Chapter

Integrating Immunotherapy with Chemotherapy: A New Approach to Drug Repurposing

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Abstract

Triple negative breast cancer (TNBC) is an aggressive breast cancer subtype lacking the three hormonal receptors namely estrogen receptor, progesterone receptor and HER2 receptor, and the only treatment option available for TNBC is chemotherapy. Chemotherapy lacks specificity since it acts on normal healthy cells as well resulting into secondary diseases in TNBC patients. In addition chemotherapy poses recurrence and relapse issues due to the development of chemoresistance among TNBC patients. Immunotherapy remarkably immune checkpoint inhibitors show a great therapeutic potential in TNBC. As TNBC contain an increased TILs (tumor infiltrating lymphocytes) infiltration making it more suitable as a therapeutic target anti-tumor immune strategy. Moreover, evidences have indicated that chemotherapy upregulates the anti-tumor immune response in TNBC. As a result, a combination of immunotherapy with chemotherapy may increase the overall relapse and recurrence free survival of TNBC patients. Therefore, in this chapter we will focus on how the immunotherapy works in TNBC, their effects and consequences. We will further be discussing the clinical studies and the importance of immune checkpoint inhibitors (ICIs) in combination with various therapeutic agents and target. Further, we will explore the processes involved.

Keywords: TNBC, PD-1, immunotherapy, immune checkpoints, immune checkpoint inhibitors, epigenetics, CTLA-4, oncolytic virus

1. Introduction

Triple negative breast Cancer (TNBC), is an aggressive breast cancer subtype characterized by the lack of hormone receptors; estrogen receptor, progesterone receptor and HER2 receptor accounting for about 15–20% of all breast cancers, with chemotherapy available as the prime systemic therapy. The treatment results into low median overall survival with earlier recurrence and metastasis posing to be a great hurdle in the control of this disease [1]. Therefore, improved therapies are urgently needed. Immunotherapy has prolonged survival in other solid tumors and represents a promising treatment strategy for TNBC (**Figure 1**). In the recent days, targeting immune checkpoint inhibitors are noted immunotherapeutic agents that are known to block immunosuppressive receptors like PD-1 (anti-programmed

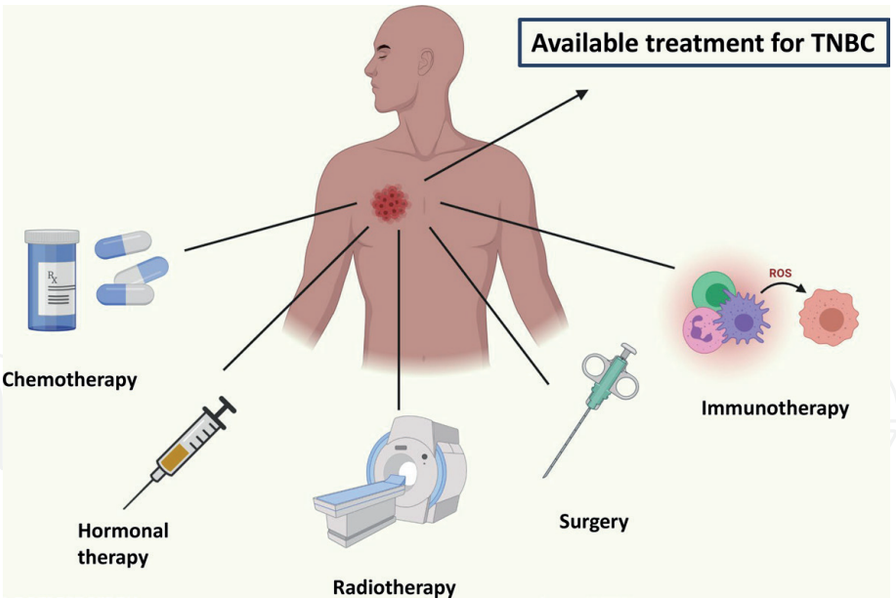

Figure 1.
Represents the available treatments for TNBC (triple negative breast cancer).

death receptor-1) and CTLA-4 (cytotoxic T lymphocyte antigen-4), which are significantly involved in tumor directed immune responses [2]. Moreover, several characteristics of TNBC make immunotherapy to be corner stone of the modern therapeutic regimens such as the presence of TILs (Tumor infiltrating Lymphocytes (TILs). The TILs are associated with better therapeutic responses increasing the disease free survival and overall prognosis in TNBC in comparison to other breast cancer subtypes. The presence of TILs as well acts as predictive biomarkers for immunotherapy response that makes immunotherapy more intriguing for TNBC treatment [3–5]. Besides, TNBC are known to possess higher PD-L1 expression levels on both tumoral and immune cells that are likely to respond to the immune checkpoint inhibitors (ICIs) such as pembrolizumab, nivolumab (monoclonal antibodies against PD-1), Ipilimumab (antibody against CTLA-4) and Atezolizumab, Avelumab (antibody against PD-L1) [2, 6, 7]. In addition, the presence of significant number of non-synonymous mutations in TNBC generate neo-antigens specific to tumors that activate robust anti-tumor immune responses that can be synergistically utilized by the current immunotherapeutic agents like ICIs [8–10]. Nevertheless, the presence of higher levels of BRCA1 and BRCA2 mutations giving rise to unstable genetics acts as a significant predictive marker for immunotherapy response [11].

The immune system plays a dual role in a way that it not only is involved in tumor initiation and progression but also acts significantly in the recognition and destruction of cancer cells. The later generates a tumor-directed immune response involving cytotoxic T lymphocytes [12, 13]. For cancer progression the tumors are known to evade the anti-tumor immune response by certain array of mechanisms like activation of pro-tumor-polarized innate inflammatory cells, activation of humoral immunity, suppression of tumor-specific antigens, infiltration by Th2 T cells, absence of major histocompatibililty complexes (MHC) on tumor cell surface and negative immune checkpoint inhibitor expression by tumor cells [13, 14]. These mechanisms followed by tumor cells to evade immune responses are known as hallmarks of cancers as these work in concordance to suppress the anti-tumor response and promote cancer progression. Therefore, in order to bring cancer control strategies targeting these specific mechanisms are utilized in immunotherapy to bring in control the tumor progression (**Figure 2**) [15].

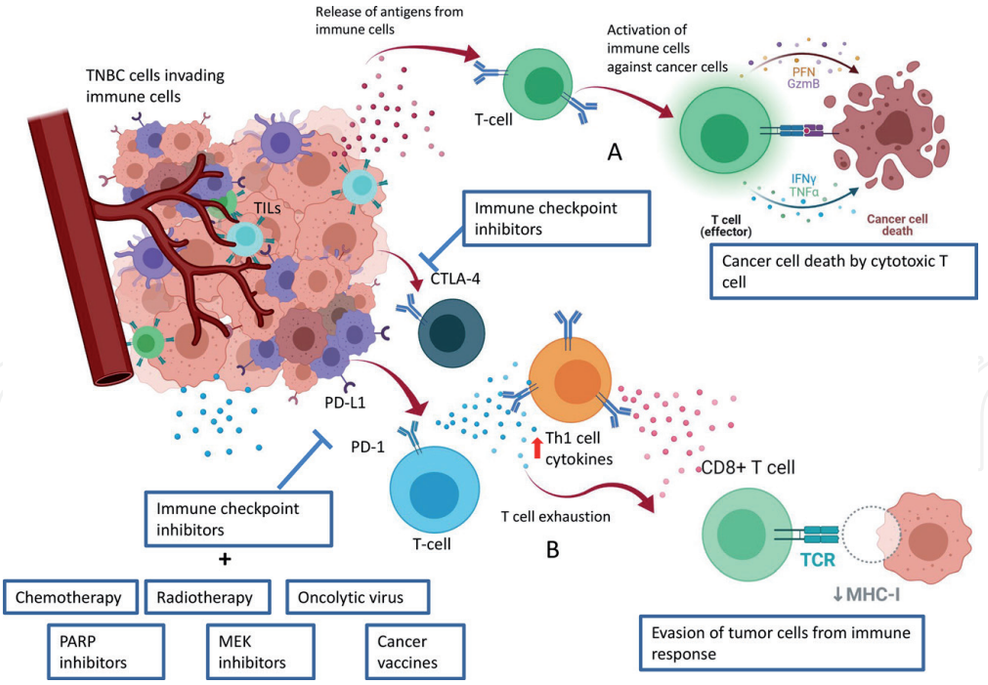

Figure 2.
Overview of involvement of immune system in TNBC with combination treatment options; A. Represents on recognition of the antigen from the tumor cell the immune cell destroying the tumor cell B. Shows that how PD-L1 from the tumor cell interacts with PD-1 and this binding causes T cell exhaustion and helps the tumor cell evading the immune response.

Therefore, actively manipulating the immune system for TNBC treatment represents to be an attractive strategy as this particular breast cancer subtype has lacked in terms of extensive clinical management. In view of that, immune checkpoint inhibitors (ICIs) has revealed promising results in TNBC patients by substantial improvement in TNBC patients overall prognosis. However, the focus of this field is to recognize the immunogenic identity of patients for the clinical management of patients and in specific to identify specific therapeutic agents to target tumor microenvironment [14]. Nevertheless, the utilization of current therapeutics like chemotherapy, radiotherapy in combination with immunotherapy will augment the immunotherapeutic response as they enhance tumors mutational load, downregulate immune suppression by tumor microenvironment and boost antigen presentation by tumor cells, henceforth making tumors more prone to immunotherapy (**Figure 2**) [16–18]. Interestingly, many clinical trials are underway and some have revealed that combination of immunotherapy with other therapeutic agents besides chemotherapy and radiotherapy has enhanced the patient responses in terms of progression free survival and standard of care [19, 20].

2. Role of immunotherapy in TNBC

The immune system is known to kill tumor cells by a process called immunosurveillance in which the immune cells target and kill the tumor cells by two ways; either directly or indirectly by releasing soluble chemicals. The cells involved are cytotoxic T lymphocytes (CTL), dendritic cells (DC), macrophages, Natural killer cells (NK) etc. As described earlier, the cancer cells are known to evade the host's immune responses in that the host's immune system identify the tumor cells as self due to which the tumor cell is favored to escape, grow, proliferate and metastasize to distant organs. Furthermore, as the tumor develops, they modify the immune cells for their own benefit like they modify TAMs and recruit them to the tumor

microenvironment to release chemicals that suppress the immune system further enhancing the suitable environment for the tumor cells to survive and proliferate [21]. Therefore, targeting this strategy of immune evasion by cancer cells i.e. modulating the immune system is imperative for the development of therapeutics against tumors. In addition, the currently available treatment options like chemotherapy, radiotherapy are known to be ineffective because of the induction of relapse and recurrence, development of resistance, lack of specificity in addition to side effects and toxicity that leads to tumor development and metastasis in secondary sites. In view of this, immunotherapy is considered to be the most reliable therapeutic approach in terms of target specificity by targeting different immune cells, their functional attributes to block the development and spread of aggressive tumors and as a non-toxic anti-cancer therapeutic strategy. Moreover, immunotherapy has emerged as the fourth most important treatment for cancer after surgery, chemotherapy and radiotherapy and has shown effective treatment responses among patients (**Figure 2**) [22].

Recently immunotherapy was developed as an effective treatment strategy against cancers with a goal to design therapeutics that can effectively enhance the immune system in terms of its specificity and strength its response towards the evading tumors [23]. In the year 2018, James P. Allison and Tasuku Honjo won the Nobel Prize in Physiology and Medicine for discovering a treatment for cancer by downregulating the negative immunomodulation. In their study, they demonstrated that the immune checkpoints like PD-1 (programmed cell death protein1) and CTLA-4 (cytotoxic T lymphocytes associated protein 4) act as "brake" in immune system as they may reactivate T cells by immune checkpoint inhibition, hence eliciting an improved immune response against malignant tumors [24]. The significance of immune checkpoint inhibitors as potential therapeutics has proven in various studies. Many studies have revealed that PD-1 inhibition promotes effective immune responses against cancers [25]. Accumulating studies on PD-1 signaling suppression has revealed that the patient's clinical response to immunotherapy depends upon the effectiveness of T-cells to penetrate the tumor [26]. In the past decade many immune system components have been explored as adoptive immunotherapies like cytotoxic T cells, TILs, anti-CD3 monoclonal antibody-induced killer cells and activated killer cells but they showed less efficiency as therapeutics because of their low anti-tumor functions [27]. However, an *in-vitro* study has suggested the cytokine-induced killer (CIK) cells to a promising target for utilization as immunotherapeutic target because of its higher proliferation rate, hence more effectiveness towards eradicating cancer [21]. CIKs contribute to sturdy cytolytic activities towards tumors as these are non-major Histocompatibility complex- restricted cells that can express both natural killer cell and T cell markers such as CD56 and CD3 [28]. Furthermore, CIKs are known to improve the immune response in patients by regulating and therefore, increase the efficacy of immune function [29]. However, study of CIK cell therapy in breast cancer, particularly in TNBC has been limited. Despite that evidences have reported that the association of CIKs with chemotherapy may result in synergistic effects, supported by an *in-vitro* and *in-vivo* study against cancer stem cells that were resistant to chemotherapy. Therefore, strongly suggests that combined therapy might improve therapeutic efficacy in patients having TNBC, as chemotherapy has shown to regulate the patient's immune status [30].

3. Immune checkpoints in immunotherapy

Immune checkpoints comprise of a collection of different regulatory proteins in the adaptive system that regulate the immune system functions i.e. anti-tumor

activity and self-tolerance. They are known to function by coordinating the frequency, magnitude and type of immune response either via positive or negative regulation. There are mainly two immune checkpoint s studied namely PD-1/PD-L1 and CTLA-4, as their presence in the TME prevents to elicit an anti-tumor response via negative regulators of immune activation [31].

3.1 PD-1

PD-1 also known as CD279 was first discovered in the year 1992 [32]. It is a 55 kDa transmembrane protein comprising of 288 amino acids with an extracellular N-terminal domain, a cytoplasmic tail at each N and C end, a transmembrane domain respectively with two tyrosine bases [33]. PD-1 are expressed on a number of immune cells like macrophages, B lymphocytes, activated T cells, Dendritic cells, natural killer cells, activated T cells and monocytes. However, they are highly expressed on specific T-cells. PD-1 is known to act as an inhibitor of both innate and adaptive immune responses [34]. It is supposed its transcription is triggered by many transcription factors such as NOTCH, nuclear factor of activated T cells (NFAT), Interferon (IFN), Forkhead box protein (FOXO1) and interferon regulatory factor 9 (IRF9) [35]. PD-1 expression is highly increased during acute infection and also when there happens to be leakage from cancer cells. PD-1 function in both beneficial and harmful manner to the immune system as it plays a significant role in maintaining immune tolerance by regulation of the harmful and inefficient immune responses while also interfering with the classical protective role of immune system by negative regulation [36–38]. A higher PD-1 expression has been seen in TNBC patients in comparison to non-TNBCs and has been associated with larger tumors, higher histological grades, increased TILs etc. [39].

3.2 PD-L1

PD-L1 is a ligand to PD-1. It belongs to the B7 series and is also known as B7-H1 and CD279. It is a transmembrane glycoprotein as is PD-1, containing

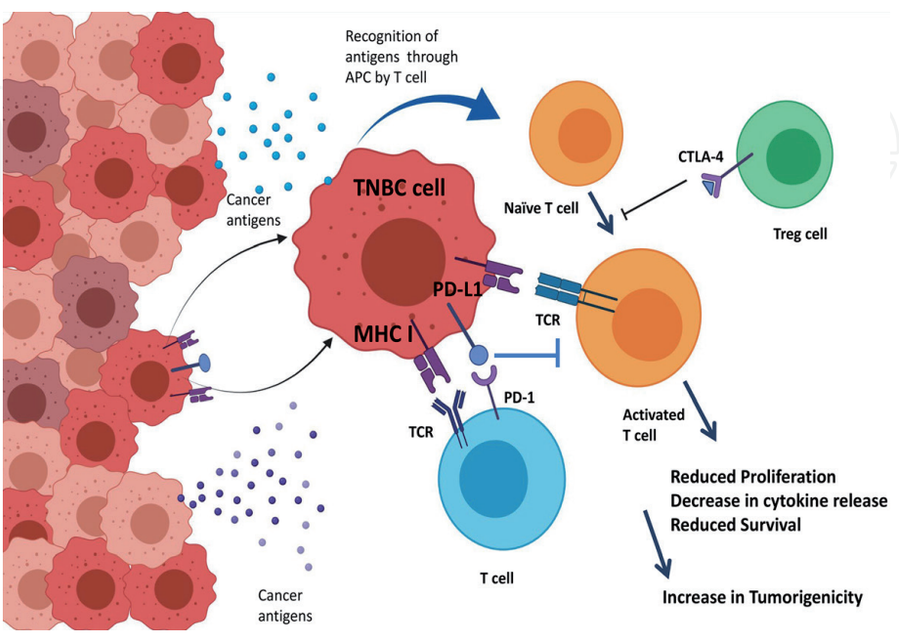

Figure 3.
Represents PD-1 mediated T cell inhibition. The binding of PD-L1 expressed on tumor cells binds to its receptor PD-1 on T cells delivering an inhibitory signal to T cells that lead to T cell exhaustion and ineffective T cells.

Similarities	Difference
Expressed by activated T cells	CTLA-4 limits T-cell responses early in an immune response, primarily in lymphoid tissues; PD-1 limits T-cell responses later in an immune response, primarily in peripheral tissues
Regulate an overlapping set of intracellular T-cell signaling proteins	CTLA-4 affects Tregs functioning; the role of PD-1 on Tregs is unclear
Level of expression affected by the strength and duration of TCR signaling	CTLA-4 is expressed by T-cells; PD-1 is expressed by T cells and other immune cells
B7 receptor family members	CTLA-4 ligands are expressed by professional APCs; PD-1 ligands are expressed by APCs and other immune cells, and can be inducibly expressed on non-immune cells, including tumor cells
Reduce T-cell proliferation, glucose metabolism, cytokine production and survival	PD-1 engagement interferes with more T-cell signaling pathways than does CTLA-4 engagement

Adapted from [40].

Table 1.
Comparison of immune checkpoints CTLA-4 and PD-1.

290 amino acids with IgC domains in its extracellular portion. The cells that express PD-L1 include: activated B and T cells, epithelial cells, macrophages and dendritic cells, particularly at the time of inflammatory responses. The PD-L1 expression is connected with the production of Th1 cytokines, presence of CD8 T cells, interferon, other chemical factors as well as expression of specific genes i.e. all these are responsible for the over expression of PD-L1 and further malignant disease progression, which we will be discussing later in the chapter. Therefore, inhibiting the particular pathways for instance, on activation the NK and T cells secrete interferon-gamma that induces PD-L1 expression on the cells including tumor cells etc. has been shown to promote strong antitumor responses among patients.

The PD-L1 is utilized by the opportunistic tumor cells to evade immune response by mimicking the "Adaptive immune process". Furthermore, PD-L1 is known to act as a pro-tumorigenic factor activating survival and proliferating signaling pathways by receptor binding, hence implicating its greater role in tumor proliferation and metastasis (**Figure 3**). In addition, PD-L1 also acts in a non- immune pattern by inducing epithelial to mesenchymal transition exerting in the tumor cells stem cell like characteristics promoting metastasis and disease progression **Table 1**[41].

3.3 CTLA-4

CTLA-4 is a member of the CD28 family and is considered to be the "leader" of the immune checkpoint inhibitors as it potentially stops autoreactive T cells in the lymph nodes at the initial stages of development [42, 43]. It is the first immune checkpoint discovered among other immune checkpoints. It is a transmembrane receptor of T cells and it is a leukocyte differentiation antigen that regulates the immune process by negative regulation by competing and binding to the B7 receptor, as it is a CD28 homolog [40]. CTLA-4 plays a significant role to prevent self-reactive immune responses particularly by increasing immunosuppressive Treg. Activity and downregulation of the T effector cell function [14].

4. Possible mechanism of action of anti-programme death receptor-1/ Ligand-1 (PD-1/PD-L1) in cancer

PD-1/PD-L1 is known to control the induction and maintenance of immune tolerance within the tumor microenvironment. It performs a significant role in cytotoxic secretion and T cell activation and proliferation in cancer to inhibit anti-tumor immune responses in host [41]. During tumor proliferation, the PD-L1 is highly expressed on tumor cells that bind to the PD-1 receptor on T cells that receive an inhibitory signal from the PD-L1 binding i.e. to inhibit the T cell's immune function that leads to T cell exhaustion making T cells ineffective (**Figure 3**).

However, monoclonal antibodies that target PD-1 and PD-L1 are being studied and used as these pathways are majorly taken by tumor cells to proliferate in host's body that are known to typically regulate activity of T cells for their own benefit that is to evade the immune responses generated against them. Accumulating evidences has suggested that by inhibiting the binding of PD-L1 to PD-1, the anti-tumor response is made stronger as the T cell exhaustion is reversed. Therefore, in view of that several monoclonal antibodies are being studied, particularly in TNBC like Atezolizumab, Avelumab and Durvalumab that specifically target PD-L1 and others such as Pembrolizumab and Nivolumab specific to target PD-1 [31].

5. Possible mechanism of action of cytotoxic T lymphocytes (CTLA-4) in cancer

CTLA-4 (Cytotoxic T lymphocyte-associated protein-4), is another regulatory pathway of T cells. During T cell activation CTLA-4 is highly upregulated. Upon T cell activation, the CTLA-4 is translocated from the intracellular granules to the plasma membrane that further amplifies the T-cell response by regulating T-cell priming and activation. It inhibits the intracellular T cell activation signaling by competitive binding for CD80/CD86 that results in downregulation of immune response. Moreover, it acts through protein tyrosine phosphates 6 and 11 to suppress

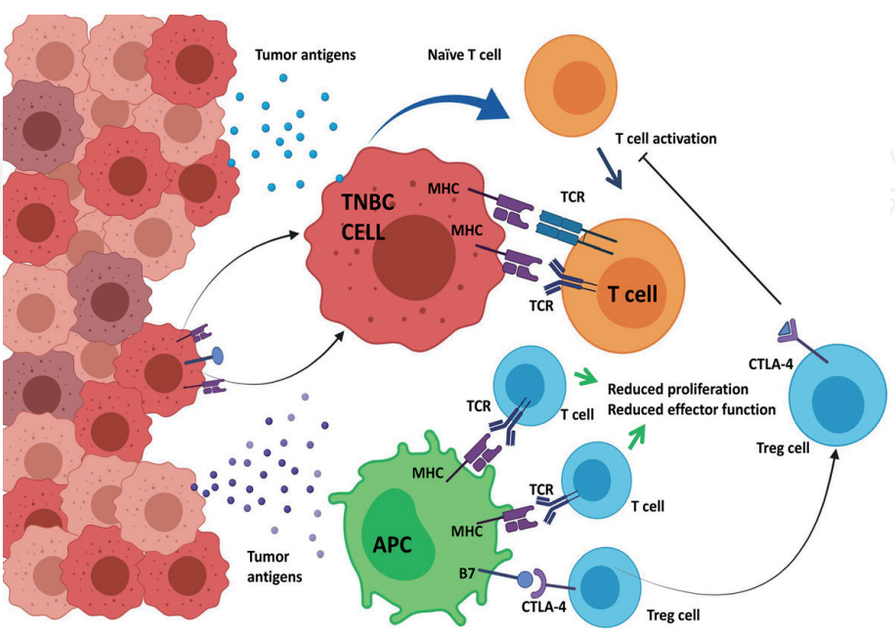

Figure 4.
Shows CTLA-4 inhibits T cell activation thereby regulating the immune responses. Therefore, tumor cells escape immune response by suppressing CTLA-4.

the TCR signal. CTLA-4 plays an important role in regulating peripheral tolerance that is an immunological process to prevent auto-immune responses by suppressing T effector cell function and further by upregulating the immunosuppressive Treg activity. Tregs express CTLA-4 constitutively unlike effector cells thereby acting as a major mechanism for immune suppression (**Figure 4**) [14].

Therefore, many monoclonal antibodies are currently being studied for instance; Tremelimumab specific for target CTLA-4 is being investigated in patients with TNBC. Limited research is available regarding CTLA-4, eagerly awaiting the need for research in discovering treatment options and other potential targets in TNBC treatment [31]. By inhibiting the CTLA-4 mediated response or the blockade of CTLA-4 results into the activation of non-specific immune cell activation and is connected with increased treatment-related adverse events (TRAEs). For instance, CTLA-4 depletion has results into rheumatoid arthritis, type I diabetes, collagen induced arthritis and systemic lupus erythematosus [14].

6. Other immune checkpoints under investigation

Apart from the above two immune checkpoints, a variety of other immune stimulatory and suppressive checkpoints are currently under investigation as immunotherapy targets that include; TIM-3, LAG-3, TIGIT, VISTA and BTLA-4, these reduce the anti-tumor immune response by regulating T cell activity like CTLA-4 and PD-1. Among them TIM 3, BTLA-4 and LAG-3 are implicated as T cell exhaustion markers in tumors same as that of PD-1. TIM 3 negatively regulates the cytotoxic CD8 T cells and Th1 CD4 T cells, thereby shifting the immune responses. TIGIT is expressed by a number of cells such as: T cells, Treg cells and NK cells

Immune checkpoints	Function
Immunoinhibitory checkpoints	
PD-1	Regulates T cell activation by binding to its ligand PD-L1 and PD-L2
CTLA-4	Acts by competitive binding with the receptors and prevent the co-stimulatory signal thereby balancing the stimulatory signals of the host immune response
TIM-3	Shifts the immune response by negatively regulation of Th1 CD4 T cells and cytotoxic CD8 T cells
TIGIT	TIGIT on T cells binds with the poliovirus receptor on the APCs and act as competitive antagonist to CD226 have suppressive effects
VISTA	Expressed by both APCs and T cells plays a role in both Treg function and myeloid cell activation
Immunostimulatory checkpoints	
ICOS	It is a member of the CD28 family. It provides the second signal in immune activation by binding with B7H/B7RP-1.
CD40L	CD40L interacts with CD40 receptor on T cells and function by promoting a proinflammatory immune response
OX40	OX40 downregulates Treg function by binding with the ligand OX40L. It also induces the expression of pro-apoptotic proteins like BCL-2 and BCL-XL

Table 2.
Immune checkpoints in immunotherapy.

and is known to bind to poliovirus receptor on APCs or tumor cells. It is supposed to perform both direct and indirect immunosuppressive effects by competitively binding to NK and T cell receptors in place of CD226; it also leads to downstream inhibition of AKT signaling in T cells [14, 44]. In addition, TIGIT increases the suppressive activity and releases inhibitory cytokines by receptor binding of TIGIT on the APCs and Tregs [45]. Moreover, VISTA is expressed by both APCs and T cells play a role in both Treg function and myeloid cell activation [14].

On the contrary, other checkpoints like OX40, ICOS and CD40L are immunostimulatory checkpoints that function in the maintenance and activation of effector T cells. The expression of OX40 is induced at the time of T-cell activation leading to the expression of anti-apoptotic factors such as BCL-2 and BCL-XL that leads to the sustenance of T cells proliferation. It also acts as a co-stimulatory signal in tumor induction and is constitutively expressed on Tregs and OX40 also decreases the Treg function by binding to its receptor on Treg [46–48]. Furthermore, ICOS leads to the activation of second signal in immune activation by binding to B7H/B7RP-1 [49]. Another immunostimulatory checkpoint CD40L interacts with CD40 on APCs induces via NF-ƙB signaling a proinflammatory immune response **Table 2** [14].

The immunostimulatory checkpoints can be inhibited by therapeutic agents targeting recombinant ligand peptides, ligands expressing viral particles or agonistic monoclonal antibody that is in contrast, to the inhibitory checkpoints where monoclonal antibodies inhibit the interaction between the respective ligand and receptors. Therefore, there is an emerging need to fully explore these biomarkers for better prognosis of patients using immunotherapy strategy [14].

7. Biomarkers in immunotherapy

Biomarkers are of significant importance in view that it predicts the clinical benefit to immunotherapy. Therefore, there is a need to bring into light several biomarkers in TNBC to distinguish that which patients is likely to get benefited from the ICIs or to build up certain therapies to overcome the hindrance in treating the respective malignancy. Until now PD-L1 was considered to be the major biomarker in TNBC. However, recent studies depicted that most of the mTNBC patients are PD-L1 negative arising the need to prospect into the immunotherapy field to find other novel biomarkers to get an insight into the patient responses to immunotherapy as a monotherapy or as a combinational therapy [50, 51]. Some of the biomarkers studied so far in TNBC include: TILs, TMB (tumor mutational burden), Gene signatures.

8. Tumor infiltrating lymphocytes as biomarkers (TILs)

Tumor infiltrating lymphocytes (TILs) have a predominant role in breast cancer as predictive and prognostic biomarker. It is present intratumorally and in adjacent stromal tissues. The increased presence of TILs in Breast cancer is associated with improved prognosis and overall survival in response to neoadjuvant chemotherapy [52, 53]. In a recent study higher number of TILs was in TNBC as compared to other breast cancer subtypes, therefore is associated with the possibility to show better responses to neoadjuvant and adjuvant chemotherapy with relapse free survival [54, 55]. The connection of TILs with anti-tumor immune response in TNBC patients also serves as a predictive biomarker, thereby making examination of immunotherapy in TNBC more interesting [14]. Furthermore, clinical trial KEYNOTE-173 trial investigating pembrolizumab in combination with chemotherapy has shown

promising results in the neoadjuvant setting of TNBC, as this trial demonstrated the presence of higher levels of TILs and higher PD-L1 expression resulting in a high combination score with increased overall response rates in TNBC patients [56].

9. Tumor mutational burden (TMB) as TNBC biomarker

TMB is defined as the measurement of non-synonymous mutations present in tumor cells. Here mutations lead to enhanced expression of neoantigens in terms of MHC I class antigens thereby increasing the visibility of cancer cells to T cells. However, limited data for TMB is reported while the frequencies of TMBs are found to be significantly higher in TNBC comparative to the other breast cancer sub types [57]. Therefore, the presence of TMB is linked with immunogenicity in several tumor types [58]. A recent study revealed no significant difference for breast cancer patients pre-treated with ICIs (immune checkpoint inhibitors) in survival. Therefore, it is assumed that TMBs alone are not supposed to represent a sole predictor as biomarker evoking the need to enrich the available information regarding TNBC biomarkers [58, 59].

10. Gene signatures as biomarkers in TNBC

A number of multiple gene signatures in correlation with TILs have been studied as surrogates of breast cancer immunogenicity. According to immune-related gene expression profiling breast cancer consisted of four categories namely ICR1–4 (immunologic constants of rejection) and these were seen to be associated with survival in a retrospective manner using in-silico analysis. Interestingly, the ICR4 (Th1 helper phenotype) was linked with the upregulation of transcripts like PD-L1, IDO1, PD-1, FOXP3 and CTLA-4 that indicated a better survival among patients, in contrast a negative regulation was showed in disruptions induced by the presence of MAPK components linked with the ICR1, an unfavorable-immune response. A study on mouse models has shown an increased anti-tumor immune response in TNBC patients that was suggested to result by the inhibition of MEK, a molecule of MAPK pathway in combination with PD-1 inhibitor due to which the MHC I and PD-1 expression on Tumor cells increased resulting in apoptosis of cancer cells. Moreover, in TNBC a four gene-signature such as CXCL13, GBP1, SULT1E1 and HLF were shown to represent an upregulation of TILs and enhanced disease free survival among patients, however their predicting response with ICIs needs to be defined [58].

11. Importance of immune checkpoint inhibitors as monotherapy

Immunotherapy stimulates the immune system by active immunization with cancer vaccines or passive immunization with tumor-specific antibodies and immune modulators, such as immune-checkpoint inhibitors. Immune checkpoints are a complex group of adaptive immune system regulatory points that play roles in self-tolerance and antitumor immunity. These checkpoints regulate the immune response in either a negative or positive way, coordinating the magnitude and form of response. Immune checkpoint inhibitors (ICIs) are regarded as the emerging immunotherapy superheroes, allowing a patient's self-immune cells to destroy tumors and remodeling cancer treatment in a board spectrum of cancers. The use of immune checkpoint inhibitors against programmed death receptor-1 (PD-1) or its ligand PD-L1 to treat a wide range of solid and hematologic tumors has dramatically altered the cancer treatment paradigm.

11.1 PD-1 inhibitors

PD-1, also known as CD279, is a CD28 family member expressed on lymphoid cells such as T cells, B cells, and natural killer (NK) cells, as well as on myeloid cells [60]. The binding of PD-1 on T cells with the ligands PD-L1 or PD-L2 suppresses signals downstream of T-cell receptor activation in the context of antitumor immunity [61, 62]. The monoclonal antibody that target the programmed death-1 receptor is Pembrolizumab, which is a humanized monoclonal antibody directed against PD-1.

11.2 Pembrolizumab

Pembrolizumab prevents immune-cell deactivation and inhibition by sterically blocking the interaction of PD-1 and its ligands. Pembrolizumab was the first immune checkpoint inhibitor to be approved as a first-line treatment, as well as the first PD-1-targeted therapy. Pembrolizumab a dose of 10 mg/kg was administered every two weeks to patients with previously treated, advanced TNBC in the KEYNOTE-012 trial, which showed efficacy and an adequate safety profile [63]. The overall response rate was 18.5 percent of the 27 patients who were assessed for antitumor activity, with 17.9 weeks an average response time (**Table 3**) [63]. The KEYNOTE-086 trial is presently examining the use of pembrolizumab (200 mg per 3 weeks) in metastatic TNBC (NCT02447003). Cohorts A and B were presented in an oral session at the 2017 ASCO conference [64, 65]. Cohort A comprises of patients with TNBC who had advanced on at least one systemic treatment. Among the 170-patient cohort, 4.7% responded, and 7.6% accomplished disease control for 24 weeks or longer, which included stable disease, partial response, and complete response [66]. In addition, 0.6% showed an absolute response to pembrolizumab monotherapy, and 27% had a decrease in the target lesion size after the first dose. The KEYNOTE-086 trial's Cohort B included metastatic TNBC with PD-L1+ tumors, without having received some systematic treatment previously. 23% of the 52 patients in this cohort showed an objective responses [64]. The use of pembrolizumab as a primary therapy and the inclusion of PD-L1+ tumors as a criterion for inclusion may have contributed to the increased response in cohort B, with only 58 percent of the patients admitted had a cumulative PD-L1 positive composite score of >1 (**Table 3**) [64].

11.3 PD-L1 inhibitors

The monoclonal antibodies atezolizumab and avelumab target the PD-L1, a transmembrane protein found on tumor cells. Avelumab is a fully human IgG1 MAB that binds to PD-L1, while as Atezolizumab is a humanized IgG1 isotype

Agent	Clinical trial id	Cancer type	Phase	Recruitment status
Pembrolizumab	NCT01848834	mTNBC	Ib	Completed
Pembrolizumab	NCT02447003	mTNBC	II	Completed
Atezolizumab	NCT01375842	mTNBC	I	Completed
Avelumab	NCT01772004	mTNBC	Ib	Completed
Tremelimumab	NCT02527434	mTNBC	II	Active, not recruiting

Table 3.
Main monotherapy clinical trials of immune checkpoint inhibitors in mTNBC.

monoclonal antibody that binds to PD-L1. The FDA has approved these PD-L1 inhibitors for the treatment of other solid tumors, and they are currently being explored further for the treatment of TNBC.

11.4 Atezolizumab

The first PD-L1 inhibitor to receive FDA approval was atezolizumab. An open-label, phase I dose-escalation analysis (NCT01375842) showed that Atezolizumab is safe in patients with locally advanced or metastatic solid tumors (**Table 3**). A cohort of 54 patients with mTNBC was evaluated for protection, and 21 patients were evaluated for efficacy in this study. 69% of the patients in the protection cohort had PD-L1 expression of at least ≥5%, and all of the patients in the efficacy cohort had PD-L1 expression of at least ≥5%. The ORR for this study was 19%. There were three patients who had pseudoprogression, but their tumors gradually shrink. A total of 63% of patients experienced drug-related side effects, with 11% experiencing grade 3 toxicity. Pneumonitis of grade 4 was diagnosed in one of the patients. Fatigue (15%), fever (15%), and nausea (15%) were the most common drug-related side effects [67].

11.5 Avelumab

In a Phase 1b JAVELIN trial, a human anti-PD-L1 IgG1 mab, Avelumab, was tested in patients with MBC [68] (**Table 3**). A total of 168 MBC previously treated patients were treated with avelumab monotherapy, including 58 TNBC patients. The confirmed ORR for the whole population was 3%, with 1 CR (complete response) and 4 PRs (partial responses). The ORR for TNBC patients was 5.2 percent. Furthermore, in both general population (16.7% vs. 1.6%) and in TNBC class (22.2% vs. 2.6%) patients with PD-L1 positive tumor-associated immune cells had a greater ORR than those with PD-L1 negative tumor-associated immune cells.

11.6 CTLA-4 inhibitors

CTLA-4 inhibits T-cell activation by interacting with its target ligand, CD80 or CD86 [69, 70]. Monoclonal antibodies (mAbs) that block CTLA-4 have been demonstrated to augment T-cell activation and thereby enhance cancer cell death.

11.7 Tremelimumab

A phase II open-label trial (NCT02527434) is evaluating the efficacy of Tremelimumab, a CTLA-4 inhibitor, in patients with advanced solid tumors such as TNBC (**Table 3**). While on treatment with tremelimumab, if the patient's develops progression in disease, they are given Durvalumab or a Durvalumab/Tremelimumab in combination. The objective response rate is the primary endpoint, with length of response, progression-free survival, and overall survival as secondary endpoints [71].

12. Drug repurposing an important aspect in immunotherapy regimen

Despite the success of disease diagnosis in modern era, the recent developments and discovery of new drug is laborious, inefficient, time consuming process and costly process [72, 73]. Not only that most drugs face high failure rates in clinical trials [74]. To overcome these problems in drug discovery a strategy namely drug repurposing (also called drug reprofiling or repurposing) came into existence which works by identifying existing drugs and using them for new purposes [75]. Several strategies

are being put to use in order to repurpose the existing drugs whether FDA approved or which are used under investigation. These include methods based on computational and non-computational strategies, also experimental based studies. However, the computational methods help in improved effectiveness in repurposing a drug. The computational methods help to select the effective candidate drugs before in-vitro-experiments [76]. Drug repurposing in breast cancer is considered an old weapon for new war. The immunotherapy approach in combination with chemotherapy is considered an important modality in TNBC treatment. As already discussed due to escape mechanisms in immunotherapy it is being combined with chemotherapy that repurposing the old school drugs for instance some FDA approved drugs like Anthracyclines and taxanes. Also these drugs are being repurposed to modulate the immune system response for better clinical outcome [74, 77]. For instance, cyclophosphamide that is an alkylating chemotherapeutic agent having well-built immunosuppressive activity and acts via cytotoxic or through immune enhancing mechanisms. However due to its high toxicity effects low-dose cyclophosphamide has been combined with immunotherapy options like immune checkpoint inhibitors, immune therapeutic agents including vaccines as well and it been tested and has shown better results in animal models [77]. Accordingly in this chapter we have provided a detailed account for the combination of immunotherapy with chemotherapy as an effective mechanism for drug repurposing that is using the different strategies to modulate existing drugs for efficient use.

13. Checkpoint inhibitors in combination with chemotherapy

In the process of immunotherapy, a combination with chemotherapy may be synergistic. Chemotherapy has been demonstrated to promote tumor cell antigen release, prompt class I MHC molecules, neoantigens, and expression of PD-L1, and stimulate activation of dendritic cells, which could improve the immune response release after or in the course of Immune Checkpoint Inhibitor treatment [78–80]. Combination therapies of checkpoint inhibitors and chemotherapy have showed significant results in TNBC. Pembrolizumab's safety profile and clinical efficacy have been examined in most of the analysis on inhibition of PD1 in TNBC. In highly positive PD-L1, untreated mTNBC patients who obtained pembrolizumab in conjunction with chemotherapy (PAX, nab-paclitaxel, carboplatin/gemcitabine), interim evaluation of the phase 3 KEYNOTE-355 (NCT02819518) trial shows a substantial increase in PFS (5.6 vs. 9.7 months) [81]. Pembrolizumab in combination with the microtubule inhibitor eribulin mesylate in the KEYNOTE-150/ENHANCE 1 (NCT02513472) trial showed a 25.6 percent ORR with an average progression free survival of 4.1 months [82]. The TONIC trial (NCT02499367) phase 2 analyzed the effectiveness of PD1 with nivolumab in previously treated mTNBC patients. The ORR for nivolumab treatment followed by doxorubicin was 35%, compared to 23% for CIS and 17% for patients who did not receive prior chemotherapy, implying that chemotherapy would cause an inflamed tumor microenvironment [83]. For LA or mTNBC patients treated with atezolizumab in conjunction with nab-paclitaxel, the clinical study GP28328 (NCT01633970) phase 1b showed an ORR of 39.4% and an average PFS of 5.5 months (**Table 4**) [84].

The first randomized Phase 3 trial to show the effectiveness of atezolizumab in conjunction with nab-paclitaxel in metastatic TNBC patients which were not treated previously was IMpassion130 (NCT02425891) [80]. The FDA and the European Medicines Agency (EMA) approved atezolizumab in conjunction with nab-paclitaxel as a primary trearment for PD-L1-positive, uneradicably, locally advanced, or mTNBC in 2019. The IMpassion131 trial (NCT03125902) phase 3 will

Trail id	Regimen	Disease setting	Phase	Recruitment status
NCT02819518	pembrolizumab + nab-paclitaxel or paclitaxel or gemcitabine/ carboplatin	Metastatic	III	Active, not recruiting
NCT02513472	pembrolizumab + eribulin mesylate	Metastatic	Ib	Active, not recruiting
NCT02499367	cyclophosphamide, cisplatin or doxorubicin followed by nivolumab	Metastatic	II	Active, not recruiting
NCT01633970	atezolizumab + nab-paclitaxel	Locally advanced, metastatic	I	Completed
NCT02425891	atezolizumab + nab-paclitaxel	Metastatic	III	Active, not recruiting
NCT03125902	atezolizumab + paclitaxel	Locally advanced, metastatic	III	Active, not recruiting
NCT03371017	atezolizumab + gemcitabine/ carboplatin or capecitabine	Locally advanced, metastatic	III	Recruiting
NCT02685059	neoadjuvant durvalumab + nab-paclitaxel + EC	early stage	II	Completed
NCT03281954	neoadjuvant atezolizumab + paclitaxel + carboplatin, followed by adjuvant atezolizumab + AC or EC	early stage	III	Recruiting
NCT03197935	neoadjuvant atezolizumab + nab-paclitaxel, followed by AC	early stage	III	Active, not recruiting

AC- doxorubin + cyclophosphamide; EC- epirubicin + cyclophosphamide.

Table 4.
Trials evaluating the use of immune checkpoint inhibitor in combination with chemotherapy.

assess the protection and effectiveness of atezolizumab in combination with PAX as a primary treatment in TNBC patients. The IMpassion 132 study (NCT03371017) examines the potential of previously treated, untreated, locally advanced and mTNBC patients who have not been eligible for the IMpassion130 trial may benefit from atezolizumab and chemotherapy (capecitabine, gemcitabine/carboplatin). Randomized study GeparNuevo (NCT02685059) phase 3 results demonstrated that durvalumab in conjunction with neoadjuvant chemotherapy based on taxane-anthracycline provides clinical benefits in early TNBC from 44% to 53% of pCR (pathological complete response) [85]. A neo-adjuvant chemotherapy (paclitaxel plus carboplatin) NSABP B-59 (NCT03281954) phase 3 is currently being recruited with atezolizumab, followed by atezolizumab adjuvant and chemotherapy. The Impassion031 (NCT03197935) trial, which combines atezolizumab neoadjuvent with concurrent nab-paclitaxel and chemotherapy based on anthracyclines in patients with an early stage TNBC, recently published interim results. Patients who were given atezolizumab in combination with chemotherapy had a pCR rate of 57.6%, compared to 41.1% in patients who obtained chemotherapy in combination with placebo [86].

14. Immune checkpoint blockade in combination with a targeted immunotherapy

14.1 Immune checkpoint inhibitors in combination with PARP inhibitors

Breast cancer patients with germline BRCA1 or BRCA2 mutations account for around 5% of all cases. While TNBC is the most common cancer with the mutation in BRCA1 gene, cancers linked to the BRCA2 mutation can turn up in any subtype of breast cancer with the same frequency as sporadic subtypes. Breast cancers with BRCA1/2 mutations have a deficiency in homologous recombination repair, a DNA double-strand break repair mechanism, the defect which has a lethal synergy with single-strand DNA repair inhibition [87]. The poly(ADP-ribose) polymerase (PARP) is involved in single-strand DNA repair, and PARP inhibitors have shown antitumor activity in patients with HER2-negative metastatic breast cancer who have BRCA1/2 germline mutations. The use of immune checkpoint inhibitors in combination with PARPi in TNBC patients has the ability to cause a powerful immune response against tumors due to the infiltrating T cell activation followed by tumor antigen release via PARPi-induced cell death. Moreover, PARPi has been shown to increase the expression of PD-L1 in cell lines, supplying additional support for combining treatment with checkpoint inhibitors [88].

The TOPACIO (NCT02657889) trail found that a combination of pembrolizumab with the PARPi niraparib resulted in an ORR of 29% in mTNBC patients [89]. The ORR was higher than what has been identified in similar patient populations for anti-PD1 monotherapy [64]. In addition, various clinical trials evaluating the PD-L1 inhibition combination with PARP inhibitors in mTNBC have been planned, two phase II studies included the combination of the PARPi olaparib with durvalumab (NCT03167619 and NCT03801369) and a phase II trial of atezolizumab in combination with olaparib (NCT02849496). In addition, triplet PD-L1 inhibition therapies with PARPi and VEGF inhibitors are currently being developed. A phase I/II analysis (NCT02484404) in case of progressive or recurring solid tumor is looking at the combination of durvalumab in conjunction with olaparib and cediranib the VEGFR inhibitor. According to preliminary findings, the recommended dosage was bearable and resulted in clinical benefit rate of 67% in 9 women having recurring solid tumors, TNBC was one of them (**Table 5**) [90].

Trail id	Intervention	Phase	Recruiting status
NCT02657889	pembrolizumab + niraparib	II	Active, not recruiting
NCT03167619	durvalumab + olaparib	II	Active, not recruiting
NCT03801369	durvalumab + olaparib	II	Recruiting
NCT02849496	atezolizumab + olaparib	II	Recruiting
NCT02484404	durvalumab + olaparib + VEGFRi	I/II	Recruiting
NCT02079636	Pembrolizumab+ Abemaciclib	I	Completed
NCT02322814	atezolizumab + taxanes + MEKi	II	Active, not recruiting

Table 5.
Combinations of PD1/PD-L1 antibody-targeted therapy in TNBC.

14.2 Immune checkpoint therapy and CDK4/6 (CDK4/6) inhibitors in combination therapy

In patients with ER-positive, HER2-negative metastatic breast cancers, pharmacological inhibitors of CDK4/6 have demonstrated remarkable activity [91–93]. Inhibitors of CDK4/6 have been demonstrated to improve anti-tumor immune response in preclinical models by manipulating two main immune evasion mechanisms in tumors [94–96]. First, CDK4/6 inhibitors elevate intracellular levels of double-stranded RNA by activating tumor cell expression of endogenous retroviral components. As a result, type III interferon synthesis is stimulated, which in turn improves tumor antigen presentation. Secondly, CDK4/6 inhibitors significantly reduce regulatory T-cell proliferation. Finally, these events facilitate tumor cell clearance by cytotoxic T cells, which can be intensified even further by the introduction of an immune checkpoint inhibitor. Abemaciclib in conjunction with pembrolizumab was studied in patients with HER2-, HR+, MBC in a phase I trial (JPBJ, NCT02079636). The main objective of the study was to determine the combination therapy's safety profile. A total of 28 patients were enrolled in the study. At the end of 24 weeks, four patients (14%) showed an analytical response. At the appropriate early time intervals in the MONARCH 1 analysis, this response was greater than the response shown by patients treated with abemaciclib monotherapy [97].

14.3 Combination of immune checkpoint inhibitors with MEK inhibitors

Suppression of the MAPK signaling pathway, which is frequently unregulated in TNBC and is correlated with enhanced proliferation of cells and shows resistance towards apoptosis, is another approach for combining immune checkpoint inhibitors with targeted therapy [98]. In the phase 2 COLET (NCT02322814) trial, cobimetinib the MEK1/2 inhibitor was combined with atezolizumab and PAX/nab-paclitaxel as a primary therapy in patients with LD or mTNBC. According to preliminary findings, paclitaxel in combination with nab-paclitaxel has a 34% ORR, while nab-paclitaxel has a 29% ORR [99]. Clinical studies of binimetinib the

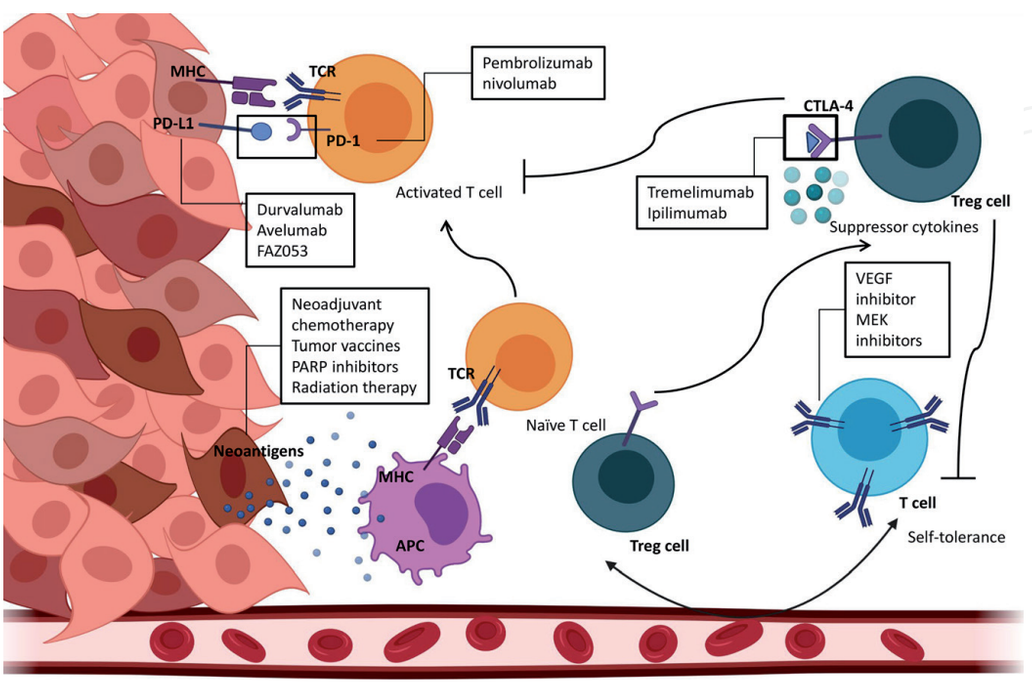

Figure 5.
Diagram representing the targets of immune checkpoint inhibitors.

Trail id	Intervention	Phase	Recruiting status
NCT03362060	pembrolizumab + PVX-410	I	Active, not recruiting
NCT02432963	pembrolizumab + p53-specific vaccine	I	Active, not recruiting
NCT03761914	pembrolizumab + WT1-specific vaccine	I/II	Recruiting
NCT03606967	durvalumab + Nab-paclitaxel+ neoantigen vaccine	II	Recruiting
NCT03199040	durvalumab + neoantigen DNA vaccine	I	Recruiting
NCT03289962	atezolizumab + neoantigen vaccine	I	Recruiting

Table 6.
Current clinical trials for cancer vaccine and immunotherapy.

MEK inhibitor in conjunction with pembrolizumab (NCT03106415) or avelumab (NCT03971409) in patients with LD or mTNBC are also underway (**Figure 5**).

14.4 Combination therapy: PD1/PD-L1 antibody and cancer vaccine

Cancer vaccines are a novel approach to cancer immunotherapy. These vaccines promote T cell priming and activation and strengthen immune recognition of cancer cells by presenting breast cancer peptides to T cells. Monovalent vaccines, which provide a single tumor-associated antigen (TAA) target for the immune system, and polyvalent peptide vaccines, which provide several TAA targets, are two types of cancer vaccines. Low response rates have hampered the application of peptide vaccines for the treatment of patients with metastatic cancer; although, making use of a multi-peptide vaccine strategy, the response rate in various cancer types has improved to 9.9%. [100, 101]. Furthermore, cancer vaccines in conjunction with immune checkpoint inhibitors can improve the vaccine's anti-tumor immune response. In advanced TNBC, a few ongoing studies are looking into the effectiveness of cancer vaccines in conjunction with pembrolizumab, making use of either the multipeptide vaccine PVX-410 (NCT03362060) or specific vaccines which target p53 (NCT02432963) or WT1 (NCT03761914). Furthermore, few clinical trials have been conducted to investigate the efficacy of durvalumab in combination with the multipeptide vaccine PVX-410 (NCT02826434) or with a neoantigen vaccine (NCT03606967, NCT03199040), as well as atezolizumab in combination with a neoantigen vaccine (NCT03289962) (**Table 6**).

15. Combining immunotherapy with epigenetics in cancer treatment

Immunotherapy arguably is one of the exciting new developments for the management of advanced human tumors, in particular the concept of immune checkpoint blockade [102–104]. Antibodies targeting PD-1, CTLA-4 and PD-L1 show robust responses in treatment of melanoma, and in high grade tumors. Although, these recent advances are very exciting and promising, however majority of the tumor patients including TNBC patients show little or no response at all to immune checkpoint therapy alone [105, 106].

Therefore raising an apparent question as to whether immunotherapy could work in combination with other therapies like immune checkpoint targeting agents to enhance the clinical response and efficiency of various sub types of cancers. Nevertheless, various clinical trials as like previously discussed are evolving while keeping in control the related toxicities [107].

Other combination strategies targeting immunotherapy in combination with chemotherapy as well targeted therapy approaches likely epigenetic therapy. As epigenetic therapy has been evidenced to strongly sensitize patients to immune checkpoint therapy.

16. Definition of epigenetic therapy

The term epigenetic therapy is now widely used, and involves use of drugs or other epigenome-influencing mechanisms for treatment of human disorders. Recent advances have delineated regulatory mechanisms of the cancer and normal epigenomes and the functional understanding of histone modifications, methylation patterns, and dynamics of nucleosomes [108, 109]. Recent studies in the field of cancer epigenetics have not only defined key targets for cancer management but also provided key insights in drug repurposing for modulating cancer epigenomes [110]. In epigenetic therapy, drugs target three specific protein categories (a) Writers, enzymes that establish epigenetic marks; (b) Readers, proteins that recognize histone and may bring in other protein complexes to change gene expression; and (c) Erasers, enzymes that remove epigenetic marks [111]. Drugs that impede writers of DNA methylation, DNA methyltransferases (DNMT), and erasers (histone deacetylases or HDAC) that regulate histone lysine acetylation are central to epigenetic therapy in cancer treatment. HDACs and DNMTs are mostly linked with transcriptional repression. Thus, inhibiting HDACs and DNMTs can upregulate expression of involved genes with many consequences for downstream pathways of this gene activation.

Cytidine analogues inhibit DNMTs by blocking their catalytic and likewise induces their degradation [112]. Also, the degradation of DNMTs can remove key scaffolding properties that may function for repression of transcription [113, 114]. Tumors show significant alterations in DNA methylation of cytosines at CpG dinucleotides such as loss of methylation at regions such as repetitive elements that must be silenced for genome stability and gain of methylation at the promoter regions of tumor suppressor and other genes [115]. Inhibitors targeting DNMTs promote reactivation of tumor suppressor, silenced by promoter DNA methylation [116]. DNA methylase inhibitors (DNMTi) showed augmented apoptosis, decreased cell cycle activity, and reduced stemness in a transient exposure to several cancer cells (**Figure 6**) [117]. DNMTis such as 5-azacytidine and 5-aza-20-deoxycytidine showed robust efficacy in treatment of hematological disorders and has been approved by FDA for the treatment of myelodysplastic syndrome (MDS) [118]. Several clinical studies are undergoing presently to study the effect of epigenetic therapy in cancer treatment **Table 7**.

Histone modifications by acetylation plays a central role in epigenetic gene regulation by altering the condensation status of chromatin, modulating the accessibility of transcription factors to target DNA sites. Histone acetyltransferases (HAT) and HDACs maintain the acetylation state of histones of nucleosomes. Inhibitors targeting HDACs known as (HDACi) are presently approved for the treatment of peripheral T-cell lymphoma (PTCL) and cutaneous T-cell lymphoma (CTCL), although it is yet to be known as why these two cancers are highly sensitive towards HDACi [119, 120]. Also, it has been observed that HDACi show dependency of, compound, dose and pleotropic characteristics. Many of the HDACi directly affect acetylation of histone proteins and modulate epigenetic changes while some affect acetylation of non-histone or cytoplasmic proteins [121]. Besides, it has been observed that transient exposure of tumor cells to low doses of DNMTs, followed by HDACi treatment increases gene expression of hypermethylated genes.

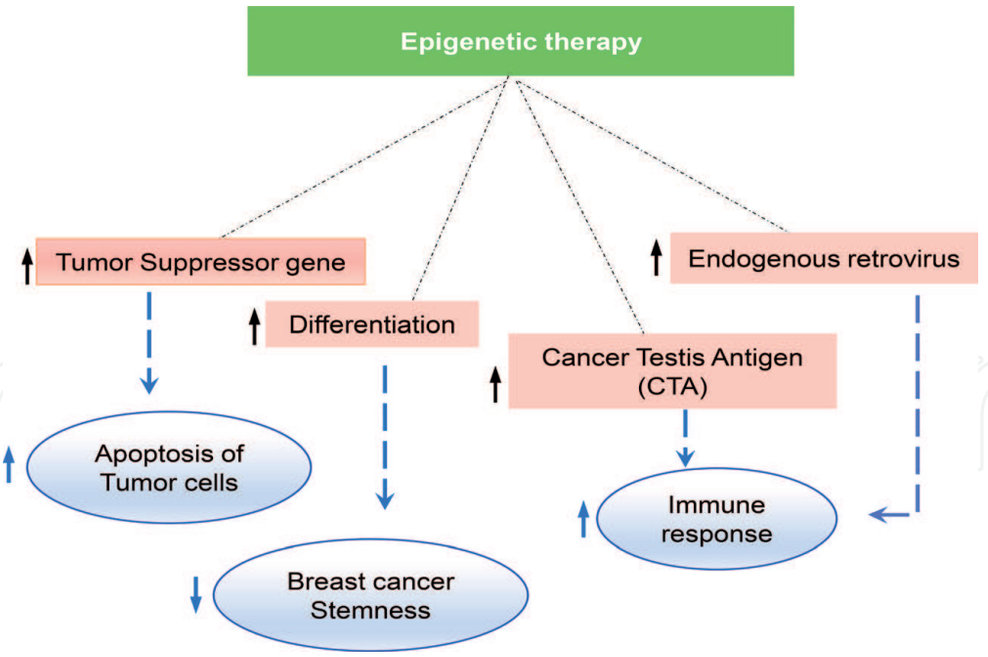

Figure 6.
Flowchart representing the overall effects of epigenetic therapy.

Epigenetic inhibitor	Target	Type of cancer
Entinostat	HDAC1/HDAC3	Recurrent or refractory solid tumors
KA2507	HDAC6	Solid tumors
Tazemetostat	EZH2	Advanced solid Tumors
AZD5153	BRD4	Advanced solid tumors and lymphomas
Triple: Entinostat, Nivolumab and Ipilimumab	HDAC/ICB	Locally advanced or metastatic HER2-negative breast cancer
Entinostat plus Pembrolizumab	HDAC/ICB	Advanced solid Tumors
CC-486 plus Durvalumab	HMA/ICB	Colorectal, ovarian, and breast tumors
CPI-1205 plus Ipilimumab	EZH2/ICB	Advanced solid tumors

Table 7.
Clinical trials for epigenetic inhibitors.

17. Connecting epigenetic modulation with immunotherapy

Over In the past two decades, the FDA approval of various DNA methyltransferase inhibitors, collectively called DNA HMAs, and histone deacetylase inhibitors (HDACi) has brought epigenetic therapy to the forefront of cancer therapies. However, the benefits of epigenetic therapy are mainly restricted to the treatment of hematological malignancies. Thus, combination strategies with standard chemotherapy and targeted therapy approaches can be considered. A recent study involving advanced NSCLC patients revealed that patients after receiving low-dose epigenetic therapy entered a trial for immune checkpoint therapy. Approximately 20% of the patients responded to the immune checkpoint therapy alone, passing 24 weeks without progression, with most achieving high-grade RECIST criteria responses [122]. This is an astounding result for immunotherapy in NSCLC. All 5

patients who had received the prior epigenetic therapy passed the 24-week point without progression with subsequent immune checkpoint therapy and three of these developed high-grade partial RECIST criteria responses that have all been durable over 2.5 years [123, 124]. Moreover, findings to date, support the hypothesis that there may be extraordinary potential for combined epigenetic and immunotherapy to increase the frequency of durable responses for immune checkpoint therapy in not only NSCLC but also other common tumor types.

18. Epigenetic therapy drugs boost immune attraction properties of epithelial cancer cells

Immunotherapy has presently become a remarkable tool to employ immune cells in tumor management. Blocking immune checkpoints to stimulate and retore immune response in the tumor immune suppressive microenvironment has showed robust clinical response. However, several patients tend to remain unresponsive towards immune checkpoints blockades. Epigenetic therapy using DNMTis and HDACis have showed potential in immune modulation properties of tumor cells and immune cells, thereby suggesting a rationale for integrating epigenetic with immunotherapy.

It is well known that cytotoxic T cells (Tc) are requisite for an anti-cancer immune response and immune check point blockade. This mechanism relies on antigen presenting cells and the quantity of antigens presented to Tc cells. Also, tumors with high mutations show robust response to immune check point blockade due to high presence of neo-antigens presented to Tc cells [125, 126]. Several studies demonstrate that high immunogenicity is followed by exposure to epigenetic therapy. DNTMis have been found to upregulate and augment expression of cancer testis antigens (CTAs) such as MAGE-A1 and NY-ESO-1 [127]. Besides, exposure to epigenetic therapy viz. HDACis and DNMTis also upregulated antigen presenting and processing related genes such as b2-microglobulin, Human leukocyte antigen (HLA)-class I genes, and TAP1 in solid tumors [128, 129]. Furthermore, it was revealed that HDAC inhibitors stimulate human endogenous retroviruses (HERVs) reactivation, which induce activation of pattern recognition receptors and a type I/III interferon response thereby enhancing antigen presentation to Tc cells [129, 130]. Together, these results paint the picture that epigenetic therapy using HDACis and DNMTis augment presentation of CTA and HERV-derived antigens, thus enhancing immune response in low mutation therapy [131]. In AML patients, epigenetic therapy with DNMTis promoted robust T cell mediated immune response by reactivation of CTAs [132]. The host immune system recognizes the CTAs with high affinity, they represent good candidates for immunotherapy, including vaccines. There is thus great potential for DNMT inhibitor treatment to upregulate CTAs on tumors, facilitating targeting by the host immune system [133]. Guo et al. demonstrated that exposure of 4T1 mammary carcinoma cells in syngeneic mice to DNMTi 5-aza-2-deoxycytidine induced demethylation and upregulation of CTA P1A. Also, the upregulated P1A was targeted by P1A–specific T cells, and combined therapy with 5- aza-20-deoxycytidine and adoptive transfer of these T cells significantly reduced lung metastases in this mouse model [134].

Additionally, synergistic relation was observed in pre-clinical models of diffuse large B cell lymphomas for combinatorial exposure to DNMTis and HDACis [135]. Increasing evidence suggests that tumors possess variable numbers of infiltrated immune cells and the quantity, type, and location of infiltration can help in predicting response to immune check point blockade [36, 136]. It is now well established that epigenetic therapy with modulates directly infiltration of immune cells in

tumor stroma. DNMTi treatment in addition to inhibiting tumor progression, increased infiltration of CD8+ T cell infiltration, and natural killer (NK) cells and reduced infiltration of immune-suppressive cells [131, 137]. Also, HDACis treatment in combination with DNMTis activates chemokine signaling networks and augments infiltration of cytotoxic T cells [138]. In preclinical studies, treatment with romidepsin, the pan-HDAC inhibitor, augmented expression of chemokines by tumor cells which elevated infiltration of T cells into the tumor stroma and reduced tumor growth by robust immune response [139].

Accumulating evidence from preclinical models of diverse solid tumors viz. breast, melanoma and colorectal cancer, revealed that combining immune check point inhibitors such as anti-CTLA4 or anti-PD1 with epigenetic therapy (DNMTis and HDACis) augmented antitumor response and reduced tumor growth and response to immunotherapy than using monotherapy of either agent [122, 136]. Also, combinational treatment with DNMTis and anti-CTLA4 antibody enhanced chemokine expression and increased survival of mice with orthotopic or subcutaneous tumors [137].

Together, these results paint the picture that combining immunotherapy with combinational therapy, greatly enhances antitumor immune responses, by augmented expression of chemokines and these act in a synergistic manner. Also, multiple clinical trials are currently testing the combination of DNMTi or HDACi with various immune check point inhibitors (**Table 7**).

19. Integrating immunotherapy with oncolytic viruses for cancer treatment

The antitumor activity of oncolytic viruses involves multiple mechanisms that encompass the natural interactions between viruses, tumor cells and the immune system [140]. During the last decade oncolytic viruses are becoming an effective means in cancer treatment. Viruses have developed sophisticated means to escape immune surveillance and which can be manipulated for therapeutic purposes to stimulate anti-cancer immune response. Likewise, nearby infusion of oncolytic virus into a tumor site can incite an abscopal impact, in which distant, uninfected tumors additionally go through insusceptible immune rejection [141]. This abscopal effect is caused by oncolytic viruses' sequential activity, multiply in cancer cells and then progresses to activation of immunogenic cell death, which results in the release of antigens and danger factors, which then enhance both innate and adaptive antitumor immune responses. Furthermore, oncolytic viruses can be genetically modified to express therapeutic genes, which can improve antitumor activity even more. In the absence of viral replication, viral encoded gene expression allows immune regulation against tumors while restricting the antiviral immune response [142]. This points out, oncolytic viruses are highly adaptable agents that offer a critical 'on' switch that enhances the migration of tumor infiltrating lymphocytes into the tumor stroma, and this can be exploited to improve antigen-specific immune responses as part of combo-immuno therapies.

20. Characteristics of oncolytic viruses

Viruses are microscopic particles that selectively replicate in the interior milieu of host cells, and inflammation and underlying pathogenicity can be associated with viral infection [143]. During the last decades, viruses have been employed in delivery of therapeutic genes for the treatment of metabolic and degenerative

illnesses, immunization against infectious diseases, and as oncolytic agents for cancer therapy [140].

The genome, which is either single-stranded or double-stranded RNA or DNA; the capsid, which is a protein coat that covers the genetic material; and the capsid, which is a protein coat that covers the genetic material, also in certain viruses, the lipidic envelope which surrounds the capsid and may enhance virus adhesion to host cell membranes, so increasing viral penetration, are the three major structural parts of most viruses. Oncolytic viruses have been developed over the last decade using both DNA and RNA viruses. DNA viruses offer several advantages: their huge genomes can be altered without interfering with viral replication; big eukaryotic transgenes may be incorporated by DNA viruses to boost therapeutic effectiveness or immunological regulation; DNA viruses express high fidelity DNA polymerases, assuring viral genome integrity and effective replication; and there is little, if any, nuclear integration of DNA viruses **Table 8** [144]. RNA viruses offer additional advantages: because they are smaller than DNA viruses, they can pass the blood–brain barrier, allowing tumors in the central nervous system to be targeted [145]. Despite the fact that their short genome restricts their capacity to encode big transgenes, because pre-existing tolerance to certain RNA viruses is poor in humans, viruses are more suited for systemic distribution, at least for the brief period before antiviral immunity is generated. Furthermore, the detection of viral double-stranded RNA by protein kinase R (PKR) that happens in normal cells may not occur in tumor cells, which often have lower levels and phosphorylation of PKR [146, 147]. Many aspects influence the selection of oncolytic viruses for tumor immunotherapy, in particular high pathogenicity, immunogenicity, cancer tropism, the potential to encode therapeutic transgenes, feasible viral concentration during synthesis, and durability. The active phase of viral infection and reproduction in host cells is described by the lytic virus life cycle [148]. Attachment, penetration and uncoating, synthesis, assembly, and release are the five different phases of the viral life cycle, which may be managed by genetic modification of the viral genome and can serve as a physiologically realistic strategy for selectively targeting tumor

	Adenovirus	**Coxsackie virus**	**Maraba virus**	**Pox virus**
Genome	dsDNA	ssRNA	ss (−) RNA	dsDNA
Genome size	Moderate (32 kb)	Small (~8 kb)	Small (11–15 kb)	Large (130–375 kb)
Cell entry mechanism	Endocytosis	Micropinocytosis via epithelial tight junctions	Endocytosis; pH dependent fusion activation	Membrane penetration and fusion
Cell entry receptors	hCAR VCAM1 CD46	CAR DAF	Unknown	GAGs EFC
Transgene capacity	Moderate	Low	Very low	High
Viral immunogenicity	Low	Low	Low	High
Ability to penetrate Blood brain barrier	Very limited	Moderate	Limited	Very limited

Table 8.
Characteristics of oncolytic virus.

cells for infection and viral replication. Viruses also display pathogenicity and immunogenicity, which vary depending on viral species, dosage, mode of administration, pre-existing host immunity, and other variables, and are characteristics that can produce effective antitumor immunity.

21. Anti-tumor activity of oncolytic viruses

Considering they influence multiple crucial phases in the cancer–immunity process, oncolytic viruses offer several benefits as cancer treatment agents [149]. These features include preferential replication in tumor cells, stimulation of immunogenic cell death and release of soluble antigens and danger signals, induction of innate immune responses by recruitment of immature dendritic cells (DCs) and innate lymphoid cells, correction of antigen processing and presentation abnormalities, and activation of adaptive immunological responses. Although the molecular and cellular intricacies of how oncolytic viruses correct these processes are not entirely known, advances in the generation of antitumor immunity employing oncolytic viruses are being achieved, and insights into rational combination therapy based on oncolytic viruses are being explored.

22. Combing oncolytic virus treatment with immune check point blockade

Immune check point blockade therapy (ICB) is extensively in cancer treatment, and long-term clinical outcomes are promising. Clinical responses are associated with pre-existing antitumor immune responses, such as an increased number of TILs, a high mutation load, and the formation of a diverse neoantigen repertoire [150, 151]. Combination therapy utilizing ICB and oncolytic viruses are appealing because the oncolytic virus can drive recruitment of TILs into immune-deficient tumors and prompt the production of soluble tumor antigens, danger signals, and pro-inflammatory cytokines, which can improve T cell recruitment and boost immune cell activation. Viral infection also raises the expression of CTLA4, PDL1, and other immunological checkpoint molecules, which would normally inhibit T cell activation (and so antitumor immunity), but also makes tumors more susceptible to ICB (**Figure 7**) [152, 153]. Preclinical research with a B16–F10 melanoma indicated that localized injection of tumors with oncolytic Newcastle disease virus caused infiltration of tumor-specific CD4+ T cells and CD8+ T cells into both the injected tumor and distant tumors, as well as improved tumor susceptibility to systemic CTLA4 inhibition 18. An oncolytic virus Maraba demonstrated therapeutic potential as a neoadjuvant in a preclinical model of triple-negative breast cancer and sensitized previously refractory tumors to ICB [154]. Several additional oncolytic viruses, including B18R-deficient vaccinia virus and vesicular stomatitis virus expressing a library of melanoma antigens (VSV- ASMEL), also shown substantial (P 0.05) therapeutic effect when used in tandem with ICB [155, 156]. Administration of T- VEC intratumorally, followed by anti-CTLA4 antibody (ipilimumab) treatment via intravenous injection, demonstrated an object response rate of 50%, with 44% of patients showing robust responses lasting more than 6 months in a phase Ib clinical trial. Also, no dose limiting toxicities were observed in the patients [157]. Additionally, a recent study reported that treatment with oncolytic poxvirus CF33-hNIS-ΔF14.5 modulates tumor microenvironment in TNBC model, and increases the response of tumor cells towards anti-PD-L1 antibody. Tumor microenvironment is one of the central plays in tumor growth, metastasis and

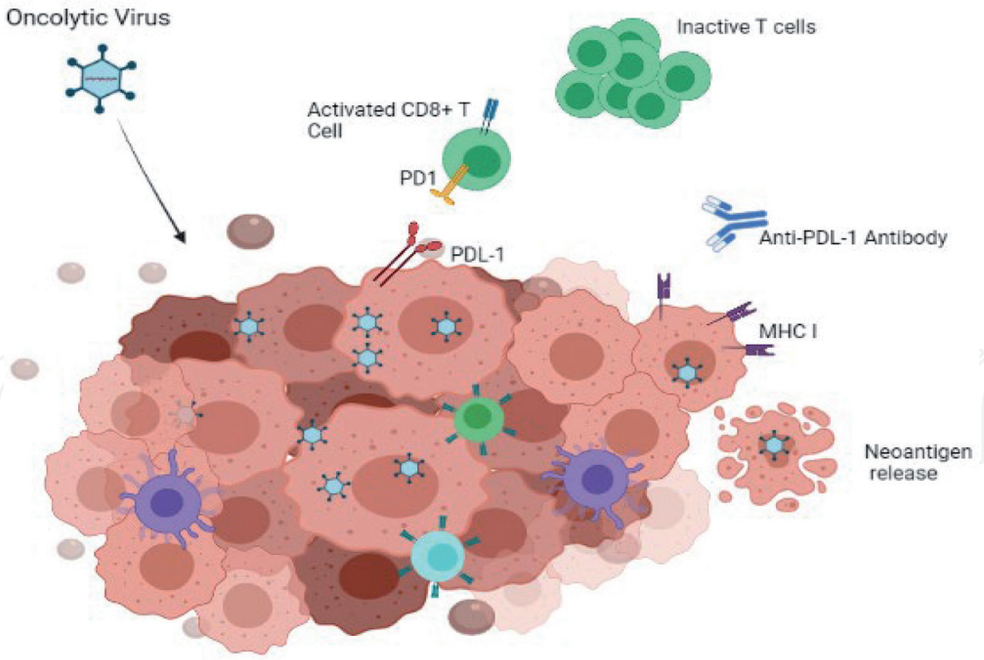

Figure 7.
Represents the role of oncolytic virus in immunotherapy.

development of resistance. Further *in vivo* and *in vitro* analysis revealed that infection with the virus stimulated expression of PD-L1 in TNBC cells. Also, exposure of mice model of TNBC to oncolytic poxvirus CF33-hNIS-ΔF14.5 enhanced infiltration of CD8+ T cells and increased expression of proinflammatory cytokines IFNγ and IL-6 by tumor cells. Combinational treatment with oncolytic poxvirus CF33-hNIS-ΔF14.5 and anti-PD-L1 antibody augmented TME modulation and induced 50% tumor regression in mice models. Administration of these as single agents failed to inhibit tumor growth. Besides, it was also observed that the recovered mice with combinational treatment did not develop tumor after re-challenge with the same cancer cells suggesting that they developed immunity against those cancer cells [158, 159].

Taken together, studies demonstrate that oncolytic virus treatment positively induces tumor immune microenvironment modulation in triple-negative breast cancer model making them responsive to the immune checkpoint inhibitors and hence warrants further studies to determine the clinical applicability of this combination approach.

23. Summary

1. Chemotherapy lacks the success in treating malignant tumors like TNBC as it lacks specificity and can act on normal healthy cells causing secondary diseases in patients.

2. Furthermore, immunotherapy have shown downfall in its efficacy due to the major problem of escape of tumor cells from the immune response against them.

3. Therefore, drug repurposing a strategy commonly used to reprofile or repurpose the existing chemotherapeutic drug has shown promising effects in targeting various diseases including malignant tumors.

4. Drug repurposing is mainly done by using both computational and non-computational methods including target-based computational studies and in vitro based experimental studies

5. These methods permit us to select an existing drug whether FDA approved or drugs that are under investigational studies before in vitro studies thus reducing time consumption and proving cost effective.

6. Because most chemotherapeutic drugs are toxic in nature and lack target specificity as well, therefore by using drug repurposing approach we can combine the chemotherapeutic drugs with target specific immunotherapeutic options to make them effective.

7. Therefore, chemotherapeutic drugs can be combined with immune checkpoint inhibitors, PD-1/PD-L1 antibody and vaccines to provide promising results in anti-tumor response

8. Various enlisted clinical trials have shown promising results in combining chemotherapy with immunotherapy.

24. Future perspective

TNBC is the most aggressive, lethal and complex subtype of breast cancer. What makes it more aggressive is the lack of targeted therapies leaving chemotherapy as the main treatment option available. However, chemotherapy itself mostly lacks target specificity and can harm normal healthy cells of an individual. Moreover, another treatment option that is immunotherapy also faces some problems showing inefficacy due to escape of tumor cells from immune surveillance. Nevertheless, a strategy known as drug repurposing has shown to be a promising strategy to overcome the inefficacy of available treatment options. In drug repurposing, an existing chemotherapeutic drug can be repurposed to modulate its efficacy. In this chapter, we have focused primarily on repurposing the available drugs whether PARP inhibitors or MEK inhibitors, vaccines including the ones under clinical trials as well by combining them with other available immunotherapeutic options like immune checkpoint inhibitors, PD-1/PD-L1 antibodies etc. Also the currently used epigenetic therapy drugs also are known to show significant efficacy in modulating immunotherapy responses in patients suffering from cancers especially TNBC. From our point of view combining drugs with other target specific drugs like drugs targeting immune system components provides a significant insight as it repurposes the drug whether chemotherapeutic or epigenetic drug making it target specific.

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