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Review

Evolving treatment landscape of immunotherapy in breast cancer: current issues and future perspectives

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Carmine Valenza, Graziella Rizzo, Maria Ilenia Passalacqua, Laura Boldrini, Chiara Corti, Dario Trapani and Giuseppe Curigliano

***Abstract*:** Immune checkpoint inhibitors (ICIs) deeply changed the treatment landscape of breast cancer (BC). In particular, anti-programmed-death (ligand) 1 antibodies were approved for the treatment of triple-negative breast cancer (TNBC), both in first line for metastatic disease and in neoadjuvant setting, on the basis of a demonstrated improvement of the survival outcomes. In light of these results, current clinical trials aim at improving this benefit investigating novel combinations and strategies, at exploring the role of ICIs beyond TNBC, and at better selecting the patients in order to spare non-responders from avoidable toxicities. This narrative review aims at summarizing and discussing the evolving landscape of immunotherapeutic treatments for BC, highlighting the current challenges and the future perspectives.

Keywords: biomarkers, breast cancer, immune checkpoint inhibitors, immunotherapy, triple-negative breast cancer

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Correspondence to:
Giuseppe Curigliano
Division of New Drugs and Early Drug Development for Innovative Therapies, European Institute of Oncology, IRCCS, via G. Ripamonti 435, Milan 20141, Italy

Department of Oncology and Haematology, University of Milan, Milan, Italy.
giuseppe.curigliano@ieo.it

Carmine Valenza
Laura Boldrini
Chiara Corti
Dario Trapani
Giuseppe Curigliano
Division of New Drugs and Early Drug Development for Innovative Therapies, European Institute of Oncology, IRCCS, Milano, Italy

Department of Oncology and Hemato-Oncology, University of Milano, Milano

Graziella Rizzo
Maria Ilenia Passalacqua
Division of New Drugs and Early Drug Development for Innovative Therapies, European Institute of Oncology, IRCCS, Milan, Italy

Department of Human Pathology "G. Barresi", Medical Oncology Unit, University of Messina, Messina, Italy

Introduction

Breast cancer (BC) is the most common cancer in women and represents the leading cause of death from all cancers.[1,2] BC is traditionally classified according to the expression of hormone receptors (HRs) and of human epidermal growth factor receptor 2 (HER2) in three subtypes: HR-positive (HR+)/HER2-negative (HER2−) BC, HER2+ BC, and HR−/HER2− or triple-negative breast cancer (TNBC), which account for approximately 70%, 15–20% and 10–15% of all BC diagnoses, respectively.[3]

immuno-oncology (IO) drugs and in particular immune checkpoint inhibitors (ICIs) have revolutionized the treatment landscape of several cancer types, including TNBC, despite BC has been for long time considered immunogenically quiescent, and less likely to derive benefit from IO approaches.[4] For example, the tumor mutational burden (TMB) in BC is on average lower than in other tumor types (e.g. melanoma and lung carcinoma) and varies by subtype, with HER2+, and triple-negative BC having higher burden than HR+ tumors: TMB has been correlated with a higher chance of immunogenicity, for a potential likelihood to mount an immunogenic, tumor-specific neoantigen restricted, immune response. Similarly, enrichment in tumor-infiltrating lymphocyte (TIL) appears higher in HER2+ and TNBC, when compared with HR+ BC and their expression is positively associated with improved prognosis.

TNBC is considered the most aggressive but immunogenic subtype: this cancer type is characterized by higher levels of programmed death-ligand 1 (PD-L1) expression on both tumor and immune cells, and non-synonymous mutations, which give rise to tumor-specific neoantigens.[5–11] ICIs, in particular anti-programmed-death (ligand) 1 (PD-[L]1) antibodies, are currently only approved for patients with TNBC, both in first line for metastatic disease and in neoadjuvant setting, on the basis of a demonstrated clinical benefit in terms of overall survival (OS) and event-free survival (EFS), respectively.[12–16]

In light of the accumulating evidence of a role of IO in BC treatments, current clinical trials aim at improving IO benefit investigating novel compounds and strategies, for exploring the role of IO in BC in other populations and disease subtypes, and at better selecting the patients to spare non-responders from toxicities.

IO agents in metastatic TNBC

A lesson from previous clinical trials: first-line chemotherapy and immunotherapy is effective in biomarker-selected TNBC

Prior clinical trials enrolling patients with locally advanced and inoperable or metastatic TNBC highlighted that ICIs work in first-line setting, in combination with chemotherapy and in PD-L1+ patients (Table 1). Attempt to develop IO in biomarker-unselected populations resulted in no added benefit, and the use of IO agents in the second and later lines showed poor general activity of IO.

Monotherapy with anti-PD-1/PD-L1 antibodies showed a limited antitumor activity [objective response rate (ORR) of 5–20%] and provided a small clinical benefit [median progression-free survival (mPFS) of 1–2 months], which was strongly influenced by the number of previous lines of therapy for metastatic disease and by the PD-L1 expression.[17–23] In other words, a monotherapy approach is limited by the high rate of primary immune resistance (i.e. 60–85% of patients experience an early progression within the first 2–3 months) undermined by a demonstrated immunosuppressing milieu that occur during metastatic progression of BC.[11]

The development of IO strategies for BC has then moved toward a combination approach with ICIs, to overcome primary and acquired immune resistance and to provide a major clinical benefit. Chemotherapy represents an ideal partner because of its immunomodulatory properties yielding immunogenic cell death and promoting antigen presentation[11,24]; in fact, both pembrolizumab plus physician's choice chemotherapy (KEYNOTE-355) and atezolizumab plus nab-paclitaxel (Impassion130) showed to improve survival in patients with PD-L1-positive TNBC in first-line setting, soon becoming a new standard of care.[13,14,16] PFS and OS were the co-primary endpoints of both trials, but, as far as OS, the Impassion130 failed to demonstrate a statistically significant benefit in the intention-to-treat (ITT) population and, according to the hierarchic design, the OS was not tested in the PD-L1+ population; anyway, an exploratory analysis in this subgroup showed a clinically significant benefit [mOS: 25.4 *versus* 17.9 months; hazard ratio (HR): 0.67, 95% CI: 0.53–0.86, median follow-up: 19 months].[25] Instead, on the basis of a different statistical plan, in the KEYNOTE-355 the OS was tested first in PD-L1+ patients [with combined positive score (CPS) $\geq$ 10 and $\geq$1] and second in the ITT only if OS in the CPS $\geq$ 1 subgroup was met; after a median follow-up of 44 months, a statistically significant OS benefit was assessed only in the CPS$\geq$10 subgroup (mOS: 23 *versus* 16 months; HR: 0.73, 95% CI: 0.55–0.95, $p = 0.0185$).

In contrast with these results, the phase III IMpassion131 trial investigating the combination of atezolizumab and paclitaxel failed to demonstrate a PFS benefit in untreated PD-L1-positive population.[26] This difference may be due to many factors, for example: the previous administration of taxanes in early setting (50% of patients enrolled), the lesser immunomodulatory properties of paclitaxel (perhaps dampened by steroid premedication), and patient heterogeneity.

Novel strategies for improving the clinical benefit of IO agents in BC

The next step to the initial demonstration of a benefit in the first-line setting with IO combined to chemotherapy aimed at potentiating the amplitude and the duration of the clinical benefit and to identify IO therapies for PD-L1-negative BCs. The deeper characterization of the tumor microenvironment (TME) of BC, the comprehension of resistance mechanisms to ICIs, and the investigation of novel ICI combination strategies, also with other IO agents have driven the formulation of innovative approaches.

In detail, resistance to ICIs in BC is strongly related to the intrinsic immune-phenotype of the tumor tissue and is characterized by poorer outcomes[27]; the emerging resistance mechanisms comprise tumor-specific alterations, loss of tumor-specific antigens, and TME reshaping driven by extrinsic immune factors.[28] These mechanisms may be bypassed by facilitating the trafficking of the expanded cytotoxic cells into the tumor mass, improving the antigen presentation, or decreasing the inhibitory functions of the

Table 1. Trials with results enrolling patients with TNBC and evaluating ICI activity or efficacy as primary endpoint.

Treatment	Trial NCT number	Ph	*n*	Patients	Study design	Primary endpoints	Activity outcomes	Efficacy outcomes	AEs⩾G3
Advanced and metastatic TNBC									
ICI monotherapy									
Pembrolizumab	KEYNOTE-012 (NCT01848834)	1b	32	PD-L1+ (⩾1%) mTNBC	Pembrolizumab iv 10 mg/kg q2w	ORR	ORR: 18.5%	mPFS: 1.9 mo; mOS: 11.2 mo	15.6%
Avelumab	JAVELIN (NCT01772004)	1	58	2–4 L; mTNBC	Avelumab iv 10 mg/kg q2w	Safety ORR	ORR: 5.2%	mPFS: 1.4 mo; mOS: 9.2 mo	13.7%
Pembrolizumab	KEYNOTE-086 (NCT02447003)	2	170	⩾2 L; mTNBC (A)	Pembrolizumab iv 200 mg q3w for up to 2 years	ORR (PD-L1+)	ORR: 5.7% (PD-L1+), 5.3% (ITT)	mPFS: 2.0 mo; mOS: 9.0 mo	12.9%
			84	1 L; PD-L1+ (CPS ⩾ 1%) mTNBC (B)		AE	ORR: 21.4%	mPFS: 2.1 mo; mOS: 18.0 mo	9.5%
Nivolumab	TONIC (NCT02499367)	2	67	1–4 L; mTNBC	Induction treatment (RT to a single lesion, low-dose cyclophosphamide, cisplatin or doxorubicin, or a 2-week waiting period) followed by nivolumab iv 3 mg/kg q2w	PFS	ORR: 20%	mPFS: 1.9 mo	19%
Durvalumab	SAFIR02-BREAST IMMUNO (NCT02299999)	2	82	1–2 L; mTNBC	Maintenance with durvalumab (10 mg/kg q2w) *versus* chemotherapy	PFS	NA	mOS: 21.2 *versus* 14 mo (ITT); 27.3 *versus* 12.1 mo (PD-L1+)	27% *versus* 20%
Pembrolizumab	KEYNOTE-119 (NCT02555657)	3	622	2–3 L; mTNBC	Pembrolizumab iv 200 mg q3w *versus* treatment of physician's choice	OS (ITT; CPS ⩾ 10; CPS ⩾ 1)	ORR: 9.6% *versus* 10.6%	mPFS: 2.1 *versus* 3.3 mo. mOS: 9.9 *versus* 10.8 mo (ITT); 10.7 *versus* 10.2 mo (CPS ⩾ 1); 12.7 *versus* 11.6 mo (CPS ⩾ 10)	20% *versus* 20%
ICIs + chemotherapy									
Pembrolizumab + CT	KEYNOTE-355 (NCT02819518)	3	847	1 L; mTNBC	Pembrolizumab/placebo 200 mg Q3W + CT (nab-paclitaxel; paclitaxel; or gemcitabine plus carboplatin)	PFS OS	ORR: 53% (CPS ⩾ 10)	mOS: 23 *versus* 16 mo (CPS ⩾ 10); mPFS: 10 *versus* 6 mo (CPS ⩾ 10)	68% *versus* 67%
Atezolizumab + nab-paclitaxel	IMpassion130 (NCT02425891)	3	902	1 L; mTNBC	Atezolizumab/placebo 840 mg D1,15 Q4W + nab-paclitaxel 100 mg/m^2 D1,8,15 Q4W	PFS (ITT; PD-L1 ⩾ 1%); OS (ITT; PD-L1 ⩾ 1%)	ORR 56% *versus* 46% (ITT); 59% *versus* 43% (PD-L1+); mDOR: 7.4 *versus* 5.6 mo (ITT); 8.5 *versus* 5.5 m (PD-L1+)	mPFS: 7.2 *versus* 5.5 mo (ITT); 7.5 *versus* 5.0 m (PD-L1+); mOS: 21.3 *versus* 17.6 mo (ITT); 25 *versus* 15.5 (PD-L1+)	49% *versus* 42%

(Continued)

Table 1. (Continued)

Treatment	Trial NCT number	Ph	*n*	Patients	Study design	Primary endpoints	Activity outcomes	Efficacy outcomes	AEs⩾G3
Atezolizumab + paclitaxel	IMpassion131 (NCT03125902)	3	651	1 L; mTNBC	(Atezolizumab/placebo iv 840 mg d1,15 + paclitaxel 90 mg/m^2 d1,8, 15) q4w	PFS (PD-L1+; ITT)*	ORR: 53.6% *versus* 47.5%	mPFS: 5.7 *versus* 5.6 mo; mOS: 19 *versus* 22.8 mo	53% *versus* 46%
Pembrolizumab + eribulin	ENHANCE 1 (NCT02513472)	1b/2	167	1–3 L; mTNBC	(Eribulin iv 1.4 mg/m^2 d1,8 + pembrolizumab iv 200 mg) q3w	Safety ORR	ORR: 23.4%	mPFS: 4.1 mo; mOS: 16.1 mo	NA
ICIs + TT/ADCs									
Atezolizumab + entinostat	ENCORE 602 – TRIO025 (NCT02708680)	2	81	⩾2 L; mTNBC	Azetolizumab iv 1200 mg qw4w + entinostat/placebo PO 5 mg qw	PFS	ORR: 10.0% *versus* 2.4%	mPFS: 1.68 *versus* 1.51 mo	NA
Atezolizumab + cobimetinib + nab-paclitaxel or paclitaxel	COLET (NCT02322814)	2	63	1 L; mTNBC	Cobimetinib (60 mg, D3-D23 q4w) + atezolizumab (840 mg, D1 and D15) + either paclitaxel (80 mg/m^2, d1,8, and 15) or nab-paclitaxel (100 mg/m^2, D1, D8, and D15)	ORR	ORR: 34.4% *versus* 29.0%	mPFS: 3.8 *versus* 7 mo	68% *versus* 70%
Niraparib + pembrolizumab	TOPACIO/ KEYNOTE-162 (NCT02657889)	1/2	55	mTNBC	Pembrolizumab 200 mg Q3W + niraparib at RP2D	ORR	ORR: 21% (ITT); 47% (gBRCAm)	mPFS: 2.3 mo (ITT); 8.3 mo (gBRCAm)	8%
Olaparib + durvalumab	MEDIOLA (NCT0273400)	1b/2	34	gBRCA1/2m, HER2– mBC (16 HR+); PD on ET and 1–2 L of CT	Olaparib 300 mg BID for 4 weeks, followed by olaparib 300 mg BID + durvalumab 1500 mg iv q4w	12wDCR Safety	12wDCR: 80%; 28wDCR: 50%; mDOR: 9.2 mo	mPFS: 8.2 mo; mOS: 21.5 mo	32%
Durvalumab + T-DXd	BEGONIA (NCT03742102)	1b/2	21	Untreated HR– HER2-low mBC	Durvalumab Q3W + T-DXd Q3W (Arm 6)	Safety	ORR: 67%	NA	38%
Ladiratuzumab vedotin + pembrolizumab	SGNLVA-002 (NCT03310957)	1b/2	51	1 L; mTNBC	Ladiratuzumab vedotin + pembrolizumab Q3W	Safety ORR	ORR: 54%	NA	>16%
ICIs + RT									
Pembrolizumab + RT	NCT02730130	2	17	mTNBC; at least two evaluable lesions	RT 30 Gy in 5 fractions + pembrolizumab 200 mg Q3W	13w-ORR	ORR: 18% (ITT)	mPFS 2.6 mo; 6-mo PFS: 18%	65%
Nivolumab + RT	TONIC (NCT02499367)	2	67	1–3 L; mTNBC	Nivolumab with or without induction (RT of a single lesion, cyclophosphamide, cisplatin, doxorubicin).	PFS	ORR: 20% (ITT), 35% (doxorubicin); 17% (no induction); 17% (RT)	mPFS 1.9 mo	3%
Tremelimumab + brain RT	NCT02563925	NA	20	mTNBC with brain metastasis and non-CNS measurable disease	Tremelimumab 10 mg/kg 5 days prior RT. Subsequent doses Q4W for 6 months, then Q3M until disease progression	12w non-CNS DCR	12w non-CNS DCR: 10%	NA	31%

(Continued)

Table 1. (Continued)

Treatment	Trial NCT number	Ph	*n*	Patients	Study design	Primary endpoints	Activity outcomes	Efficacy outcomes	AEs⩾G3
Early-stage TNBC									
Pembrolizumab + NACT	I-SPY2 (NCT01042379)	2	114	High-risk stage II/III TNBC	(Paclitaxel 80 mg/m² QW × C12 ± pembrolizumab 200 mg Q3W × C4); followed by AC Q3W × C4	pCR (ITT)	pCR: 60% *versus* 22%	NA	>9% *versus* >7%
Pembrolizumab + NACT	KEYNOTE-522 (NCT03036488)	3	1174	Stage II–III TNBC	Pembrolizumab/ placebo 200 mg Q3W × C8 + (Carboplatin AUC5 Q3W or AUC 1.5 Q1W + paclitaxel 80 mg/mq QW × C4, followed by AC or EC Q3W × C4), followed by surgery, followed by adjuvant pembrolizumab (placebo × C9)	pCR EFS	pCR: 65% *versus* 51%	3yEFS: 85% *versus* 77%	82% *versus* 79%
Atezolizumab + NACT	Impassion031 (NCT03197935)	3	333	Stage II–III TNBC	Atezolizumab/placebo 840 mg iv Q2W + (nab-paclitaxel 125 mg/mq × C12, followed by ddAC × C4), followed by surgery, followed by atezolizumab 1200 mg Q3W × C11 *versus* follow-up ± capecitabine (for non-pCR patients)	pCR (ITT; PD-L1+)	pCR: 58% *versus* 41% (ITT); 69% *versus* 49% (PD-L1+)	NA	23% *versus* 16%
Atezolizumab + NACT	NeoTRIPaPDL1 (NCT02620280)	3	280	High risk ductal TNBC	(Carboplatin AUC 2 + nab-paclitaxel 125 mg/mq) D1,8 Q3W + atezolizumab 1200 mg Q3W) × C8, followed by surgery, followed by adjuvant FEC	EFS	pCR: 49% *versus* 44%	NA	77% *versus* 70%
Durvalumab + nab-paclitaxel	GeparNuevo (NCT02685059)	2	174	TNBC with a tumor of at least 2 cm	Durvalumab /placebo iv 720 mg (window phase) followed by durvalumab/ placebo 1500 mg Q4W + (nab-paclitaxel iv 125 mg/m² QW for 12 weeks, followed EC Q2W for 4 cycles)	pCR (ITT)	pCR: 53.4% *versus* 44.2%	3yiDFS: 85% *versus* 77%; 3yDDFS: 91% *versus* 80%; 3yOS: 95% *versus* 84%	>37% *versus* >41%
Durvalumab + olaparib + NACT	I-SPY2 (NCT01042379)	II	21 *versus* 142	TNBC; stage II–III	Neoadjuvant durvalumab Q4W × C3 + olaparib W1–11 + paclitaxel QW × C12, followed by AC Q2/3W *versus* NACT	pCR	pCR: 47% *versus* 27%	NA	56% *versus* 34%

*Tested hierarchically first in the PD-L1-positive [immune cell expression 1%, VENTANA PD-L1 (SP142) assay] population, and then in the ITT population.
AC, doxorubicin + cyclophosphamide; ACT, paclitaxel QW x C12 + (anthracycline + cyclophosphamide) Q3W x C4; ADC, antibody–drug conjugate; AE, adverse events; AUC, area under curve; BID, twice a day; C, cycle; CNS, central nervous system; CPS, combined positive score; CR, complete response; CT, chemotherapy; ctDNA, circulating tumor DNA; D, day; DCR, disease control rate; DFI, disease-free interval; DFS, disease-free survival; DLT, dose limiting toxicity; EC, epirubicin + cyclophosphamide; EFS, event-free survival; ET, endocrine therapy; FEC, 5-fluorouracil + epirubicin + cyclophosphamide; G, grade; gBRCA1/2m(ut), germline BRCA1/2 mutated; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; ICI, immune checkpoint inhibitor; IO, immuno-oncology; ITT, intention to treat; iv, intravenously; L, line; mo, months; (m)BC, (metastatic) breast cancer; (m)DOR, (median) duration of response; (m)OS, median overall survival; (m)PFS, (median) progression-free survival; n, number; NA, not available; NACT, neoadjuvant chemotherapy; ORR, objective response rate; P, pembrolizumab; pCR, pathological complete response; PD, progression of disease; PD-(L)1, programmed death-(ligand) 1; Ph, phase; PLD, peghilated lyposomial doxorubicin; PO, per os; PR, partial response; pre-op, preoperative; QnW/M, every *n* weeks/months; RCB, residual cancer burden; RT, radiation therapy; SABR, stereotactic body radiation therapy; T, taxane; TAM, tumor-associated macrophage; TCb, paclitaxel + carboplatin; T-DXd, trastuzumab deructecan; TIL, tumor-infiltrating lymphocyte; TKI, tyrosine kinase inhibitor; (e/m)TNBC, (early/metastatic) triple-negative breast cancer; TRAE, treatment-related adverse event; TT, targeted therapy; W, week.

components of TME, such as tumor-associated macrophages (TAMs), regulatory T and B cells, or myeloid-derived suppressor cells, and also decreasing the activity of inhibitory cytokines.[29] Therefore, many combination strategies are under investigation to overcome these resistances and ultimately maximize ICI benefit. In particular, the 'druggability' of novel immune checkpoints, such as lymphocyte-activation gene 3 (LAG3) and T-cell immunoglobulin and mucin domain 3 (TIM3), is currently being explored at preclinical and early-phase clinical studies for patients with BC, with early signs of promising antitumor activity[30] (Table 2). Pending these data, we do not have robust evidences about biomarkers of ICI resistance.

On the basis of a strong preclinical rational and the clinical benefit demonstrated as monotherapy, combinations of antibody–drug conjugates (ADCs) with ICIs represent a promising strategy.[31,32] In particular, in two phase Ib/II trials enrolling patients with TNBC and HER2− low TNBC, respectively, the combinations of both ladiratuzumab vedotin [(LV), an antizinc transporter LIV-1 ADC] plus pembrolizumab and trastuzumab deruxtecan [(T-Dxd), an anti-HER2 ADC] plus durvalumab showed an acceptable safety profile to continue the drug development and promising ORRs (54% and 67%, respectively).[33,34] Many phase II clinical trials evaluating this strategy are ongoing; in particular, in first-line setting, the SACI-IO TNBC trial (NCT04468061) is assessing the activity of pembrolizumab plus sacituzumab govitecan, an antitrophoblast-antigen-2 (Trop-2) ADC, which has demonstrated to provide an OS benefit as monotherapy in pretreated patients with metastatic TNBC.[31]

The associations of ICIs and targeted therapy (TT) may potentiate the efficacy of ICIs too, but preliminary results were scarce. Of note, both cobimetinib, a MEK inhibitor, and entinostat, a class I-selective histone deacetylase (HDAC) inhibitor, failed to demonstrate a benefit in unselected patients with TNBC.[35,36] Other targeted agents under investigation comprise: antiangiogenic tyrosine kinase inhibitors (TKI), like apatinib or famitinib; binimetinib, a MEK inhibitor; eganelisib, a PI3K-γ inhibitor. Moreover, the combination of ICIs and Poly-(ADP-ribose)-polymerase (PARP) inhibitors (PARPi) was evaluated in two single-arm phase II trials (TOPACIO and MEDIOLA),[37,38] which showed results similar to those reported in clinical trials assessing PARPi monotherapy (OlympiAD and EMBRACA),[39,40] suggesting that biomarkers are key to identify patients who will benefit most.

The use of tumor-directed treatments has been for long viewed as promising to enhance cancer immunogenicity by exposing tumor-derived antigens to the immune system. Locoregional treatments include radiation therapy, cryoablation and microwave ablation, among others; through different mechanisms of action, they determine local tumor destruction, neoantigen release, and a subsequent enhanced and tumor-specific immune response, which can also extend outside the primary tumor (i.e. abscopal effect). Therefore, locoregional treatments represent an ideal partner of ICIs, whose action requires a pre-existing immune 'recognition' of tumor-associated antigens.[41–43] However, a clinical trial assessing the non-central nervous system disease control rate (DCR) after the administration of tremelimumab and brain radiotherapy to 20 patients with metastatic HER2− BC and brain metastasis did not meet its primary endpoint[44]; anyway, enrolled patients were heavily pretreated and non-selected according to PD-L1 status. Data from ongoing clinical trials assessing this abscopal effect in a more favorable setting of disease are awaited.

Lastly, as far as IO therapies other than ICIs are concerned, chimeric antigen receptor (CAR)-T cells have emerged as a promising immunotherapeutic strategy in TNBC: this approach combines the antigen specificity of an antibody with the effector function of T cells and is under investigation in several phase I clinical trials.[45] Despite numerous antigens have been identified as potential targets (e.g. Trop2, GD2, ROR1, MUC1, EpCAM), the target selection represents the most relevant obstacle, to minimize on-target/off-tumor toxicities, as well as to reduce tumor escape *via* antigen loss and intrinsic heterogeneity.

Current challenges for immunotherapy in BC

The evolving landscape of immunotherapy for BC presents a multitude of issues to be further clarified by the ongoing and future clinical trials (Figure 1). First, the advent of promising but toxic partners such as ADCs requires the development of novel strategies to manage the safety, such as the maintenance with only ICIs following the response to few cycles of induction treatment with only ADCs; in this regard, for example, the phase II SAFIR02-BREAST IMMUNO trial

Table 2. Ongoing clinical trials investigating ICI combinations in mTNBC.

Treatment	Trial NCT number	Ph.	Patients	Study design	Primary endpoints	Status
ICI + IO agents						
Nivolumab + ipilimumab	NCT01928394	I/II	Advanced or metastatic solid tumors (included mTNBC)	Ipilimumab + nivovolumab with different schedules	ORR	Active, not recruiting
Durvalumab + tremelimumab	NCT02527434	II	Advanced or metastatic solid tumors (included TNBC)	3 arms: tremelimumab monotherapy; durvalumab monotherapy; combination therapy	ORR	Active, not recruiting
Spartalizumab + ieramilimab (anti-LAG3) + other agents	NCT03742349	Ib	mTNBC	4 arms: Spartalizumab + ieramilimab in combination with NIR178 (oral adenosine A2a receptor antagonist), capmatinib (MET inhibitor), lacnotuzumab (anti-CSF1), or canakinumab (anti-IL1β)	Safety	Active, not recruiting
Encelimab (anti-LAG-3) + dostarlimab	NCT03250832	I	Advanced or metastatic solid tumors (included TNBC)	Encelimab + dostarlimab	Safety	Active, not recruiting
ICI + CT						
Atezolizumab + nab-paclitaxel	EL1SSAR (NCT04148911)	III	mTNBC	Atezolizumab 840 mg D1,15 Q4W + nab-paclitaxel 100 mg/m^2 D1,8,15 Q4W	Safety	Active, not recruiting
Pembrolizumab + carboplatin and gemcitabine induction, pembrolizumab + CT/olaparib	NCT04191135	II/III	mTNBC	Carboplatin (AUC) 2 + gemcitabine 1000 1000 mg/m^2 D1,8 Q3W + pembrolizumab 200 mg Q3W during the induction period for 4–6 cycles. After the induction period, pembrolizumab 200 mg Q3W + (olaparib 300 mg BID or carboplatin AUC 2 + gemcitabine 1000 mg/m^2 D1,8 Q3W)	PFS, OS	Active, not recruiting
Atezolizumab + CT	Impassion132 (NCT03371017)	III	Early relapsing recurrent TNBC; DFI ⩽12	Atezolizumab/placebo 1200 mg + gemcitabine 1000 mg/m^2, followed by carboplatin AUC2 D1,8 Q3W or with capecitabine 1000 mg/m^2, BID D1–14 Q3W	OS (ITT, PD-L1⩾1)	Recruiting
Atezolizumab + carboplatino	NCT03206203	II	mTNBC	Atezolizumab + carboplatin Q3W	PFS	Active, not recruiting
Atezolizumab + PLD + cyclophosphamide	NCT03164993	II	mTNBC	Atezolizumab/placebo + combination of anthracycline and cyclophosphamide, applied in a semi-metronomic fashion (PLD Q2W and daily cyclophosphamide for 2/4 weeks)	PFS	Active, not recruiting

(Continued)

Table 2. (Continued)

Treatment	Trial NCT number	Ph.	Patients	Study design	Primary endpoints	Status
Pembrolizumab + capecitabine/paclitaxel	NCT02734290	II	mTNBC	Pembrolizumab 200 mg IV Q3W + paclitaxel 80 mg/m^2 D1,8,15 Q3W or capecitabine 2000 mg BID D1–7 Q2W	ORR	Active, not recruiting
Pembrolizumab + cyclophosphamide	NCT02768701	II	mTNBC	Pembrolizumab 200 mg 1q21 + cyclophosphamide single dose of 300 mg/m^2 D1	PFS	Active, not recruiting
Tiragolumab + atezolizumab + nab-paclitaxel	NCT04584112	I	Previous untreated mTNBC	Tiragolumab 840 mg Q4W + atezolizumab 1680 mg Q4W + nab-paclitaxel 100 mg/m^2 D1,8,15 Q4W	ORR	Active, not recruiting
ICI + ADC						
Atezolizumab + sacituzumab govitecan or ladiratuzumab vedotin	Morpheus-TNBC (NCT03424005)	I/II	mTNBC	Umbrella study evaluating the efficacy and safety of multiple immunotherapy-based treatment combinations in patients with mTNBC	Safety ORR	Recruiting
Durvalumab + T-DXd	BEGONIA (NCT03742102)	Ib/II	Untreated mTNBC	Durvalumab + T-DXd	Safety	Recruiting
Avelumab + sacituzumab govitecan	InCITe (NCT03971409)	II	Previously Untreated mTNBC	Avelumab + sacituzumab govitecan	ORR	Active, recruiting
Pembrolizumab + sacituzumab govitecan	SACI-IO TNBC (NCT04468061)	II	Untreated mTNBC; PD-L1-negative	Sacituzumab govitecan D1,8 Q3W + pembrolizumab D1 Q3W	PFS	Recruiting
MGC018 (anti-B7-H3 ADC) + retifanlimab (anti-PD-1)	NCT03729596	I/II	TNBC	Dose escalation (3 + 3 + 3 design) followed by a cohort expansion phase	Safety	Recruiting
ICI + TT						
Olaparib + durvalumab	DORA (NCT03167619)	II	mTNBC	Olaparib 300 mg BID + durvalumab Q4W	PFS	Active, not recruiting
Olaparib + atezolizumab	NCT02849496	II	mTNBC	Olaparib BID D1–21 + atezolizumab Q3W	PFS	Suspended
Atezolizumab + nab-paclitaxel + eganelisib (PI3K-γ inhibitor)	MARIO-3 (NCT03961698)	II	Previously untreated mTNBC	Atezolizumab 840 mg D1,15 Q4W + nab-paclitaxel 100 mg/mq e1,8,15 Q4W + eganelisib 20–30–40 mg/day (depending on the results of the safety run-in phase)	CR rate	Active, not recruiting
Carelizumab (anti-PD-1) ± nab-paclitaxel ± apatinib (VEGFR2i)	NCT04335006	III	mTNBC	Arm A (carelizumab + nab-paclitaxel + apatinib) Arm B (carelizumab + nab-paclitaxel) Arm C (nab-paclitaxel)	PFS	Recruiting

(Continued)

Table 2. (Continued)

Treatment	Trial NCT number	Ph.	Patients	Study design	Primary endpoints	Status
Atezolizumab + TT	Morpheus-TNBC (NCT03424005)	I/II	mTNBC	Randomized umbrella study evaluating the efficacy and safety of multiple immunotherapy-based treatment combinations. (capecitabine, ipatasertib, SGN-LIV1A, bevacizumab, chemotherapy (gemcitabine + carboplatin or eribulin), selicrelumab, tocilizumab, nab-paclitaxel, sacituzumab govitecan)	ORR Safety	Active, recruiting
Avelumab + binimetinib + PLD	InCite (NCT03971409)	II	Previously untreated mTNBC (arm A)	Avelumab, binimetinib, liposomal doxorubicin	ORR	Active, recruiting
Camrelizumab + nab-paclitaxel + famitinib	FUTURE-C-PLUS (NCT04129996)	II	Untreated mTNBC	Nab-paclitaxel + famitinib (multi-TKI)	ORR	Active, not recruiting
ICI + RT						
SABR + atezolizumab	AZTEC (NCT03464942)	II	1 L; mTNBC	SABR 20 Gy in one fraction or 24 Gy in 3 fractions, followed by atezolizumab for up to 24 months	PFS	Not recruiting
SABR + pembrolizumab	BOSTON II (NCT02303366)	I	Oligometastatic BC (⩽5 metastasis)	SABR (20 Gy in 1 fraction) to at least 1 metastases (to a maximum of 5) followed by pembrolizumab 200 mg Q3W × C8	Safety	Completed
Pembrolizumab + ablative RT ± olaparib	NCT04683679	II	mTNBC; PD on ICI or PD-L1 negative	Pembrolizumab 200 mg 1q21 × 3 cycles + 8–9 Gy × 3 fractions. Radiation therapy will begin on C1D2–7 ± olaparib (2 × 150 mg tablets twice daily; total 600 mg daily) on continuous days without interruption for two cycles	ORR	Recruiting

AC, doxorubicin + cyclophosphamide; ACT, paclitaxel QW x C12 + (anthracycline + cyclophosphamide) Q3W × C4; ADC, antibody–drug conjugate; AE, adverse events; AUC, area under curve; BID, twice a day; C, cycle; CNS, central nervous system; CPS, combined positive score; CR, complete response; CT, chemotherapy; ctDNA, circulating tumor DNA; D, day; DCR, disease control rate; DFI, disease-free interval; DFS, disease-free survival; DLT, dose limiting toxicity; EC, epirubicin + cyclophosphamide; EFS, event-free survival; ET, endocrine therapy; FEC, 5-fluorouracil + epirubicin + cyclophosphamide; G, grade; gBRCA1/2m(ut), germline BRCA1/2 mutated; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; ICI, immune checkpoint inhibitor; IO, immuno-oncology; ITT, intention to treat; iv, intravenously; L, line; (m)BC, (metastatic) breast cancer; (m)DOR, (median) duration of response; mo, months; *n*, number; NA, not available; NACT, neoadjuvant chemotherapy; ORR, objective response rate; (m)OS, median overall survival; P, pembrolizumab; pCR, pathological complete response; PD, progression of disease; PD-(L)1, programmed death-(ligand) 1; (m)PFS, (median) progression-free survival; Ph, phase; PLD, peghilated lyposomial doxorubicin; PO, per os; PR, partial response; pre-op, preoperative; QnW/M, every *n* weeks/months; RCB, residual cancer burden; RT, radiation therapy; SABR, stereotactic body radiation therapy; T, taxane; TAM, tumor-associated macrophage; TCb, paclitaxel + carboplatin; T-DXd, trastuzumab deructecan; TIL, tumor-infiltrating lymphocyte; TKI, tyrosine kinase inhibitor; (e/m)TNBC, (early/metastatic) triple-negative breast cancer; TRAE, treatment-related adverse event; TT, targeted therapy; W, week.

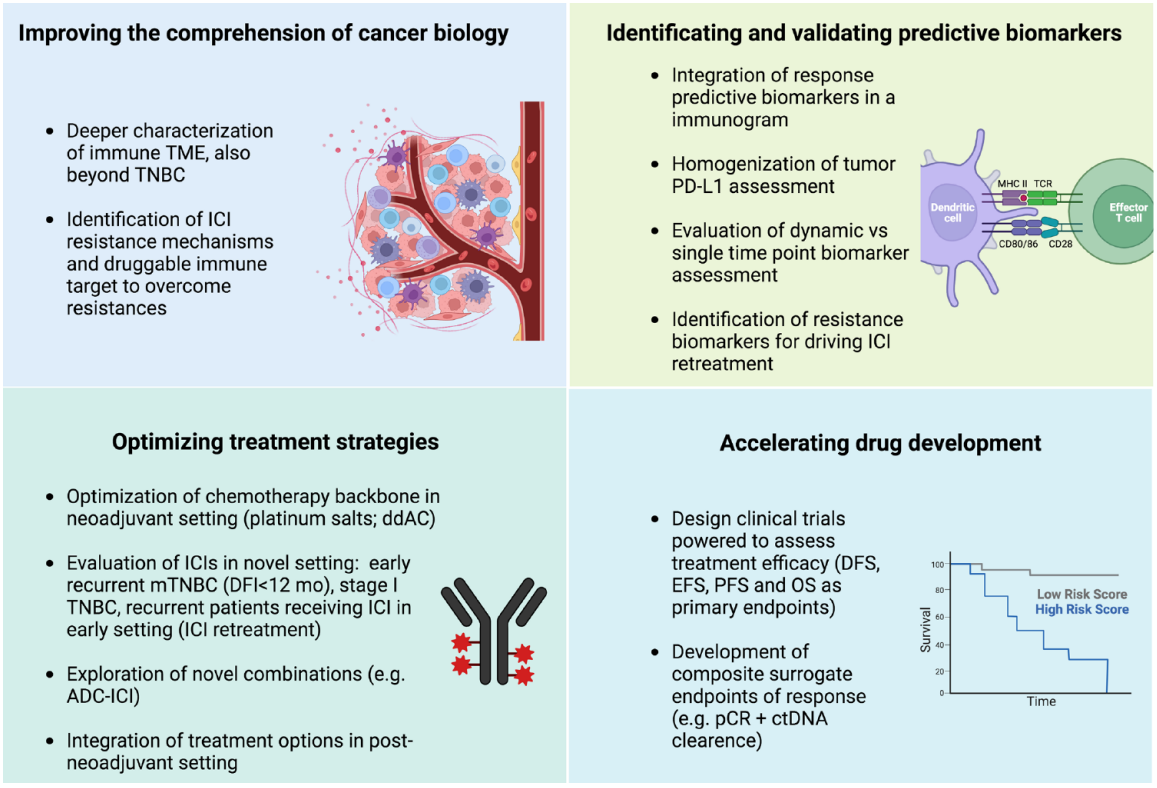

Figure 1. Immunotherapy in BC: current issues.
ADC, antibody–drug conjugate; BC, breast cancer; ctDNA, circulating tumor DNA; ddAC, dose dense doxorubicin + cyclophosphamide; DFI, disease-free survival; DFS, disease-free survival; EFS, event-free survival; ICI, immune checkpoint inhibitor; (m)TNBC, (metastatic) triple-negative breast cancer; OS, overall survival; pCR, pathological complete response; PD-L1, programmed death-ligand 1; PFS, progression-free survival; TME, tumor microenvironment.

randomized 199 patients with metastatic HER2− BC whose disease did not progress after six to eight cycles of first or second line chemotherapy to receive either durvalumab or maintenance chemotherapy.[22] Despite the trial failed to demonstrate a PFS (primary endpoint) and OS benefit in the ITT population, in an exploratory subgroup analysis, durvalumab improved OS in the 82 patients with TNBC.

Another important issue is represented by the lack of evidence of ICI efficacy in patients with early distant recurrence after neoadjuvant chemotherapy (NACT), excluded from KEYNOTE-355 and Impassion130, because a disease-free interval (DFI) of ⩾6 and ⩾12 months was respectively required. In this regard, the placebo-controlled randomized phase III IMpassion132 trial (NCT03371017) is assessing atezolizumab with first-line chemotherapy (capecitabine, mandatory in platinum-pretreated patients, or gemcitabine/carboplatin) for metastatic TNBC and a DFI of ⩽12 months.[46]

With the advent of ICIs in early setting, an emerging question is represented by the efficacy of the retreatment with ICIs and chemotherapy as first-line therapy; clinical trials with a strong translational design to identify biomarkers of ICI resistance and guide the retreatment are needed.

Lastly, a deeper comprehension of immune TME to identify response predictive biomarkers represents the most urgent need to better select the patients beyond the PD-L1 expression. In fact, according to the ICI, the PD-L1 assessment is performed with different companion diagnostic assays and is therefore burdened by a lack of homogeneity.[47]

Challenges of immunotherapy for early-stage TNBC

Trials investigating neoadjuvant immunotherapy

TNBC is diagnosed at stage II or III in more of 60% of cases[48–50]; nevertheless, because of its

aggressive behavior, it is characterized by early recurrences (3-year distant recurrence rate of 30%–35%) and the poorest prognosis among BC subtypes (5-year estimate OS of 64% for stages I–III combined).[51,52] In this regard, on the basis of positive results in advanced setting, ICIs moved to early TNBC setting, which represents the ideal context for ICI treatment, before the acquisition of multiple mechanisms of immune escape and when the host immune system is less compromised.[11]

Emerging evidences confirmed these hypotheses: a trial-based meta-analysis of five randomized trials enrolling 1496 TNBC patients showed a statistically significant association between ICI addition and pathological complete response [(pCR), odds ratio (OR): 1.72, 95% CI: 1.22–2.42].[53] Furthermore, the phase III randomized KEYNOTE-522 clinical trial randomizing 1174 patients with stage II–III TNBC to NACT with paclitaxel–carboplatin followed by doxorubicin–cyclophosphamide, with or without the addition of pembrolizumab, demonstrated a benefit in terms of EFS (primary endpoint; 3-year EFS: 84.5% *versus* 76.8%), regardless of PD-L1 expression (CPS $\geq$ 1 or $<$1) (Table 1). As far as the safety profile of this five-drug combination, 77% and 73% of patients, respectively, from the experimental and the control arm, experienced a treatment-related adverse event of grade 3 or more; furthermore, the rate of immune-mediated adverse events of grade 3 or higher was of 13% and 1%. These results led to full approval for neoadjuvant pembrolizumab in combination with chemotherapy by the Food and Drug Administration for patients with high-risk TNBC.[54]

In contrast with positive clinical trials (I-SPY2, Impassion031, KEYNOTE-522),[12,15,55,56] the phase II GeparNuevo, randomizing patients to receive nab-paclitaxel followed by dose dense anthracyclines and cyclophosphamide with either durvalumab or placebo, failed to demonstrate a statistically significant improvement in the primary endpoint of pCR (53.4% *versus* 44.2%, OR: 1.45) but showed potential clinical benefit after 42 months of follow-up in a descriptive analysis, in terms of 3-year invasive disease-free survival (iDFS, 84.9% *versus* 76.9%; HR 0.54, 95% CI 0.27–1.09), 3-year distant disease-free survival (DDFS, 91.4% *versus* 79.5%; HR 0.37, 95% CI 0.15–0.87) and 3-year OS (95.1% *versus* 83.1%, HR 0.26, 95% CI 0.09–0.79).[57,58] Unlike the other three similar positive trials, which enrolled only patients with stage II–III TNBC, 35% of patients from GeparNuevo had a TNBC in stage I and seemed to derive a lower benefit in terms of pCR from the addition of durvalumab. Although the study was formally negative for its primary endpoint, the significant difference found in iDFS represents an intriguing finding, suggesting that pCR may be not the sole driver of IO benefit in early TNBC, and that clinical outcomes may also be independent of pCR, especially when investigating IO agents.

Novel agent combinations in neoadjuvant setting

Many innovative IO combination strategies are under investigation with the aim of further improve outcomes of patients with early TNBC; they include: TT/ADC-ICI, other IO agents-ICI and locoregional treatment–ICI combinations, and cancer vaccine (CV)–chemotherapy combinations (Table 3).[59]

As it pertains TT and ADCs, on the basis of immunosuppressive properties showed by vascular endothelial growth factor (VEGF), many combinations of ICIs with antiangiogenic agents are under evaluation in clinical trials, including apatinib, a selective VEGFR2 inhibitor, and anlotinib and lenvatinib, two multi-target tyrosine kinase inhibitors (TKI) inhibiting VEGFR, FGFR, PDGFR, c-Kit, and Ret.[60–62] Morover, the phase II NeoSTAR clinical trial (NCT04230109) has investigated the activity and safety of neoadjuvant sacituzumab govitecan plus pembrolizumab on the basis of a strong preclinical rational,[63,64] reporting 30% ORR. In regard with PARPis, following the results of MEDIOLA trial,[38] the I-SPY2 study evaluated the combination of durvalumab and olaparib concurrent with weekly neoadjuvant paclitaxel, followed by AC regimen, in early-stage HER2-negative setting.[65] 73 patients, whose 21 with TNBC, were enrolled in the experimental arm and 299 in the control arm (chemotherapy only); at the final efficacy analysis, the combination improved estimated pCR rates over control from 20% to 37% in HER2-negative cancers, and from 27% to 47% in TNBC. Lastly, TNBC has been considered a poor candidate for CDK4/6 inhibitors (CDK4/6i), because it holds the loss of retinoblastoma (Rb) protein in 50% of cases, critical for CDK4/6i-induced cell cycle arrest.[66] Nevertheless, the neutrophil-preserving CDK4/6i trilaciclib has shown an antitumor activity in TNBC preclinical models and has enhanced antitumor immune responses

Table 3. Ongoing clinical trials investigating ICI combinations in early TNBC (neoadjuvant and post-neoadjuvant setting).

Treatment	Trial NCT number	Ph.	Patients	Study design	Endpoints	Status
Neoadjuvant setting						
ICI + CT						
Durvalumab post-NACT	NCT03740893 (PHOENIX DDR/ anti-PD-L1)	2	Post-NACT residual TNBC (cohort D)	PART 1: pre-operative exposure to 1500 mg durvalumab on Day 1 only of the window of opportunity. PART 2: 12 months postoperative exposure to 1500 mg durvalumab on Day 1 only of a 28 day cycle	Change in CD8+ TILs and in IFNγ+ signature post-durvalumab	Recruiting
Durvalumab + ACT (dose-dense EC)	NCT03356860 (B-IMMUNE)	1b/2	Early-stage luminal B and TNBC	Paclitaxel QW w1–12 + dose dense EC q2w w14–20 + durvalumab 1500 mg at weeks 14 and 18 *versus* paclitaxel QW w1–12 + dose dense EC q2w w14–20	pCR and AE	Recruiting
Pembrolizumab + carboplatin + docetaxel	NCT03639948 (NeoPACT)	2	Early-stage TNBC	Carboplatin AUC6 + docetaxel 75 mg/ mq + pembrolizumab 200 mg q3w for 6 cycles	pCR	Recruiting
Pembrolizumab + ACT	NCT03515798 (PELICAN)	2	Early-stage HER2-negative inflammatory BC	Pembrolizumab Q3W + ACT *versus* ACT	pCR; DLT	Recruiting
Pembrolizumab + decitabine + ACT (dose-dense AC)	NCT02957968	2	Locally advanced TNBC (cohort A)	Decitabine D1–4 + pembrolizumab D8,22 × C1, followed by neoadjuvant dose-dense AC × C4, followed by weekly paclitaxel and carboplatin AUC 1,5 × C12	Increase in TILs percentage	Recruiting
Nivolumab + ACT	NCT03742986	2	Early-stage inflammatory BC	Nivolumab Q3W × C4 + paclitaxel D1,8,15,21 Q3W × C4, followed by AC Q2W × C4	pCR	Completed
Atezolizumab + ACT	NCT04770272 (neoMono)	2	Early-stage TNBC	Window of monotherapy with Atezolizumab for 2 weeks (840 mg d1) before biopsy, followed by ACT + atezolizumab neoadjuvant *versus* ACT + atezolizumab neoadjuvant	pCR	Recruiting
Atezolizumab + carboplatin + paclitaxel	NCT02883062	2	Stage II-III TNBC	Carboplatin Q3W × C4 + paclitaxel QW × C12 + atezolizumab Q3W × C4 *versus* carboplatin Q3W × C4 + paclitaxel QW × C12	Increase in TILs percentage	Active, not recruiting
Atezolizumab + nab-paclitaxel	NCT02530489	2	Early-stage TNBC	Atezolizumab Q3W × C4 + nab-paclitaxel D1,8,15 Q3W × C4, followed by surgery, followed by adjuvant atezolizumab Q3W × C4	pCR	Active, not recruiting

(Continued)

Table 3. (Continued)

Treatment	Trial NCT number	Ph.	Patients	Study design	Endpoints	Status
atezolizumab + NACT	NCT03281954 (NSABP B-59/GBG 96-GeparDouze)	3	Early-stage TNBC	(atezolizumab/placebo Q3W + paclitaxel QW + carboplatin Q3W) × C4, followed by (atezolizumab /placebo Q3W + AC or EC Q2/3W) × C4, followed by surgery, followed by atezolizumab/placebo Q3W until 1 year after the first dose	pCR; EFS	Active, not recruiting
Camrelizumab + nab-paclitaxel + epirubicin	NCT04213898	1/2	Early-stage TNBC	Camrelizumab Q3W × C6+ nab-paclitaxel D1,8,15,21 × C6+ epirubicin D1,21 × C6	pCR	Not yet recruiting
Camrelizumab + cisplatin + vinorelbine post-NACT	NCT04848454	2	Early-stage HER2-negative BC; no PR after 2 cycles of standard NACT	Camrelizumab Q3W × C6+ vinorelbine D1,8 Q3W × C6+ cisplatin Q3W × C6, followed by surgery, followed by adjuvant camrelizumab Q3W × C11	pCR	Recruiting
Camrelizumab + ACT	NCT05088057	2	Early-stage TNBC	(Camrelizumab + AC) Q3W × C4, followed by (camrelizumab + T) Q3W × C4 OR camrelizumab Q3W × C8 + AT Q3W × C4	pCR	Recruiting
Camrelizumab + nab-paclitaxel + EC	NCT04676997	2	Early-stage TNBC	(Camrelizumab D1,15 Q2W + nab-paclitaxel D1,8,15 QW 3/4) × C4, followed by (camrelizumab Q2W + epirubicin Q2W + cyclophosphamide Q2W) × C4	pCR	Recruiting
Camrelizumab + nab-paclitaxel + carboplatin	NCT04907344	2/3	Early-stage TNBC	Camrelizumab + nab-paclitaxel + carboplatin *versus* nab-paclitaxel + carboplatin	pCR	Not yet recruiting
Camrelizumab + NACT	NCT04613674	3	Early-stage TNBC	Camrelizumab/placebo + NACT	pCR	Recruiting
Cemiplimab + PCb + AC	NCT04243616	2	Early-stage HER2-negative PD-L1+ (CPS ⩾ 1%) BC	Cemiplimab Q3W × C2 + Paclitaxel D1,8,15 Q3W × C4 + optional Carboplatin AUC6 D1 Q3W × C4, followed by AC Q2W × C4	pCR	Recruiting
Cemiplimab + ACT	NCT01042379 (I-SPY)	2	Early-stage BC	Cemiplimab Q3W × C12 + paclitaxel QW × C12, followed by AC Q2/3W × C4	pCR	Recruiting (arm is closed)
Oral paclitaxel + encequidar + dostarlimab + carboplatin + AC	NCT01042379 (I-SPY2)	2	Early-stage TNBC	Dostarlimab Q3W × C4 + oral paclitaxel D1–3 QW × C12 + oral encequidar D1–3 QW × C12 + carboplatin (AUC 1.5) QW × C12, followed by AC Q2/3W × C4	pCR	Recruiting (arm is closed)
Oral paclitaxel + encequidar + dostarlimab + AC	NCT01042379 (I-SPY2)	2	Early-stage TNBC	Dostarlimab Q3W × C4 + oral paclitaxel D1–3 QW × C12 + oral encequidar D1–3 QW × C12, followed by AC Q2/3W × C4	pCR	Recruiting (arm is closed)
Sintilimab + NACT	NCT04809779	2	Early-stage TNBC	Sintilimab Q3W × C3 + EC Q3W × C4, followed by nab-paclitaxel (QW × C12 or Q3W × C4)	pCR	Not yet recruiting

(Continued)

Table 3. (Continued)

Treatment	Trial NCT number	Ph.	Patients	Study design	Endpoints	Status
HLX10 (anti-PD-1) + NACT	NCT04301739	3	Early-stage TNBC	(HLX10/placebo + nab-paclitaxel + carboplatin) × C4, followed by (HLX10/placebo + AC or EC) × C4, followed by surgery, followed by HLX10/placebo × C9	pCR	Not yet recruiting
Toripalimab + nab-paclitaxel + dose-dense EC	NCT04418154	2	Early-stage TNBC	EC Q2W × C4, followed by nab-paclitaxel QW × C12 + toripalimab Q3W × C4	pCR	Recruiting
TT/ADC + ICI combinations						
Sintilimab (anti-PD-1) + anlotinib + NACT	NCT04877821 (NeoSACT)	2	Early-stage TNBC	Sintilimab Q3W + anlotinib d1–14 Q3W + (nab-paclitaxel QW + carboplatin Q3W) × C4, followed by EC Q3W × C4 prior to surgery. After surgery, those who exhibited residual disease were treated with capecitabine	pCR	Recruiting
Tislelizumab (anti-PD-1) + anlotinib + NACT	NCT04914390 (NeoATCT)	2	Early-stage TNBC	(Tislelizumab Q3W + anlotinib D1–14 Q3W + doxorubin or epirubicin Q3W + nab-paclitaxel Q3W) × C6	pCR	Recruiting
Sintilimab (anti-PD-1) + Apatinib + NACT	NCT04722718	2	Early-stage TNBC	(Sintilimab Q3W + apatinib d1–14 Q3W + nab paclitaxel D1,8,21 Q3W + carboplatin D1,8,21 Q3W) × C6	pCR	Recruiting
Lenvatinib + pembrolizumab	NCT04427293	1	Early-stage TNBC	Lenvatinib D1–14 × C1 + pembrolizumab D1	Measuring the infiltration of TILs	Recruiting
Pembrolizumab + sacituzumab govitecan	NCT04230109 (NeoSTAR)	2	Early-stage TNBC	Sacituzumab govitecan Q3W × C4 + pembrolizumab Q3W C4 (combination cohort)	pCR	Active, not recruiting
Durvalumab + olaparib + ACT	NCT01042379 (I-SPY2)	2	Early-stage HER2–negative BC	Durvalumab Q4W × C3 + olaparib W1–11 + paclitaxel QW × C12, followed by AC Q2/3W × C4	pCR	Completed
Durvalumab + olaparib + NACT	NCT03594396	1/2	Early-stage TNBC	Olaparib D1–28 + durvalumab D15, followed by standard NACT (biopsy at screening and on D14)	Changes of tumor biology detected by serial biopsy	Active, not recruiting
Trilaciclib + NACT + pembrolizumab	NCT05112536	2	Early-stage TNBC	Lead-in trilaciclib single-dose monotherapy, followed by trilaciclib + AC + pembrolizumab (per investigator discretion), followed by trilaciclib + paclitaxel + carboplatin (per investigator discretion; AUC 1.5)	Change in CD8 T cells/Treg ratio in tumor tissue	Recruiting

(Continued)

Table 3. (Continued)

Treatment	Trial NCT number	Ph.	Patients	Study design	Endpoints	Status
ICI + IO (+CT) combinations						
Ipilimumab + nivolumab + talimogene laherparepvec	NCT04185311	1	Early-stage HER2–negative BC	Talimogene laherparepvec intratumorally D1,22,36 + nivolumab D1,15,29,43, + ipilimumab D1,43, followed by surgery	AEs	Active, not recruiting
Talimogene laherparepvec + atezolizumab	NCT03802604 (PROMETEO)	1	Post-NACT residual early-stage BC	Intratumoral talimogene laherparepvec (Q3W × C2 then Q2W × C3) + atezolizumab Q2W × C4	RCB0/1	Recruiting
Nivolumab ± ipilimumab	NCT03815890 (BELLINI)	2	Early-stage TNBC (arm 1B, 2B, 3B)	Arm 1B: Nivolumab × C2 Arm 2B: Nivolumab × C2 + ipilimumab × C1 Arm 3B (high TIL): Nivolumab × C2 + ipilimumab × C2	Change in TME (CD8+ TILs and IFNγ+ signature) after pre-op IO	Recruiting
Pembrolizumab + NACT + IRX-2	NCT04373031	2	Early-stage TNBC	P + ACT (P single-dose induction, followed by P Q3W + T QW × C4, followed by P + AC Q3W × C4) *versus* P + IRX-2 + ACT (P single-dose + C + IRX-2 induction, followed by P Q3W + T weekly × C4, followed by IRXP2 re-induction, followed by P + AC Q3W × C4)	pCR	Recruiting
Pembrolizumab + IL-12 + L-NMMA (pan-NOS inhibitor) + docetaxel	NCT04095689 (INTEGRAL)	2	Early-stage anthracycline-refractory TNBC	Docetaxel + pembrolizumab + IL-12 gene therapy, followed by docetaxel + pembrolizumab + NG-monomethyl-L-arginine (L-NMMA)	pCR	Recruiting
Pembrolizumab + SD-101 (TLR9 agonist) + ACT	NCT01042379 (I-SPY)	2	Early-stage BC	SD-101 intratumorally QW × C4, then Q3W × C2 + pembrolizumab cycles 1,4,7,10 + paclitaxel QW × C12, followed by AC Q2/3W × C4	pCR	Recruiting (arm is closed)
Cemiplimab + fianlimab (anti-LAG3) + ACT	NCT01042379 (I-SPY)	2	Early-stage BC	Cemiplimab Q3W × C12 + fianlimab Q3W × C12 + paclitaxel QW × C12, followed by AC Q2/3W × C4	pCR	Recruiting
Nivolumab + cabiralizumab (anti-CSF-1R) + NACT	NCT04331067	1b/2	Early-stage TNBC	TCb × C4 + nivolumab Q2W × C6 + cabiralizumab Q2W × C6	AEs; increase in TILs and TAMs percentage	Recruiting
ICI + locoregional treatment combinations						
Pembrolizumab + NACT + RT boost	NCT04443348 (P-RAD)	2	Node-positive, HR+/HER2– BC or TNBC	Pembrolizumab Q6W × C4 + Paclitaxel QW × C12 + Carboplatin QW × C12 (optional) + AC Q2W × C4 + no RT boost (group A) or low-dose RT boost (group B) or high dose RT boost (group C), followed by optional adjuvant Pembrolizumab Q6W × C4 and/or Capecitabine Q3W x C6	pCR in lymph node; increase in TILs percentage	Recruiting

(Continued)

Table 3. (Continued)

Treatment	Trial NCT number	Ph.	Patients	Study design	Endpoints	Status
Pembrolizumab + RT boost	NCT03366844 (PEARL)	1/2	Early-stage TNBC T ⩾ 2 cm (cohort 2)	Pembrolizumab × C2 + RT boost, followed by standard treatment (surgery and/or chemotherapy)	Increase in TILs percentage; feasibility	Recruiting
Durvalumab + RT + Carboplatin + Paclitaxel	NCT03872505 (PANDoRA)	2	Early-stage TNBC	Durvalumab × C5 + Carboplatin QW × C12 + Paclitaxel QW × C12 ± RT (24 Gy, in conjunction with second dose of durvalumab)	pCR	Withdrawn (lack of funding)
Adebrelimab (anti-PD-L1) + SBRT + NACT	NCT05132790	NA	Early-stage TNBC	Adebrelimab Q3W + SBRT, followed by CT with adebrelimab + nab-paclitaxel and carboplatin/cisplatin	pCR	Not yet recruiting
CMP-001 + SBRT	NCT04807192	2	Early-stage TNBC	Preoperative SBRT *versus* Preoperative SBRT + intra-tumoral administrations of CMP-001	Increase in TILs percentage	Recruiting
Ipilimumab + Nivolumab + Cryoablation post-NACT	NCT03546686	2	Early-stage TNBC post-NACT	Ipilimumab + Nivolumab C1, followed by core biopsy and cryoablation, followed by definitive surgery, followed by Nivolumab Q2W × C3	EFS	Recruiting
Microwave ablation + Camrelizumab	NCT04805736	2	Early-stage BC (cT < 3cm; cN0)	Camrelizumab × C1 *versus* Microwave ablation *versus* Camrelizumab × C1 + microwave ablation, followed by surgery	TRAEs	Recruiting
CVs + CT combinations						
P10s-PADRE + NACT	NCT02938442	1/2	Early-stage TNBC	AC Q2W × C4, followed by Paclitaxel QW × C12 *versus* P10s-PADRE, followed by AC Q2W × C4, followed by Paclitaxel QW × C12	pCR; AEs	Recruiting
TriMix/placebo ± NACT	NCT03788083 (TMBA)	1	Early-stage BC	Intratumoral TriMix/placebo injection ± NACT	TRAE	Recruiting
Cell-based vaccination with autologous tumor cells engineered	NCT00880464	1	Early-stage BC– T⩾2 cm post-NACT or T⩾4 cm (2 patients with TNBC)	Vaccinations on days 1,8,15 and Q2W thereafter until the supply of vaccine has been exhausted	AEs	Completed
Autologous Dendritic cells + AC	NCT03450044 (TEBICA)	1/2	Early-stage BC	NACT with AC + transfer of autologous DCs generated *in vitro versus* NACT with AC	TRAEs	Completed
Cyclin B1/WT-1/CEF (antigen)-loaded Dendritic cell vaccine + NACT	NCT02018458	1/2	Locally advanced TNBC	(AC Q3W × C4, followed by paclitaxel + carboplatin QW × C12) + intratumoral vaccine on C1 and C3 of AC and on C1 and C3 of TCb, followed by surgery, followed 3 boost vaccination	AEs	Completed

(Continued)

Table 3. (Continued)

Treatment	Trial NCT number	Ph.	Patients	Study design	Endpoints	Status
Post-neoadjuvant setting						
Avelumab	A-Brave (NCT02926196)	3	High-risk TNBC after neoadjuvant (non pCR) or adjuvant (stage IIB–III) CT	Adjuvant avelumab for 1 year *versus* observation	DFS	
Pembrolizumab (following capecitabine)	SWOG S1418, NRG BR-006 (NCT02954874)	3	TNBC; non pCR after NACT (⩾1 cm residual in the T or ypN1mi-))	Adjuvant pembrolizumab for 1 year *versus* observation (adjuvant capecitabine is allowed, with pembrolizumab initiated after capecitabine)	Invasive DFS	
Nivolumab + Ipilimumab + RT	NCT03818685 (breast immune 03)	2	TNBC with residual disease after NACT (RCB score II or III)	Nivolumab × C8 + ipilimumab × C4 + RT *versus* capecitabine × C8 + RT	DFS	
Atezolizumab + capecitabine	NCT03756298	2	TNBC with residual disease after NACT	Atezolizumab + capecitabine × C8 *versus* capecitabine × C8	Invasive DFS	
Nivolumab + capecitabine	OXEL (NCT03487666)	2	TNBC with residual disease after NACT	Nivolumab × C6 cycles *versus* capecitabine × C6 *versus* nivolumab + capecitabine × C6	Changes in a immunoscore at week 6	
Atezolizumab + capecitabine ± inavolisib/ tatazoparib	PERSEVERE (NCT04849364)	2	TNBC with residual disease after NACT (RCB score II or III)	Patients will be allocated to each arm based on the positivity of ctDNA and the presence of a genomic target • Arm 1b (ICI target): atezolizumab + capecitabine • Arm 1c: (PI3K target) inavolisib + capecitabine, followed by atezolizumab • Arm 1d (DNA repair target + ICI): talazoparib + atezolizumab + capecitabine	DFS	
Sacituzumab govitecan + atezolizumab	ASPRIA (NCT04434040)	2	TNBC with residual disease after NACT	Sacituzumab govitecan + atezolizumab × C6	Rate of undetectable ctDNA	

AC, doxorubicin + cyclophosphamide; ACT, paclitaxel QW × C12 + (anthracycline + cyclophosphamide) Q3W × C4; ADC, antibody–drug conjugate; AE, adverse events; AUC, area under curve; BID, twice a day; C, cycle; CNS, central nervous system; CPS, combined positive score; CR, complete response; CT, chemotherapy; ctDNA, circulating tumor DNA; D, day; DCR, disease control rate; DFI, disease-free interval; DFS, disease-free survival; DLT, dose limiting toxicity; EC, epirubicin + cyclophosphamide; EFS, event-free survival; ET, endocrine therapy; FEC, 5-fluorouracil + epirubicin + cyclophosphamide; G, grade; gBRCA1/2m(ut), germline BRCA1/2 mutated; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; ICI, immune checkpoint inhibitor; IO, immuno-oncology; ITT, intention to treat; iv, intravenously; L, line; (m)BC, (metastatic) breast cancer; (m)DOR, (median) duration of response; mo, months; (m)OS, median overall survival; (m)PFS, (median) progression-free survival; *n*, number; NA, not available; NACT, neoadjuvant chemotherapy; ORR, objective response rate; P, pembrolizumab; pCR, pathological complete response; PD, progression of disease; PD-(L)1, programmed death-(ligand) 1; Ph, phase; PLD, peghilated lyposomial doxorubicin; PO, per os; PR, partial response; pre-op, preoperative; QnW/M, every *n* weeks/months; RCB, residual cancer burden; RT, Radiation Therapy; SABR, Stereotactic Body Radiation Therapy; T, taxane; TAM, tumor-associated macrophage; TCb, paclitaxel + carboplatin; T-DXd, trastuzumab deructecan; TIL, tumor-infiltrating lymphocyte; TKI, tyrosine kinase inhibitor; (e/m)TNBC, (early/metastatic) triple-negative breast cancer; TRAE, treatment-related adverse event; TT, targeted therapy; W, week.

through T-cell effector stimulation and regulatory T-cell suppression,[67–69] demonstrating also an intriguing and unexpected OS benefit in association with chemotherapy in an unpowered phase II trial.[70,71]

Combinations of IO agents are also under investigation in early TNBC; they comprise ipilimumab,[72] an antibody anticytotoxic t-lymphocyte antigen (CTLA)-4; fianlimab, an antibody anti-LAG-3 which enhances cytotoxic T-lymphocyte (CTL)-mediated tumor cell lysis[73]; cabiralizumab, an antibody anticolony-stimulating factor 1 receptor (CSF-1R) which reduces immunosuppressive TAMs and promotes a proinflammatory TME, stimulating T-cell responses[74]; IRX-2, a mixture of cytokines which increases the immune activation in TME[75]; IL-12 and monomethylated L-arginine (L-NMMA), a pan-NOS-inhibitor which showed the ability to remodel the immune TME[76]; SD-101, a toll-like receptor 9 (TLR9) agonist which promotes a T helper 1-like chemokine milieu in TME and induces an antitumor CD8+ T-cell response.[77]

As stated, the combination of ICI with locoregional treatments is based on a strong preclinical rational and may represent an opportunity to de-escalate NACT and optimize its toxicity; in fact, in the phase Ib/II PEARL clinical trial, enrolling patients with early-stage TNBC, the administration of pembrolizumab and RT prior to physician's choice NACT provided a 56% of pCR rate, overlapping results from control arm of KEYNOTE-522, despite only 1/3 of study participants received the same chemotherapy regimen.[78]

Lastly, after almost two decades of limited benefit from clinical trial investigating CVs, the coronavirus disease 2019 pandemic boosted a significant technological advancement and many neoantigens are emerging as the preferred targets to develop therapeutic CVs.[79,80] Moreover, in light of a deeper comprehension of immune escape mechanisms, clinical trials moved from metastatic disease, where the TME is more likely compromised by inhibitory mechanisms, to premalignant or adjuvant setting and investigated combination treatments (i.e. CV plus ICI). Examples of CVs under investigation in patients with TNBC comprise: P10s-PADRE, a peptide-based CVs contains a carbohydrate mimetic peptide P10s fused to the pan-HLA-DRe binding epitope (PADRE) peptide, with a CTL-stimulating activity,[81] TriMix, a cell-based CVs consisting of a mRNA encoding a mixture of three immune modulating molecules (TLR-4, CD40L, and CD70) with a T-cell stimulatory capacity.[82,83]

Post-neoadjuvant setting

For patients with early TNBC, NACT is preferred to adjuvant therapy in order to *in vivo* test the sensitivity to chemotherapy and to personalize treatments in post-neoadjuvant setting on the basis of pCR, which represents a valid patient-level prognosticator of better outcomes.[84] For patients with high-risk TNBC, for example those who do not experience pCR after NACT, the use of treatment intensification in the adjuvant setting has showed to portend survival benefits: specifically, capecitabine and Olaparib in patients without and with germline BRCA (gBRCA) pathogenetic mutations.[85,86]

Of interest, the KEYNOTE-522 scheduled included nine cycles of post-neoadjuvant pembrolizumab, regardless of the pCR, and neither concomitant olaparib nor capecitabine were admitted, because the trial was designed before the approval of these indications.[87]

Therefore, there is an urgent need to incorporate these treatment options and to adapt them to the risk of recurrence. In particular, the role of ICI in post-neoadjuvant setting has to be clarified, both in ICI-naïve patients and in those who received neoadjuvant immunotherapy.[88]

In this regard, many clinical trials are investigating the role of ICIs as alternative or in association (sequential or concomitant) to capecitabine in ICI-naïve patients with high-risk TNBC and residual disease after NACT (Table 3). Furthermore, ASPRIA trial is investigating another escalation option with sacituzumab govitecan plus atezolizumab or alone in patients with residual disease after NACT.

However, on the basis of safety profile assessed in other settings, the combination of post-neoadjuvant pembrolizumab plus capecitabine or olaparib, according to gBRCA status, may be considered in clinical trials for patients not reaching the pCR and at higher risk of recurrence.[88]

Contrarily, the 3-year EFS rate of patients enrolled in KEYNOTE-522 who reached the pCR (94.4% *versus* 92.5%, HR: 0.73, 95% CI:

0.39–0.36) suggests the exploration of de-intensification strategies to spare post-neoadjuvant pembrolizumab and, consequently, clinical, psychological, and financial toxicities; this hypothesis is corroborated by GeparNuevo trial, which demonstrated an underpowered but significant OS benefit and did not include post-neoadjuvant durvalumab.[15,58]

Current issues

The introduction of ICIs for the treatment of patients with early TNBC raises many interesting issues warranting new translational studies and clinical trials[59,89,90] (Figure 1).

First, the choice of the best backbone chemotherapy is not fully understood, in particular platinum salts demonstrated to provide an EFS benefit when added to standard NACT but GeparNuevo trial showed a survival benefit also without carboplatin[58,91]; therefore, the need for platinum in the presence of pembrolizumab has to be assessed. Furthermore, KEYNOTE-522 did not include dose-dense anthracyclines and cyclophosphamide (AC) regimen, which has demonstrated to be superior to standard AC[92]; hence, also the role of dose dense AC in association to ICIs needs to be clarified.

As far as predictive biomarkers, pembrolizumab demonstrated to provide a clinical benefit regardless of PD-L1 status (CPS ≥ 1 or <1); there is an urgent need to optimize the patient selection in order to develop de-escalation and escalation strategies for the upfront responders to ICI and non-responders, respectively. Also in this setting, PD-L1 assessment is burdened by a lack of homogeneity: in fact, three different companion diagnostic assays with different thresholds were used. Furthermore, the role of TILs is not fully understood because their high expression is predictive of response also to chemotherapy alone.[57] Other biomarkers predictive of ICI response in other tumor histology, but not yet evaluated and validated in early TNBC, are circulating tumor cells and circulating tumoral DNA (ctDNA), mismatch repair deficiency/microsatellite instability, CD274 amplification.[59] In particular, a recent meta-analysis has demonstrated that the detection of ctDNA, both at baseline and after completion of NACT, is significantly associated to worse recurrence-free survival [(RFS), HR 4.22, 95%CI: 1.29–13.82 and HR: 5.67, 95%CI: 2.73–11.75, respectively] and worse OS (HR 19.1, 95% CI: 6.9–53.04 and HR 4.00, 95% CI: 1.90–8.42, respectively).[93] This dynamic and circulating biomarker represents an interesting tool for monitoring tumor evolution, predicting treatment response and determining prognosis. Probably, the ideal prognostic and predictive immune biomarker will derive from the integration of different features into a unique immunogram and will require a dynamic assessment before, during and after the neoadjuvant treatment.

Another issue regards the role of neoadjuvant ICIs in patients with stage I TNBC; in fact these patients were not included in KEYNOTE-522 and currently cannot receive pembrolizumab. Anyway, they represent the 35% of patients enrolled in GeparNuevo trial, which demonstrate to provide a benefit in terms of iDFS regardless of the stage (stage 0 or I: HR for iDFS: 0.55, 95% CI: 0.09–3.31; stage IIA or higher: HR for iDFS: 0.51; 95% CI: 0.24–1.12).[58]

Lastly, there is an urgent need for surrogate endpoints to optimize and accelerate the drug development. In fact, in early TNBC setting, the pCR has shown to be an adequate endpoint of response to NACT, being associated with EFS (HR 0.24, 95% CI 0.18–0.33) and OS (HR 0.16; 95%CI 0.11–0.25) only at patient level, so regardless of treatment group.[84] Instead, at a trial level (i.e. considering trial arms), an increase in pCR rate between treatment groups does not predict improvements in EFS and OS; therefore, pCR may not represent a perfect surrogate endpoint for clinical outcomes, maybe because it does not capture the whole effect of treatment upon the true endpoint: for example, pCR does not consider pathological partial responses [measured by residual cancer burden (RCB)] and the clearance of micrometastatic systemic disease, which could be measured with liquid biopsy.[84,94,95] This complex scenario is further complicated by the advent of ICIs, whose kinetic of action is even less framed by a response endpoint such as pCR.[11] In fact, in both KEYNOTE-522 and GeparNuevo, survival outcomes improved to an extent greater than expected on the basis of pCR rate increases (by 7.5% and 9.2%, respectively)[11]; furthermore, GerparNuevo showed an improvement in DFS, EFS, and OS (secondary endpoints), despite the primary endpoint (pCR) was not met. On the basis of these evidences, a solution may be the validation of new composite response endpoints (RCB, the clearance of ctDNA and/or the dynamic modification of tissue immune biomarkers), in

order to test and eventually validate a response surrogate endpoint for long-term outcomes.

Immunotherapy in other subtypes beyond TNBC

After the demonstration of a clinical benefit in patients with TNBC, the next step was moving IO agents to other BC subtypes, namely HR+/HER2− BC and HER2+ BC (Tables 4 and 5). Furthermore, the finding of some cases of non-TNBCs expressing high level of TMB, TILs, and PD-L1 supported this expansion.[96]

HR+/HER2− BC

HR+/HER2− BC is characterized by an immune suppressive TME, with low TIL infiltration, low HLA class I expression and abundant TAMs, which limit the antitumor immune activity.[97] In fact, only 6% of HR+ BC is characterized by high (⩾50%) TILs-infiltration and the prognostic role of TILs in this subtype is controversial, probably mirroring a disease that is less differentiated and more aggressive.[98] Anyway, on the basis of these limited signs of immune response, the activity and efficacy of IO agents was investigated also in this subtype, which is the most prevalent.

The first proof of concept phase I/II clinical trial demonstrated that in metastatic endocrine-resistant non-selected patients ICIs as monotherapy or in combination with endocrine therapy are associated with very limited ORRs of 0–3% and mPFS lower than 3 months [18,99,100]; furthermore, the KEYNOTE-028 showed that neither the patient selection on the basis of PD-L1 (CPS ⩾ 1) provides clinically significant results (ORR 12%)[101] (Table 4).

As in TNBC, ICI–chemotherapy combination were then explored, but without any evidence of clinical benefit in terms of PFS in unselected patients[22,102,103] (Table 4). For example, on the basis of preclinical data demonstrating an upregulation of immune-related genes in invasive lobular BC (ILBC),[104] the phase II GELATO trial enrolled patients with metastatic ILBC of any type, of which 18 had endocrine-resistant HR+ BC and investigated the activity of carboplatin plus atezolizumab, but only 2 (11%) patients had a clinical benefit in terms of DCR.[105]

Moreover, the randomized phase II SAFIR02-IMMUNO clinical trial investigated the maintenance with durvalumab or chemotherapy also in patients with HER2− mBC who did not progress on 6–8 cycles of chemotherapy; in fact, 108 of 199 enrolled patients had an endocrine-resistant HR+ disease. Despite the trial failed to demonstrate a benefit (mPFS: 2.7 *versus* 4.6 months), an exploratory analysis showed that seven of 67 (10%) patients with HR+ disease presented *CD274* gene gains/amplifications, six received durvalumab and five were alive after 15, 16, 19, 24, and 26 months, thus providing a strong rational for further exploring this predictive biomarker also beyond TNBC subtype.[22]

As far as early setting, the phase II GIADA trial investigated the activity in terms of pCR of neoadjuvant epirubicin, cyclophosphamide, nivolumab, exemestane, and triptoreline in 43 premenopausal patients with stage II–IIIA luminal B-like BC.[106] This trial did not meet its primary endpoint (pCR: 16%) but showed that pCR was associated with higher TILs at baseline (15% in pCR subgroup *versus* 2% in non-pCR) and basal subtype at PAM50, and that chemotherapy determined an increase of TILs and CD8+ cells and a decrease of intratumoral stromal CD4+ cells.

In contrast, the randomized phase II I-SPY2 clinical trial evaluating the activity of the combination of pembrolizumab with standard NACT in HER2− early BC demonstrated a pCR benefit (30% *versus* 13%) in patients with HR+ disease.[56] As in TNBC, these data highlight the importance of investigating ICIs in early setting, in association with chemotherapy and possibly in selected patients; in this sense, the ongoing randomized phase III CheckMate 7FL clinical trial is assessing the benefit in terms of pCR and EFS from the addition of Nivolumab to standard NACT in 1200 patients with stage II–III high-grade HR+/HER2− BC.[107]

Chemotherapy-free options were also explored. On the basis of preclinical evidences demonstrating an increase of immunogenic cell death driven by CDK4/6 inhibitors (CDK4/6i) and, in general, a synergistic activity of ICIs and CDK4/6i through modulation of the TME, this combination was investigated both in early and in metastatic setting.[69,108] Anyway, because of safety concerns, in particular interstitial lung disease and severe liver enzyme abnormalities, the enrolment was discontinued in both settings.[109–111] HDAC inhibitors represented another potential partner for ICIs,

Table 4. Results from clinical trials investigating ICIs in HR+/HER2– and HER2+ BC.

Treatment	Trial NCT number	Ph	*n*	Patients	Study design	Primary endpoints	Response outcomes	Efficacy outcomes	Safety outcomes
HR+/HER2– BC									
ICI ± ET									
Avelumab	JAVELIN (NCT01772004)	Ib	72	HR+/HER2– mBC; <4 L of CT; prior taxane, anthracycline	Avelumab 10 mg/kg q2w	Safety ORR	ORR: 3%	mPFS: 1,35 mo	TRAEs G⩾3: 14%
Tremelimumab + Exemestane	NCT02997995	I	26	HR+/HER2– mBC; ⩾2 L	Tremelimumab (3–10 mg/kg) every 28 days or every 90 days + Exemestane 25 mg daily	Safety	ORR: 0% DCR: 42%	Not evaluated	5 DLT (diarrhea, transaminitis)
Tremelimumab + Durvalumab	NCT02536794	**II**	18	HER2– mBC (11 HR+, 7 TNBC); PD on ET and at least 1 L of CT	Durvalumab + Tremelimumab q4w × C4, for responders maintenance with durvalumab q2w × C18	ORR	ORR: 0% (HR+); 43% (TNBC)	mPFS: 2.2 mo (HR+); NR (TNBC) mOS: NR (HR+); NR (TNBC)	TRAEs ⩾G4: 0%
Pembrolizumab	KEYNOTE 028 (NCT02054806)	Ib	25	ER+/HER2– mBC; PD-L1+ (CPS ⩾1); ⩾1 L	Pembrolizumab 10 mg/kg q2w for up to 24 months	mDOR	ORR: 12% mDOR: 12 mo	mPFS: 1.8 mo mOS: 8.6 mo	TRAEs: 64% TRAEs G⩾3: 16% irAEs 20%
ICI + CT									
Pembrolizumab + Capecitabine	NCT03044730	II	30	HER2– mBC; ⩾1 L (16 TNBC, 14 HR+/HER2–)	Pembrolizumab 200 mg q2w + capecitabine 1000 mg/m^2 BID on D1–14	mPFS	ORR: 14% 1yPFS: 21%	mPFS: 4.0 mo (ITT), 5.2 mo (HR+) mOS: 15.4 mo (ITT), NR (HR+)	TRAEs: 33% TRAEs G⩾3: 10%
Eribulin ± Pembrolizumab	NCT03051659	II	88	HR+/HER2– mBC, 37% PD-L1+ (CPS > 1); PD on ET	Eribulin 1,4 mg/mq D1,8 q3w + Pembrolizumab 200 mg q3w (EP) *versus* Eribulin 1,4 mg/mq D1,8 q3w (E)	mPFS	ORR: 27% (EP); 34% (E) mDOR: 0.6 mo (EP); 2.1 months (E)	mPFS (ITT): 4.1 mo (EP); 4.2 mo (E) mPFS (PD-L1+): 4.2 mo (EP); 4.3 mo (E)	AEs: 100% TRAEs G5 (EP): 2 pts
Atezolizumab + Carboplatin	GELATO (NCT03147040)	II	18	Lobular mBC; (18 ER+/HER2–)	Carboplatin AUC 1.5 qw × C12 + Atezolizumab 1200 mg q3w starting from C3	6mPFS	ORR: 19% CBR: 29% mDOR: 12 w	mPFS: 15 w	NA
Durvalumab maintenance after CT	SAFIR02-IMMUNO (Arm B) (NCT02299999)	II	199 (108 HR+)	HER2– mBC (108 HR+); disease control after C6 of CT; 1–2 L of CT; PD on ET (for HR+)	Maintenance Durvalumab 10 mg/kg q2w *versus* maintenance chemotherapy	PFS	NA	mPFS: 2.7 mo *versus* 4.6 mo mOS: 21.7 *versus* 17.9 mo	Serious TRAEs: 8.5%
Nivolumab + AC + ET	GIADA (NCT04659551)	II	43	Stage II–IIIA; premenopausal pts with luminal B-like BC	Neodjuvant EC Q3W + Triptorelin, followed by nivolumab Q2W × C14 + exemestane	pCR	pCR: 16%	NA	NA
Pembrolizumab + NACT	I-SPY2 (NCT01042379)	II	40	HR+/HER2– early BC; stage II–III	Paclitaxel QW × C12, followed by AC Q2/3W × C4 ± pembrolizumab 200 mg Q3W × C4	pCR	pCR: 30% *versus* 13%	NA	NA

(Continued)

Table 4. (Continued)

Treatment	Trial NCT number	Ph	*n*	Patients	Study design	Primary endpoints	Response outcomes	Efficacy outcomes	Safety outcomes
ICI + TT									
Abemaciclib + nivolumab + fulvestrant or letrozole	WJOG11418B NEWFLAME	II	17 (12F/5 L)	HR+/HER2− mBC; ⩽1 L; CT naive	Nivolumab 240 mg D1,15 + Abemaciclib 150 mg BID + Fulvestrant 500 mg D1,15,29, and q4w thereafter (F) or letrozole 2.5 mg once daily (L)	ORR	ORR: 55% (F); 20% (L) DCR: 91% (F); 80% (L)	PFS and OS were undetermined due to the discontinuation	Aes G⩾3: 92% (F); 100% (L) irAEs G⩾3: 67% (F); 60% (L)
Pembrolizumab + abemaciclib ± anastrozole	NCT02779751	Ib	28	HR+/HER2− mBC; CDK4/6i naive; 1–2 prior lines	Nivolumab 240 mg D1,15 + Abemaciclib 150 mg BID, + Fulvestrant (F) 500 mg D1,15,19 and Q4W or letrozole (L) 2.5 mg die	Safety	ORR: 29% CBR: 46%	mPFS: 9 mo mOS:26 mo	AST increase G⩾3: 18%
Nivolumab + Palbociclib + Anastrozole	CheckMate 7A8 (NCT04075604)	II	21	ER+/HER2− early BC; T ⩾ 2; postmenopausal	Neoadjuvant nivolumab 480 mg Q4W IV + palbociclib 100–125 mg QD PO 3 weeks on/1 week off + anastrozole 1 mg QD PO × C5	Safety	pCR: 5%	NA	9/21 pts discontinued treatment due to toxicity
Pembrolizumab (P) + Vorinostat (V) + Tamoxifene (T)	NCT02395627	II	38	HER2− mBC; PD on ET (ER+: 34 pts – arms A and B; ER−: 4 pts – arm C)	Arm A: T 20 mg/die + V 400 mg for 5 day qw + P 200 mg qw3. Arm B: T 20 mg/die + V 400 mg for 5-day qw (from C2) + P 200 mg q3w (from C2) Arm C: V 400 mg for 5 day qw + P 200 mg qw3	ORR Safety	ORR: 4% CBR: 19%	mPFS: 8.6 mo (pts with increased CTLA4+/PD-1+/CD8+ T cells in either blood or tumor) *versus* 2.8 mo	NA
Olaparib + Durvalumab	MEDIOLA (NCT02734004)	Ib/II	34	gBRCA1/2-mut, HER2− mBC (16 HR+); PD in ET and 1–2 L of CT	Olaparib 300 mg BID for 4 weeks, followed by Olaparib 300 mg BID + durvalumab 1500 mg iv q4w	12wDCR Safety	12wDCR: 80% 28wDCR: 50% mDOR: 9.2 mo	mPFS: 8.2 mo (ITT), 9.9 mo (HR+) mOS: 21.5 mo (ITT), 22.4 mo (HR+)	Aes G⩾3: 32% irAEs: 29%
Durvalumab + Olaparib + NACT	I-SPY2 (NCT01042379)	II	52	HR+/HER2− early BC; stage II–III	Neoadjuvant durvalumab Q4W × C3 + olaparib W1–11 + paclitaxel QW × C12, followed by AC Q2/3W *versus* NACT	pCR	pCR: 28% *versus* 14%	NA	AEs G⩾3: 56% *versus* 34% irAEs G⩾3: 12%*versus* 1%
HER2+ BC									
ICI ± anti-HER2									
Avelumab	JAVELIN (NCT01772004)	Ib	26	mBC (26 HER2+); <4 L of CT; prior taxane, anthracycline	Avelumab 10 mg/kg q2w	Safety ORR	ORR: 3% (ITT); 0% (HER2+)	mPFS: 1,35 mo	TRAEs G⩾3: 14%
Durvalumab + Trastuzumab	NCT02649686	I	15	HER2 + mBC; prior taxane and T (0% PD-L1+)	Durvalumab 1125 mg Q3W + Trastuzumab 8 mg/kg loading then 6 mg/kg Q3W	RP2D	ORR: 0% DCR: 29%	NA	irAEs G⩾3: 7%

(Continued)

Table 4. (Continued)

Treatment	Trial NCT number	Ph	*n*	Patients	Study design	Primary endpoints	Response outcomes	Efficacy outcomes	Safety outcomes
Trastuzumab + Pembrolizumab	PANACEA KEYNOTE-014 (NCT02129556)	Ib/II	58	HER2+ mBC; prior T and taxanes	Pembrolizumab 200 mg iv + Trastuzumab 6 mg/kg iv q3w for 24 months	ORR Safety	ORR: 15% (PD-L1+) DCR: 25%	mPFS: 2,7 mo (PD-L1+); 2.5 mo (PD-L1–) 1yOS: 65% (PD-L1+); 12% (PD-L1–)	AEs G⩾3: 50% TRAEs G⩾3: 29%
ICI + CT/ADCs ± anti-HER2									
Atezolizumab + Trastuzumab + Pertuzumab + NACT	IMpassion050 (NCT03726879)	III	454	Stage II–III HER2 + BC	Neoadjuvant ddAC, followed by TP + paclitaxel + atezolizumab/ placebo, followed by surgery, followed by TP + atezolizumab/ placebo	pCR	pCR: 62% *versus* 63% (ITT); 64% *versus* 73% (PD-L1)	NA	AEs G⩾3: 52% *versus* 44%
Atezolizumab + T-DM1	KATE 2 (NCT02924883)	II	202	HER2+ mBC; prior T+CT or PD within 6 mo after adjuvant therapy	T-DM1 3.6 mg/kg + atezolizumab/ placebo 1200 mg q3w	PFS Safety	ORR: 45% *versus* 43%	HR PFS (ITT): 0.82 PFS (PD-L1+): 8.5 mo *versus* 4.1 mo (HR 0.60)	AEs G⩾3: 53% *versus* 45% SAEs: 39% *versus* 23%
Pembrolizumab + T-DM1	NCT03032107	Ib	27	HER2 + mBC; previous T and taxane; T-DM1 naïve	T-DM1 3.6 mg/kg q3w + Pembrolizumab 200 mg q3w	ORR Safety	ORR: 29% (PD-L1+); 33% (–) CBR: 43% (PD-L1+); 67% (–)	mPFS: 2.9 mo (PD-L1+); 8.7 mo (PD-L1–)	AE G3: 20% TRAEs G > 1: 85%
T-Dxd + Nivolumab	NCT03523572	Ib	32	HER2+ mBC; ⩾2 L with anti-HER2	T-Dxd + nivolumab 360 mg q3w	ORR Safety	ORR: 66% mDOR: NR	mPFS: 11.6 mo mOS: NR	TRAEs: 100% TRAEs G⩾3: 50%
		16	HER2-low mBC; ⩾2 L with anti-HER2	ORR: 50% mDOR: 5.5 mo			mPFS: 7 mo mOS: 19.5 mo		

AC, doxorubicin + cyclophosphamide; ACT, paclitaxel QW × C12 + (anthracycline + cyclophosphamide) Q3W × C4; ADC, antibody–drug conjugate; AE, adverse events; AUC, area under curve; BID, twice a day; C, cycle; CNS, central nervous system; CPS, combined positive score; CR, complete response; CT, chemotherapy; ctDNA, circulating tumor DNA; D, day; DCR, disease control rate; DFI, disease-free interval; DFS, disease-free survival; DLT, dose limiting toxicity; EC, epirubicin + cyclophosphamide; EFS, event-free survival; ER, estrogen receptor; ET, endocrine therapy; FEC, 5-fluorouracil + epirubicin + cyclophosphamide; G, grade; gBRCA1/2m(ut), germline BRCA1/2 mutated; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; ICI, immune checkpoint inhibitor; IO, immuno-oncology; ITT, intention to treat; iv, intravenously; L, line; mo, months; (m)BC, (metastatic) breast cancer; (m)DOR, (median) duration of response; (m)OS, median overall survival; (m)PFS, (median) progression-free survival; *n*, number; NA, not available; NACT, neoadjuvant chemotherapy; NR, not reached; ORR, objective response rate; P, pembrolizumab; pCR, pathological complete response; PD, progression of disease; PD-(L)1, programmed death-(ligand) 1; Ph, phase; PLD, peghilated lyposomial doxorubicin; PO, per os; PR, partial response; pre-op, preoperative; QnW/M, every *n* weeks/months; RCB, residual cancer burden; RT, Radiation Therapy; SABR, Stereotactic Body Radiation Therapy; T, taxane; TAM, tumor-associated macrophage; TCb, paclitaxel + carboplatin; T-DXd, trastuzumab deructecan; TIL, tumor-infiltrating lymphocyte; TKI, tyrosine kinase inhibitor; (e/m)TNBC, (early/metastatic) triple-negative breast cancer; TRAE, treatment-related adverse event; TT, targeted therapy; W, week.

Table 5. Ongoing clinical trials investigating IO agents in HR+/HER2– or HER2+ BC.

Treatment	Trial NCT number	Ph	Patients	Study design	Primary endpoints	Status
HR + BC						
ICI ± ET						
Nivolumab + Ipilimumab	NIMBUS (NCT03789110)	II	HER2– (ER+: 70%) mBC; TMB > 10 mut/Mb; 0–3 L of prior CT and 1 L of ET	Nivolumab q6w + ipilimumab 2qw	ORR	Not recruiting
Pembrolizumab + Fulvestrant	NCT03393845	II	HR+/HER2– mBC; <3 L of ET or CT	Pembrolizumab 200 m q3W + Fulvestrant	ORR	Recruiting
ICI + CT						
Nivolumab + ipiliumab + PLD + cyclophsphamide	ICON Trial (NCT03409198)	IIb	mBC ER+/HER2– mBC; maximum 1 L of CT	PLD 20 mg/m^2 q2w + cyclophosphamide 50 mg/die D1–14 q4w + ipilimumab 1 mg q6w + nivolumab 240 mg q2w (arm B)	PFS Safety	Not recruiting
Pembrolizumab + paclitaxel	TATEN study (NCT04251169)	II	HR+/HER2– mBC; prior CT and CDK4/6	Pembrolizumab 200 mg q3w + paclitaxel 80 mg/m^2 D1,8,15 q3w	ORR	Recruiting
Pembrolizumab + nab-paclitaxel	NCT02752685	II	HER2– (TNBC: 30; HR+: 20) mBC; 0–2 L of CT	Pembrolizumab + nab-paclitaxel	ORR	Recruiting
Pembrolizumab + doxorubicin or ET	NCT02648477	II	HER2– mBC; 1 L	Pembrolizumab + doxorubicin q3w × C6; followed by maintenance with pembrolizumab for up to 24 months (Cohort 1) Pembrolizumab q3w + AI QD for 24 months (Cohort 2)	ORR	Not recruiting
Atezolizumab + Bevacizumab + Paclitaxel	AMBITION (NCT04732598)	III	HR+/HER2– mBC; PD on ET; CT naive	Bevacizumab 10 mg/k, D1,15 + Paclitaxel 90 mg/m^2, D1,8,15 ± atezolizumab 840 mg/m^2 D1,15 q4w	PFS	Recruiting
Nivolumab + neoadjuvant CT	CheckMate 7FL (NCT04109066)	III	Localized ER+/HER2– BC; G3 or G2 with ER 1–10%; stage II–III	Neoadjuvant nivolumab/placebo 360 mg q3w + paclitaxel 80 mg/m^2 D1,8,15 q3w x C4, followed by nivolumab/placebo 360 mg q3w + doxorubicin 60 mg/m^2 or epirubicin 90 mg/m^2 and cyclophosphamide 600 mg/m^2 q3w or q2w for C4. Surgery. Adjuvant phase with Nivolumab/placebo 480 mg q4w × C7 + investigator's choice of ET per local SoC	pCR EFS	Not recruiting
ICI + TT/ADCs						
Avelumab + talazoparib	NCT03330405	Ib/II	HR+/HER2– mBC; prior ET and 0–2 L of CT; no PD on platinum-based CT (cohort 2B)	Avelumab 800 mg q2w + talazoparib 1.0 mg QD	ORR	Not recruiting
Sacituzumab govitecan + Pembrolizumab	SACI-IO HR+ (NCT04448886)	II	HR+/HER2– mBC; PD on ET; 0–1 prior L of CT; PD-L1-positive	Sacituzumab twice per cycle ± pembrolizumab once per cycle	PFS	Recruiting
T-Dxd + other agents	DESTINY 08 (NCT04556773)	Ib	HER2-low mBC; ⩾2 L (part 1) 0–1 L (part 1)	T-DXd + other agents (durvalumab with paclitaxel, capivasertib, anastrozole, fulvestrant, capecitabine)	Safety	Recruiting

(Continued)

Table 5. (Continued)

Treatment	Trial NCT number	Ph	Patients	Study design	Primary endpoints	Status
Pembrolizumab + palbociclib + ET	NCT02778685	II	ER+ postmenopausal mBC; PD on CDK4/6i + IA	Letrozole + palbociclib + pembrolizumab (Cohorts 1 and 2); Letrozole/ fulvestran + palbociclib + pembrolizumab (Cohort 3)	ORR	Recruiting
Avelumab + palbociclib + tamoxifen	ImmunoADAPT (NCT03573648)	II	HR+/HER2– BC; stage II–III	Neoadjuvant Tamoxifen ± palbociclib for 1 cycle; then, avelumab × C3 + tamoxifen ± palbociclib × C4	Clinical CR	Recruiting
IO-based treatment combinations	MORPHEUS (NCT03280563)	Ib/II	HR+/HER2– mBC; PD on CDK4/6i in 1–2 L setting	Fulvestrant *versus* atezolizumab + fulvestrant/ entinostat/ipatasertib *versus* triplet combination (atezo + ipatasertib + fulvestrant/atezo + beva + ET). At PD, a new triplet combination treatment	ORR	Recruiting
HER2 + BC						
ICI ± anti-HER2						
Durvalumab + Trastuzumab	NCT02649686	Ib	HER2 + mBC; prior taxane, T and P	Durvalumab q3w until PD + T q3w × C6	RD2P	Completed
Atezolizumab + Pertuzumab + Trastuzumab	NCT03417544	II	HER2 + mBC; CNS metastasis	Atezolizumab q3w + P q3w + high-dose T for 24 w then T q3w	ORR in CNS	Not recruiting
Pembrolizumab + trastuzumab + pertuzumab	Keyriched-1 (NCT03988036)	II	HER2-enriched (PAM50) eBC	Neoadjuvant pembrolizumab + trastuzumab + pertuzumab	pCR	Recruiting
Durvalumab + tremelimumab + trastuzumab	DIAmoND (ACTRN12617001325392)	II	HER2 + mBC; PD on T	Durvalumab 1500 mg q4w × C4 + Tremelimumab 75 mg q4w × C4 + T 2 mg/kg qw × C16, followed by maintenance with (durvalumab 1120 mg + trastuzumab 6 mg/kg) q3w × C12	1yPFS	Recruiting
Pembrolizumab + tucatinib + trastuzumab	TOPAZ (NCT04512261)	I/II	HER2 + mBC; brain metastasis	Tucatinib + pembrolizumab + T	24-week CNS DCR	Withdrawn
ICI + CT ± anti-HER2						
Atezolizumab + trastuzumab + vinorelbine	ATREZZO (NCT04759248)	II	HER2 + mBC; prio T, P and T-DM1	Atezolizumab + trastuzumab + vinorelbine	pCR	Recruiting
Pembrolizumab + tucatinib + trastuzumab + capecitabine	TUGETHER (NCT04789096)	II	HER2 + mBC	Pembrolizumab + tucatinib + trastuzumab (± capecitabine)	ORR	Not yet recruiting
Atezolizumab + paclitaxel + trastuzumab + pertuzumab	NCT03125928	IIa	HER2 + mBC	Atezolizumab + paclitaxel + trastuzumab + pertuzumab	Safety ORR	Recruiting
Pembrolizumab + trastuzumab + pertuzumab + paclitaxel	NCT03747120	II	HER2 + eBC; cT > 2 cm and/or N+, no prior treatment	Neoadjuvant pembrolizumab + trastuzumab + pertuzumab + weekly paclitaxel	pCR	Recruiting
Taxane + pertuzumab + trastuzumab ± atezolizumab	NRGBR004 (NCT03199885)	III	HER2+ mBC; 1 L setting	P D1,22 + T D1,22 + Paclitaxel D1,8,15,22,29,36 or docetaxel D1,22 q6w + atezolizumab/placebo D1,22 q6w for 2 years	PFS	Not recruiting
Atezolizumab + trastuzumab + pertuzumab + NACT	APTneo (NCT03595592)	III	HER2 + BC; stage II–III	Neoadjuvant atezolizumab + trastuzumab + pertuzumab + carboplatin + paclitaxel → surgery → adjuvant atezolizumab + trastuzumab + pertuzumab	EFS	Not recruiting

(Continued)

Table 5. (Continued)

Treatment	Trial NCT number	Ph	Patients	Study design	Primary endpoints	Status
ICI + ADCs						
Atezolizumab + T-DM1	KATE 3 (NCT04740918)	III	HER2 + mBC; prior T + CT or PD within 6 mo after adjuvant therapy	T-DM1 3.6 mg/kg + Atezolizumab/placebo 1200 mg q3w	PFS OS	Recruiting
Atezolizumab + T-DM1	ASTEFANIA (NCT04873362)	III	HER2 + eBC, PDL-1 positive, patients with RD, after neoadjuvant T	Atezolizumab/placebo + T-DM1	IDFS	Recruiting
Atezolizumab + pertuzumab + trastuzumab or atezolizumab + T-DM1	NCT02605915	Ib	HER2 + mBC	Atezolizuma + T + P ± docetaxel or atezolizumab + TDM1	Safety	Completed
T-Dxd + pembrolizumab	NCT04042701	Ib	HER2 + or HER2-low mBC; previously treated (PD on T-DM1 for HER2 + BC)	T-DXd + pembrolizumab 200 mg IV q3w	Safety	Recruiting
T-Dxd + Durvalumab	DESTINY 07 (NCT04538742)	Ib/II	HER2 + mBC; ⩾1 L	T-DXd + other agents (durvalumab ± paclitaxel, paclitaxel, tucatinib, pertuzumab)	Safety	Recruiting

AC, doxorubicin + cyclophosphamide; ACT, paclitaxel QW x C12 + (anthracycline + cyclophosphamide) Q3W x C4; ADC, antibody–drug conjugate; AE, adverse events; AUC, area under curve; BID, twice a day; C, cycle; CNS, central nervous system; CPS, combined positive score; CR, complete response; CT, chemotherapy; ctDNA, circulating tumor DNA; D, day; DCR, disease control rate; DFI, disease-free interval; DFS, disease-free survival; DLT, dose limiting toxicity; EC, epirubicin + cyclophosphamide; EFS, event-free survival; ET, endocrine therapy; FEC, 5-fluorouracil + epirubicin + cyclophosphamide; G, grade; gBRCA1/2m(ut), germline BRCA1/2 mutated; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; ICI, immune checkpoint inhibitor; IO, immuno-oncology; ITT, intention to treat; iv, intravenously; L, line; mo, months; (m)BC, (metastatic) breast cancer; (m)DOR, (median) duration of response; (m)OS, median overall survival; (m)PFS, (median) progression-free survival; *n*, number; NA, not available; NACT, neoadjuvant chemotherapy; ORR, objective response rate; P, pembrolizumab; pCR, pathological complete response; PD, progression of disease; PD-(L)1, programmed death-(ligand) 1; Ph, phase; PLD, peghilated lyposomial doxorubicin; PO, per os; PR, partial response; pre-op, preoperative; QnW/M, every *n* weeks/months; RCB, residual cancer burden; RT, Radiation Therapy; SABR, Stereotactic Body Radiation Therapy; T, taxane; TAM, tumor-associated macrophage; TCb, paclitaxel + carboplatin; T-DXd, trastuzumab deructecan; TIL, tumor-infiltrating lymphocyte; TKI, tyrosine kinase inhibitor; (e/m)TNBC, (early/metastatic) triple-negative breast cancer; TRAE, treatment-related adverse event; TT, targeted therapy; W, week.

but the triplet of vorinostat, pembrolizumab and tamoxifen showed initial signs of a clinical benefit only in patients with increased CTLA4+/PD-1+/CD8+ T cells in either blood or tumor (mPFS: 8.6 *versus* 2.8 months).[112]

As far as the combination with PARPi, on the basis of preclinical evidences showing a synergistic activity, the phase I/II MEDIOLA clinical trial investigated the combination of olaparib and durvalumab in patients with germline BRCA-mutated HER2-negative mBC and showed results similar to those reported in OlympiAD and EMBRACA trials, with PARPi monotherapy.[38–40,113,114] In particular, considering the 13 evaluable for ORR patients with HR+/HER2− disease, nine of them (70%) had partial response as best response.

Furthermore, the I-SPY2 study demonstrated an improvement in terms of pCR (28% *versus* 14%) by the addition of olaparib and durvalumab to the standard NACT in 52 patients with stage II-III HR+/HER2− BC.[65] Interestingly, these patients were classified as MammaPrint High1 (MP1) or MammaPrint (ultra) High2 (MP2), with MP2 defined as MammaPrint score ⩽0.154, and MP2 cases benefited selectively from durvalumab and olaparib (pCR: 64% *versus* 22%), while no benefit was seen in MP1 cancers (pCR: 9% *versus* 10%).

Lastly, with the advent of ADCs in HR+ endocrine-resistant mBC and in light of ADCs immunomodulatory activity by interacting with cancer and immune cells, there is a strong rationale to combine ICIs with ADCs in this setting[32,115,116]; in this regard, two ongoing clinical trials (NCT04448886 and NCT04556773) are evaluating the safety and the active of anti-PD-(L)1 in association with sacituzumab govitecan and T-DXd, respectively (Table 5).

In conclusion, there is an urgent need to better characterize the immunologic aspects of the TME in HR+ BC and to identify novel IO agents impacting also on immune cells other than T cells, such as TAMs.

HER2+ BC

In comparison with HR+ subtype, HER2+ BC is characterized by a less immunosuppressive TME. In fact, in this subtype, PD-L1 is expressed in more than 50% of patients and high (⩾50%) TILs infiltration is found in 16% of cases. In general, TILs have a median level of 15–20% and are associated with improved prognosis and response to chemotherapy and trastuzumab[4,98,117–119]; in fact, trastuzumab is also characterized by immune-mediated mechanisms of action, and preclinical studies suggest immune-mediated mechanisms of trastuzumab resistance that can be overcome with checkpoint inhibition combinations.[120] These evidences provide the rational to combine anti-HER2 therapies with ICIs in this BC subtype.

The single arm phase Ib/II PANACEA clinical trial investigated the activity of trastuzumab and pembrolizumab in patients with metastatic HER2+ BC progressing on trastuzumab or TDM-1; 58 patients were enrolled, 46 of them had a PD-L1-positive disease.[121] The activity of this combination was limited (ORR: 15%) and observed only in PD-L1-positive cases; anyway, the biomarker analysis showed a statistical significant correlation between TILs and PD-L1 status and an association between stromal TILs at baseline and better response and DC. Interestingly, a significant difference in 12-month OS rate emerged between PD-L1+ and PD-L1 tumors (65% *versus* 12%), but, because of the very limited population and the absence of a control arm, it is difficult to define the prognostic or predictive value of PD-L1 expression.

The combination of ICIs and chemotherapy was also investigated in early-stage setting, in the Impassion-050 trial.[122] It was a phase III clinical trial assessing the clinical benefit in terms of pCR provided by the addition of atezolizumab to standard neoadjuvant therapy. The trial was stopped prematurely due to an unfavorable risk–benefit ratio for patients receiving atezolizumab: despite the absence of a pCR benefit, also in PD-L1+ patients, as stated, a clinical benefit in terms of EFS cannot be excluded; the similar ongoing phase III APTneo trial is powered for EFS and its result may clarify this issue (Table 5).

As far as the combination of ICIs and ADCs, the randomized phase II KATE2 trial investigated the addition of atezolizumab to trastuzumab emtansine (T-DM1) in patients with HER2+ mBC progressing to trastuzumab and taxanes.[123] It did not meet its primary endpoint (PFS in ITT), but in PD-L1+ subgroup (84/202, 42% of patients) a significant difference emerged (mPFS: 8.5 *versus* 4.1 months; HR: 0.60, 95% CI: 032–1.11); no differences in OS were observed (secondary endpoint). The ongoing phase III KATE3 clinical trial is investigating this combination in

the same setting but only in PD-L1-positive patients, with PFS and OS as coprimary endpoints; moreover, the benefit of the addition of atezolizumab to T-DM1 is under investigation also in the post-neoadjuvant setting, in patients with residual disease following neoadjuvant therapy (ASTEFANIA trial).

Another promising ADC is T-DXd, which demonstrated an extraordinary antitumor activity both in HER2+ and in HER2-low BC[115,124]; the safety profile of the combination of T-DXd and Nivolumab was evaluated in a phase Ib trial enrolling 48 patients (32 HER2+ and 16 HER2 low) of whom 50% experienced a TRAE of grade 3 or more, and 37% discontinue the treatment because of TEAEs (25% related to T-DXd, 21% to nivolumab).[125] The ongoing phase Ib/II DESTINY-Breast 07 trial is assessing the combination of T-Dxd with durvalumab and paclitaxel.

Novel promising IO strategies other than ICIs comprise the CAR T cell therapy, which seems to be promising in HER2+ BC on the basis of preclinical data but is burdened by serious adverse events, and vaccines, which – on the basis of initial negative trials – have been moved from heavily pretreated patients to early setting, where an immune engagement is eventually more predictable.[118]

Conclusions

The pharmacopoeia of TNBC deeply changed after the advent of ICIs, which demonstrated to improve outcomes in terms of OS and EFS in metastatic and early setting, respectively. Clinical trials confirmed that IO agents should be administrated as upfront therapy in both setting, when the immune microenvironment is more permissive and the degree of tumor evasion is lower.

In order to increase this benefit among nonresponders and to expand this treatment approach beyond triple-negative subtype, which accounts only for 15% of all BCs, a deeper characterization of BC immune landscape and a better definition of BC immunogram are paramount. In fact, escalation and de-escalation strategies require the identification and prospective validations of biomarkers of response and resistance.

Moreover, many clinical trials investigating IO agents and aiming at improving clinical outcomes are ongoing; in this regard, the ADC-ICI combination seems to be the most promising escalation strategy, despite burdened by important toxicities. Instead, as far as IO beyond ICIs, CAR-T-cell therapy and CVs represent the most intriguing and innovative strategies in advanced and in early setting, respectively.

Lastly, because efficacy endpoints (e.g. EFS, PFS, OS) better measure the immune-mediated antitumor effects over time compared to response endpoints (e.g. pCR, ORR), the acceleration of drug development requires the identification and validation of novel composite surrogate endpoints considering the mechanism of action of IO agents.

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Author contribution(s)

Carmine Valenza: Conceptualization; Writing – original draft; Writing – review & editing.

Graziella Rizzo: Conceptualization; Writing – original draft; Writing – review & editing.

Maria Ilenia Passalacqua: Conceptualization; Writing – original draft; Writing – review & editing.

Laura Boldrini: Conceptualization; Writing – original draft; Writing – review & editing.

Chiara Corti: Conceptualization; Writing – original draft; Writing – review & editing.

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