BreastCare
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Online Supplemental Tables

Supplemental Table 1. Immune checkpoints and co-stimulatory molecules and their targeting agents

Target	Agent[a]	Ligand	Agent[a]
Inhibitory molecules			
CTLA-4 (CD152)	Ipilimumab (trade name Yervoy) Tremelimumab (Ticilimumab, CP-675,206)	B7-1 (CD80), B7-2 (CD86)	NA
PD-1 (CD279)	Nivolumab (ONO-4538, BMS-936558, or MDX1106; trade name Optivo) Pembrolizumab (MK-3475 lambrolizumab, trade name Keytruda) Pidilizumab (CT-011)	PD-L1 (CD274; B7-H1)	BMS-936559 (MDX-1105); Atezolizumab (MPDL3280A); Durvalumab (MEDI4736); Avelumab (MSB00170718C);
		PD-L2 (CD273; B7-DC)	NA
LAG-3 (CD223)	BMS-986016	MHC class II	NA
BTLA (CD272)	NA	HVEM, TNFRSF14, CD270	NA
CD160	NA	HVEM (CD270)	NA
TIM-3 (CD366)	NA	Galectin-9	NA
VISTA (B7-H5, PD-1 homolog, Dies 1)	NA	Unknown	
B7-H3 (CD276)	MGA271	Unknown	
Unknown		B7-H4	NA
CD200R	NA	CD200 (OX2)	Samalizumab (ALXN6000)
TIGIT (Visig 9, Vstm3, WUCAM)	NA	PVR (CD155); PVRL2 (nectin-2; CD112)	NA
Co-stimulatory molecules			
CD226	NA	PVR (CD155); PVRL2, nectin-2 (CD112)	NA
CD28	TGN1412	B7-1 (CD80), B7-2 (CD86)	NA
OX-40	GSK3174998	OX40-L	NA
CD27 (TNFRSF7)	Valrilumab (CDX1127-06)	CD70	NA
4-1BB (CD137, TNFRSF9)	PF-05082566 BMS-66513	4-1BBL	NA
ICOS (CD278)	NA	ICOSL, B7-H2 (CD275)	NA
LIGHT (TNFSF14)	NA	HVEM	NA
LT	NA	HVEM	NA

[a]Clinically developed or under development agents.
NA= not available.

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Supplemental Table 2. Expression of PD-L1 in breast cancer lesions

Breast cancer lesion	Number of samples analyzed	Frequency of PD-L1 [a]	Clinical outcome relationship	Association with immune infiltrate [b]	Assay	PD-L1 positivity definition	Bibliography
Primary, stage I – III	44	Tumor: 22/44 (50) [c] TIL [d]: 18/44 (41)	No association with clinical outcome	Positive	IHC	Tumor: ≥1% membrane or cytoplasm staining TIL: ≥1%	Ghebeh et al., 2006
Primary, Stage I-III	650	152/650 (23.4 %) including the following subtypes: Luminal A: 10/83 (12.1) Luminal B HER2^{-}: 63/309 (20.4) Luminal B HER2^{+}: 21/73 (28.8) HERBB2-enriched: 19/56 (33.9) TNBC: 39/127 (30.7)	PD-L1 was an independent negative prognostic factor for OS ($p < 0.0001$) for different tumor subtypes except for luminal A breast cancer.	Positive	IHC	Modified H-score with a range of possible scores from 0 to 300, using a cut-off score of ≥ 100	Muenst et al., 2014
TNBC	105	20/105 (19)	NA	Positive	IHC	Tumor : ≥5% membrane staining	Mittendorf et al., 2014
Primary, Stage I-III	636	382/636 (60)	PD-L1 expression was associated with longer RFS (log-rank P = 0.01),	Positive	In situ mRNA hybridization	Automatic quantitative fluorescence (QIF) score of mRNA [e]	Schalper, et al., 2014
Primary, Stage I-III [f]	116	Luminal A: 11/33 (33) Luminal B: 8/25 (32) HERBB2-enriched: 1/5 (20) TNBC: 31/53 (59)	NA	Positive [g]	IHC	Histo-Score (H-Score) from 1+ to 3+, membrane and/ or cytoplasm staining	Gatalica et al., 2014
Invasive breast cancer, Stage I-III	870	189/870 (21,7) including the following subtypes: Luminal A: 37/321 (11.5) Luminal B HER2^{-}:27/314 (8.6) Luminal B HER2^{+}: 1/13 (7.7) TNBC: 124/222 (55.9)	PD-L1^{+} patients had poorer 5-year DFS (78.6% vs. 84.9%; P = 0.012), and OS (88% vs. 91.5%, P < 0.001) than those five-year of PD-L1-negative patients;	No correlation	IHC	Tumor : ≥5% membrane staining with or without cytoplasm staining	Qin et al., 2015
Primary, Stage I-III	3916	All cohort Tumor: 66/3916 (1.7) [h] TIL: 235/3916 (6%) TNBC	PD-L1 expression in >10% of immune cells was associated with reduced disease-specific mortality (***P*** = 0.08)	Positive	IHC	Tumor : ≥1% membrane staining with or without cytoplasm staining	Ali et al., 2015

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		Tumor: 24/302 (8%) TIL: 56/302 (19%)					
TNBC	161	Tumor: 103/161 (64) membrane staining; 129/161 (80) cytoplasm staining; TIL: 150/161 (93)	Both tumor cytoplasmic and TIL expression of PD-L1 was associated with OS (0.032 and 0.0042, respectively)	Positive	IHC	Tumor: ≥1% membrane or cytoplasm staining TIL: ≥1% [i]	Beckers et al., 2015
Primary Invasive, Stage I-III	5454	1076/5454 (20) including the following subtypes: Luminal A: 133/1515 (8.7) Luminal B: 186/1243 (15) Basal-like: 453/1205 (37.6) ERBB2-enriched: 227/841 (27)	PDL1 expression influenced MFS and OS (at 5 years 63 and 82%, respectively) in the basal-like subtype (P,<0.00005 and 0.0000003)	Positive	mRNA expression by microarray analysis	Expression in T was compared with mean expression in NB: upregulation was defined by a T/NB ratio ≥2 [l]	Sabatier et al., 2015
Invasive pre-treatment primary, stage I-III	306	non-IBC: 54/194 (28) IBC: 42/112 (38)	*PDL-1* expression was associated with pCR: 50% in the "PDL1-high" group *versus* 22% in the "PDL1-low" groups (p = 0.03). No association with OS.	Positive	mRNA expression by microarray analysis	Expression in T was compared with mean expression in NB: upregulation was defined by a T/NB ratio ≥2 [l]	Bertucci, et al., 2015
Primary, stage I – III	189	107/189 (56,6)	PD-L1+ patients had improved OS (p=0.04)	NA	IHC	An arbitrary score ranging from 0 to 8 was determined by the sum of scores for staining intensity and the percentage of stained cells	Baptista et al., 2016
	43	Tumor: 9/43 (21); TIL: 35/43 (81)	No patient with PD-L1+ disease recurred, 15% of PD-L1− patients developed a distant recurrence	Positive	IHC	Tumor : ≥5% membrane staining TIL: ≥5%	Cimino-Mathwes et al., 2016
Primary, stage I-III (treatment							

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naïve)							
Matched primary and metastatic	26	Primary Tumor: 2/24 (8) TIL: 14/26 (54); high concordance with PD-L1+ TIL in metastatic lesions	NA	No correlation	IHC	Tumor : ≥5% membrane staining TIL: ≥5%	Cimino-Mathwes et al., 2016
Primary, Stage I-III	316	292/316 (92.4)	No significant association between PD-L1 expression and DFS or OS	Negative	IHC	Histo-Score (H-Score) from 1+ to 3+, membrane or cytoplasm staining	Park et al., 2016

[a]frequency of PD-L1 in specific subtypes is reported only for large studies; [b]association between PD-L1 expression and immune infiltration, positive= positive correlation; negative = inverse correlation; [c]number in parenthesis is the % of positive lesions; [d]tumor infiltrating lymphocytes (TIL); [e]automatic quantitative fluorescence (QIF) score of mRNA signal in the tumor was calculated by dividing the target mRNA pixel intensities with the area of the tumor compartment defined by the staining with cytokeratin. A cutoff for PD-L1 mRNA positivity was defined as the noise threshold of the system, determined by using the average QIF score of negative control bacterial gene; [f]the correlation was observed only for TNBC; [f]this analysis has been performed in tumors with different histological origin; [g]only for TNBC; h: for additional tumors (METABRIC cohort) genomic data were available: among 44 of the previously defined IntClust10/Basal-like, 17 (39%) were PD-L1 immune cell positive; [i]the staining score of ≥5% was also used for PD-L1 staining and similar results were obtained (PD-L1 expression: 60% tumors with membrane staining, 77% tumors with cytoplasm staining and 93% of TILs; [l]T, tumor; NB, normal breast tissue.

IHC= Immunohistochemistry; OS= overall survival; NA= Not available; RFS: Recurrence-free survival; MFS: Metastasis free survival; IBC= Inflammatory breast cancer; non-IBC= non inflammatory breast cancer; pCR: pathological complete response.

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Supplemental Table 3. Immune checkpoint agents under clinical development in breast cancer

Target	Agent	Stage of clinical development	Tumor stage	Combination therapy	Status
CTLA-4	Ipilimumab (trade name Yervoy)	Phase I	Stage III and IV disease[a]	Following allogeneic hematopoietic stem cell transplantation	Completed
		Phase I	Early stage/resectable disease	Single-dose Ipilimumab and/or cryoablation	Completed
	Tremelimumab (Ticilimumab, CP-675,206)	Phase I	Advanced hormone-responsive disease	Exemestane	Completed
		Phase II	Advanced disease[a]	No	Active
PD-1	Nivolumab (ONO-4538, BMS-936558, or MDX1106; trade name Opti-vo)	Phase I and II	TNBC[a]	Etinostat (benzamide histone deacetylase inhibitor) and Ipilimumab	Active
		Phase I	Metastatic disease[a]	Nab-paclitaxel	Active
	Pembrolizumab (MK-3475 Lambrolizumab, trade name Keytruda)	Phase III	Metastatic TNBC	No	Active
		Phase I	HER-2$^-$ metastatic disease	No	Active
		Phase II	Metastatic TNBC	No	Active
		Phase I and II	TNBC[a]	IDO inhibitor (INCB024360)	Active
		Phase I and II	TNBC[a]	PLX3397 (multitargeted tyrosine kinase inhibitor)	Active
		Phase I and II	Metastatic TNBC	Eribulin mesylate (microtubule inhibitor)	Active
		Phase II	Metastatic or recurrent inflammatory disease	**No**	Active
		Phase II	Stage IV ER$^+$	Tamoxifen citrate and Vorinostat	Active
		Phase I	P53$^+$ TNBC[a]	p53MVA vaccine	Active
		Phase I	Metastatic disease	Hypofractionated radiotherapy	Active
	Atezolizumab (MPDL3280A)	Phase I	Metastatic TNBC	No	
		Phase III	Previously untreated metastatic TNBC	Chemotherapy	Active
		Phase I	Advanced disease[a]	Bevacizumab and/or chemotherapy	Active
		Phase I	Advanced TNBC[a]	IDO inhibitor GDC0919	Active
		Phase I	HER-2$^+$ metastatic or locally advanced disease	Trastuzumab, and Pertuzumab or Atezolizumab and Trastuzumab Emtansine	Active
		Phase I and II[a]	Relapsed or refractory Her-2$^+$ or TNBC	Ibrutinib	Active
		Phase II	TNBC before surgery	Paclitaxel albumin-stabilized nanoparticles	Approved-not yet active

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Target	Agent	Stage of clinical development	Tumor stage	Combination therapy	Status
	Durvalumab (MEDI4736)	Phase I	Metastatic relapsed or refractory disease[a]	Hypofractionated radiotherapy and Tremelimumab	Active
		Phase I and II	Advanced measurable disease with accessible biopsy[a]	Toll-like receptor agonist PolyICLC and Tremelimumab	Approved-not yet active
		Phase I and II	Stage I-III TNBC	Nab-paclitaxel followed by MEDI4736 concomitant with ddAC neoadjuvant chemotherapy	Approved-not yet active
	Avelumab (MSB00170718C)	Phase I	Metastatic or locally advanced disease[a]	No	Active
OX-40	GSK3174998	Phase I	Advanced TNBC[a]	Pembrolizumab	Active
CD27	Valrilumab (CDX1127-06)	Phase I and II	Advanced disease[a]	Atezolizumab	Active

TNBC= Triple negative breast cancer.
[a]Enrolling subjects with different types of tumor.