SUPPLEMENTARY MATERIALS

Emens LA, Molinero L, Loi S, et al. Atezolizumab and *nab*-Paclitaxel in Advanced Triple-Negative Breast Cancer: Biomarker Evaluation of the IMpassion130 Study

Supplementary methods

Further detail on PD-L1 evaluation

Five pathologists from HistoGeneX who were trained by Roche Tissue Diagnostics/Ventana participated in scoring samples from the IMpassion130 study. HistoGeneX was required to be College of American Pathologists (CAP)/ Clinical Laboratory Improvement Amendments (CLIA) certified, among other standard operating procedural requirements. All pathologists were required to be board-certified anatomic pathologists (or equivalent), to undergo training and pass a proficiency test with a score of 85% or more at the PD-L1 IC 1% and IC 5% cutoffs. Further specifics on the training and validation can be found in the VENTANA PD-L1 (SP142) Assay package insert (https://www.accessdata.fda.gov/cdrh_docs/pdf16/p160002s009c.pdf).

Further detail on CD8 evaluation: CD8 immunohistochemistry and quantification

Stained slides were scanned using the Pannoramic SCAN (3DHISTECH, Budapest, Hungary) to obtain whole-slide images. A 20x Plan Apo objective (0.80 NA) and Hitachi (HV-F22CL) 3CCD progressive scan color camera with a resulting image resolution of 0.23 µm / pixel were used. JPEG image encoding with quality factor 80 and an interpolated focus distance of 15 with stitching in the scan options were chosen. Scanned images were examined using the Pannoramic Viewer (3DHISTECH, Budapest, Hungary) to check for image quality and to confirm that the entire tissue section was captured. Automated image analysis on the whole-slide images was configured in Definiens™ Architect (version 2.1.1; Definiens AG, Munich, Germany). Manual delineation of a central tumor region (CnTumor) was done based on hematoxylin and eosin evaluation of a serial section by a anatomic pathologist. With the help of a self-written Definiens™ Action Library, at the interface between malignant and adjacent normal tissue, a 1000-µm wide border (invasive margin region [IM]) centered around the perimeter was created. Tissue processing and imaging artifacts such as ruptured tissue, dirt or out-of-focus regions were excluded. To reduce analysis time, tiling was performed for CnTumor and IM, followed by systematic random sampling of minimum 35% of the tiles (at least 100 tiles need to be sampled). For both CnTumor and IM, the relative area of

marker-positive cells is calculated in Definiens™ Tissue Studio® (version 3.5.1) at 6.6X.

Supplementary Table 1. Comparison of baseline characteristics in biomarker-evaluable populations

Characteristic	ITT (n = 902)	CD8-BEP (n = 720)	sTILs-BEP (n = 892)	BRCA1/2-BEP (n = 612)	PD-L1 TC-BEP (n = 900)
Disease status, n (%)					
Locally advanced unresectable	88 (9.8)	77 (10.7)	88 (9.9)	60 (9.8)	88 (9.8)
Metastatic	812 (90.0)	641 (89.0)	802 (90.0)	550 (90.0)	810 (90.0)
N/A	2 (0.2)	2 (0.3)	2 (0.2)	2 (0.3)	2 (0.2)
Age group, n (%)					
<65 years	683 (75.7)	536 (74.4)	674 (75.6)	459 (75.0)	681 (75.7)
≥65 years	219 (24.3)	184 (25.6)	218 (24.4)	153 (25.0)	219 (24.3)
ECOG PS, n (%)					
0	526 (58.3)	418 (58.1)	522 (58.5)	363 (59.3)	525 (58.3)
≥1	374 (41.5)	300 (41.7)	368 (41.3)	248 (40.5)	373 (41.4)
N/A	2 (0.2)	2 (0.3)	2 (0.2)	1 (0.2)	2 (0.2)
Presence of liver metastases, n (%)					
Yes	238 (26.4)	181 (25.4)	234 (26.2)	145 (23.7)	238 (26.4)
No	664 (73.6)	537 (74.6)	658 (73.8)	467 (76.3)	662 (73.6)
Prior taxane treatment, n (%)					
Yes	464 (51.4)	347 (48.2)	457 (51.2)	306 (50.0)	462 (51.3)
No	438 (48.6)	373 (51.8)	435 (48.8)	306 (50.0)	438 (48.7)
Tumor PD-L1 status, n (%)					
IC0	533 (59.1)	317 (44.0)	526 (59.0)	310 (50.7)	532 (59.1)
IC1/2/3	369 (40.9)	403 (56.0)	366 (41.0)	302 (49.3)	368 (40.9)
Race, n (%)					
American Indian or Alaska Native	40 (4.4)	32 (4.4)	40 (4.5)	29 (4.7)	40 (4.4)
Asian	161 (17.8)	144 (20.0)	161 (18.0)	117 (19.1)	161 (17.9)
Black or African American	59 (6.5)	42 (5.8)	58 (6.5)	33 (5.4)	59 (6.6)
Multiple	5 (0.6)	4 (0.6)	5 (0.6)	1 (0.2)	5 (0.6)
Native Hawaiian/Pacific Islander	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.2)	1 (0.1)
White	609 (67.5)	475 (66.0)	601 (67.4)	409 (66.8)	607 (67.4)
Unknown/other	27 (3.0)	22 (3.1)	26 (2.9)	22 (3.6)	27 (3.0)
Presence of brain metastases, n (%)					
Yes	60 (6.7)	47 (6.5)	61 (6.8)	40 (6.5)	61 (6.8)
No	841 (93.2)	673 (93.5)	831 (93.2)	572 (93.5)	839 (93.2)
Nodal only disease, n (%)					
Yes	56 (6.2)	42 (5.8)	56 (6.3)	41 (6.7)	56 (6.2)
No	843 (93.5)	676 (93.9)	833 (93.4)	569 (93.0)	841 (93.4)
N/A	3 (0.3)	2 (0.3)	3 (0.3)	2 (0.3)	3 (0.3)
Number of metastatic sites, n/n (%)					
0-3	673 (74.6)	535 (74.3)	666 (74.7)	450 (73.5)	671 (74.6)
≥4	226 (25.1)	183 (25.4)	223 (25.0)	160 (26.1)	226 (25.1)
N/A	3 (0.3)	2 (0.3)	3 (0.3)	2 (0.3)	3 (0.3)
Time from last surgery until diagnosis of metastatic/unresectable disease, n (%)					
<24 months	276 (30.6)	220 (30.6)	271 (30.4)	202 (33.0)	274 (30.4)
≥24 months	289 (32.0)	218 (30.3)	286 (32.1)	179 (29.2)	289 (32.1)
N/A	337 (37.4)	282 (39.2)	335 (37.6)	231 (37.7)	337 (37.4)

BEP = biomarker-evaluable population; ECOG PS = Eastern Cooperative Oncology Group performance status; IC = tumor-infiltrating immune cells; ITT = intention to treat; N/A = not available; PD-L1 = programmed death-ligand 1; sTIL = stromal tumor-infiltrating lymphocyte.
Summary statistics are based on the full population indicated in the header. If the baseline characteristic was not available for all patients, the total number of patients evaluable for this characteristic is presented.

Supplementary Table 2. Baseline characteristics by PD-L1 IC status

Characteristic	PD-L1 IC <1% (n = 532)	PD-L1 ≥1% (n = 368)	*P* value
Disease status, n (%)			0.02
Locally advanced unresectable	41 (7.7)	47 (12.8)	
Metastatic	490 (92.1)	320 (87.0)	
N/A	1 (0.2)	1 (0.3)	
Age group, n (%)			0.63
<65 years	399 (75.0)	282 (76.6)	
≥65 years	133 (25.0)	86 (23.4)	
ECOG PS, n (%)			0.602
0	307 (57.7)	218 (59.2)	
1/2	307 (57.7)	218 (59.2)	
N/A	2 (0.4)	0	
Presence of liver metastases, n (%)			0.034
Yes	155 (29.1)	83 (22.6)	
No	377 (70.9)	285 (77.4)	
Prior taxane treatment, n (%)			0.725
Yes	192 (36.1)	270 (73.4)	
No	176 (33.1)	262 (71.2)	
Race, n (%)			0.811
Asian	95 (17.9)	66 (17.9)	
Black or African American	36 (6.8)	23 (6.3)	
White	356 (66.9)	251 (68.2)	
Unknown/other	47 (8.8)	26 (7.1)	
Presence of brain metastases, n (%)			0.88
Yes	35 (6.6)	26 (7.1)	
No	497 (93.4)	342 (92.9)	
Nodal only disease, n (%)			0.053
Yes	25 (4.7)	31 (8.4)	
No	505 (94.9)	336 (91.3)	
N/A	2 (0.4)	1 (0.3)	
Number of metastatic sites, n (%)			0.077
0-3	383 (72.0)	288 (78.3)	
≥4	147 (27.6)	79 (21.5)	
N/A	2 (0.4)	1 (0.3)	
Time from last surgery until diagnosis of metastatic/unresectable disease, n (%)			0.001
<24 months	136 (25.6)	138 (37.5)	
≥24 months	179 (33.6)	110 (29.9)	
N/A	217 (40.8)	120 (32.6)	

ECOG PS = Eastern Cooperative Oncology Group performance status; IC = tumor-infiltrating immune cells; N/A = not available; PD-L1 = programmed death-ligand 1.
Summary statistics are based on the full population indicated in the header. If the baseline characteristic was not available for all patients, the total number of patients evaluable for this characteristic is presented.

Supplementary Table 3. PD-L1 IC status in matched paired samples collected at the same time

PD-L1 IC status	Matched paired samples, n (%)	
Unchanged (IC <1%)	33 (48.5)	63 (92.6)
Unchanged (IC ≥1%)	30 (44.1)	
Changed	5 (7.4)	
Total	68 (100.0)	

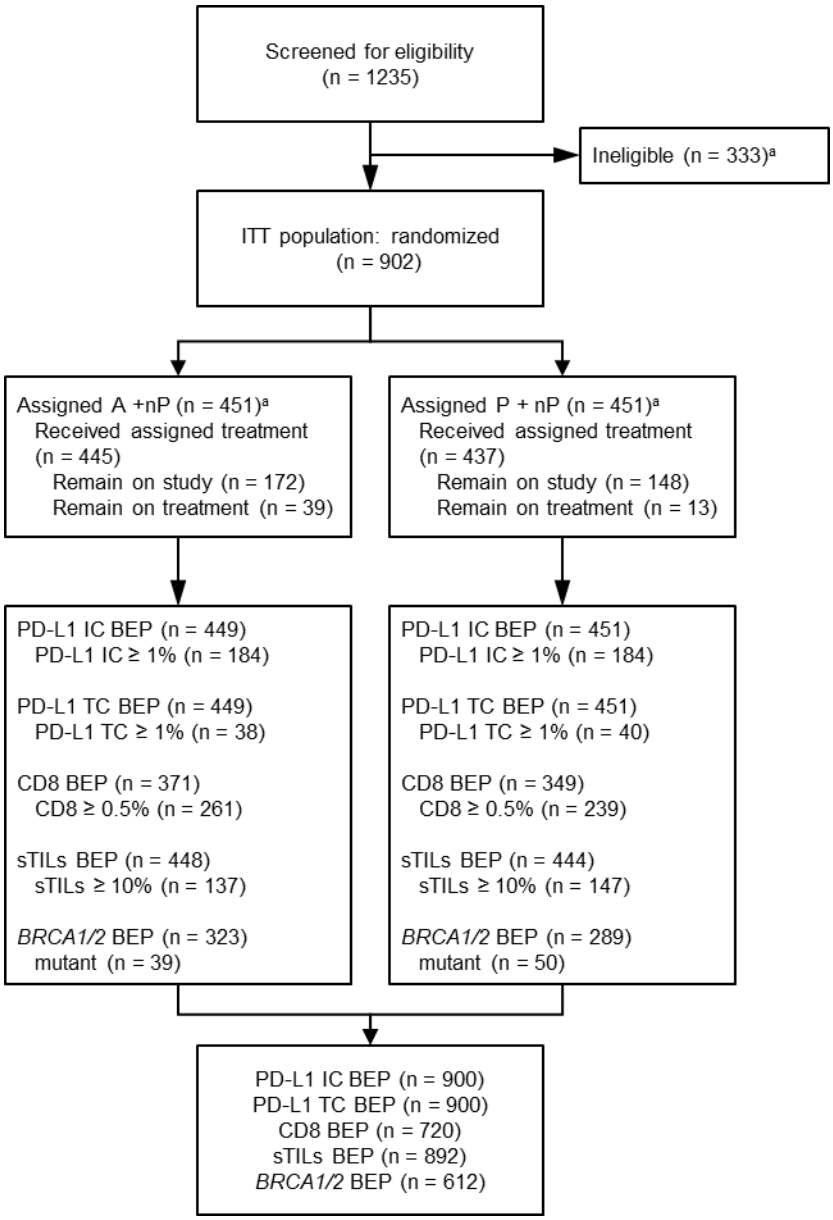
IC = tumor-infiltrating immune cell; PD-L1 = programmed death-ligand 1.
PD-L1 IC status agreement in synchronous samples: 92.6%.
Primary:metastases: 3 pairs; primary:primary: 50 pairs; metastases:metastases: 15 pairs.

Supplementary Table 4. PD-L1 IC status in matched primary:metastasis paired samples collected on different days

PD-L1 status		Metastases		
		IC <1%	IC ≥1%	Total
Primary tumor	IC <1%, n (%)	16 (43.2)	7 (18.9)	23 (62.2)
	IC ≥1%, n (%)	10 (27.0)	4 (10.8)	14 (37.8)
	Total, n (%)	26 (70.0)	11 (30.0)	37 (100.0)

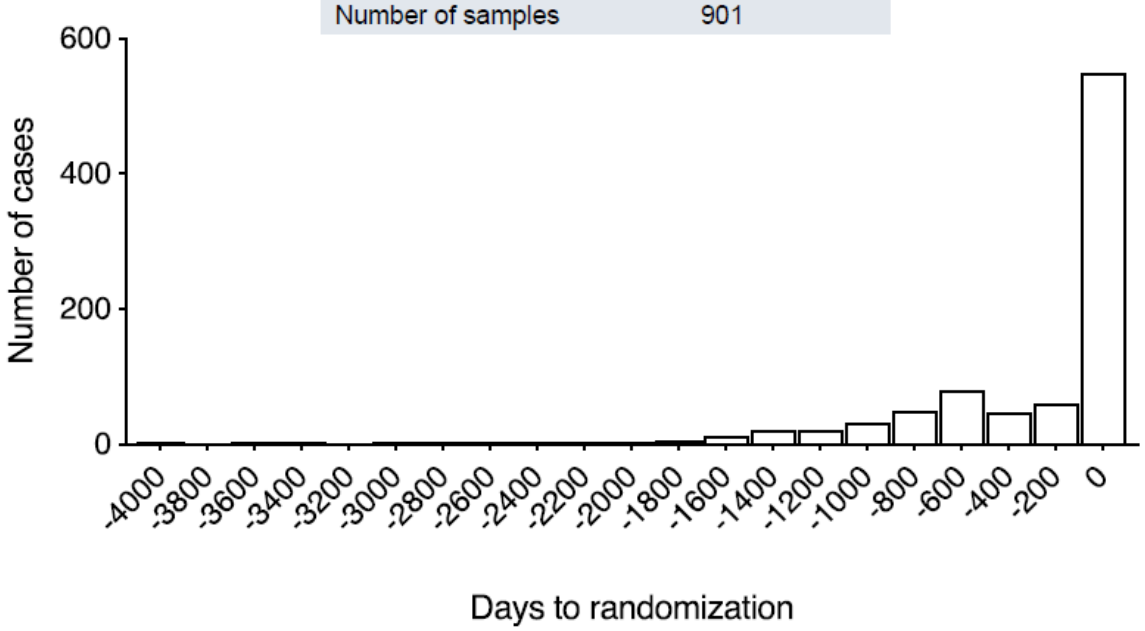
IC = tumor-infiltrating immune cell; PD-L1 = programmed death-ligand 1.
PD-L1 IC status agreement in asynchronous primary:metastases pairs: 54.1%.

Supplementary Figure 1. Study flowchart. BEPs include patients with evaluable tumor samples for the given biomarker; the main reasons for lack of biomarker-evaluable samples were exhaustion of slides or blocks and technical artifacts. [a] For additional details on screening ineligibility, treatment assignment, study and treatment discontinuation, death, and loss to follow-up, please see Schmid P, et al. *N Engl J Med*. 2018;379(22):2108-2121 and Schmid P, et al. *Lancet Oncol*. 2020;21(1):44-59. A = atezolizumab; BEP = biomarker-evaluable population; IC = tumor-infiltrating immune cells; ITT = intention to treat; nP = *nab*-paclitaxel; P = placebo; PD-L1 = programmed death-ligand 1; sTIL = stromal tumor-infiltrating lymphocyte; TC = tumor cells.



Supplementary Figure 2. Timing of sample collection. Time from collection to randomization is plotted in days.

	Collection relative day
Minimum	−3984
25th Percentile	−555
Median	**−61**
75th Percentile	−35
Maximum	−7
Number of samples	901



Supplementary Figure 3. Clinical validation of BEP vs intention-to-treat population (All). Kaplan-Meier plots for PFS and OS are shown for the indicated BEPs. A = atezolizumab; BEP = biomarker-evaluable population; nP = *nab*-paclitaxel; OS = overall survival; P = placebo; PFS = progression-free survival; TIL = tumor-infiltrating lymphocyte.