MINIREVIEWS

Immunotherapy in triple-negative breast cancer: A literature review and new advances

Guillermo Arturo Valencia, Patricia Rioja, Zaida Morante, Rossana Ruiz, Hugo Fuentes, Carlos A Castaneda, Tatiana Vidaurre, Silvia Neciosup, Henry L Gomez

Guillermo Arturo Valencia, Patricia Rioja, Zaida Morante, Rossana Ruiz, Hugo Fuentes, Carlos A Castaneda, Tatiana Vidaurre, Silvia Neciosup, Henry L Gomez, Department of Medical Oncology, Instituto Nacional de Enfermedades Neoplásicas, Lima 15036, Peru

Corresponding author: Guillermo Arturo Valencia, MD, Medical Assistant, Department of Medical Oncology, Instituto Nacional de Enfermedades Neoplásicas, Angamos Este Avenue 2520, Surquillo, Lima 15036, Peru. guillermo.valencia.mesias@gmail.com

Abstract

Triple-negative breast cancer (TNBC) is a highly complex, heterogeneous disease and historically has limited treatment options. It has a high probability of disease recurrence and rapid disease progression despite adequate systemic treatment. Immunotherapy has emerged as an important alternative in the management of this malignancy, showing an impact on progression-free survival and overall survival in selected populations. In this review we focused on immunotherapy and its current relevance in the management of TNBC, including various scenarios (metastatic and early -neoadjuvant, adjuvant-), new advances in this subtype and the research of potential predictive biomarkers of response to treatment.

Core Tip: Triple-negative breast cancer (TNBC) is an exceptionally heterogeneous disease and historically a cancer with limited treatment options other than chemotherapy. Recent advances in immunotherapy has changed the standard of care in selected groups, especially in metastatic TNBC. This article review continues the detailed, updated and comprehensive literature review regarding immunotherapy in TNBC, including the discussion of clinical trials in different scenarios (metastatic, neoadjuvant, adjuvant) and potential biomarkers to provide useful knowledge for medical oncologists and the medical community. Our goal is sharing updated information for TNBC which is considered an overlooked population with an enormous necessity of novel treatments and biomarkers.

INTRODUCTION

Triple-negative breast cancer (TNBC) which effects approximately 15 - 20% of all patients, is a heterogeneous, complex disease with a more aggressive behavior than other subtypes of breast cancer. It is associated with a high incidence of visceral metastasis (predominance of hepatic, pulmonary and central nervous system metastasis), a high risk of early recurrence and a worse prognosis[1]. Unlike other subtypes, historically, TNBC has had no other systemic treatment options other than chemotherapy which has been the cornerstone of treatment for many years. However, this has recently changed with the introduction of immunotherapy in patients with programmed death ligand 1 (PD-L1) expressing tumors, both in unresectable locally advanced/metastatic disease. In the neoadjuvant setting, the use of immunotherapy has recently been approved[1].

Based on efforts in genetic studies, breast cancer was divided into molecular subtypes. Perou *et al*[2] proposed a classification based on expression patterns, subdivided into 4 clinical molecular subtypes (luminal A, luminal B, HER2 enriched and basal-like). Most basal-like tumors are included in TNBCs (they represent 70%-80% of the TNBCs)[3]. Lehmann *et al*[4] identified 6 different subtypes using DNA and RNA profiles in TNBC ["basal-like 1" (BL1), "basal-like 2" (BL2), "immunomodulatory" (IM), "mesenchymal" (M), "mesenchymal stem-like" (MSL) and "luminal androgen receptor" (LAR)] each with particular characteristics. BL1 and IM tumors have a higher sensitivity to DNA-damaging agents such as platinum and are associated with a young age at diagnosis. They are also the subtype with the highest pathological complete response (pCR) rate (65.6%) followed by BL2 (36.4%) in a cohort of patients treated with platinum-based neoadjuvant therapy (n = 97). The LAR subtype has the lowest pCR rate (21.4%)[4].

Although breast cancer has traditionally been considered a non-immunogenic tumor, multiple studies have shown that TNBC can stimulate the immune system. Compared with luminal breast cancer, TNBC has a higher tumor mutational burden (TMB), elevated levels of PD-L1 expression and increased levels of tumor infiltrating lymphocytes (TILs) in the tumor microenvironment which are associated with higher rates of pCR to neoadjuvant chemotherapy and efficacy to immunotherapy which justifies the use of immunotherapy in this subtype[5].

Due to advances in the molecular characterization of TNBC, with addition of immunotherapy, new therapeutic agents including poly ADP-ribose polymerase-1 (PARP) inhibitors, tyrosine kinase inhibitors (TKI), checkpoint inhibitors, antiandrogens, antibody-drug conjugates (ADC) and other targeted therapies are being researched. Moreover, ongoing trials are evaluating immunotherapy (immune checkpoint inhibitors) in combination with PARP inhibitors in a series of cancers including BC [6].

IMMUNOTHERAPY AGENTS APPROVED IN TNBC

The high mutational burden of the TNBC was determined to lead to the synthesis of abnormal proteins, acting as "neoantigens" which will be recognized by the antigen presenting cells and would initiate an antitumor immune response[7].

Early-stage TNBC has a high TIL infiltrate but breast cancer has not traditionally been considered immunogenic. Recent trials demonstrate TIL infiltrate has a high expression of PD-1 (and other inhibitory checkpoint molecules). TNBC has potential therapeutic targets such as immune checkpoint inhibitors (ICIs) (anti-PD-1/PD-L1 agents) in metastatic and the early-stage scenario[8] (Table 1).

Table 1 Randomized phase II/III immunotherapy trials en triple-negative breast cancer

Scenario	Trial	Phase	n	Intervention	Recruitment Status	Magnitude of clinical benefit		
						PFS (mo)	OS (mo)	Additional information
Neoadjuvant	NCT03639948	II	100	Carboplatin + Docetaxel + Pembrolizumab	Recruiting			
	NCT03289819	II	50	Pembrolizumab + Nab-paclitaxel → Pembrolizumab + Epirubicin and Cyclophosphamide	Recruiting			
	NCT03356860 (B-IMMUNE)	II	57	Paclitaxel + Epirubicin + Cyclophosphamide + Durvalumab	Recruiting			
	NCT02685059 (GeparNuevo) (June 2018)	II	174	Epirubicin + Nab-paclitaxel + Cyclophosphamide + Durvalumab	Active, no recruiting	-	-	
				Population: Early TNBC				pCR was increased to 53.4% with Durvalumab *vs* 44.2% with chemotherapy alone, not being statistically significant (P = 0.048)
								In the PD-L1 (+) subgroup: pCR 58% *vs* 50.7% (P = 0.363)
								pCR was increased in patients with high levels of TILs y TMB-H (P < 0.01)
								3-yr iDFS was 84.9% with durvalumab *vs* 76.9% with placebo (HR: 0.54, 0.27-1.09, P = 0.0559); 3-yr DDFS 91.4% *vs* 79.5% (HR: 0.37, 0.15-0.87, P = 0.0148); 3-yr OS: 95.1% *vs* 83.1% (HR: 0.26, 0.09-0.79, P = 0.0076)
Neoadjuvant/Adjuvant	NCT03036488 (KEYNOTE-522) (August 2020)	III	1174	Carboplatin + Paclitaxel + AC (anthracycline + cyclophosphamide) +/- Pembrolizumab → Adjuvant Pembrolizumab	Active, no recruiting	-	-	Co-primary endpoints were pCR and EFS
				Population: Early TNBC				pCR: 64.8% in Pembro group *vs* 51.2% with placebo (P < 0.001)
								The benefit of Pembro in pCR was consistent in all subgroups, including PD-L1 (+): pCR 68.9% *vs* 54.9% (P < 0.001)
								A statistically benefit was observed in EFS (HR: 0.63, 0.48-0.82)
								Pembro showed a favorable trend in OS (HR: 0.72, 0.52-1.02)
	NCT02620280 (NeoTRIPaPDL1)	III	280	Carboplatin/nab-paclitaxel +/- Atezolizumab → anthracycline (AC/EC)	Active, no recruiting	-	-	Primary endpoint was pCR

	(December 2019)			Population: Early TNBC		The pCR rates were not statistically significant between both groups: 43.5% with atezolizumab *vs* 40.8% with chemotherapy alone
						A multivariate analysis showed that the only variable associated with pCR was the PD-L1 (+) status: pCR 51.9% *vs* 48% ($P < 0.0001$)
						These results differ from KEYNOTE-522, where pembrolizumab achieved significant rates of pCR in a similar population
	NCT03281954	III	1520	Doxorubicin + Cyclophosphamide + Paclitaxel + Carboplatin +/- Atezol-izumab → Atezolizumab	Recruiting	
	NCT03197935 (IMpassion031) (September 2020)	III	204	AC (doxorubicin + cyclophosphamide) + Nab-paclitaxel +/- Atezolizumab → Adjuvant Atezolizumab	Active, no recruiting	pCR was 58% in Atezolizumab group *vs* 41% in placebo group ($P = 0.0044$)
				Population: Early TNBC		In the PD-L1 (+) population, pCR was 68.8% in the Atezolizumab group *vs* 49.3% in the placebo group ($P = 0.021$)
						A favorable trend was obtained in EFS (immature data) (HR: 0.76, 0.40 -1.44)
						In patients with early TNBC, neoadjuvant treatment of Atezolizumab + nab-paclitaxel and an anthracycline-based regimen achieve higher rates of pCR, with an acceptable safety profile
Adjuvant (for patients with residual disease after neoadjuvant chemotherapy)	NCT02954874	III	1000	Pembrolizumab *vs* observation	Recruiting	
	NCT03756298	II	284	Capecitabine +/- Atezolizumab	Recruiting	
Adjuvant	NCT03498716 (IMpassion030)	III	2300	Paclitaxel → dd Doxorubicin/Epirubicin + Cyclophosphamide +/- Atezolizumab	Recruiting	Primary endpoint was iDFS
						Secondary endpoints were iDFS according to PD-L1 status and nodal affectation, OS, safety, y health related to a QoL
	NCT02926196 (A-Brave)	III	335	Avelumab *vs* observation	Recruiting	This trial evaluates patients in two groups: (1) Primary TNBC patients who completed surgery followed by adjuvant therapy; and (2) Primary TNBC patients with residual disease after neoadjuvant chemotherapy (did not achieve pCR)
						The first and second co-primary endpoints are DFS in all patients and DFS in B group
Locally advanced or mTNBC	NCT02768701	II	40	Cyclophosphamide + Pembrolizumab	Active, no recruiting	

NCT03121352	II	30	Carboplatin, Nab-paclitaxel y Pembrolizumab	Recruiting			
NCT02499367 (TONIC)	II	67	Control or irradiation 3 x 8 Gy or oral cyclophosphamide or Cisplatin or Doxorubicin → anti-PD-1 (Nivolumab)	Active, no recruiting			Five cohorts were included in the randomization, all followed by nivolumab Overall, the ORR was 20% Most responses were observed with cisplatin (ORR: 23%) and doxorubicin (ORR: 35%)
NCT02819518 (KEYNOTE-355) (December 2020)	III	858	Nab-paclitaxel or Paclitaxel or Carboplatin/Gemcitabine +/- Pembrolizumab Population: First-line mTNBC	Active, no recruiting	9.7 *vs* 5.6 (HR: 0.82) in CPS ≥ 10	-	Co-primary endpoints were PFS and OS (this latter is pending outcome) Pembro treatment was statistically significant only for patients with high levels of PD-L1 (expressed in CPS ≥ 10) Pembro + chemotherapy showed a significant increase in PFS among mTNBC patients A recent update showed that KEYNOTE-355 trial met primary endpoint of OS in patients with mTNBC whose tumors expressed PD-L1 (CPS ≥ 10)
NCT02555657 (KEYNOTE-119) (September 2019)	III	600	Capecitabine, Eribulin, Gemcitabine, or Vinorelbine *vs* Pembrolizumab Population: Second and third-line mTNBC	Active, no recruiting	2.1 *vs* 2.1 (HR: 1.14)	12.7 *vs* 10.7 (HR: 0.78)	Pembro did not show improvement in OS or PFS as 2L/3L of treatment for mTNBC *vs* chemotherapy (OS: 9.9 mo *vs* 10.8 mo, HR: 0.97, 0.82- 1.15) OS in tumors with CPS > 10: 12.7 mo *vs* 11.6 mo (HR: 0.78, 0.57-1.06) A greater benefit was obtained in OS/PFS in tumors with high levels of PD-L1 (expressed in the CPS score) Pembro was well tolerated and had less adverse events compared with chemotherapy
NCT02447003 (KEYNOTE-086) (March 2019)	II	285	Pembrolizumab monotherapy	Active, no recruiting	-	-	Primary endpoint: ORR in the total population and PD-L1 (+) ORR was 5.3% in the total population, and 5.7% in the PD-L1 (+) population Pembro demonstrated antitumor activity in patients previously treated with mTNBC (≥ 1 systemic treatments)
NCT02425891 (IMpassion130) (November 2018)	III	902	Atezolizumab + Nab-paclitaxel (comparator: placebo + Nab-paclitaxel)	Active, no recruiting	7.5 *vs* 5.5 (HR: 0.62, *P* < 0.001)	25.0 *vs* 15.5 (HR: 0.62)	In the analysis of the ITT population, the median PFS was 7.2 mo *vs* 5.5 mo (HR: 0.80, *P* = 0.002). In PD-L1 (+) patients, the median PFS was 7.5 mo *vs* 5.5 mo (HR: 0.62, *P* < 0.001)

			Population: First-line mTNBC				In the analysis of the ITT population, the median OS was 21.3 mo *vs* 17.6 mo (HR: 0.84, *P* = 0.08). In PD-L1 (+) patients, the median OS was 25.0 mo *vs* 15.5 mo (HR: 0.62)
							Final analysis showed that OS benefit with atezolizumab + nab-paclitaxel in the ITT population was not statistically significant, but a clinically meaningful OS benefit was observed in PD-L1 IC-(+) patients
	NCT03125902 (IMpassion131) (September 2020)	III	600	Paclitaxel +/- Atezolizumab (comparator: placebo + paclitaxel)	Active, no recruiting	5.7 *vs* 6.0 (HR: 0.82, *P* = .20) in PD-L1 (+) population	22.1 *vs* 28.3 (HR: 1.12) in PD-L1 (+) population	Primary endpoint was PFS
				Population: First-line mTNBC				In the ITT population, the median PFS was 5.7 mo in atezolizumab group *vs* 5.6 mo in placebo group (HR: 0.86)
							OS: 19.2 mo *vs* 22.8 mo (HR: 1.11, 0.87-1.42)
							The 2-yr OS rates were 51% and 49% in placebo and atezolizumab groups, respectively
	NCT03371017 (IMpassion132) (early recurrence)	III	350	Carboplatin + Gemcitabine or Capecitabine +/- Atezolizumab	Recruiting			Primary endpoint was OS
							Estimated completion date: July 2023

CPS: Combined positive score; dd: Dense dose; DDFS: Distant-disease free survival; DFS: Disease-free survival; EFS: Event-free survival; HR: Hazard ratio; IC: immune cells; iDFS: Invasive disease-free survival; ITT: Intention to treat; mTNBC: Metastatic triple-negative breast cancer; pCR: Pathological complete response; PFS: Progression-free survival; ORR: Objective response rate; OS: Overall survival; QoL: Quality of life; TNBC: Triple-negative breast cancer.

In the early stage scenario there are considerations for the addition of immunotherapy to chemotherapy in the neoadjuvant setting: the benefit of improving the pCR rates (KEYNOTE-522, IMpassio031), and the risks regarding toxicities (immune related adverse events in a potentially curable setting) and costs.

Atezolizumab

Atezolizumab is a humanized anti-PD-L1 monoclonal antibody, non-glycosylated IgG1 that binds to PD-L1 and blocks interaction with PD-1 and B7.1 (a co-stimulatory protein on the cell surface) that induces a reactivation of the antitumor immune response without antibody-induced cellular cytotoxicity [9].

Atezolizumab monotherapy in mTNBC: A phase I study (Schmid *et al*[10], 2017) that evaluated the safety and tolerability of atezolizumab single-drug (primary endpoints), demonstrated an antitumor activity and safety with the use of atezolizumab in patients with mTNBC (*n* = 116). It was also observed that the greatest benefit was in patients who received atezolizumab in the first line and among those with high levels of TILs and PD-L1 immune cells (IC)[10].

Other measured endpoints were overall survival (OS) (41% at 1 year, 19% at 2 years, and 16% at 3 years) and the PD-L1 IC ≥ 1% was associated with a higher objective response rate (ORR) (12% *vs* 0%) and higher OS (10.1 mo *vs* 6 mo, respectively). Atezolizumab was well tolerated and provides clinical

benefit in patients with mTNBC. 100% of the patients who responded to atezolizumab were alive at 1 year *vs* 38% of non-responders[10].

Atezolizumab + nab-paclitaxel in mTNBC (IMpassion130): The IMpassion130 (November 2018), phase III, randomized trial evaluated patients with mTNBC or unresectable locally advanced disease without previous treatment (*n* = 902) and regardless of PD-L1 expression, who were randomized (in a 1:1 ratio) to receive nab-paclitaxel (100 mg/m^2 on days 1, 8 and 15 every 28 d) in association with atezolizumab (840 mg IV on days 1 and 15 every 28 d) or with placebo until disease progression or limiting toxicity [11]. The two primary end points were progression-free survival (PFS) [in the intention-to-treat (ITT) population and PD-L1 positive subgroups] and OS (tested in the ITT population; if the finding was significant, it would be tested in the PD-L1 (+) subgroup). Stratification factors were: receipt or nonreceipt neoadjuvant or adjuvant taxane therapy, presence or absence of liver metastases at baseline, and PD-L1 expression at baseline (positive *vs* negative) according to immunohistochemical testing (Ventana SP142). The trial was initially designed to assign 350 patients for the evaluation of primary end point (PFS), but during the course of trial, enrollment was expanded to about 900 patients to accommodate the addition of OS as a second primary end point. 41% of the patients were PD-L1 (+) [11]. The possible rationale for using taxane-based chemotherapy is that it can enhance tumor antigen release and antitumor response to checkpoint inhibitors. Furthermore, nab-paclitaxel can promote dendritic cell activity and was used to avoid the interaction between atezolizumab and corticosteroids (under the rationale that the use of corticosteroids could decrease the immune response of anti-PD-L1 therapy). In addition, nab-paclitaxel has a decreased risk of hypersensitivity reactions and does not require corticosteroid treatment[12].

After a median follow-up of 12.9 mo in the ITT population, the addition of atezolizumab to nab-paclitaxel increased the median PFS (7.2 mo with atezolizumab + nab-paclitaxel *vs* 5.5 mo with placebo + nab-paclitaxel, hazard ratio [HR]: 0.80, 95% confidence interval [CI]: 0.69-0.92, *P* = 0.002), although this did not increase OS (21.3 mo with atezolizumab + nab-paclitaxel *vs* 17.6 mo with placebo + nab-paclitaxel, HR: 0.84, 95%CI: 0.69-1.02, *P* = 0.08). However, in the subgroup of PD-L1 (+) patients (defined as PD-L1 expression in tumor infiltrating immune cells ≥ 1% of the tumor area), the median PFS (7.5 mo *vs* 5.0 mo, HR: 0.62, 95%CI: 0.49-0.78, *P* < 0.001) and OS (25 mo *vs* 15.5 mo, HR: 0.62, 95%CI: 0.45-0.86) was improved with the combination of atezolizumab + nab-paclitaxel compared to placebo + nab-paclitaxel[11].

Regarding adverse events, the frequency of grade ≥ 3 adverse events (AEs) was 48.7% in the atezolizumab + nab-paclitaxel group and 42.2% in the placebo + nab-paclitaxel group, with neutropenia (8%), peripheral neuropathy (6%), fatigue (4%) and anemia (3%) being the most common events in both groups. Grade ≥ 3 immune-related events (irAEs) occurred in 7.5% and 4.5% of the atezolizumab + nab-paclitaxel and placebo + nab-paclitaxel groups, respectively. Authors conclude that atezolizumab + nab-paclitaxel prolonged PFS among patients with mTNBC in both ITT population and PD-L1 (+) subgroup [11].

An OS data update from a second interim analysis of a median follow-up of 18 mo showed an OS of 21.0 mo in the atezolizumab + nab-paclitaxel group *vs* 18.7 mo in the placebo + nab-paclitaxel group (*P* = 0.0777) on ITT. In the PD-L1 (+) subgroup, OS was 25.0 mo *vs* 18.0 mo (HR: 0.71). This update confirms the benefit in OS of the population with PD-L1 (+)[13]. Very recently, a final OS analysis from the IMpassion130 trial was published: final OS data from IMpassion130 agree with prior interim analysis. The OS benefit in the ITT population was not statistically significant (21.0 mo *vs* 18.7 mo, HR: 0.87, 95%CI: 0.75-1.02, *P* = 0.077). Data showed clinically meaningful OS benefit with the combination of atezolizumab + nab-paclitaxel in the PD-L1 IC-positive population (25.4 mo *vs* 17.9 mo, HR: 0.67, 95%CI: 0.53-0.86), 3-year OS rates in the PD-L1 group were 35.8% using atezolizumab + nab-paclitaxel *vs* 22.2% in the placebo group and no new safety events were reported with longer follow-up. The authors conclude that although OS benefit in the ITT population was not statistically significant, a clinical meaningful OS benefit was reported in PD-L1 IC-positive patients with atezolizumab + nab-paclitaxel. The statistical results of this trial (ITT population) were negative[14].

In conclusion, the combination of atezolizumab plus nab-paclitaxel prolongs PFS and OS in the mTNBC subgroup with PD-L1 (+) but not in the intention-to-treat (ITT) population, changing the treatment paradigm with patients in the metastatic setting. This combination has been initially included in international clinical practice guidelines (currently NCCN guidelines removed this option)[15] (IB, ESMO guidelines)[16] and the FDA (Food and Drug Administration) accelerated approval in March 2019 for its use in the treatment of patients with mTNBC or unresectable locally advanced disease with PD-L1 positive using a validated test[7]. This was the first approval of atezolizumab and of an immuno-therapy regimen for the treatment of breast cancer[17]. It is important to note that the FDA has granted accelerated approvals to oncology medicines on the basis of evidence that suggests a benefit to patients, however many immunotherapies (atezolizumab, pembrolizumab, nivolumab, durvalumab) approval are under evaluation since the approval is based on a surrogate endpoint and it requires a confirmatory trial with a clear benefit. In addition, four indications were voluntarily withdrawn by manufacturers (nivolumab in metastatic small cell lung cancer, durvalumab in locally advanced or metastatic urothelial carcinoma, pembrolizumab for metastatic small cell lung cancer and atezolizumab for metastatic urothelial carcinoma)[18]. Although in April 2021 the FDA Oncologic Drugs Advisory Committee

(ODAC) voted 7 to 2 in favour of maintaining accelerated approval of atezolizumab in combination with nab-paclitaxel for the treatment of adults with unresectable locally advanced or mTNBC whose tumours express PD-L1. In August 2021, the manufacturer announced that it was voluntarily withdrawing atezolizumab indication for BC in United States. Due to recent changes in the treatment landscape (including IMpassion131 results) the FDA will no longer consider it appropriate to maintain the accelerated approval for atezolizumab in BC. The indication received accelerated approval based in benefit in PFS and OS of IMpassion130, but there was no difference in survival advantage in PD-L1 (+) nor ITT population of IMpassion131[19,20].

Ventana SP142: In the IMpassion130 study, not only was the approval of atezolizumab in combination with chemotherapy achieved, but the FDA also approved the antibody diagnostic measurement test "Ventana PD-L1 SP142 assay", to select TNBC patients to receive treatment with atezolizumab, and perhaps it could be considered a predictive biomarker[21]. Tumor samples were evaluated by immuno-histochemistry to evaluate the expression of PD-L1 (Ventana SP142) in tumor infiltrating immune cells (PD-L1 IC), using a 2-level system: "a percentage of tumor area" < 1% (= PD-L1 negative) or > 1% (= PD-L1 positive). The study revealed that patients whose tumors were positive for PD-L1 (approximately 41%) and received atezolizumab + nab-paclitaxel had a better median PFS compared to placebo + nab-paclitaxel (7.2 mo *vs* 5.5 mo)[11]. In the PD-L1 (+) subgroup, the ORR was 59% with atezolizumab + nab-paclitaxel compared to 43% in the placebo + nab-paclitaxel group. Furthermore, 10% of the patients in the atezolizumab group achieved complete response (CR) compared to only 1% in the placebo group [17] (Table 2)**.**

Atezolizumab + paclitaxel in TNBC (IMpassion131): IMpassion131, a phase III randomized trial, evaluated the combination of atezolizumab + paclitaxel compared with placebo + paclitaxel in patients with unresectable locally advanced disease or mTNBC who had not received prior therapy or ≥ 12 mo since neoadjuvant chemotherapy) (*n* = 651). Forty-five percent of patients were PD-L1 (+), 48% were treated with taxanes, 31% had mTNBC, and 27% had liver metastases. The primary endpoint of IMpassion131 was PFS, and there was no significant difference in PFS between the atezolizumab group *vs* placebo in PD-L1 (+) patients: 5.7 mo *vs* 6.0 mo, respectively (HR: 0.82, *P* = 0.20) or in the ITT population: median PFS was 5.6 *vs* 5.7 in the atezolizumab and placebo groups, respectively (HR: 0.86). Even in the OS analysis, no benefit was demonstrated with atezolizumab in the ITT population or in the PD-L1 (+) population. Regarding AEs, grades 3-4 were similar in both groups (43% *vs* 49%)[22].

In IMpassion130 trial, atezolizumab + nab-paclitaxel did not improve OS in ITT but resulted in a "clinically significant" improvement in OS in PD-L1 (+) patients. The results of the IMpassion130 trial demonstrated the benefit of atezolizumab in combination with nab-paclitaxel. However, the results were divergent in the IMpassion131. Potential reasons for the divergent results between the two studies are under investigation. Tumor heterogeneity could be a reason. Other reasons could be the use of concomitant corticosteroids (necessary for paclitaxel infusion) may have a negative effect on the immunotherapy activity (checkpoint inhibitors); likewise, the differences in the study populations may have a role, as well as the cremophor associated with paclitaxel.

In July 2021, primary results from IMpassion131 have been published. Neither PFS or OS were improved with the combination of atezolizumab + paclitaxel in PD-L1 (+) nor ITT population. The baseline characteristics of the populations in both trials were similar, including median PFS in control groups (5.6 mo with paclitaxel alone *vs* 5.5 mo with nab-paclitaxel alone). Ongoing research may be valuable to explain possible reasons for the IMpassion131 results; authors said the lack of information on BRCA status could be a limitation, as imbalances between treatment arms for this prognostic biomarker may not be detected. In addition, findings from IMpassion131 differ with KEYNOTE-355 results, which evaluated pembrolizumab and more chemotherapy backbones (nab-paclitaxel, paclitaxel, gemcitabine/carboplatin). Despite the main goal of KEYNOTE-355 was similar to that of IMpassion131 and there were important differences regarding eligibility, statistical design, PD-L1 testing and chemotherapy regimens[22].

Atezolizumab + adjuvant chemotherapy (Impassion 030): A pending question is to determine the effectiveness of anti-PD-1/PD-L1 in the adjuvant setting. Several studies are underway including IMpassion030, a phase II study evaluating atezolizumab + adjuvant chemotherapy *vs* placebo + chemotherapy[23].

Pembrolizumab
Pembrolizumab monotherapy in mTNBC (KEYNOTE-119): Pembrolizumab showed antitumor activity and a manageable toxicity profile in TNBC in the umbrella study KEYNOTE-012 (June 2017), a phase Ib study that evaluated the use of immunotherapy in advanced solid tumors. In the subgroup of patients with TNBC, an ORR of 18.5%, a stable disease rate (SD): 25.9%, partial response (PR): 14.8% and complete response (CR): 3.7% rates were obtained[24].

Then, the KEYNOTE-086 (March 2019) phase II study, which evaluated the use of pembrolizumab for up to 2 years as a second or subsequent line of treatment in patients with mTNBC (that previously received anthracyclines and taxanes). The primary endpoint was ORR in the subgroup of patients with PD-L1 (+). As results, an ORR of 4.7%, SD of 20.6%, PR of 4.1% and CR of 0.6% were obtained. In the

Table 2 Common commercially monoclonal programmed death ligand 1 antibodies for immunohistochemical analysis to assess the expression of programmed death ligand 1 (considering Food and Drug Administration approvals)

PD-L1 antibody	Immunotherapy	IHC assay	Cut-off	Line
22C3	Pembrolizumab	DAKO	TPS ≥ 1%	1L
			TC ≥ 1%	2L
28-8	Nivolumab	DAKO	TC ≥ 1%	2L
SP142	Atezolizumab	Ventana	TC ≥ 50% and/or IC ≥ 10%	1L
			TC ≥ 1% and/or IC ≥ 1%	2L
SP263	Durvalumab	Ventana	TC ≥ 1%	1L maintenance, in unresectable stage III after chemoradiation therapy
	Nivolumab		TC ≥ 1%	2L
	Pembrolizumab		TC ≥ 50%	1L
73-10	Avelumab	DAKO	TC ≥ 1%	2L (not approved)

Notes: (1) Atezolizumab in combination with nab-paclitaxel is approved as 1L of treatment for patients with unresectable locally advanced or metastatic triple-negative breast cancer (mTNBC) whose tumors express programmed death ligand 1 (PD-L1) immune cells (IC) (PD-L1 stained tumor-infiltrating IC of any intensity covering ≥ 1% of the tumor area), as determined by a Food and Drug Administration (FDA) approved test (Ventana SP142); and (2) Pembrolizumab with chemotherapy is approved as 1L of treatment for patients with locally recurrent unresectable or mTNBC whose tumors express PD-L1 CPS ≥ 10, as determined by an FDA approved test (PD-L1 IHC 22C3 PharmDx). CPS: Combined positive score; IC: Immune cell; IHC: Immunohistochemistry; TC: Tumor cell; TPS: Tumor proportion score; 1L: First-line; 2L: Second-line; PD-L1: Programmed death ligand 1.

latter, the response was independent of PD-L1 expression [4.8% in patients with PD-L1 (+) *vs* 4.7% PD-L1 (-)][25].

Subsequently, the KEYNOTE-119 (September 2019), phase III, open-label, randomized study was presented which used pembrolizumab monotherapy (*n* = 312) *vs* single agent chemotherapy (*n* = 310) in previously treated mTNBC patients (1-2 prior systemic treatments). The patients were stratified in PD-L1 (+) and (-). The primary endpoint was OS in patients with a combined positive score (CPS) ≥ 10, patients with CPS ≥ 1, and all patients. Secondary endpoints were PFS, ORR and safety. As results, pembrolizumab did not improve OS in patients with CPS ≥ 10 or CPS ≥ 1. In an exploratory analysis of patients with CPS ≥ 20, the median OS was 14.9 mo *vs* 12.5 with chemotherapy (HR: 0.58, 95%CI: 0.38-0.88), no improvement in PFS was observed. Grade 3-5 AEs were 14% *vs* 36% with chemotherapy. In conclusion, this monotherapy treatment did not improve significantly as a second or third line of treatment for mTNBC *vs* chemotherapy, but it was well tolerated and had a lower toxicity than chemotherapy[26].

Pembrolizumab + chemotherapy in mTNBC (KEYNOTE-355): Since pembrolizumab monotherapy showed antitumor activity in mTNBC patients, the KEYNOTE-355 (December 2020), phase III, randomized study evaluated the addition of pembrolizumab to chemotherapy in previously untreated patients with inoperable disease or mTNBC (*n* = 847), in two groups: pembrolizumab (200 mg IV every 21 d) plus nab-paclitaxel (100 mg/m^2 on days 1, 8 and 15 of a 28-d cycle), paclitaxel (90 mg/m^2 on days 1, 8 and 15 of a 28-d cycle), or gemcitabine (1000 mg/m^2) with carboplatin (AUC 2 on days 1 and 8 of a 21-d cycle) *vs* placebo plus chemotherapy. The co-primary endpoints were PFS and OS, evaluated in the PD-L1 subgroup with CPS ≥ 10, CPS ≥ 1, and in the ITT population[27]. As results, among patients with CPS ≥ 10, the median PFS was 9.7 mo in the pembrolizumab group *vs* 5.6 mo in the placebo group (statistically significant) (HR: 0.65, 0.49-0.86, *P* = 0.0012). Among patients with CPS ≥ 1, median PFS was 7.6 mo *vs* 5.6 mo (HR: 0.74, 0.61-0.90, *P* = 0.0014) (not significant) and in the ITT population, median PFS was 7.5 mo *vs* 5.6 mo (HR: 0.82, 0.67-0.97). The effect of pembrolizumab was increased in the enriched PD-L1 population (CPS ≥ 10). In the subgroup analysis, in the ITT population there was more benefit when pembrolizumab is used with paclitaxel, followed by nab-paclitaxel and gemcitabine/carboplatin, showing an asymmetry of chemotherapy regimens used with anti-PD-1 therapy. Similar results were observed in the population with CPS ≥ 1. Regarding AEs, grades 3-5 were 68% in the pembrolizumab group *vs* 67% in the placebo group, including death in < 1% in the pembrolizumab group *vs* 0% in the placebo group. In conclusion, pembrolizumab associated with chemotherapy showed a significant clinical improvement in PFS *vs* placebo in mTNBC patients with CPS of 10 or more[27].

The authors suggest a role in adding pembrolizumab to standard first-line chemotherapy in mTNBC. In fact, NCCN guidelines recommend pembrolizumab (associated to chemotherapy) as first-line treatment options in mTNBC (category 1, preferred as first-line therapy)[15].

It should be noted that, to date, ESMO guidelines do not recommend the use of immunotherapy in subsequent lines for mTNBC due to its low response rates (IB, ESMO)[16].

In the San Antonio Breast Cancer Symposium (SABCS) 2020, new findings from the KEYNOTE-355 trial were presented. Pembrolizumab plus chemotherapy improved PFS, ORR, durable CR and duration of response for patients with locally recurrent, unresectable or mTNBC with tumors expressing PD-L1 and a CPS ≥ 10. This additional endpoint results showed the PFS benefit for the addition of pembrolizumab to chemotherapy, regardless of which chemotherapy partner was chosen, particularly in PD-L1 enriched (CPS ≥ 10) patients[28].

In the ITT population, the median PFS in the pembrolizumab and placebo groups was 7.5 mo *vs* 5.4 mo when given with nab-paclitaxel, 8.0 mo *vs* 3.8 mo with paclitaxel, and 7.4 mo *vs* 7.4 mo with gemcitabine plus carboplatin. The hazard ratios (HRs) favored pembrolizumab over placebo, at a significant HR: 0.69 and HR: 0.57 for nab-paclitaxel and paclitaxel, respectively, and a nonsignificant HR: 0.93 for gemcitabine plus carboplatin. When stratified by PD-L1 expression, patients with a CPS ≥ 10 or CPS ≥ 1 had longer PFS with pembrolizumab. The trial was not powered to compare efficacy among treatment groups by different chemotherapy regimens[28].

In patients with CPS ≥ 10, secondary endpoints favored pembrolizumab plus chemotherapy compared with chemotherapy alone (ORR: 53.2% *vs* 39.8%, disease control rate: 65% *vs* 54.4%). The authors conclude these findings support a role of addition of pembrolizumab to standard chemotherapy for the first-line treatment of mTNBC[28].

In ESMO Congress 2021 (September 2021) final results from the KEYNOTE-355 confirmed pembrolizumab + chemotherapy met dual primary endpoints (PFS and OS) in patients with mTNBC whose tumors expressed PD-L1 (CPS ≥ 10). For all endpoints, the pembrolizumab effect increased with PD-L1 enrichment. No new safety signals were identified[29].

Recently, in SABCS 2021 (December 7-10th, 2021), final results of pembrolizumab plus chemotherapy in mTNBC were presented and demonstrated that the addition of pembrolizumab yielded significant survival over placebo. The authors suggested that a CPS ≥ 10 is considered a "reasonable" cutoff to determine expected treatment benefit[30].

PD-L1 IHC 22C3 pharmDx: The determination of PD-L1 status in the KEYNOTE-355 trial was assessed the PD-L1 IHC 22C3 pharmDx assay and characterized by the CPS, defined as the number of PD-L1 positive cell (tumour cells, lymphocytes and macrophages) divided by total number of tumour cells x 100. PD-L1 (+) tumours are classified as CPS ≥ 10 and CPS ≥ 1, and PD-L1 (-) tumours are classified as CPS < 1. The PFS and OS analysis in the KEYNOTE-355 trial was stratified using CPS ≥ 10, CPS ≥ 1 and the ITT population[27].

Based on KEYNOTE-355 results, in November 2020, the FDA granted accelerated approval to pembrolizumab (200 mg IV every 3 wk or 400 mg every 6 wk prior to chemotherapy) in combination with chemotherapy for the treatment of patients with locally recurrent unresectable or mTNBC whose tumors express PD-L1 (CPS ≥ 10) as determined by an FDA approved test. The FDA also approved the use of PD-L1 IHC 22C3 pharmDx (Dako North America Inc.) as a companion diagnostic test for selecting patients with TNBC who may be appropriate for treatment with pembrolizumab[31].

Pembrolizumab + neoadjuvant chemotherapy (KEYNOTE-522): Pembrolizumab associated to neoadjuvant chemotherapy demonstrated antitumor activity and safety in patients with early TNBC in the I-SPY 2 and the KEYNOTE-173 studies. The I-SPY 2 (September 2017), phase II randomized study designed to test new treatments by identifying therapies based on molecular characteristics included patients with HER2 negative, stage II - III breast cancer who were randomized to receive weekly neoadjuvant paclitaxel with or without pembrolizumab (200 mg IV every 3 wk x 4 cycles) followed by AC (every 3 wk x 4 cycles). In the TNBC subgroup (*n* = 118), it was demonstrated that the combination in the neoadjuvant setting increases pCR up to 3 times more (62.4% *vs* 22.3%, respectively) compared to the control[32].

Subsequently, the results of the KEYNOTE-522 (August 2020), phase III study, which included patients with non-metastatic TNBC, without previous treatment (*n* = 1174), were randomized 2:1 to receive pembrolizumab (200 mg every 3 wk) or placebo, both given with 4 cycles of paclitaxel + carboplatin, followed by 4 cycles of doxorubicin or epirubicin + cyclophosphamide (neoadjuvant phase). After surgery, patients received either pembrolizumab or placebo for 9 cycles until recurrence or unacceptable toxicity (adjuvant phase). The co-primary endpoints were pCR and event-free survival (EFS). As results, a pCR was achieved in 64.8% of the pembro group *vs* 51.2% with placebo ($P < 0.001$). The benefit in pCR with pembrolizumab was consistent across all subgroups, including those with PD-L1 (+). After a median of 15.5 mo, 7.4% of the pembro group and 11.8% of the placebo group had disease progression, local or distant recurrence, or death from any cause (HR: 0.63). The safety of pembrolizumab was consistent with previous studies. In conclusion, pCR was higher in patients receiving pembro + neoadjuvant chemotherapy compared with placebo[33]. A post-hoc analysis showed a better pCR difference in pembrolizumab group *vs* placebo group in clinical stages (CS) IIIA (66.7% *vs* 42.1%, Δ 24.6) and IIIB (48.6% *vs* 23.1%, Δ 25.6), also a better pCR difference by lymph node involvement: positive (64.8% *vs* 44.1%, Δ 20.6) *vs* negative (64.9% *vs* 58.5%, Δ 6.3).

An update of the KEYNOTE-522 trial (presented in ESMO virtual plenary, 15-16 July 2021) showed that at the median follow-up of 39 mo, pembrolizumab had a statistically and clinically significant EFS benefit (HR: 0.63, 95%CI: 0.48-0.82, *P* = 0.0003) compared with chemotherapy alone. At a 3-year follow-up, EFS was 84.5% in the pembrolizumab group compared with 76.8% in the placebo group. The most common event was distance recurrence (7.7% with pembrolizumab group *vs* 13.1% with placebo group). Moreover, pembrolizumab showed a favorable trend in overall survival (OS) (HR: 0.72, 95%CI: 0.51-1.02). Regarding the adverse events (AEs), the immune-mediated AEs (IMAEs) of any grade were found in 43.6% of pembrolizumab group *vs* 21.9% in the placebo group. The most common AEs reported with pembrolizumab were infusion reactions and hypothyroidism[34]. Based on results of the KEYNOTE-522, on July 2021, the FDA approved pembrolizumab for high-risk early-stage TNBC in combination with chemotherapy as neoadjuvant treatment. This is the first immunotherapy approved for early-stage TNBC[35].

DIFFERENCES BETWEEN IMPASSION130 AND KEYNOTE-355 TRIALS IN mTNBC

To clarify, the IMpassion130 and KEYNOTE-355 trials have similar designs and results are consistent. The overall survival results are expected to be similar and the benefit was in PD-L1 (+) patients, suggesting that the direction is identifying the presence of a biomarker (PD-L1 status).

One difference is the way the authors define PD-L1 (+) by immunohistochemistry at a central laboratory and the companion diagnostic methods: in IMpassion130 trial they look at PD-L1 expression of ICs (IC score: greater than 1% of the area of tumor, using SP142 assay), meanwhile, in the KEYNOTE-355, the authors used a different antibody (22C3) to look at PD-L1 expression (CPS: a combination of PD-L1 staining on ICs and the tumor cells, looking for immune cell staining and tumor cell staining greater than 10% of the area). SP142 was seen in 41% of PD-L1 (+) patients but the 22C3 is much higher (is close to 80%)[13,29]. Diagnostic companions can link or homogenize cut-off points of validated tests in order to obtain similar results.

Another difference is that the KEYNOTE-355 includes several standard chemotherapy regimens as taxanes (paclitaxel, nab-paclitaxel) or gemcitabine-carboplatin (IMpassion130 only used nab-paclitaxel as chemotherapy regimen) and patients with early recurrences, thereby offering more treatment options to a population with a high unmet medical need. It is important to note that the KEYNOTE-355 trial was not designed to compare chemotherapy regimens but the last update shows a trend of benefit using taxanes instead of gemcitabine-carboplatin in addition to pembrolizumab[29].

EMERGING BIOMARKERS OF RESPONSE TO IMMUNOTHERAPY IN TNBC

The results obtained in the PD-L1 (+) subgroup of the IMpassion130 trial confirm the benefit of immunotherapy in mTNBC. However, PD-L1 is not the ideal biomarker to select patients for anti-PD-1/anti-PD-L1 therapies as it has been shown in other cancers. Therefore, there is an urgent need for identification and implementation of emerging biomarkers that can predict response to immunotherapy.

TILs

High levels of TILs have been shown to have a prognostic value in patients with HER2 (+) breast cancer and TNBC, as a predicting factor of pathological complete response (pCR) to chemotherapy and its high expression seems to be linked to a better prognosis after adjuvant therapy as well as a reduction in the risk of recurrence[36].

TILs are frequently present in TNBC (around 20%) and they are associated with a good prognosis[37, 38]. The characterization of the immune lymphocytic infiltrates, with the presence of a high number of T lymphocytes (CD8+ TILs), defines a better prognosis for neoadjuvant (higher pCR) and adjuvant chemotherapy (higher DFS and OS). The evidence indicates that in the neoadjuvant setting of TNBC, intratumoral TILs, as well as stromal ones, are predictive of pathological response to platinum-based chemotherapy[39]. However, currently, TILs score should not be used to make treatment decisions nor to escalate or de-escalate. TILs score can be used as a prognostic marker, providing a relative improvement of 15% to 20% in survival due to a 10% increase in TILs, and its use as a prognostic factor is supported by the 2019 St. Gallen Consensus[40,41].

Various studies on neoadjuvant and adjuvant therapies have measured TILs both at the intratumoral and stromal levels[42]. Some studies used immunohistochemistry while others evaluated molecular markers using immunohistochemistry and gene expression. At present, there is no specific cut-off point for TILs (+) established[43,44].

Stromal TIL score

A biomarker of interest is the stromal TIL score which is known to be prognostic and predictive in the

neoadjuvant setting. In the IMpassion130 analysis, the stromal TIL score or CD8+ cell count (T cells) did not predict the benefit of the use of atezolizumab. It also appears that a dearth of stroma in metastatic breast cancer samples could contribute to an inability to detect an association between stromal TILs and the benefit of atezolizumab[45]. Another study that compared the number of TILs in primary and metastatic tumors showed that TILs decrease in metastasis compared to primary breast tumors[46].

PD-L1

PD-L1, which can be expressed in tumor cells and/or in tumor infiltrating immune cells, contributes to the inhibition of the antitumor immune response in the tumor microenvironment[47].

TNBC can present a higher expression of PD-L1 (in a range of 21-56%) compared to the other subtypes, predominantly in inflammatory immune cells and occasionally in neoplastic cells[48].

PD-L1 expression is considered a useful biomarker of response to treatment pf anti-PD-1 or anti-PD-L1 therapies[49]. PD-L1 expression in immune cells (IC) has been estimated in a range from 40%-65% in TNBC patients[50,51]. In the IMpassion130 trial, the expression of PD-L1 IC ≥ 1% was used to define PD-L1 (+)[11].

It has recently been shown that the expression of PD-L1 IC along with TILs influence the prognosis of TNBC and can predict the response to immunotherapy with pembrolizumab and atezolizumab in breast cancer[52]. In the KEYNOTE-086 study, TNBC patients with PD-L1 (+) IC and high TILs had a better response to immunotherapy[53]. Furthermore, an exploratory analysis of the KEYNOTE-173 study investigating the combination of pembrolizumab and neoadjuvant chemotherapy in TNBC, shows that high levels of stromal TILs prior to treatment and the expression of PD-L1, reported in a combined score, were significantly associated with a higher pCR and overall response rates in TNBC patients who received chemotherapy and immunotherapy combined[54,55].

PD-L1 detection in tumor cells and immune cells (IC) varied by antibody clone and is easily evaluated using IHC. The most common commercially available monoclonal PD-L1 antibodies for immunohisto-chemical analysis to assess the expression of PD-L1 are the following: 22C3, 28-8, SP142, SP263 and 73-10. While many PD-L1 assays are available, only Ventana SP142 and PD-L1 IHC 22C3 pharmDx are licensed companion diagnostic tests for selecting patients with mTNBC who are candidates for treatment with atezolizumab and pembrolizumab, respectively[56].

Other emerging biomarkers in TNBC: PD-L1 has been mentioned as a biomarker to select patients to receive anti-PD1/PD-L1 therapies, being an imperfect marker as has been demonstrated in trials (in IMpassion031 and KEYNOTE-522 the benefit not confined to PD-L1 group). PD-L1 has some limitations: the difficult and subjective scoring (tissue types, cell types, antibodies), the expense for 22C3 validation for independent laboratories, the dependence on immune content of biopsy (number of immune cells), also it is not considered a great marker in most disease types. There is a great need for better predictive biomarkers for response to immunotherapy and many of them are under investigation, including: TILs, genetic signatures, TMB, microsatellite instability [microsatellite instability-high (MSI-H)/mismatch repair (MMR) deficiency], major histocompatibility complex (MHC) class I and II, *etc*.

TMB: The mutational burden of the tumor has been correlated with response to immunotherapy in various types of neoplasms; however, a high mutational burden is rare in breast cancer. In the study only 3.1% of breast cancers had high TMB (TMB-H) (≥ 10 mutations/Mb) when compared to 39.7% of melanomas and 24.3% of lung cancer[25]. TMB could be a potential biomarker in TNBC with TMB-H, but this could exclude patients that can benefit from immunotherapy[57,58].

TMB has an indication but clinically is not a great marker and is probably mostly driven by MSI.

MSI-H or deficient MMR: MSI-H or deficient MMR (dMMR) could be a predictive marker of response or benefit with anti-PD-1 therapy, taking into consideration that pembrolizumab is FDA approved for adult and pediatric patients with unresectable or metastatic, MSI-H or dMMR solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options. However, MSI is infrequent in TNBC with an approximate frequency of 0.7%-2%[59].

Other potential biomarkers in TNBC: In view of the above, the research of new predictive biomarkers or risk factors (*e.g*., LDH levels, visceral liver disease) are underway to identify a group of patients that could benefit from atezolizumab as monotherapy or in combination, and thus optimize the treatment of mTNBC[60]. In the KEYNOTE-086 study, it was observed that patients with elevated levels of lactate dehydrogenase and visceral liver disease had little or no response to immunotherapy. Another study reports that patients with liver metastases derive limited benefit from immunotherapy independent of other established biomarkers of response: liver metastases create a systemic immune desert in preclinical models (apoptosis of CD8 T cells) and reduction of peripheral T cell numbers and diminished tumoral T cell diversity and function[61].

MHC-I and II are new potential biomarkers under analysis: most tumor cells (including BC) express MHC-I, whereas MHC-II is expressed by only a fraction of tumor/tumor cells (MHC-II is considered a professional antigen-presenting cell). A previous trial showed tumors which express high levels of MHC-I or II have high counts of CD4 and CD8 T lymphocytes ($P < 0.001$). Positive expression of MHC-II in tumor cells is associated with better disease-free survival (DFS) in patients who have lymph node

metastases (P = 0.009). Also, the expression of MHC-II in tumor cells was associated with an increased level of TILs[62]. A recent study reported MHC-II predicts early-stage HER2-negative breast cancer response to immunotherapy + neoadjuvant chemotherapy[63].

In general, the evolution of treatment with immunotherapy can be divided into "three waves":

The "first wave" includes the use of immunotherapy as monotherapy, which has shown antitumor activity and modest results in advanced disease.

The "second wave" used immunotherapy in combination with chemotherapy. Cytotoxic therapy can induce increased antigen release from tumor cells, change in tumor microenvironment, upregulation of PD-L1 and increased expression of cell surface markers (*e.g.*, MHC I). All of these effects can increase immunotherapy effectiveness. Despite evaluations of which would be the ideal (safest or most effective) chemotherapy for combination therapy with immunotherapy, several questions remain. Nab-paclitaxel was used in the IMpassion130 because it facilitates the reduction of corticosteroid use. However, other chemotherapy agents have also been evaluated to improve the immunogenicity of breast cancer, including anthracyclines, taxanes, platinum salts, among others[64].

The TONIC, phase II trial compared the effects of induction chemotherapy associated with immunotherapy (nivolumab). Objective response rate (ORR) was 20%, and the highest ORR rates were observed in the cisplatin (ORR: 23%) and doxorubicin (ORR: 35%) cohorts. Initial and post-induction biopsies analysis showed an upregulation of immune-related genes in PD-1/PD-L1 and T-cell cytotoxicity in the cisplatin and doxorubicin cohorts[65].

The lymphocyte depleting effect of combination therapy should also be considered. A comparison of chemotherapy (capecitabine or paclitaxel) associated with pembrolizumab showed a profound and significant depletion of T cells (including CD4+ and CD8+). This could explain the decrease in efficacy of anti-PD-1/PD-L1 in later lines of chemotherapy in TNBC[66].

The "third wave" includes immunotherapy in combination with targeted therapies (as PARP inhibitors). Currently, a phase II/III trial (KEYLYNK-009) of olaparib + pembrolizumab compared with chemotherapy (carboplatin/gemcitabine) + pembrolizumab after initial treatment with chemotherapy + pembrolizumab in TNBC (n = 932) is ongoing. The aim is evaluating if combination of olaparib and pembrolizumab is effective and safe. Co-primary endpoints are PFS and OS and results are ongoing[67].

In this setting, the use of antibody-drug conjugates (ADCs) can be included. Sacituzumab govitecan (a new ADC) is approved by the FDA for treatment of adult patients with mTNBC who received at least two prior therapies for metastatic disease based in results of ASCENT trial[68].

CONCLUSION

The treatment of TNBC has evolved in the last decade with the application of immunotherapy, which has become the new standard of treatment and is changing the management paradigm, mainly in advanced disease, where there were only limited treatment options such as systemic chemotherapy. Knowledge of the molecular profile of TNBC and immunogenicity has made it possible to identify characteristics that differentiate them from other subtypes. Likewise, immunotherapy was evaluated and approved for more TNBC scenarios (metastatic, neoadjuvant).

TNBC is considered a more immunogenic subtype compared to the other subtypes of breast cancer due to the higher expression of TILs and PD-L1. According to the analysis of IMpassion130, PD-L1 has been shown to be a discussible predictive biomarker of response in selected patients [subgroup with PD-L1 (+)]. Other potential biomarkers are under investigation (LDH levels, presence of visceral disease, TMB, MSI-H) to identify and select patients who may benefit from immunotherapy alone or in combination in the different scenarios of TNBC.

New advances have been made with immunotherapy in mTNBC. First, progression-free survival (PFS) and overall survival (OS) benefit have been demonstrated in selected populations (PD-L1 positive subgroups) with immunotherapy + chemotherapy (nab-paclitaxel) in metastatic stage (mTNBC), locally advanced or unresectable disease (IMpassion130 trial). Furthermore, the approval of anti-PD-1 also led to the approval of a companion diagnostic test (Ventana SP142) for selecting patients who are candidates for atezolizumab. However, the benefit of atezolizumab (PFS and OS) could not be demonstrated in combination with paclitaxel (study IMpassion 131). The reasons for the divergent results between IMpassion130 and IMpassion131 trials are currently under investigation. Second, the KEYNOTE-355 trial results are consistent with Impassion130 trial and pembrolizumab is considered as a first-line option of treatment in mTNBC. Moreover, there is another companion diagnostic test approved (PD-L1 IHC 22C3 PharmDx) as an aid to identify patients with TNBC who are candidates for pembrolizumab.

In the neoadjuvant setting of TNBC, pembrolizumab has achieved the 2 co-primary endpoints evaluated (KEYNOTE-522): a higher pCR when combined with chemotherapy and a statistically significant event-free survival (EFS) benefit compared with chemotherapy alone. In the metastatic setting, benefit has been shown with the use of pembrolizumab + chemotherapy (KEYNOTE-355 study) as the first-line of treatment in those patients with enriched expression of PD-L1 (CPS ≥ 10).

Finally, in adjuvant disease, ongoing studies (such as IMpassion030) are evaluating the benefit of immunotherapy. It should be noted that, for TNBC in early disease, the standard of treatment continues to be neoadjuvant chemotherapy, as this is considered a systemic disease.

The evolution of immunotherapy in TNBC began with immunotherapy as monotherapy ("first wave"), followed by combination of immunotherapy + chemotherapy ("second wave") that is considered the new standard of care as first line in selected mTNBC PD-L1 (+). Currently, there are ongoing trials evaluating the combination of immunotherapy (immune checkpoint inhibitors) plus targeted therapies (as PARP inhibitors) for several cancers including TNBC and the development of antibody-drug conjugates (as sacituzumab govitecan) which had demonstrated benefit in refractory mTNBC ("third wave").

FOOTNOTES

ORCID number: Guillermo Arturo Valencia 0000-0002-4234-4448; Patricia Rioja 0000-0003-3141-7418; Zaida Morante 0000-0001-9725-9278; Rossana Ruiz 0000-0001-5199-6686; Hugo Fuentes 0000-0002-2747-7381; Carlos A Castaneda 0000-0001-6200-0856; Tatiana Vidaurre 0000-0003-1995-4560; Silvia Neciosup 0000-0002-2657-9853; Henry L Gomez 0000-0003-2660-1843.

REFERENCES

1 **Waks AG**, Winer EP. Breast Cancer Treatment: A Review. *JAMA* 2019; **321**: 288-300 [PMID: 30667505 DOI: 10.1001/jama.2018.19323]
2 **Perou CM**, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. *Nature* 2000; **406**: 747-752 [PMID: 10963602 DOI: 10.1038/35021093]
3 **Yersal O**, Barutca S. Biological subtypes of breast cancer: Prognostic and therapeutic implications. *World J Clin Oncol* 2014; **5**: 412-424 [PMID: 25114856 DOI: 10.5306/wjco.v5.i3.412]
4 **Lehmann BD**, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest* 2011; **121**: 2750-2767 [PMID: 21633166 DOI: 10.1172/JCI45014]
5 **Franzoi MA**, Romano E, Piccart M. Immunotherapy for early breast cancer: too soon, too superficial, or just right? *Ann Oncol* 2021; **32**: 323-336 [PMID: 33307202 DOI: 10.1016/j.annonc.2020.11.022]
6 **Garufi G**, Palazzo A, Paris I, Orlandi A, Cassano A, Tortora G, Scambia G, Bria E, Carbognin L. Neoadjuvant therapy for triple-negative breast cancer: potential predictive biomarkers of activity and efficacy of platinum chemotherapy, PARP- and immune-checkpoint-inhibitors. *Expert Opin Pharmacother* 2020; **21**: 687-699 [PMID: 32052646 DOI: 10.1080/14656566.2020.1724957]
7 **Caparica R**, Lambertini M, de Azambuja E. How I treat metastatic triple-negative breast cancer. *ESMO Open* 2019; **4**: e000504 [PMID: 31231572 DOI: 10.1136/esmoopen-2019-000504]
8 **Kassam F**, Enright K, Dent R, Dranitsaris G, Myers J, Flynn C, Fralick M, Kumar R, Clemons M. Survival outcomes for patients with metastatic triple-negative breast cancer: implications for clinical practice and trial design. *Clin Breast Cancer* 2009; **9**: 29-33 [PMID: 19299237 DOI: 10.3816/CBC.2009.n.005]
9 **Food and Drug Administration**. Atezolizumab (Tecentriq®) label. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/Label/2019/761034s018 Lbl.pdf
10 **Schmid P**, Cruz C, Braiteh FS, Eder JP, Tolaney S, Kuter I, Nanda R, Chung C, Cassier P, Delord JP, Gordon M, Li Y, Liu B, O'Hear C, Fasso M, Molinero L, Emens LA. Atezolizumab in metastatic TNBC (mTNBC): Long-term clinical outcomes and biomarker analyses. AACR Annual Meeting 2017. Available from:

https://www.abstractsonline.com/pp8/#!/4292/presentation/1296

11   **Schmid P**, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, Diéras V, Hegg R, Im SA, Shaw Wright G, Henschel V, Molinero L, Chui SY, Funke R, Husain A, Winer EP, Loi S, Emens LA; IMpassion130 Trial Investigators. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. *N Engl J Med* 2018; **379**: 2108-2121 [PMID: 30345906 DOI: 10.1056/NEJMoa1809615]

12   **Emens LA**, Middleton G. The interplay of immunotherapy and chemotherapy: harnessing potential synergies. *Cancer Immunol Res* 2015; **3**: 436-443 [PMID: 25941355 DOI: 10.1158/2326-6066.CIR-15-0064]

13   **Schmid P**, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, Dieras V, Henschel V, Molinero L, Chui SY, Husain A, Winer EP, Loi S, Emens LA. IMpassion130: updated overall survival (OS) from a global, randomized, double-blind, placebo-controlled, Phase III study of atezolizumab (atezo) + nab-paclitaxel (nP) in previously untreated locally advanced or metastatic triple-negative breast cancer (mCMTN). *J Clin Oncol* 2019; **37**: 1003 [DOI: 10.1200/JCO.2019.37.15_suppl.1003]

14   **Emens LA**, Adams S, Barrios CH, Diéras V, Iwata H, Loi S, Rugo HS, Schneeweiss A, Winer EP, Patel S, Henschel V, Swat A, Kaul M, Molinero L, Chui SY, Schmid P. First-line atezolizumab plus nab-paclitaxel for unresectable, locally advanced, or metastatic triple-negative breast cancer: IMpassion130 final overall survival analysis. *Ann Oncol* 2021; **32**: 983-993 [PMID: 34272041 DOI: 10.1016/j.annonc.2021.05.355]

15   **National Comprehensive Cancer Network**.   NCCN Clinical Practice Guidelines in Oncology: Breast Cancer. Version 2. 2022 [cited December 20, 2021] Available from: https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf

16   **Cardoso F**, Paluch-Shimon S, Senkus E, Curigliano G, Aapro MS, André F, Barrios CH, Bergh J, Bhattacharyya GS, Biganzoli L, Boyle F, Cardoso MJ, Carey LA, Cortés J, El Saghir NS, Elzayat M, Eniu A, Fallowfield L, Francis PA, Gelmon K, Gligorov J, Haidinger R, Harbeck N, Hu X, Kaufman B, Kaur R, Kiely BE, Kim SB, Lin NU, Mertz SA, Neciosup S, Offersen BV, Ohno S, Pagani O, Prat A, Penault-Llorca F, Rugo HS, Sledge GW, Thomssen C, Vorobiof DA, Wiseman T, Xu B, Norton L, Costa A, Winer EP. 5th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 5). *Ann Oncol* 2020; **31**: 1623-1649 [PMID: 32979513 DOI: 10.1016/j.annonc.2020.09.010]

17   **Cyprian FS**, Akhtar S, Gatalica Z, Vranic S. Targeted immunotherapy with a checkpoint inhibitor in combination with chemotherapy: A new clinical paradigm in the treatment of triple-negative breast cancer. *Bosn J Basic Med Sci* 2019; **19**: 227-233 [PMID: 30915922 DOI: 10.17305/bjbms.2019.4204]

18   **Food and Drug Administration**.   FDA in Brief: FDA Oncologic Drugs Advisory Committee to Review Status of Six Indications Granted Accelerated Approval. [cited March 11, 2021] Available from: https://www.fda.gov/news-events/fda-brief/fda-brief-fda-oncologic-drugs-advisory-committee-review-status-six-indications-granted-accelerated

19   **The ASCO Post**.   FDA ODAC Votes in Favor of Maintaining Accelerated Approval of Atezolizumab/Nab-paclitaxel for PD-L1–Positive, Metastatic Triple-Negative Breast Cancer. Last updated April 30th, 2021. Available from: https://ascopost.com/news/april-2021/fda-odac-votes-in-favor-of-maintaining-accelerated-approval-of-atezolizumabnab-paclitaxel-for-pd-l1-positive-metastatic-triple-negative-breast-cancer/

20   **F. Hoffmann-La Roche Ltd**.   Roche provides update on Tecentriq US indication for PD-L1-positive, metastatic triple-negative breast cancer. [cited August 27, 2021] Available from: https://www.roche.com/media/releases/med-cor-2021-08-27.htm

21   **Schmid P**, Cruz C, Braiteh F, et al Atezolizumab in metastatic TNBC (mTNBC): Long-term clinical outcomes and biomarker analyses.   AACR Annual Meeting 2017. Available from: https://www.abstractsonline.com/pp8/#!/4292/presentation/1296

22   **Miles D**, Gligorov J, André F, Cameron D, Schneeweiss A, Barrios C, Xu B, Wardley A, Kaen D, Andrade L, Semiglazov V, Reinisch M, Patel S, Patre M, Morales L, Patel SL, Kaul M, Barata T, O'Shaughnessy J; IMpassion131 investigators. Primary results from IMpassion131, a double-blind, placebo-controlled, randomised phase III trial of first-line paclitaxel with or without atezolizumab for unresectable locally advanced/metastatic triple-negative breast cancer. *Ann Oncol* 2021; **32**: 994-1004 [PMID: 34219000 DOI: 10.1016/j.annonc.2021.05.801]

23   **ClinicalTrials.gov**.   A study comparing atezolizumab (Anti PD-L1 Antibody) in combination with adjuvant anthracycline/taxane-based chemotherapy vs chemotherapy alone in patients with operable triple-negative breast cancer (IMpassion030). Available from: https://clinicaltrials.gov/ct2/show/NCT03498716

24   **Nanda R**, Chow LQ, Dees EC, Berger R, Gupta S, Geva R, Pusztai L, Pathiraja K, Aktan G, Cheng JD, Karantza V, Buisseret L. Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study. *J Clin Oncol* 2016; **34**: 2460-2467 [PMID: 27138582 DOI: 10.1200/JCO.2015.64.8931]

25   **Adams S**, Schmid P, Rugo HS, Winer EP, Loirat D, Awada A, Cescon DW, Iwata H, Campone M, Nanda R, Hui R, Curigliano G, Toppmeyer D, O'Shaughnessy J, Loi S, Paluch-Shimon S, Card D, Zhao J, Karantza V, Cortes J. Phase 2 study of pembrolizumab (pembro) monotherapy for previously treated metastatic triple-negative breast cancer (mCMTN): KEYNOTE-086 cohort A. *J Clin Oncol* 2017; **35**: 1008-1008 [DOI: 10.1200/JCO.2017.35.15_suppl.1008]

26   **Cortes J**, Lipatov O, Im S, Gonçalves A, Lee KS, Schmid P, Tamura K, Testa L, Witzel I, Ohtani S, Zambelli S, Harbeck N, André F, Dent R, Zhou X, Karantza V, Mejia JA, Winer EP. 1103- KEYNOTE-119: Phase 3 study of Pembrolizumab (Pembro) vs single-agent chemotherapy (chemo) for metastatic triple-negative breast cancer (mCMTN). *Ann Oncol* 2019; **30**: v851-v934 [DOI: 10.1093/annonc/mdz394]

27   **Cortes J**, Cescon DW, Rugo HS, Nowecki Z, Im SA, Yusof MM, Gallardo C, Lipatov O, Barrios CH, Holgado E, Iwata H, Masuda N, Otero MT, Gokmen E, Loi S, Guo Z, Zhao J, Aktan G, Karantza V, Schmid P; KEYNOTE-355 Investigators. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. *Lancet* 2020; **396**: 1817-1828 [PMID: 33278935 DOI: 10.1016/S0140-6736(20)32531-9]

28   **Rugo HS**, Schmid P, Cescon DW, Nowecki Z, Im SA, Md yusof M, Gallardo C, Lipatov ON, Barrios CH, Perez-Garcia J, Iwata H, Masuda N, Otero MT, Gokmen E, Loi S, Guo Z, Zhao J, Karantza V, Aktan G, Cortes J.   Additional efficacy endpoints from the phase 3 KEYNOTE-355 study of pembrolizumab plus chemotherapy vs placebo plus chemotherapy as first-line therapy for locally recurrent inoperable or metastatic triple-negative breast cancer. Presented at: 2020 Virtual San Antonio Breast Cancer Symposium; December 8-11, 2020. Abstract GS3-01. Available from:

https://www.cancernetwork.com/view/pembrolizumab-chemo-combo-demonstrates-improvement-in-pfs-for-advanced-tnbc

29   **Rugo HS**, Cortés J, Cescon DW, et al LBA16 -KEYNOTE-355: Final results from a randomized, double-blind phase III study of first-line pembrolizumab + chemotherapy vs placebo + chemotherapy for metastatic TNBC.   Annals of Oncology (2021) 32 (suppl_5): S1283-S1346 [DOI: 10.1016/annonc/annonc741]

30   **Cortes J**, Cescon DW, Rugo HS, et al.   Final results of KEYNOTE-355: randomized, double-blind, phase 3 study of pembrolizumab + chemotherapy vs placebo + chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer. Presented at: SABCS 2021; December 7-10, 2021; San Antonio, TX. Abstract GS1-02. Available from: https://www.cancertherapyadvisor.com/home/news/conference-coverage/san-antonio-breast-cancer-symposium-sabcs/sabcs-2021/breast-cancer-mtnbc-keynote-355-pdl1-expression-pembrolizumab/

31   **Food and Drug Administration**.   FDA grants accelerated approval to pembrolizumab for locally recurrent unresectable or metastatic triple negative breast cancer. [cited November 13th, 2020] Available from: https://www.fda.gov/drugs/drug-approvals-and-databases/fda-grants-accelerated-approval-pembrolizumab-locally-recurrent-unresectable-or-metastatic-triple

32   **Esserman L**.   I-SPY 2 trial: Neoadjuvant and Personalized Adaptive Novel Agents to Treat Breast Cancer. University of California, San Francisco. NCT01042379. Available from: https://clinicaltrials.gov/ct2/show/NCT01042379

33   **Schmid P**, Cortes J, Pusztai L, McArthur H, Kümmel S, Bergh J, Denkert C, Park YH, Hui R, Harbeck N, Takahashi M, Foukakis T, Fasching PA, Cardoso F, Untch M, Jia L, Karantza V, Zhao J, Aktan G, Dent R, O'Shaughnessy J; KEYNOTE-522 Investigators. Pembrolizumab for Early Triple-Negative Breast Cancer. *N Engl J Med* 2020; **382**: 810-821 [PMID: 32101663 DOI: 10.1056/NEJMoa1910549]

34   **Schmid P**, Cortes J, Dent R, et al VP7-2021: KEYNOTE-522: Phase III study of neoadjuvant pembrolizumab + chemotherapy vs.   placebo + chemotherapy, followed by adjuvant pembrolizumab vs. placebo for early-stage TNBC. ESMO virtual plenary abstracts. Annals of Oncology. July 15, 2021 [DOI: 10.1016/j.annonc.2021.06.014]

35   **Food and Drug Administration**.   FDA approves pembrolizumab for high-risk early-stage triple-negative breast cancer. [cited July 26, 2021] Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-pembrolizumab-high-risk-early-stage-triple-negative-breast-cancer?utm_medium=email&utm_source=govdelivery

36   **Carbognin L**, Pilotto S, Nortilli R, Brunelli M, Nottegar A, Sperduti I, Giannarelli D, Bria E, Tortora G. Predictive and Prognostic Role of Tumor-Infiltrating Lymphocytes for Early Breast Cancer According to Disease Subtypes: Sensitivity Analysis of Randomized Trials in Adjuvant and Neoadjuvant Setting. *Oncologist* 2016; **21**: 283-291 [PMID: 26865589 DOI: 10.1634/theoncologist.2015-0307]

37   **Liu Z**, Li M, Jiang Z, Wang X. A Comprehensive Immunologic Portrait of Triple-Negative Breast Cancer. *Transl Oncol* 2018; **11**: 311-329 [PMID: 29413765 DOI: 10.1016/j.tranon.2018.01.011]

38   **Zeichner SB**, Terawaki H, Gogineni K. A Review of Systemic Treatment in Metastatic Triple-Negative Breast Cancer. *Breast Cancer (Auckl)* 2016; **10**: 25-36 [PMID: 27042088 DOI: 10.4137/BCBCR.S32783]

39   **Solinas C**, Gombos A, Latifyan S, Piccart-Gebhart M, Kok M, Buisseret L. Targeting immune checkpoints in breast cancer: an update of early results. *ESMO Open* 2017; **2**: e000255 [PMID: 29177095 DOI: 10.1136/esmoopen-2017-000255]

40   **Dieci MV**, Radosevic-Robin N, Fineberg S, van den Eynden G, Ternes N, Penault-Llorca F, Pruneri G, D'Alfonso TM, Demaria S, Castaneda C, Sanchez J, Badve S, Michiels S, Bossuyt V, Rojo F, Singh B, Nielsen T, Viale G, Kim SR, Hewitt S, Wienert S, Loibl S, Rimm D, Symmans F, Denkert C, Adams S, Loi S, Salgado R; International Immuno-Oncology Biomarker Working Group on Breast Cancer. Update on tumor-infiltrating lymphocytes (TILs) in breast cancer, including recommendations to assess TILs in residual disease after neoadjuvant therapy and in carcinoma in situ: A report of the International Immuno-Oncology Biomarker Working Group on Breast Cancer. *Semin Cancer Biol* 2018; **52**: 16-25 [PMID: 29024776 DOI: 10.1016/j.semcancer.2017.10.003]

41   **Balic M**, Thomssen C, Würstlein R, Gnant M, Harbeck N. St. Gallen/Vienna 2019: A Brief Summary of the Consensus Discussion on the Optimal Primary Breast Cancer Treatment. *Breast Care (Basel)* 2019; **14**: 103-110 [PMID: 31798382 DOI: 10.1159/000499931]

42   **Loi S**, Drubay D, Adams S, Pruneri G, Francis PA, Lacroix-Triki M, Joensuu H, Dieci MV, Badve S, Demaria S, Gray R, Munzone E, Lemonnier J, Sotiriou C, Piccart MJ, Kellokumpu-Lehtinen PL, Vingiani A, Gray K, Andre F, Denkert C, Salgado R, Michiels S. Tumor-Infiltrating Lymphocytes and Prognosis: A Pooled Individual Patient Analysis of Early-Stage Triple-Negative Breast Cancers. *J Clin Oncol* 2019; **37**: 559-569 [PMID: 30650045 DOI: 10.1200/JCO.18.01010]

43   **Salgado R**, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, Wienert S, Van den Eynden G, Baehner FL, Penault-Llorca F, Perez EA, Thompson EA, Symmans WF, Richardson AL, Brock J, Criscitiello C, Bailey H, Ignatiadis M, Floris G, Sparano J, Kos Z, Nielsen T, Rimm DL, Allison KH, Reis-Filho JS, Loibl S, Sotiriou C, Viale G, Badve S, Adams S, Willard-Gallo K, Loi S; International TILs Working Group 2014. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol* 2015; **26**: 259-271 [PMID: 25214542 DOI: 10.1093/annonc/mdu450]

44   **Vinayak S**, James R, Adams S, Jensen KC, Manola J, Goldstein AAJ, Ford JM, Badve SS, Telli ML. Association of increased tumor-infiltrating lymphocytes (TILs) with immunomodulatory (IM) triple-negative breast cancer (TNBC) subtype and response to neoadjuvant platinum-based therapy in PrECOG105. *J Clin Oncol* 2014; **32**: 1000-1000 [DOI: 10.1200/jco.2014.32.15_suppl.1000]

45   **Emens LA**, Loi S, Rugo HS, Schneeweiss A, Diéras V, Iwata H, Barrios CH, Nechaeva M, Molinero L, Nguyen Duc A, Funke R, Chui SY, Husain A, Winer EP, Adams S, Schmid P. Abstract GS1-04: IMpassion130: Efficacy in immune biomarker subgroups from the global, randomized, double-blind, placebo-controlled, phase III study of atezolizumab + nab-paclitaxel in patients with treatment-naïve, locally advanced or metastatic triple-negative breast cancer. *Cancer Res* 2019; **79**: GS1-04 [DOI: 10.1158/1538-7445.Sabcs18-gs1-04]

46   **Ogiya R**, Niikura N, Kumaki N, Bianchini G, Kitano S, Iwamoto T, Hayashi N, Yokoyama K, Oshitanai R, Terao M, Morioka T, Tsuda B, Okamura T, Saito Y, Suzuki Y, Tokuda Y. Comparison of tumor-infiltrating lymphocytes between primary and metastatic tumors in breast cancer patients. *Cancer Sci* 2016; **107**: 1730-1735 [PMID: 27727484 DOI: 10.1111/cas.13101]

47   **Sun C**, Mezzadra R, Schumacher TN. Regulation and Function of the PD-L1 Checkpoint. *Immunity* 2018; **48**: 434-452 [PMID: 29562194 DOI: 10.1016/j.immuni.2018.03.014]

48   **Zhang M**, Sun H, Zhao S, Wang Y, Pu H, Zhang Q. Expression of PD-L1 and prognosis in breast cancer: a meta-analysis. *Oncotarget* 2017; **8**: 31347-31354 [PMID: 28430626 DOI: 10.18632/oncotarget.15532]

49   **Gibney GT**, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. *Lancet Oncol* 2016; **17**: e542-e551 [PMID: 27924752 DOI: 10.1016/S1470-2045(16)30406-5]

50   **Beckers RK**, Selinger CI, Vilain R, Madore J, Wilmott JS, Harvey K, Holliday A, Cooper CL, Robbins E, Gillett D, Kennedy CW, Gluch L, Carmalt H, Mak C, Warrier S, Gee HE, Chan C, McLean A, Walker E, McNeil CM, Beith JM, Swarbrick A, Scolyer RA, O'Toole SA. Programmed death ligand 1 expression in triple-negative breast cancer is associated with tumour-infiltrating lymphocytes and improved outcome. *Histopathology* 2016; **69**: 25-34 [PMID: 26588661 DOI: 10.1111/his.12904]

51   **Li X**, Li M, Lian Z, Zhu H, Kong L, Wang P, Yu J. Prognostic Role of Programmed Death Ligand-1 Expression in Breast Cancer: A Systematic Review and Meta-Analysis. *Target Oncol* 2016; **11**: 753-761 [PMID: 27422273 DOI: 10.1007/s11523-016-0451-8]

52   **Noske A**, Möbus V, Weber K, Schmatloch S, Weichert W, Köhne CH, Solbach C, Ingold Heppner B, Steiger K, Müller V, Fasching P, Karn T, van Mackelenbergh M, Marmé F, Schmitt WD, Schem C, Stickeler E, Loibl S, Denkert C. Relevance of tumour-infiltrating lymphocytes, PD-1 and PD-L1 in patients with high-risk, nodal-metastasised breast cancer of the German Adjuvant Intergroup Node-positive study. *Eur J Cancer* 2019; **114**: 76-88 [PMID: 31075727 DOI: 10.1016/j.ejca.2019.04.010]

53   **Loi S**, Adams S, Schmid P, Cortés J, Cescon DW, Winer EP, Toppmeyer DL, Rugo HS, De Laurentiis M, Nanda R, Iwata H, Awada A, Tan A, Wang A, Aktan G, Karantza V, Salgado R. Relationship between tumor infiltrating lymphocyte (TIL) levels and response to pembrolizumab (pembro) in metastatic triple-negative breast cancer (mCMTN): results from KEYNOTE-086. *Ann Oncol* 2017; **28**: v605-v649 [DOI: 10.1093/annonc/mdx440]

54   **Loi S**, Schmid P, Cortés J, ark YH, Munoz-Couselo E, Kim SB, Sohn J, Im SA, Holgado E, Foukakis T, Kuemmel S, Dent R, Wang A, Aktan G, Karantza V, Salgado R. Relationship between tumor infiltrating lymphocytes (TILs) and response to pembrolizumab (Pembro)+chemotherapy (Chemo) as neoadjuvant treatment (NAT) for triple-negative breast cancer (CMTN): phase Ib KEYNOTE-173 trial. *Cancer Res* 2019; **79**: Abstract nr P3-10 [DOI: 10.1158/1538-7445.SABCS18-P3-10-09]

55   **Marra A**, Viale G, Curigliano G. Recent advances in triple negative breast cancer: the immunotherapy era. *BMC Med* 2019; **17**: 90 [PMID: 31068190 DOI: 10.1186/s12916-019-1326-5]

56   **Peg V**, López-García MÁ, Comerma L, Peiró G, García-Caballero T, López ÁC, Suárez-Gauthier A, Ruiz I, Rojo F. PD-L1 testing based on the SP142 antibody in metastatic triple-negative breast cancer: summary of an expert round-table discussion. *Future Oncol* 2021; **17**: 1209-1218 [PMID: 33289433 DOI: 10.2217/fon-2020-1100]

57   **Samstein RM**, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, Barron DA, Zehir A, Jordan EJ, Omuro A, Kaley TJ, Kendall SM, Motzer RJ, Hakimi AA, Voss MH, Russo P, Rosenberg J, Iyer G, Bochner BH, Bajorin DF, Al-Ahmadie HA, Chaft JE, Rudin CM, Riely GJ, Baxi S, Ho AL, Wong RJ, Pfister DG, Wolchok JD, Barker CA, Gutin PH, Brennan CW, Tabar V, Mellinghoff IK, DeAngelis LM, Ariyan CE, Lee N, Tap WD, Gounder MM, D'Angelo SP, Saltz L, Stadler ZK, Scher HI, Baselga J, Razavi P, Klebanoff CA, Yaeger R, Segal NH, Ku GY, DeMatteo RP, Ladanyi M, Rizvi NA, Berger MF, Riaz N, Solit DB, Chan TA, Morris LGT. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet* 2019; **51**: 202-206 [PMID: 30643254 DOI: 10.1038/s41588-018-0312-8]

58   **Karn T**, Denkert C, Weber KE, Holtrich U, Hanusch C, Sinn BV, Higgs BW, Jank P, Sinn HP, Huober J, Becker C, Blohmer JU, Marmé F, Schmitt WD, Wu S, van Mackelenbergh M, Müller V, Schem C, Stickeler E, Fasching PA, Jackisch C, Untch M, Schneeweiss A, Loibl S. Tumor mutational burden and immune infiltration as independent predictors of response to neoadjuvant immune checkpoint inhibition in early TNBC in GeparNuevo. *Ann Oncol* 2020; **31**: 1216-1222 [PMID: 32461104 DOI: 10.1016/j.annonc.2020.05.015]

59   **Obeid E**, Ellerbrock A, Handorf E, Goldstein L, Gatalica Z, Arguello D, Swain SM, Isaacs C, Vacirca J, Tan A, Schwartzberg L. Abstract PD6-03: Distribution of microsatellite instability, tumor mutational load, and PD-L1 status in molecularly profiled invasive breast cancer. *Cancer Res* 2018; **78**: PD6-03 [DOI: 10.1158/1538-7445.SABCS17-PD6-03]

60   **Kim I**, Sanchez K, McArthur HL, Page D. Immunotherapy in Triple-Negative Breast Cancer: Present and Future. *Curr Breast Cancer Rep* 2019; **11**: 259-271 [DOI: 10.1007/s12609-019-00345-z]

61   **Yu J**, Green MD, Li S, Sun Y, Journey SN, Choi JE, Rizvi SM, Qin A, Waninger JJ, Lang X, Chopra Z, El Naqa I, Zhou J, Bian Y, Jiang L, Tezel A, Skvarce J, Achar RK, Sitto M, Rosen BS, Su F, Narayanan SP, Cao X, Wei S, Szeliga W, Vatan L, Mayo C, Morgan MA, Schonewolf CA, Cuneo K, Kryczek I, Ma VT, Lao CD, Lawrence TS, Ramnath N, Wen F, Chinnaiyan AM, Cieslik M, Alva A, Zou W. Liver metastasis restrains immunotherapy efficacy *via* macrophage-mediated T cell elimination. *Nat Med* 2021; **27**: 152-164 [PMID: 33398162 DOI: 10.1038/s41591-020-1131-x]

62   **Park IA**, Hwang SH, Song IH, Heo SH, Kim YA, Bang WS, Park HS, Lee M, Gong G, Lee HJ. Expression of the MHC class II in triple-negative breast cancer is associated with tumor-infiltrating lymphocytes and interferon signaling. *PLoS One* 2017; **12**: e0182786 [PMID: 28817603 DOI: 10.1371/journal.pone.0182786]

63   **Gonzalez-Ericsson PI**, Wulfkhule JD, Gallagher RI, Sun X, Axelrod ML, Sheng Q, Luo N, Gomez H, Sanchez V, Sanders M, Pusztai L, Petricoin E, Blenman KRM, Balko JM; I-SPY2 Trial Team. Tumor-Specific Major Histocompatibility-II Expression Predicts Benefit to Anti-PD-1/L1 Therapy in Patients With HER2-Negative Primary Breast Cancer. *Clin Cancer Res* 2021 [PMID: 34315723 DOI: 10.1158/1078-0432.CCR-21-0607]

64   **Pérez-García J**, Soberino J, Racca F, Gion M, Stradella A, Cortés J. Atezolizumab in the treatment of metastatic triple-negative breast cancer. *Expert Opin Biol Ther* 2020; **20**: 981-989 [PMID: 32450725 DOI: 10.1080/14712598.2020.1769063]

65   **Voorwerk L**, Slagter M, Horlings HM, Sikorska K, van de Vijver KK, de Maaker M, Nederlof I, Kluin RJC, Warren S, Ong S, Wiersma TG, Russell NS, Lalezari F, Schouten PC, Bakker NAM, Ketelaars SLC, Peters D, Lange CAH, van Werkhoven E, van Tinteren H, Mandjes IAM, Kemper I, Onderwater S, Chalabi M, Wilgenhof S, Haanen JBAG, Salgado R, de Visser KE, Sonke GS, Wessels LFA, Linn SC, Schumacher TN, Blank CU, Kok M. Immune induction strategies in

metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial. *Nat Med* 2019; **25**: 920-928 [PMID: 31086347 DOI: 10.1038/s41591-019-0432-4]

66   **Page DB**, Chun B, Pucilowska J, Kim I, Sanchez K, Redmond WL, Sun Z, Wu Y, Feryn A, Martel M, Abaya CD, Wadell D, Mellinger SL, Moxon N, Urba WJ, McAndrew P, El-Masry M, Basho RK, Conlin AK, McArthur HL. Pembrolizumab (pembro) with paclitaxel (taxol) or capecitabine (cape) as early treatment of metastatic triple-negative breast cancer (mCMTN). *J Clin Oncol* 2019; **37**: 1015 [DOI: 10.1200/JCO.2019.37.15_suppl.1015]

67   **ClinicalTrials.gov**.   Study of olaparib plus pembrolizumab vs chemotherapy plus pembrolizumab after induction with first-line chemotherapy plus pembrolizumab in triple-negative breast cancer (TNBC) (MK-7339-009/KEYLYNK-009). NCT04191135. National Cancer Institute. Available from: https://clinicaltrials.gov/ct2/show/NCT04191135

68   **Bardia A**, Mayer IA, Vahdat LT, Tolaney SM, Isakoff SJ, Diamond JR, O'Shaughnessy J, Moroose RL, Santin AD, Abramson VG, Shah NC, Rugo HS, Goldenberg DM, Sweidan AM, Iannone R, Washkowitz S, Sharkey RM, Wegener WA, Kalinsky K. Sacituzumab Govitecan-hziy in Refractory Metastatic Triple-Negative Breast Cancer. *N Engl J Med* 2019; **380**: 741-751 [PMID: 30786188 DOI: 10.1056/NEJMoa1814213]