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Mini-review

Immunological therapy: A novel thriving area for triple-negative breast cancer treatment

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ABSTRACT

Triple-negative breast cancer (TNBC) refers to cancers that are low in expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). TNBC tends to behave more aggressively than other types of breast cancer. Unlike other breast cancer subtypes (ie, ER-positive, HER2-positive subtypes), there are no approved targeted treatments available, other than the administration of chemotherapy. Immunotherapy is a new kind of treatment approach for TNBC when compared with the surgical treatment, chemotherapy, endocrine therapy, and molecular targeting therapy. The present article reviews the research progresses of immunotherapy for TNBC in recent years. The full text structure covers molecular classification of TNBC, active immunotherapy of TNBC, passive immunotherapy of TNBC, oncolytic immunotherapy and the prospect of immunotherapy for TNBC.

1. Introduction

According to the UpToDate which is a clinical decision support system based on evidence-based medicine (http://www.uptodate.cn/), TNBC accounts for approximately 20% of breast cancers diagnosed worldwide, amounting to almost 200,000 cases every year [1]. TNBC is more commonly diagnosed in women younger than 40 years compared with hormone-positive breast cancer. In one study, there was a twofold higher attributable risk of TNBC in women under 40 years compared with women over 50 years (odds ratio [OR] 2.13, 95% CI 1.34–3.39) [2]. In addition, TNBC appears to be more common among black women compared with white women (OR 2.41, 95% CI 1.81–3.21) [2]. Risk factors associated with the diagnosis of TNBC include: positive BRCA mutation status, race (African-American women have a higher risk of TNBC compared with non-African American women), premenopausal status, obesity and a few maternal-related factors. TNBC is usually high grade, and the most common histology is infiltrating ductal carcinoma [3], although a rare histologic subtype, medullary carcinoma, is generally triple negative. TNBCs can exhibit geographic necrosis, a pushing border of invasion, and a stromal lymphocytic response. By definition, TNBC lacks immunohistochemical (IHC) expression of the estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2). Cut-offs used for ER, PR, and HER2 to make this diagnosis are discussed below. So, it is impossible for TNBC patients to benefit from endocrine therapy and molecular targeting therapy. The pace of discovery in the fields of immunology and cancer biology is accelerating due to the foundation laid decades ago. As our understanding of the role of the immune system in tumor initiation, progression, and metastasis evolves, continued progress is likely in the treatment of malignancy including TNBCs. Immunotherapy, also called biologic therapy, is a type of cancer treatment that boosts the body's natural defenses to fight cancer. It uses substances made by the body or in a laboratory to improve or restore immune system function. Immunotherapy may work by: stopping or

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slowing the growth of cancer cells, stopping cancer from spreading to other parts of the body, and helping the immune system work better at destroying cancer cells. There are several types of immunotherapy, including: monoclonal antibodies, non-specific immunotherapies, oncolytic virus therapy, T-cell therapy and cancer vaccines. The present study reviewed the recent research progress of immunotherapy for TNBC.

2. Molecular classification of TNBC

According to the UpToDate, the TNBC mostly comprises the basal-like molecular subtype, although TNBC and basal breast cancer are not synonymous and there is substantial heterogeneity within TNBCs. Burstein et al. found that four stable subtypes of TNBC could be identified when utilizing DNA and RNA profiling of TNBCs: luminal androgen receptor, mesenchymal, basal-like immunosuppressed, and basal-like immune-activated [4]. In Bertucci et al.'s study, 172 TNBCs based on IHC staining were correlated with gene expression profiles that defined the basal subtype [5]. Only 71 percent of TNBCs were assigned the basal subtype. In a converse analysis of 160 tumors conducted by Shah et al., 77% of basal cancers were triple-negative by IHC [6]. Also based on the UpToDate, Basal breast cancer is characterized by the genomic expression of the “basal cluster,” a unique cluster of genes that includes the epidermal growth factor receptor (EGFR, also called HER1), basal cytokeratins 5/6, c-Kit, the proliferation cluster, and low expression of the hormone receptor- and HER2-related genes [3,7,8]. Separate subtypes of TNBC have been characterized by gene expression, including two basal-like subtypes (BL1 and BL2), as well as immunomodulatory, mesenchymal, mesenchymal stem-like, and luminal androgen subtypes [9]. Additional subtypes that have been characterized include claudin-low and interferon-rich subtypes [10,11].

3. Active immunotherapy of TNBC

3.1. Immune checkpoint blocking

Immune checkpoint is a protective molecule in the human immune system that prevents normal tissue injury caused by the excessive activation of T cells, maintains tolerance to “self” tissues, and avoids autoimmune reactions. Importantly, tumor cells can overexpress immune checkpoint molecules and related ligands to resist human immune responses and escape immune surveillance and immune killing, which promotes their growth. Therefore, immune checkpoint blocking can reactivate T cell immune responses to tumor cells and break tumor immune suppression. The most extensively used immune checkpoint inhibitors for the study and application of cancer therapy include PD-1 and inhibitors of its ligand PD-L1, as well as CTLA-4 [12].

3.1.1. CTLA-4 inhibition

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is a leucocyte differentiation antigen and a transmembrane receptor expressed by activated $CD4^+$ regulatory T cells and $CD8^+$ T cells that express Foxp3 and CD25. It shares the B7 ligand with CD28. The combination of CTLA-4 and B7 molecules inhibits the activity of T cells and participates in the negative regulation of immune responses by binding to CD86 (B7-2) and CD80 (B7-1) on dendritic cells (DCs) with a greater affinity compared with CD28 [13,14]. Consequently, CTLA-4-antibody induces anti-tumor immunity by the blockade of Foxp3+ Treg cells in the TME and the Akt phosphorylation pathway, allowing for the potent expansion of T-cells [15,16], resulting in the enhanced rejection of tumor cells.

Ipilimumab, an anti-CTLA-4 antibody developed by Bristol-Myers Squibb, was approved by the USA FDA for metastatic melanoma in 2011. It showed a potentially curative and durable efficacy in tumor regression [13,17]. Currently, ipilimumab and tremelimumab (another promising anti-CTLA-4 antibody) have been used in clinical trials related to TNBC (Table 1). Furthermore, in a study of the melanoma antigen family, Karn et al. found that MAGE-A antigen defined an aggressive subgroup of TNBC, characterized by a lack of immune infiltration in the cancer microenvironment [18]. New immunostimulatory drugs, such as CTLA-4 inhibitors, might achieve stronger immune responses for the treatment of TNBC patients with MAGE-A expression.

3.1.2. PD-L1/PD-1 inhibition

PD-1 is an inhibitory transmembrane receptor expressed on the surface of immune effector cells including B cells, T cells, DCs, NK cells, and many TILs [19]. Two ligands of PD-1 have been identified: PD-L2 and PD-L1 [20,21]. PD-L1 is mainly expressed on B-cells, T-cells, macrophages, NK cells, epithelial cells, DCs, and vascular endothelial cells following stimulation with IFN-γ [22]. The expression of PD-1 can be induced by the action of tumor-derived IL-18 on immunosuppressive CD16 (dim) CD56 (dim) NK cells. PD-1 is generally activated in combination with the B7 globulin family ligand PD-L1 and these interactions restrict the activity of T cells, thereby inhibiting overactive immunity and autoimmunity. Of note, in normal tissues, PD-1/PD-L1 interactions protect against excessive tissue damage by confining inflammatory reactions regulated by T-cells and other components of the immune system during infections [23]. However, previous evidence showed that PD-1/PD-L1 interactions with tumor cells led to immune exhaustion and a downregulation of local immune responses [24].

Additionally, PD-1/PD-L1 interactions occurred in TNBC cells [25–27]. Mittendorf et al. detected the transcriptional and protein expression of PD-L1 in TNBC and other breast cancers by qRT-PCR and immunohistochemical staining [27]. They found that 20% of TNBC patients expressed PD-L1, which was higher than for other breast cancer patients. The expression of PD-L1 was positively associated with the degree of TNBC malignancy because a higher expression of PD-1 or PD-L1 was associated with increased Foxp3+ Treg infiltration, indicating they may work synergistically during immune evasion [27,28]. Furthermore, PD-1/PD-L1 interactions on T cells were reported to decrease the proliferation of T cells and accelerate apoptosis, which suggests PD-L1/PD-1 might be a potential therapeutic target for the killing of TNBC cells [29,30].

Using a mouse-model of breast cancer, Loi et al. found that MEK inhibitors enhanced the expressions of MHC and PD-L1 on the surface of TNBC cells *in vitro* and *in vivo*. A combination of MEK and PD-L1/PD-1 inhibitors significantly enhanced the anti-tumor immune response of TNBC mice [31]. Li et al. demonstrated a PD-L1 glycosylation-based mechanism in TNBC that enhanced immunosuppression by improving interactions with PD-1, and a recently discovered antibody, STM108, targeting gPD-L1 (glycosylated PD-L1), induced PD-L1 internalization and degradation in lysosomes [32]. Thus, the use of drug-conjugated antibodies to target glycosylated PD-L1 has identified new potential therapeutic strategies [28]. In addition, Adams et al. reported that in many TNBC cases, the expression of PD-L1 was closely related to the existence of FOXP3+ regulatory T-cells and CD163-positive macrophages [33]. This finding indicates that the combined targeting of several immunological features of TNBC may require a valid anti-tumor response.

Antibodies targeting PD-1 or PD-L1 were confirmed to elicit objective and durable responses in patients with NSCLCs, melanomas, and renal cell carcinomas [12,14]. Regarding TNBC, some agents including anti-PD-1 antibodies (nivolumab, PDR001, JS001, and pembrolizumab) and anti-PD-L1 antibodies (atezolizumab and durvalumab) are currently being tested in multiple clinical studies. Of note, the anti-PD-L1 antibodies (atezolizumab and durvalumab) are yielding promising results [25]. Additionally, Nanda reported that pembrolizumab has good antitumor activity and safety in TNBC patients.

These data suggest that blocking PD-1/PD-L1 together with modalities that deplete regulatory T-cells might be a feasible and promising therapeutic approach although the efficacy of immune checkpoint inhibitors remain to be improved. To validate whether targeting PD-1/

Table 1
Some ongoing clinical trials of anti-CTLA-4 immunotherapeutic interventions of TNBC.

Drug	NCT Number	Title	Status	Conditions	Interventions
Ipilimumab	NCT02983045	A Dose Escalation and Cohort Expansion Study of CD122Biased Cytokine (NKTR-214) in Combination with Anti-PD-1 Antibody (Nivolumab) or in Combination with Nivolumab and Anti-CTLA4 Antibody (Ipilimumab) in Patients With Select Advanced or Metastatic Solid Tumors	Recruiting	Triple Negative Breast Cancer	• Drug: Combination of NKTR-214 + nivolumab • Drug: Combination of NKTR-214 + nivolumab + ipilimumab
Tremelimumab	NCT02527434	Study of Tremelimumab in Patients with Advanced Solid Tumors	Recruiting	Triple Negative Breast Cancer	• Biological: Tremelimumab monotherapy • Biological: MEDI4736 monotherapy • Biological: MEDI4736 + tremelimumab combination therapy
Tremelimumab	NCT02658214	Durvalumab and Tremelimumab in Combination with First-Line Chemotherapy in Advanced Solid Tumors	Recruiting	Triple Negative Breast Cancer	• Drug: paclitaxel + carboplatin • Drug: carboplatin + etoposide • Drug: gemcitabine + carboplatin • Drug: nabpaclitaxel (paclitaxel-albumin) + carboplatin • Drug: oxaliplatin + 5-fluorouracil + leucovorin (calcium folinate/folinic acid) • Biological: durvalumab • Biological: tremelimumab • Drug: nabpaclitaxel (paclitaxel-albumin) + gemcitabine • Drug: cisplatin + 5fluorouracil

Drug	Characteristics				Population			Mechanisms of action	Sponsor/Collaborators
	Study Type	Phase	Study Design	Outcome Measures	Enrollment	Age	Sex		
Ipilimumab	Interventional	Phase 1 Phase 2	• Allocation: Nonrandomized • Intervention Model: Parallel Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Primary outcome measure: Safety and tolerability: Primary Outcome Measures: incidence of drugrelated Adverse Events (AEs), incidence of Dose Limiting Toxicities (DLTs), Serious Adverse Events (SAEs), and adverse events leading to discontinuation, deaths, and clinical laboratory test abnormalities. Efficacy: assessed by the Objective Response Rate (ORR) • Secondary Outcome Measures: Best Overall Response (BOR), Duration Of Response (DOR), Progression-Free Survival (PFS), Clinical Benefit Rate (CBR), Median Time to	480	18 Years and older (Adult, Older Adult)	All	All three drugs target the immune system and may act synergistically to promote anti-cancer effects.	Nektar Therapeutics Bristol-Myers Squibb

(*continued on next page*)

Table 1 (*continued*)

Drug	Characteristics			Population			Mechanisms of action	Sponsor/Collaborators
	Study Type	Phase	Study Design	Outcome Measures	Enrollment	Age	Sex		
Tremelimumab	Interventional	Phase 2	● Intervention Model: Single Group Assignment ● Masking: None (Open Label) ● Primary Purpose: Treatment	Response (MTR), Overall Survival (OS) ● Objective response rate (ORR) ● Duration of response (DoR) ● Disease control rate (DCR) ● Progression-free survival (PFS) ● Overall survival (OS) ● Best objective response (BoR) ● Objective Response Rate (ORR)	64	18 Years and older (Adult, Older Adult)	All	Tremelimumab is an immune checkpoint blocker, which aims to stimulate an immune system attack on tumors. Tremelimumab binds to the protein CTLA-4, which is expressed on the surface of activated T lymphocytes and inhibits the killing of cancer cells.	AstraZeneca
Tremelimumab	Interventional	Phase 1	● Allocation: NonRandomized ● Intervention Model: Parallel Assignment ● Masking: None (Open Label) ● Primary Purpose: Treatment	● Laboratory findings (including: clinical chemistry, hematology, and urinalysis) ● Incidence of Adverse Events ● Tumor assessment based on RECIST 1.1 (for cohort 6 only)	42	18 Years–99 Years (Adult, Older Adult)	All	These two drugs target the immune system and may act synergistically to promote anti-cancer effects.	AstraZeneca

PD-L1 might be a treatment choice for TNBCs, pembrolizumab/atezolizumab, which are used in metastatic/adjuvant/neoadjuvant settings for TNBC treatment, are being studied in a series of ongoing phase II/III clinical trials (Tables 2 and 3). Thus, targeting PD-1/PD-L1 might be an effective option for the treatment of TNBCs, although the efficacy of immune checkpoint inhibitors remains to be enhanced.

3.1.3. CD47 checkpoint blockade

CD47 is a cell surface transmembrane protein which is highly expressed on various of cancer cells and cancer stem cells. By binding to its ligands, phagocytosis is blocked by signal regulatory protein α (SIRPα) and thrombospondin-1 (TSP-1), which is on the surface of macrophages and dendritic cells. Thus, a strong “don't eat me” signal is delivered. According to a study conducted by Noman MZ et al., CD47 was highly upregulated in Epithelial-to-mesenchymal transition (EMT)-activated mesenchymal breast cancer cells [34]. By direct binding of SNAI1 and ZEB1 (both belong to EMT-inducing transcription factors) to human CD47 proximal promoter, CD47 blockade efficiently increased and restored their phagocytosis. All these results come up with a rationale for a novel preclinical combination immunotherapy based on PD1/PD-L1 and CD47 blockade along with EMT inhibitors in patients with highly aggressive, mesenchymal, and metastatic breast cancer [34].

3.1.4. Combination of immune checkpoint inhibitors with targeted treatments

It is widely acknowledged that the function of checkpoint inhibitors is remarkable and that combinations of checkpoint inhibitors as well as combination with other targeted treatments might be beneficial. Several clinical trials demonstrated that the combined application of immune checkpoint inhibitors (blockade of PD-L1/PD-1 and CTLA-4) improved the response rate indicating they are a promising therapy strategy for TNBC. Furthermore, a combination of checkpoint inhibitors with targeted treatments improved the efficacy of immunotherapy to reduce the out-growth and metastasis of primary tumors, which are specific manifestations in a neoadjuvant background, and simultaneously improve the response of tumor-specific T-lymphocytes (Table 4).

3.2. Vaccines

3.2.1. DR5 DNA vaccination

TNF-Related Apoptosis Inducing Ligand Receptor 2 (TRAIL-R2; also known as DR5), is overexpressed in a wide range of solid cancers. It regulates cell apoptosis by TRAIL or agonist antibodies. Using TNBC cells, which are TRAIL-sensitive, Marie et al. observed that apoptosis induced by anti-DR5 antibodies was related to the cleavage of caspase-3 and PARP. In addition, DNA vaccination induced DR5-specific IFN-γ secreting T cells. These findings all support DR5 as a feasible immunotherapeutic target for TNBC and other DR5 positive tumors [15].

3.2.2. Combined vaccinations

It was previously reported that a combination of IGF-1R/HER-1 antibodies improved anti-cancer effects in TNBC cell lines. MDA-MB-231 has anti-tumor functions related to the induction of apoptotic cell death, suppression of cell proliferation, and intensification of antibody-dependent cell-mediated cytotoxicity [35]. IGF-1R, which is overexpressed in a broad range of malignant cancers, is a critical component in combination treatments. Thus, combined vaccinations might increase the efficacy of HER-1 targeted immunotherapeutic treatment (e.g., cetuximab) against TNBCs.

3.2.3. DC-based vaccines

Dendritic cells (DC) are bone marrow derived antigen-presenting cells (APC) that induce and regulate immune responses. In studies of tumor vaccines, DCs are frequently used as a natural adjuvant to induce

Table 2
Some ongoing clinical trials of anti-PD-1 immunotherapeutic interventions of TNBC.

Drug	NCT Number	Title	Status	Conditions	Interventions
Spartalizumab (PDR001)	NCT03499899	A Study of Efficacy and Safety of LAG525 in Combination with Spartalizumab, or With Spartalizumab and Carboplatin, or With Carboplatin, in Patients With Advanced Triple-negative Breast Cancer	Recruiting	Triple-negative Breast Cancer	• Drug: LAG525 • Drug: spartalizumab • Drug: carboplatin
Spartalizumab (PDR001)	NCT02807844	Phase Ib/II Study of MCS110 in Combination with PDR001 in Patients With Advanced Malignancies	Recruiting	Triple Negative Breast Cancer	• Drug: MCS110 • Drug: PDR001
Spartalizumab (PDR001)	NCT02404441	Phase I/II Study of PDR001 in Patients with Advanced Malignancies	Recruiting	Triple Negative Breast Cancer	Biological: PDR001
Nivolumab	NCT02393794	Cisplatin Plus Romidepsin & Nivolumab in Locally Recurrent or Metastatic Triple Negative Breast Cancer (TNBC)	Recruiting	• Triple-Negative Breast Cancer • Breast Cancer	• Drug: Romidepsin • Drug: Cisplatin • Drug: Nivolumab
Nivolumab	NCT03487666	OXEL: Pilot Study of Immune Checkpoint or Capecitabine or Combination Therapy as Adjuvant Therapy for TNBC With Residual Disease	Recruiting	• Triple Negative Breast Cancer	• Drug: Nivolumab • Drug: Capecitabine
Nivolumab	NCT02834247	A Study of TAK-659 in Combination with Nivolumab in Participants With Advanced Solid Tumors	Recruiting	• Triple-Negative Breast Neoplasms	• Drug: TAK-659 • Drug: Nivolumab
Nivolumab	NCT03435640	A Study of NKTR-262 in Combination With NKTR-214 and With NKTR-214 Plus Nivolumab in Patients With Locally Advanced or Metastatic Solid Tumor Malignancies	Recruiting	• Triple Negative Breast Cancer	• Drug: NKTR-262 • Drug: NKTR-214 • Drug: nivolumab
Pembrolizumab	NCT02977468	Effects of MK-3475 (Pembrolizumab) on the Breast Tumor Microenvironment in Triple Negative Breast Cancer	Recruiting	•Triple Negative Breast Cancer	• Drug: Merck 3475 Pembrolizumab • Radiation: Intraoperative radiation therapy (IORT)
Pembrolizumab	NCT03121352	Carboplatin, Nab-Paclitaxel and Pembrolizumab for Metastatic Triple-Negative Breast Cancer	Recruiting	•Metastatic Triple Negative Breast Cancer	•Drug: Carboplatin •Drug: Nabpaclitaxel •Drug: Pembrolizumab
Pembrolizumab	NCT02734290	Standard of Care Chemotherapy Plus Pembrolizumab for Breast Cancer	Recruiting	•Triple Negative Breast Cancer	• Drug: Pembrolizumab • Drug: Paclitaxel • Drug: Capecitabine
Pembrolizumab	NCT03184558	Bemcentinib (BGB324) in Combination with Pembrolizumab in Patients With TNBC	Recruiting	•Triple Negative Breast Cancer	•Drug: Bemcentinib; pembrolizumab
Pembrolizumab	NCT01676753	Phase 1b Trial of Dinaciclib with Pembrolizumab for Advanced Breast Cancer	Recruiting	• Advanced or Metastatic Breast Cancer • Triple Negative Breast Cancer	• Drug: Dinaciclib • Drug: Pembrolizumab
Pembrolizumab	NCT03197389	Effect of Pembrolizumab (Keytruda®) on Biomarkers in Early ER/PR Negative Breast Cancer.	Recruiting	• Breast Cancer • Triple Negative Breast Cancer	•Drug: Pembrolizumab

Drug	Characteristics				Population			Mechanisms of action	Sponsor/ Collaborators
	Study Type	Phase	Study Design	Outcome Measures	Enrollment	Age	Sex		
Spartalizumab (PDR001)	Interventional	Phase 2	• Allocation: Randomized • Intervention Model: Parallel Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Overall response rate (ORR) per RECIST v1.1 per investigators' assessment • Duration of response (DOR) • Overall Survival (OS) • Pharmacokinetics (PK) parameter, Ctrough, of LAG525, spartalizumab and carboplatin • Time to response (TTR) • Progression free survival (PFS) • Clinical Benefit Rate (CBR)	96	18 Years and older (Adult, Older Adult)	All	LAG525 and spartalizumab are two immuno-agents targeting different immune checkpoints and have been tested as single agents and in combination.	• Novartis Pharmac-euticals • Novartis

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Table 2 (*continued*)

Drug	Characteristics				Population			Mechanisms of action	Sponsor/ Collaborators
	Study Type	Phase	Study Design	Outcome Measures	Enrollment	Age	Sex		
				• PK parameter, Cmax of LAG525, spartalizumab and carboplatin • PK parameter, AUC, of LAG525, spartalizumab and carboplatin • Anti-drug antibodies (ADA) prevalence at baseline for LAG525 and spartalizumab • Anti-drug antibodies (ADA) incidence on treatment for LAG525 and spartalizumab					
Spartalizumab (PDR001)	Interventional	• Phase 1 • Phase 2	• Allocation: NonRandomized • Intervention Model: Parallel Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• (phase 1) Number of patients with adverse events, as a measure of safety • (phase 2): Overall Response rate (ORR) • Phase 1: Overall Response Rate (ORR) • Phase 1: progression free survival (PFS) • Phase 2 Overall response rate per immune related Response Criteria • Phase 1: clinical benefit rate (CBR) • Phase 1: duration of response (DOR) • Phase 1: disease control rate (DCR) • Phase 2: Progression Free Survival (PFS) • Phase 2 Duration Of Response (DOR) • Phase 2: Disease Control Rate (DCR) • Phase 2: Clinical Benefit Rate (CBR)	175	18 Years and older (Adult, Older Adult)	All	Tumor-associated macrophages mediate intrinsic/acquired resistance to programmed death-1 (PD-1) inhibitors; these cells can be reduced by inhibiting the colony-stimulating factor-1 (CSF-1)/receptor pathway. Targeting CSF-1 with lacnotuzumab (MCS110), a high-affinity, humanized mAb, combined with spartalizumab (PDR001), a humanized anti-PD-1 mAb, is hypothesized to result in synergistic antitumor activity.	• Novartis Pharmaceuticals • Novartis
Spartalizumab (PDR001)	Interventional	• Phase 1 • Phase 2	• Allocation: NonRandomized • Intervention Model: Single Group Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Part l: The exposure (AUC(0–336 h)) after first dose of treatment • Part l: Incidence of dose limiting toxicities (DLTs) • Part ll: Overall response Rate (ORR) • Safety and Tolerability as assessed by incidence and severity of adverse events, dose interruptions, reductions and dose intensity • Presence and/or concentration of antiPDR001	318	18 Years and older (Adult, Older Adult)	All	By blocking the interaction between PD-1 and its ligands, PD-L1 and PD-L2, PDR001 inhibits the PD-1 immune checkpoint, resulting in activation of an antitumor immune response by activating effector T-cells and inhibiting regulatory T-cells.	• Novartis Pharmaceuticals • Novartis

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Table 2 (*continued*)

Drug	Characteristics				Population			Mechanisms of action	Sponsor/ Collaborators
	Study Type	Phase	Study Design	Outcome Measures	Enrollment	Age	Sex		
				• Overall Response Rate (ORR) - Phase l only • Progression Free Survival (PFS) - Phase l/ll • Duration of Response (DOR) - Phase l/ll • Disease Control Rate (DCR) - Phase l/ll • Overall Response Rate (ORR) per immune related Response Criteria - Phase ll only • Composite Serum pharmacokinetics (PK) parameters • Serum concentration vs. time profiles					
Nivolumab	Interventional	• Phase 1 • Phase 2	• Allocation: Nonrandomized • Intervention Model: Single Group Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Phase I: Recommended Phase II Dose of romidepsin in combination with cisplatin • Phase II: Objective response rate of treated subjects according to RECIST v1.1 criteria • Phase II: Clinical Benefit Rate at 16 weeks of study treatment for subjects treated at the recommended phase II dose of romidepsin plus cisplatin and nivolumab • Pharmacokinetics romidepsin plasma concentration vs time profile when given with cisplatin and nivolumab • Pharmacokinetics - cisplatin plasma concentration vs time profile when given with romidepsin • Median ProgressionFree Survival and Overall Survival	54	18 Years and older (Adult, Older Adult)	Female	This study will determine if taking romidepsin (at the dose determined in Phase I) in combination with cisplatin and nivolumab is safe and effective in treating patients with breast cancer.	• Priyanka Sharma • Celgene Corporation • Bristol-Myers Squibb • University of Kansas Medical Center
Nivolumab	Interventional	Phase 2	• Allocation: Randomized • Intervention Model: Parallel Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Immune activation measured by changes in the peripheral immunoscore (PIS) at week 6 • Immune activation measured by changes of PIS at week 12 • Grade 3 and 4 toxicities according to the National Cancer Institute Common	45	18 Years and older (Adult, Older Adult)	All	Capecitabine, sold under the brand name Xeloda among others, is a chemotherapy medication used to treat breast cancer. Nivolumab is a human IgG4 anti-PD-1 monoclonal antibody. Nivolumab works as a	• Georgetown University • Bristol-Myers Squibb

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Table 2 (*continued*)

Drug	Characteristics				Population			Mechanisms of action	Sponsor/ Collaborators
	Study Type	Phase	Study Design	Outcome Measures	Enrollment	Age	Sex		
				Toxicity Criteria for Adverse Events Version 4.0 [NCI CTCAE v4.03] ● Distant recurrence free survival (DRFS) and Overall Survival ● Immune activation in the tumor by IHC ● Immune activation in the tumor by flow cytometry ● Immune activation in the tumor by ELISA ● Intracellular cytokine staining and $CD8^+$ T-cell clonal expansion ● Circulating tumor DNA				checkpoint inhibitor, blocking a signal that would have prevented activated T cells from attacking the cancer, thus allowing the immune system to clear the cancer.	
Nivolumab	Interventional	Phase 1	● Allocation: NonRandomized ● Intervention Model: Parallel Assignment ● Masking: None (Open Label) ● Primary Purpose: Treatment	● Part 1: Maximum Tolerated Dose (MTD) ● Part 1: RP2D ● Part 2: Overall Response Rate (ORR) ● Percentage of Participants Experiencing 1 or More Treatment-Emergent Adverse Events (TEAEs) ● Percentage of Participants with 1 or More Grade 3 and Grade 4 Adverse Events (AEs) ● Percentage of Participants Experiencing Serious Adverse Events (SAEs) ● Percentage of Participants With TEAEs Resulting in Study Drug Discontinuation ● Number of Participants With Clinically Significant Laboratory Values ● Number of Participants with Clinically Significant Vital Sign Measurements ● Part 2: Percentage of Participants With Disease Control	126	18 Years and older (Adult, Older Adult)	All	TAK-659 has demonstrated activity against SYK and FLT3 *in vitro* and has shown activity in multiple DLBCL xenograft tumor models.	● Millennium Pharmaceuticals, Inc. ● Takeda
Nivolumab	Interventional	● Phase 1 ● Phase 2	● Allocation: NonRandomized ● Intervention Model: Parallel Assignment ● Masking: None (Open Label) ● Primary Purpose: Treatment	● Safety of NKTR-262 in combination with NKTR-214/nivolumab as evaluated by incidence of drug-related Adverse Events (AEs), Serious Adverse Events (SAEs), and AEs leading to discontinuation, deaths, and clinical laboratory	393	18 Years and older (Adult, Older Adult)	All	NKTR-262 is a small molecule agonist of toll-like receptors (TLRs) 7/8 designed to be retained in the tumor micro-environment in order to activate antigen-presenting cells (APC), such as dendritic cells, to create new antigen-specific	● Nektar Therapeutics

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Table 2 (*continued*)

Drug	Characteristics				Population			Mechanisms of action	Sponsor/ Collaborators
	Study Type	Phase	Study Design	Outcome Measures	Enrollment	Age	Sex		
				abnormalities per CTCAE 4.03 • Tolerability of NKTR-262 in combination with NKTR-214/nivolumab as evaluated by incidence of Dose Limiting Toxicities (DLTs), drug-related AEs, SAEs, AEs leading to discontinuation, deaths, clinical laboratory abnormalities per CTCAE 4.03 • Efficacy of NKTR-262 in combination with NKTR-214/nivolumab as assessed by the Objective Response Rate (ORR) based on RECIST 1.1				cytotoxic T cells. As a CD122-biased agonist, NKTR-214 monotherapy increases newly proliferative $CD8^+$ T cells in tumors.	
Pembrolizumab	Interventional	Phase 1	• Intervention Model: Single Group Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	Number of subjects with significant mean percent change in TILs	15	21 Years–80 Years (Adult, Older Adult)	Female	Investigators hope to determine if MK-3475 exposure will change the molecular signature of breast stroma from "normal" adjacent breast tissue (non-tumor tissue) obtained prospectively at the time of breast conserving surgery (BCS) in TNBC patients.	• Eileen Connolly • Merck Sharp & Dohme Corp. • Columbia University
Pembrolizumab	Interventional	Phase 2	• Intervention Model: Single Group Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Determine overall response rate (ORR) in patients treated with CNP • Determine progressionfree survival (PFS) in patients treated with CNP • Determine disease control rate (DCR) in patients treated with CNP • Determine duration of response in patients treated with CNP	30	18 Years and older (Adult, Older Adult)	All	Pembrolizumab is an investigational (experimental) drug that works by reinvigorating the immune system, allowing it to target and destroy cancer cells.	•Case Comprehens-ive Cancer Center
Pembrolizumab	Interventional	• Phase 1 • Phase 2	• Allocation: NonRandomized • Intervention Model: Parallel Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Treatment-Associated Adverse Events • Number of patients who complete chemotherapy without a dose delay of more than 21 days. • Overall response rate	88	18 Years and older (Adult, Older Adult)	All	Pembrolizumab is a humanized antibody used in cancer immunotherapy. It is an IgG4 isotype antibody that blocks a protective mechanism of cancer cells and allows the immune system to destroy those cancer cells. It targets the programmed cell death	• Providen-ce Health & Services • Merck Sharp & Dohme Corp

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Table 2 (*continued*)

Drug	Characteristics				Population			Mechanisms of action	Sponsor/ Collaborators
	Study Type	Phase	Study Design	Outcome Measures	Enrollment	Age	Sex		
Pembrolizumab	Interventional	Phase 2	• Intervention Model: Single Group Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Objective Response Rate • Disease Control Rate • Duration of Response • Time to Progression • Overall Survival • Number of patients with Adverse Events (as assessed by CTCAE v4.03)	56	18 Years and older (Adult, Older Adult)	All	1 (PD-1) receptor of lymphocytes. Bemcentinib, also known as BGB324 or R428, is an experimental oral small molecule that is an inhibitor of AXL kinase. Bemcentinib targets and binds to the intracellular catalytic kinase domain of AXL receptor tyrosine kinase and inhibits its activity.	• BerGenBio ASA • Merck Sharp & Dohme Corp
Pembrolizumab	Interventional	Phase 1	• Intervention Model: Single Group Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Maximum tolerated dose (MTD) • Define dose-limiting toxicities (DLTs) • Anti-tumor activity in patients with advanced triple negative breast cancer	32	18 Years–85 Years (Adult, Older Adult)	All	Dinaciclib (SCH727665) inhibits the unfolded protein response (UPR) through a CDK1 and CDK5-dependent mechanism.	• Jo Chien • Merck Sharp & Dohme Corp. • University of California, San Francisco
Pembrolizumab	Interventional	Early Phase 1	• Allocation: NonRandomized • Intervention Model: Single Group Assignment • Masking: None (Open Label) • Primary Purpose: Basic Scienc	• PD-1 expression • Increase in the amount of TILs • PD-L1 expression	34	18 Years–100 Years (Adult, Older Adult)	All	Pembrolizumab is an investigational (experimental) drug that works by reinvigorating the immune system, allowing it to target and destroy cancer cells.	•Universitaire Ziekenhuizen Leuven

Table 3
Some ongoing clinical trials of anti-PD-L1 immunotherapeutic interventions of TNBC.

Drug	NCT Number	Title	Status	Conditions	Interventions
Avelumab (MSB0010718C)	NCT02926196	Adjuvant Treatment for High-risk Triple Negative Breast Cancer Patients with the Anti-PD-l1 Antibody Avelumab	Recruiting	•Triple Negative Breast Neoplasms	• Drug: MSB0010718C
Avelumab (MSB0010719C)	NCT03387085	QUILT-3.067: NANT Triple Negative Breast Cancer (TNBC) Vaccine: Molecularly Informed Integrated Immunotherapy in Subjects with TNBC Who Have Progressed on or After Standard-of-care Therapy.	Recruiting	•Triple Negative Breast Cancer	• Drug: Aldoxorubicin HCl • Biological: ALT-803 • Biological: ETBX-011 • Biological: ETBX-051 • Biological: ETBX-061 • Biological: GI-4000 • Biological: GI-6207 • Biological: GI-6301 • Biological: haNK for Infusion • Biological: avelumab • and 8 more
Durvalumab	NCT03199040	Neoantigen DNA Vaccine Alone vs. Neoantigen DNA Vaccine Plus Durvalumab in Triple Negative Breast Cancer Patients Following Standard of Care Therapy	Recruiting	• Triple Negative Breast Cancer • Triple Negative Breast Neoplasms • TNBC - TripleNegative Breast Cancer • Triple-negative Breast Carcinoma	• Drug: Durvalumab • Biological: Neoantigen DNA vaccine • Device: TDS-IM system (Inchor Medical Systems) • Procedure: Peripheral blood draw
Durvalumab	NCT03356860	Safety and Efficacy of Durvalumab Combined to Neoadjuvant Chemotherapy in Localized Luminal B HER2(−) and Triple Negative Breast Cancer.	Recruiting	• Breast Cancer • Triple Negative Breast Cancer • Luminal B	• Drug: Paclitaxel • Drug: Epirubicin • Drug: Cyclophosphamide • Drug: Durvalumab
Durvalumab	NCT03544125	Olaparib and Durvalumab in Treating Participants with Metastatic Triple Negative Breast Cancer	Recruiting	• Triple-Negative Breast Carcinoma • Anatomic Stage IV Breast Cancer AJCC v8 • Estrogen Receptor Negative • HER2/Neu Negative • Progesterone Receptor Negative • Prognostic Stage IV Breast Cancer AJCC v8	• Drug: Olaparib • Biological: Durvalumab
Atezolizumab (MPDL3280A)	NCT03281954	Clinical Trial of Neoadjuvant Chemotherapy with Atezolizumab or Placebo in Patients with Triple-Negative Breast Cancer Followed After Surgery by Atezolizumab or Placebo	Recruiting	• Triple Negative Breast Cancer	• Drug: Placebo • Drug: Atezolizumab
Atezolizumab (MPDL3281A)	NCT03164993	Atezolizumab Combined with Immunogenic Chemotherapy in Patients With Metastatic Triplenegative Breast Cancer	Recruiting	• Cancer, Breast • Triple Negative Breast Cancer	• Drug: Atezolizumab • Drug: Pegylated liposomal doxorubicin • Drug: Cyclophosphamide • Other: Placebo
Atezolizumab (MPDL3282A)	NCT03498716	A Study Comparing Atezolizumab (Anti PD-L1 Antibody) In Combination with Adjuvant Anthracycline/TaxaneBased Chemotherapy	Recruiting	• Triple Negative Breast Cancer	• Drug: Atezolizumab • Drug: Paclitaxel

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Table 3 (*continued*)

Drug	NCT Number	Title	Status	Conditions	Interventions
		Versus Chemotherapy Alone In Patients With Operable Triple-Negative Breast Cancer			• Drug: Dosedense Doxorubicin or dose-dense Epirubicin • Drug: Cyclophosphamide
Atezolizumab (MPDL3283A)	NCT03256344	A Safety Study of Talimogene Laherparepvec Combined with Atezolizumab for Triple Negative Breast Cancer and Colorectal Cancer With Liver Metastases	Recruiting	• Metastatic Triple Negative Breast Cancer	• Biological: Talimogene Laherparepvec • Biological: Atezolizumab
Atezolizumab (MPDL3284A)	NCT03206203	Carboplatin with or Without Atezolizumab in Treating Patients With Stage IV Triple Negative Breast Cancer	Recruiting	• Triple Negative Breast Cancer • Stage IV Breast Cancer • HER2 Negative • Invasive Breast Cancer	• Drug: Atezolizumab • Drug: Carboplatin • Other: Laboratory Biomarker • Other: Quality-of Life Assessment
Atezolizumab (MPDL3285A)	NCT03424005	A Study Evaluating the Efficacy and Safety of Multiple Immunotherapy-Based Treatment Combinations in Patients with Metastatic Triple-Negative Breast Cancer (Morpheus-TNBC)	Recruiting	• Triple Negative Breast Cancer	• Drug: Capecitabine • Drug: Atezolizumab • Drug: Ipatasertib • Drug: SGN-LIV1A • Drug: Bevacizumab • Drug: Cobimetinib • Drug: Chemotherapy (Gemcitabine + Carboplatin or Eribulin)

Drug	Characteristics				Population				Sponsor/Collaborators
	Study Type	Phase	Study Design	Outcome Measures	Enrollment	Age	Sex		
Avelumab (MSB0010718C)	Interventional	Phase 3	• Allocation: Randomized • Intervention Model: Parallel Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Disease free survival • Disease free survival in PD-L1-positive patients • Overall survival • Safety profile	335	18 Years and older (Adult, Older Adult)	All	Avelumab is a whole monoclonal antibody of isotype IgG1 that binds to the programmed death-ligand 1 (PD-L1) and therefore inhibits binding to its receptor	• Istituto Oncologico Veneto IRCCS • University of Padova • Dipartimento di scienze chirurgiche, Oncologiche e Gastroenterologic
Avelumab (MSB0010719C)	Interventional	• Phase 1 • Phase 2	• Intervention Model: Single Group Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Incidence of treatmentemergent Adverse Events (AEs) and Serious Adverse Events (SAEs), graded using the NCI CTCAE Version 4.03. • Objective response rate by RECIST • Objective response rate by irRC • Progression-free survival by RECIST during Phase 1b • PFSl by irRC during Phase 1b • Overall survival • Duration of response by RECIST and irRC • Disease control rate (confirmed complete response, partial response, or stable disease lasting	79	18 Years and older (Adult, Older Adult)	All	programmed cell death 1 (PD-1). Formation of a PD-1/PD-L1 receptor/ligand complex leads to inhibition of $CD8^+$ T cells, and therefore inhibition of an immune reaction. Immunotherapy aims at ceasing this immune blockage by blocking those receptor ligand pairs. In the case of avelumab, the	• NantKwest, Inc.

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Table 3 (*continued*)

Drug	Characteristics				Population				Sponsor/Collaborators
	Study Type	Phase	Study Design	Outcome Measures	Enrollment	Age	Sex		
				zfor at least 2 months) by RECIST and irRC. • Patient-reported outcomes of pancreatic cancer symptoms • PFS by RECIST during Phase 2 • Progression-free survival by irRC during Phase 2				formation of PD-1/PDL1 ligand pairs is blocked and $CD8^+$ T cell immune response should be increased.	
Durvalumab	Interventional	Phase 1	• Allocation: Randomized • Intervention Model: Parallel Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Safety of neoantigen DNA vaccines given alone or in combination with durvalumab as measured by number of adverse events experienced by patient • Immune response to neoantigen DNA vaccines given alone or in combination with durvalumab as measured by luminex assay • Immune response to neoantigen DNA vaccines given alone or in combination with durvalumab as measured by ELISPOT • Immune response to neoantigen DNA vaccines given alone or in combination with durvalumab as measured by multiparametric flow cytometry	24	Age: 18 Years and older (Adult, Older Adult)	Female	Durvalumab is a human immunoglobulin G1 kappa (IgG1κ) monoclonal antibody that blocks the interaction of programmed cell death ligand 1 (PD-L1) with the PD-1 and CD80 (B7.1) molecules.	• Washington University School of Medicine • MedImmune LLC
Durvalumab	Interventi-onal	• Phase 1 • Phase 2	• Allocation: Randomized • Intervention Model: Parallel Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Adverse events • Pathological response	57	18 Years and older (Adult, Older Adult)	All		• Grand Hôpital de Charleroi • Cliniques universitaires Saint-LucUniversité Catholique de Louvain
Durvalumab	Interventional	Phase 1	• Intervention Model: Single Group Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Proportion of completion of Clinical Laboratory Improvement Amendments (CLIA) analytics on pretreatment biopsy before the planned for 4-week biopsy • Incidence of ≥ grade 3 adverse events per Common Terminology Criteria for Adverse Events (CTCAE) version (v) 4.03 • Overall response rate (ORR) for olaparib in combination with durvalumab • Clinical benefit rate (CBR) for olaparib in combination with durvalumab • Duration of response (DOR) for olaparib in combination with durvalumab	8	18 Years and older (Adult, Older Adult)	All		•OHSU Knight Cancer Institute

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Table 3 (*continued*)

Drug	Characteristics				Population				Sponsor/Collaborators
	Study Type	Phase	Study Design	Outcome Measures	Enrollment	Age	Sex		
				• Progression-free survival (PFS) for olaparib in combination with durvalumab • Overall survival (OS) olaparib in combination with durvalumab					
Atezolizumab (MPDL3280A)	Interventional	Phase 3	• Allocation: Randomized • Intervention Model: Parallel Assignment • Masking: Triple (Participant, Care Provider, Investigator) • Primary Purpose: Treatment	• Pathologic complete response in the breast and lymph nodes (ypT0/Tis ypN0) • Event-free survival (EFS) • Pathologic complete response in the breast (ypT0/Tis) • Pathologic complete response in the breast and lymph nodes (ypT0 ypN0) • Positive nodal status conversion rate • Overall survival (OS) • Recurrence-free interval (RFI) • Distant disease-free survival (DDFS) • Brain metastases free survival • Frequency of Adverse Events	1520	18 Years and older (Adult, Older Adult)	All	Atezolizumab blocks the interaction of PD-L1 with programmed cell death protein 1 (PD-1) and CD80 receptors (B7-1Rs). PD-L1 can be highly expressed on certain tumors, which is thought to lead to reduced activation of immune cells (cytotoxic T-cells in particular) that might otherwise recognize and attack the cancer. Inhibition of PD-L1 by atezolizumab can remove this inhibitor effect and thereby engender an anti-tumor response. It is one of several ways to block inhibitory signals related to T-cell activation, a more general strategy known as "immune checkpoint inhibition."	• NSABP Foundation Inc • Genentech, Inc. • Hoffmann-La Roche
Atezolizumab (MPDL3281A)	Interventional	Phase 2	• Allocation: Randomized • Intervention Model: Parallel Assignment • Masking: Double (Participant, Investigator) • Primary Purpose: Treatment	• Assessment of toxicity of combined treatment with Atezolizumab, pegylated liposomal doxorubicin and cyclophosphamide • Progression-free survival (PFS) • Objective tumor response rate • Overall survival • Duration of response • Durable tumor response rate (DRR; > 6 months) • Patient reported outcome	75	18 Years and older (Adult, Older Adult)	All		• Oslo University Hospital • Hoffmann-La Roche • Norwegian Cancer Society • St. Olavs Hospital • Helse Stavanger HF • University Hospital of North Norway
Atezolizumab (MPDL3282A)	Interventional	Phase 3	• Allocation: Randomized • Intervention Model: Parallel Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Invasive Disease-Free Survival (iDFS) • Overall Survival (OS) • Disease-Free Survival (DFS) • Recurrence-Free Interval (RFI) • Distant RFI • Percentage of participants with adverse events • Serum concentration of Atezolizumab • Invasive Disease-Free Survival (iDFS) in PDL1Selected Patients • Invasive Disease-Free Survival (iDFS) in Node Positive Disease • Invasive Disease Free Survival (iDFS) including second primary non-breast invasive cancer	2300	18 Years and older (Adult, Older Adult)	All		•Hoffmann-La Roche •Breast International Group •Alliance Foundation Trials (AFT) •Institut Jules Bordet/ Clinical Trials Support Unit (IJB/CTSU) •Frontier Science and Technology Research Foundation Inc (FS)
Atezolizumab (MPDL3283A)	Interventional	Phase 1	• Intervention Model: Single Group Assignment	• Subject's incidence of dose limiting toxicities (DLTs) • Subject's incidence of treatment-emergent adverse events	36	18 Years–99 Years (Adult, Older Adult)	All		• Amgen • Genentech/Roche

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Table 3 (*continued*)

Drug	Characteristics				Population			Sponsor/Collaborators
	Study Type	Phase	Study Design	Outcome Measures	Enrollment	Age	Sex	
			• Masking: None (Open Label) • Primary Purpose: Other	• Number of subject with clinically relevant laboratory abnormalities • Objective response rate (ORR) • Best overall response (BOR) • Duration of response (DOR) • Disease control rate (DCR) • Durable response rate (DRR) • Progression-free survival (PFS) • Overall survival (OS) • Lesion level responses in injected and uninjected tumor lesions				
Atezolizumab (MPDL3284A)	Interventional	Phase 2	• Allocation: Randomized • Intervention Model: Crossover Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Progression free survival (PFS) • Overall response rate (ORR) • Clinical benefit rate (CBR) • Duration of response (DOR) • Overall survival (OS)	185	18 Years and older (Adult, Older Adult)	All	• VanderbiltIngram Cancer Center • National Cancer Institute (NCI) • Genentech, Inc.
Atezolizumab (MPDL3285A)	Interventional	• Phase 1 • Phase 2	• Allocation: Randomized • Intervention Model: Parallel Assignment • Masking: None (Open Label) • Primary Purpose: Treatment	• Objective Response Rate (ORR) • Progression Free Survival (PFS) • Disease Control Rate (DCR) • Overall Survival (OS) • Overall Survival (at specific time-points) • Duration of Response (DOR) • Percentage of Participants with Adverse Events • Serum Concentration of Atezolizumab • Plasma Concentration of Ipatasertib • Plasma or Serum Concentration of SNGLIV1A • Plasma Concentration of Cobimetinib • Plasma Concentration of Capecitabine	260	18 Years and older (Adult, Older Adult)	All	• Hoffmann-La Roche • Seattle Genetics, Inc.

tumor antigen-specific effects and memory cells [20]. DC-based vaccines can be classified into tumor antigen peptide-loaded, gene-modified, and whole tumor cell antigen-loaded vaccines [21]. Immunotherapy related tumor vaccines mainly include the latter two for the treatment of TNBC.

3.2.3.1. Whole tumor cell antigen-loaded DC-based vaccines. Whole tumor cell antigen-loaded DC-based vaccines present numerous tumor antigen epitopes to enhance T cell immunization and prevent immune escape by tumor cells. Zhang et al. prepared a whole tumor cell antigen-loaded DC-based vaccine by fusing DC cells derived from peripheral blood and TNBC cells (MDA-MB-231) using cell electrofusion technology [36]. They reported that the fusion vaccine induced many immune active factors such as IL-12 and IFN-γ. Simultaneously, it was found that effector cells induced by the fusion vaccine stimulated the proliferation of T lymphocytes and increased their cytotoxicity activity against breast cancer cells, indicting this approach might be useful against TNBC cells [36].

3.2.3.2. Gene-modified DC-based vaccines. Gene-modified DC-based vaccines are transfected into DC cells by overexpressing genes in tumor cells, which enhances the immune function of T cells and induces specific cellular immune killing effects. Tang et al. prepared gene-modified DC-based vaccines by the transfection of DCs following the preparation of a Runx2-overexpressing lentivirus. The secretion of IL-12 and IFN-γ by T cells was increased significantly after the co-culture of gene-modified DC-based vaccines and T cells. In addition, induced cytotoxic T cells (CTL) had a targeted killing effect on TNBC [22].

3.2.4. Peptide vaccination

Personalized peptide vaccination (PPV) therapy selects different peptides for different patients, based on their active immunity, to trigger immune responses to cancer cells. Experimental studies showed that PPV has clinical efficacy for the treatment of recurrent or progressive glioblastoma, unresectable pancreatic cancer, melanoma, lung cancer, colorectal cancer, cervical cancer, and other advanced tumors [37–42]. A phase II study of 18 cases of metastatic recurrent TNBC (mrTNBC) treated with PPV reported that the levels of CTL and IgG were elevated in most PPV patients [43]. Moreover, one patient with TNBC had a complete immune response and another patient produced a partial immune response. No drug related side effects were noted [43]. The above results suggest that PPV might have beneficial effects for the treatment of TNBC. AE37 is a HER-2 derived peptide vaccine and clinical trials reported that it had peptide specificity and induced continuous immune responses to prevent the recurrence of breast cancer [35]. In addition, Mittendorf et al. carried out a clinical trial of AE37 in 298 patients, among which 145 received granulocyte macrophage colony-stimulating factor (GM-CSF) alone, and the other 153 cases received a combined treatment of AE37 and GM-CSF [44]. Prior evidence indicated that AE37 is safe and has good clinical efficacy for patients with a low expression of HER-2, especially patients with TNBC.

In addition, a study by Overholser et al., demonstrated that a designed peptide vaccine or mimic is a feasible therapeutic-strategy to block abnormal molecular signaling-pathways with high specificity, potency, safety, and affinity. Furthermore, they showed that a combination of HER-1 and IGF-1R or HER-1 and HER-2 peptide mimics/vaccine-antibodies inhibited tumorigenesis in MDA-MB-231 TNBC and OE19 EC cell lines, supporting dual targeting with HER-1 and IGF-1R or HER-2 as a reformative treatment regimen for different cancers [19].

In conclusion, PPV might be beneficial for patients with TNBC, especially mrTNBC patients, on the basis of its induced immune responses and safety.

3.3. Cancer-testis antigen

Cancer-testis antigen is a tumor associated antigen, mainly expressed in the cells of the testis and embryonic tissues. Although it has a low expression or is not expressed in other normal tissues, it is expressed by malignant tumor cells of different tissue types. Cancer-testis antigen is highly immunogenic, and it induces anti-tumor immune responses; thus, it might have an important role in tumor immunotherapy. Recent studies demonstrated that TNBC might be a "CTA-rich" cancer, suggesting CTA-based tumor vaccines might be a therapeutic option for TNBC patients [45,46].

Table 4
Recent advances of the combination of immune checkpoint inhibitors with targeted treatments in TNBC.

Combination schedules	Recent advances
Dual application (anti-PD-1 and anti-CTLA-4)	① It possesses more than twice the efficacy of either alone in melanoma and lung cancer. ② It can overcome tumor immunosuppression and effectively treats TNBC, with a regression of ~80% of tumor, allowing inactivated tumor-specific T lymphocytes to continue to expand and carry out effector functions, and this shifts the TME from suppressive to inflammatory. ③ It is able to restore T lymphocyte rejection function in tumors, especially when combined with GVAX vaccination (consisting of GM−CSF−expressing irradiated tumor cells).
Anti-PD-1/PD-L1 with EGFR inhibitors (Cetuximab and Panitumumab)	① Inhibiting EGFR by either mAbs or EGFR-TKIs could decrease the expression of PD-L1 in tumors, and it will probably exert a synergetic effect. ② Delayed tumor growth and increased survival were demonstrated in preclinical EGFR mutant lung cancer models treated with anti-PD-1 mAbs.
Anti-PD-1/PD-L1 with VEGF inhibitors (Bevacizumab)	① VEGF-A produced in the TME enhances expression of PD-1 and other immune checkpoint molecules. ② High levels of VEGF-A might be involved in resistance to PD-1 blockades, which could be reverted by targeting the VEGF-A/VEGFR pathway. ③ Blocking the VEGF pathway could potentiate anti-PD-L1 mAb (Atezolizumab) therapy and improve antigen-specific T-cell migration. ④ A phase I study evaluating atezolizumab in combination with Bevacizumab showed an overall response rate of 40% in metastatic renal cell carcinoma and was well-tolerated in these patients without synergistic toxicity. ⑤ Synergistic anti-tumor effect *in vivo* can also be induced successfully by combining PD-1/PD-L1 and VEGF-A/VEGFR blockade.
Anti-PD-1/PD-L1 with PARP inhibitors	① PARP inhibitors can upregulate PD-L1 expression and enhance cancer-associated immunosuppression. ② Anti-PD-1/PD-L1 mAbs may exert a supplementary and increased anti-tumor effect in combination with a PARP inhibitor.
Anti-PD-1/PD-L1 with anti-MMP-14 antibodies (Fab 3369, Fab R2C7, DX 2400)	① Immunotherapeutic strategy targeting MMP-14 can restrict immune suppression, tumor progression, and metastasis in TNBC patients, and it can suppress extracellular matrixc (ECM) degradation and invasion of TNBC cell. ② At this point, there have been no reports or clinical trials on targeting MMP-14 together with anti-PD-1/PD-L1 treatment.

Table 5
Recent studies about the CAR-T immunotherapy for triple negative breast cancer (TNBC).

Author	Year	Country	Affiliation	Journal	Key Findings and Significance
Song et al.	2016	USA	University of Pennsylvania	J Hematol Oncol	FRα CAR T cells can mediate antitumor activity against established TNBC tumor, particularly when FRα is expressed at higher levels.
Tchou et al.	2017	USA	University of Pennsylvania	Cancer Immunol Res	1) The cell-surface molecule c-Met was expressed in about half of breast tumors, prompting the construction of a CAR T cell specific for c-Met, which halted tumor growth in immune-incompetent mice with tumor xenografts. 2) RNA c-Met-CAR T cell injections were well tolerated, as none of the patients had study drug-related adverse effects greater than grade 1. 3) Intratumoral injections of mRNA c-Met-CAR T cells are well tolerated and evoke an inflammatory response within tumors.
Byrd et al.	2018	USA	Baylor College of Medicine	Cancer Res	The authors developed a CAR T cell-based immunotherapeutic strategy to target TEM8, a marker initially defined on endothelial cells in colon tumors that was discovered recently to be upregulated in TNBC.They offered a preclinical proof of concept for immunotherapeutic targeting of an endothelial antigen that is overexpressed in triple-negative breast cancer and the associated tumor vasculature.
Bajgain et al.	2018	USA	Texas Children's Hospital and Houston Methodist Hospital	J Immunother Cancer	This study demonstrated the feasibility of targeting breast cancer using transgenic T cells equipped to thrive in the suppressive tumor milieu and highlight the importance of providing transgenic T cells with signals that recapitulate physiologic TCR signaling - [activation (signal 1), co-stimulation (signal 2) and cytokine support (signal 3)] - to promote *in vivo* persistence and memory formation.
Han et al.	2018	USA	University of Pennsylvania	J Hematol Oncol	This study demonstrated that CD27 or 4-1BB costimulated, self-enriched NKG2D CAR-redirected T cells mediate anti-tumor activity against TNBC tumor, which represent a promising immunotherapeutic approach to TNBC treatment.

3.3.1. CTA NY-ESO-1-targeted immunotherapy

A study of 215 TNBC patients by Ademuyiwa et al. reported that after treatment, 16% of the patients expressed NY-ESO-1 in pathological sections of the tumor and in serum samples taken before their treatment [24]. Of these patients, 72.7% of the serum samples from NY-ESO-1 positive TNBC patients reacted with NY-ESO-1 antibody [24]. Significantly higher numbers of $CD8^+$ T cells infiltrated NY-ESO-1 positive TNBC tissues compared with NY-ESO-1 negative tissues. Furthermore, the expression of NY-ESO-1 was positively correlated with the number of $CD8^+$ T cells infiltrating the tumor tissues. These results indicate that NY-ESO-1 might induce cellular immunity and humoral immunity, enhance anti-tumor immunity, reduce tumor load, and become a new therapeutic target for TNBC tumor vaccines. In addition, Badovinac et al. suggested that NY-ESO-1 together with MAGE-A10, a highly immunogenic CTA, might be a highly attractive specific immunotherapy. However, no clear correlation between NY-ESO-1 and MAGE-A10 expression and tumor grade, tumor size, lymph node status, or Ki67 was reported [20].

3.3.2. CTA SP17-targeted immunotherapy

Sperm protein 17 (SP17), a CTA, is abnormally expressed in many neoplasms, such as esophageal and ovarian cancers, and multiple myeloma and nervous system tumors. SP17 was proposed as a candidate target for tumor immunotherapy. Mirandola et al. evaluated SP17 expression levels and estimated whether SP17 induced SP17-specific cytotoxic T lymphocytes *in vitro* [47]. SP17 is expressed by primary breast cancers, breast cancer cell lines, and TNBC cells, but not unaffected tissues and adjacent non-cancer breast tissues in addition to male germinal cells [47]. Anti-SP17 antibodies detected in patient sera stimulated the production of HLA class-I restricted, SP17 specific, cytotoxic T-lymphocytes that effectively destroyed breast cancer cells [47]. Given that immunotherapeutic treatments directed against specific tumor-associated antigens (TAAs) and mediated by specific cytotoxic T lymphocytes (CTL) are currently unavailable for TNBC, SP17-targeted immunotherapy might be a novel and low toxic treatment for TNBC.

3.3.3. MAGE-A/B-targeted immunotherapy

MAGE-A has functions in chromosomal alignment and centrosome duplication, and is a CTA and a marker for CIN in TNBC. Raghavendra et al. found that NY-ESO-1 and MAGE-A were expressed at a high level and frequency in TNBC (approximately 17% and 47% of TN cases, respectively). Most NY-ESO-1 positive cases also expressed MAGE-A ($p = 2.06 \times 10^{-9}$), and both were closely related to the TN phenotype ($p < 0.0001$), indicating these might be important therapeutic targets for TNBC. Compared with approaches that only target a single antigen, therapies that target multiple CTAs may induce more efficacious antitumor responses. Regarding the heterogeneity of expression, several studies reported that most cases expressed NY-ESO-1 and MAGE-A in > 75% of tumor cells, which suggests it might be useful as a therapeutic adjunct[51]. Considering the benefit of current immunotherapies for TNBC patients, this provides the basis of targeting CTAs for TNBCs [29].

MAGE-B is also a tumor-associated antigen (TAA). A study conducted by Singh et al. suggested that curcumin enhanced the efficacy of the Listeria^{at}-MAGE-B vaccine against metastases in TNBC patients. Curcumin mediates this effect through the reversal of tumor-induced immune suppression via the reduction of IL-6, which increases the effects of vaccinations against TNBCs by improving T-cell responses [30,37]. Although many achievements have been made in the researches of MAGE-A/B-targeted immunotherapy, it should be noted that while some targeting antigens like MAGE and other tumor-associated antigens may be readily targetable in theory, it is unlikely to be targetable in practice since some of these antigens are not tumor-specific.

3.4. Cancer-relevant targeted immunotherapy

3.4.1. MMP-14 blockade

Matrix-metalloproteinase 14 (MMP-14) is a clinically-correlative target in metastatic tumors because of its important role in the progression and metastasis of tumors. MMP-14 is localized on the surface of cells, and the activation of MMP-14 induces antibody mediated blockade in cancer.

Ling et al. developed an inhibitory anti-MMP-14 antibody, Fab 3369, which inhibits the catalytic domain of MMP-14 [48]. An immunotherapeutic strategy targeting MMP-14 might restrict immune suppression, cancer progression, and metastases in TNBC patients. MMP-14 blockade by Fab 3369 suppressed extracellular matrix (ECM) degradation and tissue invasion by TNBC cells. However, further clinical studies using a series of markers are still necessary to adequately demonstrate the effect of MMP-14 inhibitory antibody immunotherapy for TNBC patients, as well as immune checkpoint inhibitor therapies in other cancers.

3.4.2. CSPG4 protein

Chondroitin sulfate proteoglycan 4 (CSPG4) is a cell surface proteoglycan and a target for monoclonal-antibody (mAb) based immunotherapy for numerous types of cancers including TNBC. Clinical research conducted by Wang et al. reported that CSPG4 protein was expressed in 32 of 44 (72.7%) primary tissues of TNBC, including TNBC cell lines and tumor cells in the pleural effusion from 12 metastatic TNBC patients [49]. CSPG4-specific mAb significantly inhibited the growth, adhesion, and migration of TNBC cells *in vitro*. Its other anti-tumor effects include enhanced apoptosis, reductive mitotic activation in tumor cells, reduced blood vessel density in the tumor microenvironment, and the decreased activation of important signaling pathways involved in the survival, proliferation, and metastasis of tumor cells. In conclusion, CSPG4 is expressed in TNBC tissues and might be a target for CSPG4-specific mAb. Although the molecular mechanisms responsible for the preferential expression of CSPG4 protein in TNBC tissues are currently unclear, it is encouraging that the results demonstrate the potential function of CSPG4 protein as a diagnostic biomarker and immunotherapeutic target for TNBC.

3.4.3. Melanoma-associated antigen-3 (MAGE-3)

MAGE-3, expressed by 40% of TNBC patients, has been verified as a tumor vaccine. MAGEA-3-associated tumor vaccines targeting lung cancer and melanoma have already entered phase II and phase III clinical trials, and their therapeutic benefit and safety have been confirmed [17]. Thus, MAGEA-3-associated tumor vaccines might be effective immunotherapeutic approaches for TNBC patients that express MAGE-3.

3.4.4. α-Lactalbumin antigen

α-Lactalbumin is a breast-specific protein that is present in the breasts of females during late pregnancy and lactation, but not in aging females. However, it is expressed in many tissues of TNBC patients and induces anti-cancer immunity. The stable expression of α-lactalbumin genes were detected in early and late stage TNBC patients. According to a previous study, healthy, cancer-free, and adult females can mount abundant proinflammatory T cell responses to α-lactalbumin. A history of breastfeeding and lactation had no effect on α-lactalbumin-induced immunity and on the preservation of immunity against the progression of breast cancer. Regarding therapy and the primary immunoprevention of TNBC, α-lactalbumin vaccination might be efficient. TNBC is the most aggressive type of breast cancer and is dominant in women with a high genetic risk related to mutations in BRCA1 genes [13]; therefore, α-lactalbumin might be a novel immunotherapeutic target for TNBC vaccines.

3.4.5. Mesothelin

Mesothelin, a cell-surface glycoprotein, is overexpressed in the majority of TNBC cases (67%) but only rarely (< 5%) in ER (+) or Her2-neu (+) breast cancers [50]. This suggests that a range of therapeutic options may be available for women with TNBC. Genetically-modified T-cells expressing a chimeric-antibody receptor (CAR) specific for mesothelin (mesoCAR T cells) had a higher anti-cancer cytotoxicity for mesothelin expressing primary breast cancer cells compared with non-transduced T-cells (31.7% vs 8.7%, $p < 0.001$), indicating mesothelins might be a novel immunotherapeutic target for TNBC.

4. Passive immunotherapies of TNBC

4.1. CAR-T treatment

Chimeric antigen receptor (CAR) T cell therapy uses genetic engineering technology to modify T cells to recognize and kill tumor cells via a chimeric antibody specific for the tumor cells. Song et al. constructed an FRα specific chimeric antigen receptor and inserted the gene encoding this receptor into T lymphocytes. When these T cells were amplified and purified *in vitro* they killed TNBC cells both *in vivo* and *in vivo*, and inhibited the growth and invasion of tumor cells [16]. Han et al. used the chimeric antigen receptor (CAR) approach to target NKG2DLs expressed on human TNBCs. Lentiviral vectors were used to express the extracellular domain of human NKG2D that binds various NKG2DLs, which was fused to signaling domains derived from T cell receptor CD3 zeta alone or with CD27 or the 4-1BB (CD137) co-stimulatory domain. They demonstrated that CD27 or 4-1BB co-stimulated, self-enriched NKG2D CAR-redirected T cells mediated anti-tumor activity against TNBC tumors, and therefore represent a promising immunotherapeutic approach to TNBC treatment [51]. Table 5 summarizes recent studies of CAR-T immunotherapy for TNBC. It should be noted that CAR-T therapy will only work in patients who have an intact immune system. In patients with breast cancer receiving the standard cytotoxic chemotherapy, immunosuppression is inevitable, and all leukocytes and subsets will be low in the peripheral blood, so CAR-T therapy would need to occur at a time when patients are not immunosuppressed. In addition, although many patients will achieve a complete response following the CAR-T treatment, the broad applicability of this therapy is hampered by severe cytokine release syndrome (CRS) and neurotoxicity. CRS is usually characterized by respiratory insufficiency, hypotension, and fever associated with elevated serum cytokines, including interleukin-6 (IL-6). CRS and neurotoxicity often occur within several days of T cell infusion at the peak of CAR-T-cell expansions. These adverse reactions might respond to IL-6 receptor-blockade but can require further treatments with corticosteroids (high dose) for curbing potential lethal severities [52,53].

4.2. CIK treatment

Cytokine-induced killer (CIK) is a heterogeneous cell type with numerous phenotypes that kills tumor cells. It has strong proliferative ability, cytotoxic effects, and specific immune characteristics. Pan et al. carried out a study of 90 patients with TNBC after surgical excision, and showed that overall survival (OS) and disease-free survival (DFS) were significantly increased in 45 TNBC patients treated with CIK. Moreover, multivariate survival analyses showed that CIK assisted therapy was an independent prognostic factor for OS in TNBC patients. The sub-group analysis showed that the extension of OS and DFS was more prominent in patients with stage III TNBC [54]. Wang et al. provided two cycles of chemotherapy consisting of anthracycline and taxanes in 23 young TNBC patients. Then, 50 mg cyclophosphamide was maintained every day, and 3 DC-CIK extracts were given during the chemotherapy. Some patients had an immune response, and the median OS and PFS was 15.2 months and 13.5 months, respectively. No treatment related deaths were noted during the treatment [55]. These above mentioned studies

demonstrate that adjuvant chemotherapy with CIK can prevent TNBC recurrence and prolong the survival of these patients.

5. Oncolytic immunotherapy (chimeric parapoxvirus CF189)

Oncolytic virus, which has a unique property to selectively infect tumor cells, causes the oncolysis of cancer cells with minimal toxicity to healthy tissues. Oncolytic virus functions as a biological agent by inducing local immunological responses, tumor cell lysis, and enhancing systemic anti-tumor immunity. Talimogene laherparepvec (T-Vec) is the first live virus to be approved by the US Food and Drug Administration for the treatment of cancer. T-Vec preferentially infects and lyses tumor cells and, in some cases, induces a systemic immune response against the tumor [56]. For TNBC, the chimeric parapoxvirus CF189 is an oncolytic virus that is effective. Choi et al. reported that Chimeric parapoxvirus (CF189) induced efficient *in vitro* cytotoxicity in four TNBC cell lines of nonmetastatic and metastatic origin, and had *in vivo* anti-tumor effects at doses as low as 103 PFU [57]. This evidence is encouraging for the clinical development for these extremely effective agents against TNBC. The immune stimulatory properties of oncolytic virus suggest it might be a novel treatment for TNBC patients.

6. The prospect of immunotherapy for TNBC

Currently, breast cancer is the most common malignant tumor in women, with devastating effects on health. Because of the special phenotype of TNBC, endocrine therapy is ineffective, and there is lack of drugs for its targeted therapy. Considering the limitations of traditional treatment methods, immunotherapy might improve the survival rate and prognosis of TNBC patients based on its high specificity and immune memory, hopefully providing a new turning point for the treatment of TNBC. At present, there have been few studies on immunotherapy for TNBC patients. Most studies are still at the basic research stage, and many problems remain to be solved. Researchers need to further explore and carry out clinical validation studies. Nevertheless, we are convinced that immunotherapy will create a new era for TNBC treatment on the basis of basic research, the reform of science and technology, the discovery and screening of highly specific antigens isolated from tumors, and combined immunotherapy. This will also be an era of “individualized medicine” and “precision medicine”.

Conflicts of interest statement

I would like to declare on behalf of my co-authors that we have no conflicts of interest.

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