.384230397	5.363446283	$1.45 \times 10^{-6}$	0.000353343	5.119307099
<i>CD79A</i>	1.398772653	6.883134059	5.902172681	$1.92 \times 10^{-7}$	0.000123025	7.007260681
<i>CXCR2P1</i>	1.384830625	6.064015095	6.05509216	$1.07 \times 10^{-7}$	$8.67 \times 10^{-5}$	7.550705343
<i>PIM2</i>	1.383637253	7.001465215	5.567678968	$6.79 \times 10^{-7}$	0.000232898	5.829458072
<i>TRAV12-2</i>	1.375753055	5.361220858	5.097362895	$3.88 \times 10^{-6}$	0.00062071	4.206589283
<i>YME1L1</i>	1.370614713	4.836545567	5.825355882	$2.57 \times 10^{-7}$	0.000138742	6.735358162
<i>IGHG1</i>	1.351807848	5.847076781	4.467101487	$3.68 \times 10^{-5}$	0.003291861	2.116500313
<i>UBD</i>	1.335481789	7.503789199	4.853240859	$9.39 \times 10^{-6}$	0.001184665	3.383797741
<i>TENT5C</i>	1.334892171	7.181763181	4.605384024	$2.27 \times 10^{-5}$	0.002378397	2.565076177
<i>FAM30A</i>	1.265925483	6.835648924	5.825294045	$2.57 \times 10^{-7}$	0.000138742	6.735139598
<i>IL2RG</i>	1.255929871	7.464312792	6.776048603	$6.69 \times 10^{-9}$	$2.36 \times 10^{-5}$	10.14048779
<i>CCL19</i>	1.22677208	7.651253468	3.861529069	0.00028406	0.014944919	0.233984184
<i>IGLC1</i>	1.219948285	7.607714531	4.548842393	$2.77 \times 10^{-5}$	0.002734128	2.380911705
<i>SLAMF7</i>	1.213199925	6.574147652	6.280428912	$4.53 \times 10^{-8}$	$5.79 \times 10^{-5}$	8.356010201
<i>MZB1</i>	1.197273079	7.35465191	4.944604621	$6.75 \times 10^{-6}$	0.000941908	3.689948804
<i>TRAV20</i>	1.165609208	3.733066918	5.33860379	$1.60 \times 10^{-6}$	0.000360407	5.03346172
<i>SLAMF6</i>	1.152327487	4.772127079	5.888984425	$2.02 \times 10^{-7}$	0.000123025	6.960524665
<i>TRAV20</i>	1.130564502	5.911463894	6.07646435	$9.90 \times 10^{-8}$	$8.56 \times 10^{-5}$	7.626869084
<i>GBP5</i>	1.118917883	6.130462332	4.805756945	$1.11 \times 10^{-5}$	0.001366469	3.225575643
<i>LAX1</i>	1.114841438	5.691522904	5.47767354	$9.51 \times 10^{-7}$	0.000269933	5.515558511
<i>ANKRD44-IT1</i>	1.114619595	5.398345928	7.186071885	$1.36 \times 10^{-9}$	$2.14 \times 10^{-5}$	11.62370392
<i>OR6C4</i>	1.099240147	6.444723972	5.30191346	$1.83 \times 10^{-6}$	0.000394399	4.906901709
<i>YME1L1</i>	1.096860683	5.04075177	4.765745683	$1.28 \times 10^{-5}$	0.001540635	3.092743426
<i>YME1L1</i>	1.093800838	4.617918367	3.811595136	0.000334065	0.016933924	0.085480333
<i>GPR174</i>	1.093060811	4.102078365	6.292250112	$4.33 \times 10^{-8}$	$5.79 \times 10^{-5}$	8.398384236
<i>SEL1L3</i>	1.090322848	7.044401313	5.533272873	$7.73 \times 10^{-7}$	0.000250206	5.709297909
<i>CCR2</i>	1.078606635	6.115620598	5.275981805	$2.01 \times 10^{-6}$	0.000407161	4.817619124
<i>VCAM1</i>	1.065525166	6.15650004	4.501200371	$3.27 \times 10^{-5}$	0.003083731	2.226533783
<i>BTG2</i>	1.054206604	9.076613637	5.35724828	$1.49 \times 10^{-6}$	0.000356297	5.097877918
<i>IGKC</i>	1.039223904	7.412084324	4.303013122	$6.49 \times 10^{-5}$	0.005084913	1.592595057
<i>YME1L1</i>	1.037853606	6.770696289	4.975084582	$6.05 \times 10^{-6}$	0.000872718	3.792570132
<i>TRDV2</i>	1.037646389	3.670155434	5.215321289	$2.51 \times 10^{-6}$	0.000458124	4.609318714
<i>MIR142</i>	1.033700489	4.774926725	4.763015112	$1.30 \times 10^{-5}$	0.001548397	3.083694906

<i>CCL5</i>	1.033222203	7.59358146	5.876897681	$2.12 \times 10^{-7}$	0.000123025	6.917711683
<i>IFNG</i>	1.029312304	4.65923508	6.399562831	$2.87 \times 10^{-8}$	$5.61 \times 10^{-5}$	8.783557791
<i>YME1L1</i>	1.028751756	3.414385854	5.601535183	$5.98 \times 10^{-7}$	0.000215255	5.94789292
<i>CD2</i>	1.027183095	7.216852064	5.808298453	$2.74 \times 10^{-7}$	0.000138742	6.675088041
<i>SLFN12L</i>	1.017642293	5.541069054	6.150462534	$7.46 \times 10^{-8}$	$7.92 \times 10^{-5}$	7.890941865
<i>IL23A</i>	1.013598461	5.190110719	4.729454739	$1.46 \times 10^{-5}$	0.001689798	2.972659022
<i>TRAV12-2</i>	1.012090486	5.930724244	4.299928847	$6.56 \times 10^{-5}$	0.005084913	1.582839135
<i>ITGAL</i>	1.008762873	6.514419207	6.115592128	$8.52 \times 10^{-8}$	$8.28 \times 10^{-5}$	7.766432672
<i>IGKC</i>	1.008642719	5.57773397	5.540534135	$7.52 \times 10^{-7}$	0.000249032	5.734640302
<i>BCL2A1</i>	1.007547625	5.561778583	4.024974657	0.000165882	0.009729212	0.727703982
<i>YME1L1</i>	1.006633651	4.265723623	4.872486364	$8.76 \times 10^{-6}$	0.001116699	3.448102231
<i>CTSS</i>	1.005838028	7.306051985	5.04659995	$4.66 \times 10^{-6}$	0.000708075	4.03426944

LOFFC: Log Fold chance; AVEEXP: average expression; ADJ: adjusted.

**Table S4.** Clinical and pathological characteristics of TNBC patients with IM and non-IM subtypes.

Clinical Characteristics	IM	Non-IM	<i>p</i> -Value
	%( <i>n</i> / <i>n</i> )	%( <i>n</i> / <i>n</i> )	
Hormonal Status			
Premenopausal	73.3(11/15)	45.2(19/42)	-
Postmenopausal	26.7(4/15)	54.8(23/42)	0.057
Vascular Invasion			
Yes	66.7(10/15)	33.3 (14/42)	-
No	33.3(5/15)	40.4(28/42)	0.026 *
PCR			
Yes	45.5(5/11)	23.3(7/30)	-
No	54.5(6/11)	76.6(23/30)	0.160
Recurrence			
Yes	33.3 (5/15)	76.2(32/42)	-
No	66.7 (10/15)	23.8 (10/42)	0.004 *
Lung Metastases			
Yes	0(0/15)	50 (21/42)	-
No	100 (15/15)	50 (21/42)	<0.001 *
Liver Metastases			
Yes	0 (0/15)	26.2 (11/42)	-
No	100 (15/15)	73.8 (31/42)	0.023 *
Bone Metastases			
Yes	6.7 (1/15)	26.2 (11/42)	-
No	93.3 (14/15)	73.8 (31/42)	0.106
CNS Metastases			
Yes	20 (3/15)	47.6 (20/42)	-
No	80 (12/15)	52.4 (22/42)	0.022 *

Bivariate analysis by chi-square/Fisher exact for the association between the dependent variable (molecular subtypes) and independent variables (clinical and pathological variables). PCR: complete pathological response; CNS: central nervous system; EGFR: epidermal growth factor receptor; \*  $p < 0.05$ .

**Table S5.** Univariate and multivariate analysis with RFS and OS of TNBC patients.

Variable	RFS				OS			
	Univariate Analysis		Multivariate Analysis		Univariate Analysis		Multivariate Analysis	
	HR (95%CI)	<i>p</i> -value	HR (95% CI)	<i>p</i> -value	HR (95%CI)	<i>p</i> -value	HR (95%CI)	<i>p</i> -value
Stage								
III	-		-		Reference	-	-	
IV					2.4 (1.2–4.7)	0.014 *		
Mastectomy								
Done	Reference	-	Reference	-	Reference	-	Reference	-
Not Done	2.7 (1.3–5.5)	0.007 *	3.6 (1.6–7.7)	0.001 *	2.3(1.3–4.1)	0.004 *	1.9(1.1–3.5)	0.037 *
Recurrence								
No	Reference		-		Reference	-	-	
Yes	36.8 (4.9–276.4)	<0.001 *			36.8(4.9–276.4)	<0.001 *		
RT to SNC								
No	Reference	-	-	-	Reference	-	Reference	-
Yes	3.73 (1.9–7.3)	<0.001 *	2.6 (1.3–5.5)	0.007 *	3.5(1.9–6.3)	<0.001 *	2.4(1.3–4.5)	0.008 *
Metastasis								
None	Reference	-			Reference	-		
Visceral	3.2 (1.5–6.8)	0.004 *			2.6 (1.4–5.1)	0.004 *		
Non Visceral	3.7 (1.03–13.5)	0.045 *	-		1.9 (0.5–6.5)	0.328	-	
Both	3.3 (1.2–9)	0.019 *			2.9(1.2–7.1)	0.017 *		
Immunomodulator								
Non-IM	Reference	-	Reference	-	Reference	-	Reference	-
IM	0.3 (0.1–0.8)	0.011 *	0.3 (0.1–0.9)	0.031 *	0.3(0.1–0.7)	0.007 *	0.4(0.2–0.9)	0.038 *
Bone Metastasis								
No	Reference	-			Reference	-	-	
Yes	2.01 (0.96–4.24)	0.065	-		1.6 (0.9–3)	0.124		
Lung Metastases								
No	Reference	-			Reference	-	-	
Yes	1.91 (1–3.7)	0.054	-		2.2 (1.2–3.8)	0.007 *		
Liver Metastases								
No	Reference	-			Reference	-	-	
Yes	1.8 (0.84–3.83)	0.13	-		2.3(1.3–4.1)	0.006 *		
CNS Metastases								
No	Reference	-			Reference	-	-	
Yes	4.74 (2.3–9.8)	<0.001 *	-		4.5 (2.3–8.5)	<0.001 *		

TNBC: Triple negative breast cancer; rfs: recurrence-free survival; OS: overall survival (OS); HR: hazard ratio; RT: radiotherapy; CNS: central nervous system; IM: immunomodulatory (IM); \*  $p < 0.05$ .



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