Chapter 63

Perception on Immune Checkpoint Inhibitor Vanquishing Standard Treatment Paradigm for Triple-negative Breast Cancer

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ABSTRACT

Triple-negative breast cancer/ TNBC has an apprehended challenge in the field of oncology because of its agonistic nature and limitation to its treatment. Some conventional treatments have efficacy to a limited extent and have a short durable return. In the context of this literal disease, the revolutionary immune checkpoint inhibitors have transformed the disease paradigm. The studies have concluded the potential of combining immunotherapy with several existing modules of treatments. The challenges regarding these algorithms are also discussed. Combining immune checkpoint inhibitors with taxanes, anthracyclines, and platinum therapy shows promise in treating Triple Negative Breast Cancer. This multi-agent approach enhances immune response, leading to improved outcomes, particularly in cases with BRCA mutations. Despite potential side effects, this comprehensive strategy offers an effective therapeutic intervention for TNBC. The amalgamation of vaccines with immune checkpoint inhibitors has ensured the enhanced activation of T cells to combat triple-negative breast cancer by incorporating DNA-based vaccines. Adjuvants are advanced strategic tools used to boost vaccine quality and functions. Certain adverse effects arise due to the suppression of T cells, referred to as immune-related adverse events which may induce immune toxicity. Progress in triple-negative breast cancer (TNBC) immunotherapy involves the exploration of predictive biomarkers, particularly focusing on TNBC subtypes like immune-modulatory (IM) and basal-like immune-activated (BLIA). Research is ongoing into TNBC subtypes for subgroup analysis. The dynamic nature of PD-L1 expression and the examination of CD274 amplification underscore the evolving landscape of TNBC immunotherapy. This article underscores the focal role of immunotherapy and ICI in comparison with standard treatment ideas. Its target is to get improved and effective outcomes for patients of Triple negative breast cancer (TNBC).

KEYWORDS
Triple-negative Breast Cancer, Treatment, Paradigm

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INTRODUCTION

Breast cancer is the second leading deadly cancer to occur in women. It is categorized into four types: hormone receptor-positive, HER2-positive, and triple-negative breast cancer (TNBC). Among them, Triple-negative breast cancer is a highly heterogeneous type of breast cancer with wide intertumoral and intratumor heterogeneity (Zhang et al., 2022). It has a high ratio of malignancy in most of the young women. From all breast neoplasms, TNBC accounts for 24% of the latest diagnoses (Rakha and Ellis 2009). TNBC is categorized by insufficient expression of Estrogen receptor (ER), progesterone receptor (PR), and amplification of antihuman epidermal growl factor receptor 2 (HER2) (Lin et al., 2019). Widespread interest in research on TNBC can be attributed to difficult prognosis along with limited therapeutic agents because hormone receptors and HER2 expression are not targetable (Wu et al., 2020). Only in the initial 5 years of disease diagnosis does a high mortality rate of 40% appear in patients (Bauer, et al., 2011; Gerratana et al., 2014). According to currently available evidence, TNBC is the term encompassing various entities with specifically unique genetics, histologic, clinical, and transcriptional changes. (Smith, et al., 2010) Breast cancer heterogeneity and genomic subtypes are revealed by looking at breast tumors from a molecular viewing approach (Sobolewski et al., 2019). The recently modified classifications of breast cancer based on the expression pattern of its gene differentiated the tumors in the breast into four

intrinsic subtypes namely: luminal androgen receptor(LAR) gene expression of estrogen receptor categorizes this, (basal-like BL1, BL2) tumor that is positive for myoepithelial and basal markers and fewer hormone receptors and HER2 gene amplification, HER2 subtype identified by HER2 gene amplification and lastly normal Breast like subtype including the phenotype of triple-negative. However, the cellular derivation is typical of normal breast epithelium (Krings and Chen, 2018). 45% of Patients with the mature stage of TNBC are with a high probability of metastasizing the TNBC to visceral organs and the brain (Claus et al., 2008). However, to high chances of malignancy, heterogeneity, drug resistance and increased risk of tumor recurrence of TNBC have contributed to convolution towards its treatment. TNBC patients will not benefit much from drugs like endocrine and HER-targeted drugs because of the absence of relative receptor markers. Many standard treatment therapies like surgeries, chemotherapy regimens, radiation therapy, combinational therapies, and conventional postoperative adjuvant chemotherapy remain incompetent due to toxicity, the resistance of some drugs, and the inability to identify molecular targets (Numan et al., 2021). Immunotherapy is, however, an emerging strategy for the treatment of TNBC. Immunotherapy uses the patient's immune system to defeat cancerous cells (Bertoni et al., 2016). Immunotherapy gives an efficient response by T- lymphocytes which provides CD8+ cytotoxic effectors. In addition to immunotherapy, a more favorable and modern phenomenon for treating TNBC is immune checkpoint inhibitors (ICI). They can relieve immunosuppression and anti-tumor effects of Tells. The microenvironment of the tumor is managed via high CD 4+ regulatory T cell levels (Diamandis et al., 2016).

In this article, we will discuss the prospects of immunotherapy with further advancements in immune response and the role of biomarkers. It is also important to highlight the challenges of immunotherapies and designing checkpoint blockages for TNBC.

TNBC Molecular Subtype Glossary

To design a specified therapeutic approach it is necessary to address molecular subtyping of TNBC with hallmarking causative factors of each of the types, specific chemotherapies for each type, their response to multiple treatments, and their complexities.

Clinical approaches and trials have shown that TNBC has molecular subtypes based on the specificity of the expression patterns. The subtypes are basal-like (BL1 and BL2), mesenchymal-stem-cell-like (MSL), immunomodulatory (IM), mesenchymal (M), and luminal androgen receptor (LAR) (Bauer et al., 2011; Li et al., 2012; Jovanović et al., 2016).

Basal-like (BL1) has greater expression of genes for proliferation signaling, DNA damage response, and cell cycle checkpoint loss. basal-like immunosuppressed (BLIS) subtype, has immunosuppressive molecule expression. BL2 has greater glycolysis/gluconeogenesis, growth factor [GF], and myoepithelial surface receptors. Thus, the BL1/2 subtype cells could be of either myoepithelial or basal origin. basal-like immune-activated (BLIA) subtype, exhibits signal transducer and transcription activators.

The M subtype has raised pathways of cell motility, extracellular matrix (ECM), cell differentiation pathways, and receptor interaction. The MSL subtype has the same expression as that of the M subtype, but also exclusively expresses the paths related to platelet-derived growth factor (PDGF), calcium (CA2+), G-protein-coupled receptor (GPCR), extracellular-related kinase (ERK 1 AND 2), GF signaling (inositol phosphate metabolism), epithelial-mesenchymal transition (EMT), and Wnt/B- catenin. There is a lowered gene expression for cell proliferation in the MSL subtype.

Both M and MSL subtypes have higher gene expression of genes in pathways of cell differentiation and growth factors.

IM subtype is engaged in the regulation of immune cells in a way by modulating cytokine signaling, T and B cell receptor signaling (immune cell signaling), antigen processing/presentation, and immune signal transduction.

Lastly, the LAR subtype has increased gene expression of pathways associated with hormone regulation, particularly with androgen receptor signaling (which could be about nine times the amount as the other TNBC subtypes) and AR antagonism (Finetti et al., 2006; Jovanović et al., 2016; Ghorayeb et al., 2022).

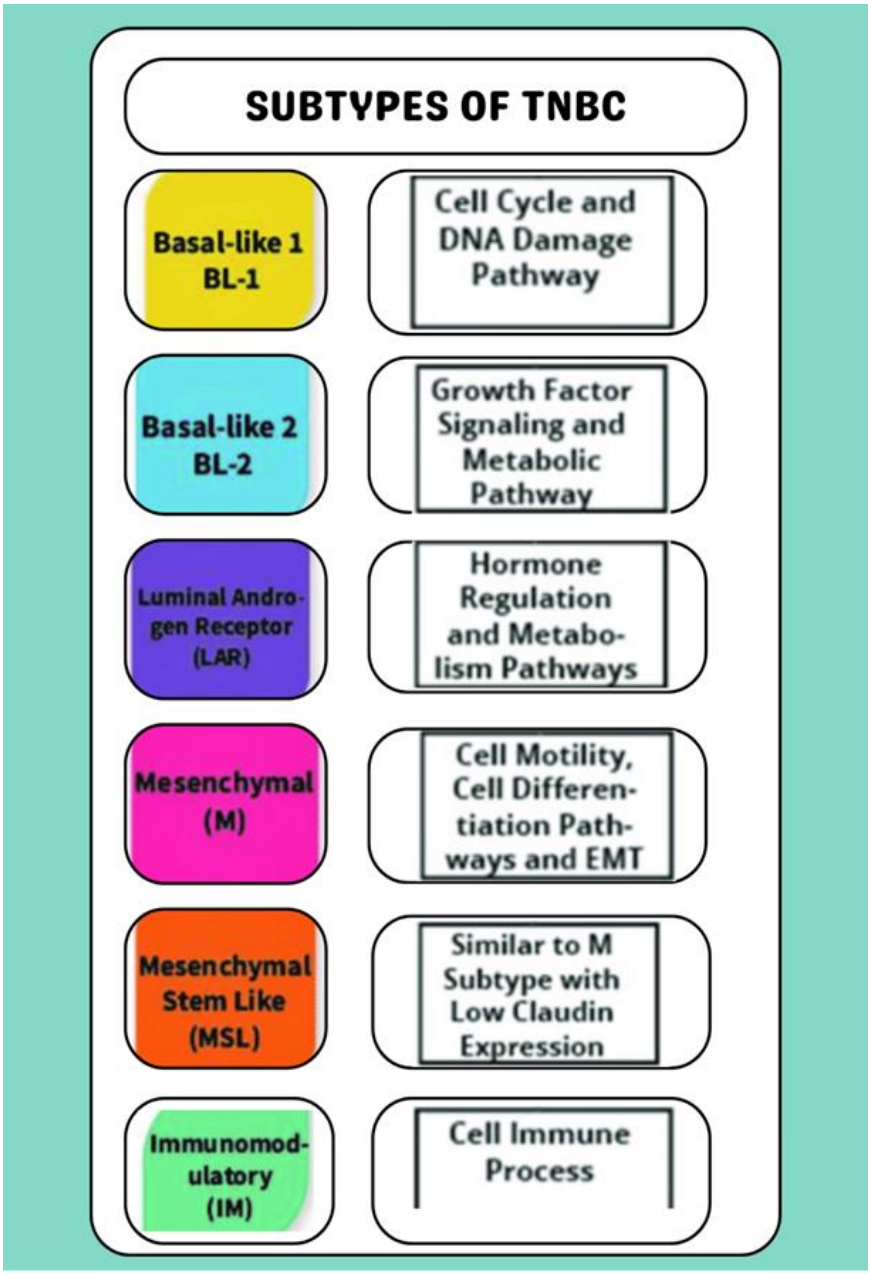
Introduction in Relevance with Immunotherapy and ICI

The tumor microenvironment consisting several components such as tumor cells, lymphocytes (B and T), NK cells, tumor-related macrophages, cytokines, chemokine, and fibroblasts interact with each other (Buisseret, et al., 2020). These interactions have the potential to change the internal environment, providing ease for developing resistance in TNBC. The tumor cells in combination with stromal cells aid metastasis, survival, and advancing tumor, drug resistance (De Angelis et al., 2022). Cancer-related fibroblasts promote tumor progression and resistance to chemokine, cytokines, etc. The cytokines secreted cause immunosuppression and proliferation of tumors (Jones et al., 2021; Wu et al., 2023). However, lipid-associated macrophages induce immunosuppression in TNBC (Gueguen et al., 2022). Macrophages activate PCAT 6 and secrete growth factor Vascular endothelial (VEGF) which enhances cancer metastasis and growth. Macrophage-enriched, neutrophil-enriched, myeloid cell subtypes have mechanisms for resistance to immunotherapy (Gao et al., 2019).

Furthermore, the failure of drug delivery, chemotherapy, and immunotherapy as treatment options is due to several reasons (Gibbons et al., 2020). The cancer tissues show serious fibrosis and extracellular matrix accumulation causing vascular compression and reduced passage of fluid. This leads to drug delivery failure (Fan and He, 2022). Altering the TME is important to versatile treatment options e.g. improving oxygen, blood flow to tumor sites/vacuolization, functional vasculature, the anti-tumor response through enhancing dendritic cells and TAM, activating immunosuppression (Nguyen et al., 2023).

The chemotherapeutic agents are far less effective against TNBC. Because cancer-associated fibroblasts and their

secretions including IL-6, IL10, and TGF- β act as a physical blockade. It inhibits the activity of CD 8+ and causes immunosuppression against cancer (Kellokumpu-Lehtinen et al., 2012). promotes extracellular matrix destruction. The TGF-β promotes CD8+ cells and destructs the extracellular matrix. Thereby, the effectuality of immunochemotherapy is increased due to immune cell infiltration and blood perfusion, hence, promoting drug delivery (Qin et al., 2022).



Gut microbiota plays a crucial role in enhancing immune checkpoint inhibitor (ICI) response by modulating innate and adaptive immunity, influencing anti-tumor immune responses. In TNBC, there is a subtype called IM, which subtype composed of more than 20% of cases and this also shows more commending prognosis and benefits from immunotherapy interventions. This subtype is characterized by the enhancement of Clostridium species also trimethylamine oxide (TMAO) in cancer cells, and TMAO also promotes anti-tumor immunity and increases CD8+ T cell infiltration (Yuan et al., 2022). Clinical trials using choline, a TMAO precursor, show promise for precision immunotherapy in TNBC. Despite the success of therapeutic strategies combining intestinal microbiome interventions with ICIs, (Zhou et al., 2021) the molecular mechanisms linking gut microbiota and their metabolites to ICI efficacy in TNBC require further exploration in future studies.

Interferons for Activation of Anti-tumor Immunity

Interferons in tumor immune cell therapy first activate CD8+ T cells by stimulating dendritic cells. However, long-term exposure has immunosuppressive effects and adverse feedback, which means that adjusting IFN responses may improve anti-tumor immunity (McGaha et al., 2017). Studies reveal that Interferon signaling in cancer cells hinders immune cell responses, while IFN signaling in innate and adaptive immune cells enhances the immune response (Johnson et al., 2019).

Type I IFNs (IFN-α, IFN-β) positively impact NK cells, promoting cytotoxicity and IFN-γ secretion, and aiding antigen-presenting cell differentiation, maturation, and migration. IFN-γ provides immunomodulatory purposes (Cardoso, et al., 2018). In TNBC, MYC overexpression inhibits the IFN signaling pathway, (Brambillasca, et al., 2022) leading to immunosuppression; targeting MYC may signal to reinstate MHC-I expression, CD8+ T cell infiltration, and enhance anti-PD-L1 responses.

CpG oligodeoxynucleotides, TLR9 agonists, stimulate IFN α and β production in plasmacytoid DCs, activating T cells and B cells, recruiting natural killer cells, and enhancing antibody-mediated (humoral) and cell-mediated immunity(cellular) (Yang et al., 2022). CpG-B, at low doses, significantly inhibits tumor growth and synergizes with PD-1 inhibitors, while CpG-C, even at higher doses, demonstrates greater efficacy in combination with anti-PD-1 suppressors (Hua et al., 2020). The difficulty in Triple-negative breast cancer treatment is successfully directing IFNs to re-adapt the immune microhabitat for rational combination treatment.

Addressing Resistance to Immunotherapy in Triple-negative Breast Cancer (TNBC)

ICI is beneficial for some Triple Negative Breast Cancer patients, but others show no betterment or develop resistance (Schoenfeld and Hellmann, 2020). The limited knowledge of resistance mechanisms hinders the development of next-gen immunotherapy.

p53 Delivery Combined with PD-1 Inhibitors

Several studies indicate that cancers with higher CD8+ T cell presence exhibit increased effectiveness in response to different PD-1 and PD-L1 inhibitors, exploring CD8+ T cells as potential prognostic and therapeutic indicators for anti-PD-1 therapy in the context of treatment (Zitvogel et al., 2019; Shrimali et al., 2019).

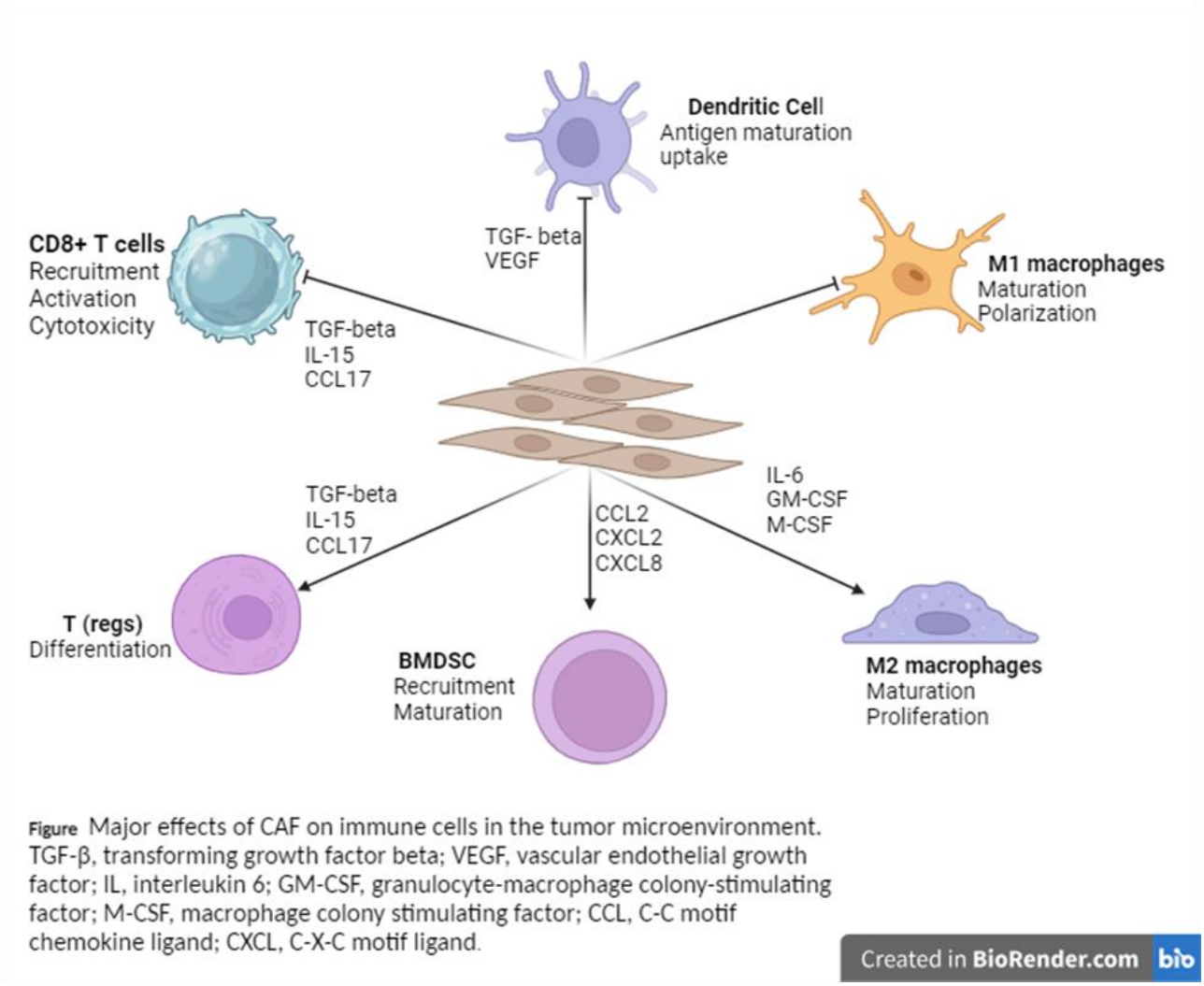


Figure Major effects of CAF on immune cells in the tumor microenvironment. TGF-β, transforming growth factor beta; VEGF, vascular endothelial growth factor; IL, interleukin 6; GM-CSF, granulocyte-macrophage colony-stimulating factor; M-CSF, macrophage colony stimulating factor; CCL, C-C motif chemokine ligand; CXCL, C-X-C motif ligand.

In advanced TNBC cases, which do not show CD8+ T cell infiltration, there has been observed resistance to inhibitors targeting PD-1/PD-L1. With a high mutation frequency of up to 80% in TNBC, the tumor suppressor protein p53 emerges as a potential biomarker (Synnott et al., 2018). Utilizing the natural Pos3Aa protein crystal in Bt bacteria as a carrier, the delivery of p53 restores its purpose in deficient tumor cells, overcoming immune escape. The combination of Pos3Aa-p53

crystals along with anti-PD-1 antibodies demonstrates safety, notably increases interferons and memory T cells, presenting a promising strategy for enhancing the efficacy of anti-PD-1 therapy in p53 mutant TNBC (Sun et al., 2022). Further studies and evidence-based clinical research are essential for future clinical applications

CXCR2 Inhibitor Combined with ICIs

TNBC, with heightened CXCR2 expression mainly in neutrophils (Jacot et al., 2020) faces drug resistance due to CSC stemness. Combining AZD5069 with doxorubicin reduces chemoresistance, and synergy with atezolizumab shows promising cytotoxic effects, emphasizing the need for clinical trials to assess potential breakthroughs in improving TNBC survival rates. This also suggests that beyond diminishing chemoresistance, CXCR2 inhibitors also improve the effectiveness of PD-L1 inhibitors (Eissa et al., 2022).

Revising Chemotherapy and Immune Checkpoint Inhibitors Combined with Chemotherapy

After analyzing the response ratio of immune checkpoint inhibitors as an independent treatment, there is needed to shift the focus to combined therapies. The motive is to induce an effective response to patients unresponsive to immunotherapies by using any additional agent that fabricates an inflamed tumor microenvironment (Emens and Middleton, 2015). Generally, chemotherapy is appealing due to its property to lessen the suppressive immune cells and upgrade response to PD-L1 by increasing the ratio of antigens affecting it (Zitvogel et al., 2017).

Patients with TNBC are usually treated with a mix of surgeries and chemotherapy since this is a very efficient way to eliminate cancer cells all over the body. The patient's condition and the tumor's stage determine the chemotherapy plan. The gold standard for treating Triple Negative Breast Cancer is systemic chemotherapy, which employs medications like taxanes (like paclitaxel) and anthracyclines (like doxorubicin) in the adjuvant or neoadjuvant (NACT) settings (Furlanetto and Loibl, 2020). To render inoperable tumors, the first NACT was carried out on patients with locally progressed breast tumors in the 1980s (Calabuig-Fariñas et al., 2021). Chemotherapy is considered to be the primary therapy for TNBC; however, tumor size should determine how it is administered, according to research. TNBCs that lack involvement of lymph nodes and have tumor diameters between 0.5 and 1.0 cm have decent future outcomes. (Gupta et al., 2020). Another team of researchers believed that there was no discernible benefit to treatment for TNBC tumors less than 1 cm in size (Vaz-Luis et al., 2014) Small tumors can attain full pathological cure with treatment alone. Durvalumab was found to have a greater pCR rate in those people with tumors grade IIA and above than placebo. This was shown in a phase 2 study that was randomized, double-blind, and inactive drug-controlled and examined the pCR rate of NACT in primary non-metastatic TNBC patients. The experiment included nab-paclitaxel accompanied by dose-dense epirubicin/cyclophosphamide with durvalumab against placebo (Loibl et al., 2019).

However, metastatic triple-negative breast cancer is treated by chemotherapy. The response ratio using only a single agent is less (10 to 30%) as compared to combined multi-agents (63 %) (Wang et al., 2015). The statistical analysis of early-stage TNBC patients showed the escalation in pathologic response rate induced by taxanes and anthracyclines. The immune checkpoint inhibitor in junction with chemotherapy is highly responsive and has less poor effects (Peng et al., 2021).

Taxanes and anthracyclines are often included in the conventional chemotherapy treatment for TNBC patients (Collier et al., 2020)Anthracyclines treat TNBC by destabilising DNA by insertion, which results in repaired DNA cascade degeneration (Yadav, Sharma et al., 2014) According to research, anthracycline effectively destroys cancerous tissues and stimulates the body's defence system by boosting CD8+ T cells (Katz and Alsharedi, 2017) Anthracyclines, an chemotherapeutic agent cause immunogenic cell death. As a result, the dendritic cells causing the multiplication of CD 8+ T cells are activated (Buqué, et al., 2017).

Anthracyclines, such as doxorubicin and epirubicin, have been demonstrated to improve cure rates and lifespan for longer periods. These medicines have shown advantages and increased responsiveness when administered either alone or as a neoadjuvant. The most common treatments for patients with ferine BRCA1/2 who have not had these medications administered in neoadjuvant or adjuvant contexts are taxane- or anthracycline-based treatments. Given the evidence that individuals may respond to anthracycline therapy, a lot of doctors refuse to retest their patients because of the accumulated cardiac damage they have seen (Sendi, et al., 2021)

Furthermore, the retaliation rate for anthracycline-based treatment is greater. However, its usage is limited due to its association with greater risks of reappearance and a low average survival rate. Short-term toxicity from the medication can also result in alopecia, permanent cardiopathy, myelosuppression, and nausea (Ghosh et al., 2021)

Taxanes are an important family of chemotherapy medications due to their distinct mode of activity and several uses in cancer treatment. Taxanes' primary molecular functions include suppression of new blood vessels, mitotic slippage, and rupture of the mitotic apparatus (Ilari et al., 2021) Taxanes, a class of chemotherapy, increase immune cells TIL (tumor-infiltrating lymphocyte), decrease T regulatory (Treg) and MDSC (Myeloid-derived suppressor cells). The Treg and MDSC suppress the immune response to tumors (Volm et al., 2001).In contrast with other subtypes of breast tumors, TNBC has proof of benefiting from either taxanes independently or in combination with anthracyclines, with pCR rates of no less than 40%. For therapeutic usage, three taxanes—paclitaxel, docetaxel, and cabazitaxel—have been authorized. among the oldest and most successful chemotherapy drugs for treating cancer is docetaxel, which was licensed in the 1980s. However, because of its negative impact on the body's natural microbiota, docetaxel may also contribute to resistant bacteria (Catalano et al., 2022).

Paclitaxel is one of the most well-liked chemotherapeutic drugs due to its selective, flexible, and saturable bonding characteristics to molecules and small tubules. Pneumothorax inhibits mitosis, which results in apoptosis in tumor tissues. It is an effective chemotherapeutic agent for several malignancies, including ovarian, and breast tumors (Blanchard, Paul et al., 2015). Treatments for many different cancers have all been shown to benefit from paclitaxel's antitumor properties (Reddy and Bazile, 2014). A combination of therapies is necessary for successfully treating Triple Negative Breast Cancer since PTX's cell death can have drawbacks, such as the cell's use-dependent tolerance and toxicity to normal cells (Cho, et al., 2020). Nab-paclitaxel, also known as albumin-bound paclitaxel, was created to enhance the medication's safety aspect.

Cyclophosphamide, being another chemotherapeutic drug causes suppression of T regulatory cells, boasts division of CD8+ T cells, and causes immunogenic death of cells. However, platinum therapy does the same but it additionally enhances the presentation of major histocompatibility complex Class 1 on tumor cells. This is for the downregulation of MDSC and activating T cells (Majewski et al., 2012).

Platinum agents have well-established effects on breaks in DNA and cell death. Because of their distinct mode of action, they are especially useful against cancerous cells that lack functional DNA repair systems, such as those harboring harmful BRCA mutations (Tovey et al., 2018). TNBC is typified by abnormalities in DNA repair. Since it is generally known that anthracyclines, platinum, and cyclophosphamide directly cause the degradation of DNA, these treatments have received a lot of attention in the treatment of malignant TNBC, particularly in patients with germline mutations of BRCA. The possibility that BRCA1/2 germline mutations are linked to susceptibility to platinum treatment has been the subject of several inquiries. Better results and higher susceptibility to chemotherapy are linked to gBRCAm and BRCAness statuses (Tutt et al., 2018) Tumours that have characteristics with BRCA-mutated tumors but do not contain hereditary mutations in BRCA1/2 are referred to as "BRCAness" (Mehanna et al., 2019). Individuals with homologous recombination abnormalities and BRCA1/2 mutant TNBC have demonstrated noteworthy advantages from platinum-based treatment plans (Sendi et al., 2021).

Combination of Immune Checkpoint Inhibitors with Vaccines

Breast cancer manifests numerous tumor-associated antigens, notably HER2 and mucin 1 (MUC1), serving as focal points in vaccine development (Cruz et al., 2019).

Preliminary vaccine trials demonstrated safe administration and elicited antigen-specific rise of immune responses, but the quality of clinical protocols seems appropriate. A significant constraint may arise from targeting common tumor antigens, suggesting a potentially more efficacious strategy of formulating vaccines incorporating mutation-specific antigens exclusive to the tumor. Furthermore, synergizing vaccines with checkpoint inhibitors holds promise in enhancing efficacy through heightened T-cell activation and mitigation of immunosuppressive pathways. a new approach to administrating DNA-based neoantigen vaccine has been introduced which involves gemcitabine and carboplatin along with nabpaclitaxel and durvalumab.

Immunizations have been instrumental in preserving millions of lives and safeguarding many others from illness. Throughout history, the efficacy of immunization practices in preventing infectious diseases has been evident. In ancient times, the utilization of these practices was based on empirical knowledge, as the protective mechanisms induced by early formulations were not fully understood, leading to occasional failures. (Jakubowska et al., 2015)

Adjuvants have been used as an effective strategic tool to obtain target-specific vaccines. These substances when incorporated into vaccines expedite the immunological activities by enhancing the functional efficacy and quality of vaccines. The therapeutic strategy of using peptide vaccines to target metastatic cancer seems to have low acceptance, so this challenge is sorted by using multi-peptide vaccines with a response rate of 9.9%. (Sukumar et al., 2015)

The amalgamation of vaccines with immune checkpoint inhibitors has proved to be a significant factor for the heightened immune response against tumor growth. The execution of clinical trials which are focused on determining the effectiveness of cancer vaccine and PD-L1 antibody combination is approved. A few trials are subjected to TNBC to unveil the efficacy results induced by the amalgamation of vaccines and pembrolizumab. incorporated vaccines are multi peptides and those that target p53 or WT1. In addition, the research trial involving the combination of dervalumab and multi-peptide (PVX-410)or neo-antigen vaccine (fused with atezolizumab) is under process.

The implementation of tumor vaccines in therapeutic measures for cancer prevention has assured the boosted immunological empowerment of the host. This advanced approach to tumor vaccines is much more efficient than the conventional therapeutic approaches used to combat cancer. as it offers high specificity and fewer side effects (DeCristo et al., 2017).

α-lactalbumin is a protein present in most TNBCs, although absent in normal and aging tissues post-lactation. The expression of this surplus protein is retarded with the activation of the innate immune system against it. It is done by amalgamation of vaccines with adjuvants that mediate targeted immune response and stop the advancement of tumors.

Immunotherapy Mediated Toxicity

Immune checkpoint proteins like PD-L1 play a role in inhibiting T-cell function. The use of immunotherapeutic techniques has successively reduced the suppression in T cell functionality, regulated by tumors. Such immune activations can mediate many associated toxicities as they are not solely targeted toward tumor sites in treated patients. Prevailed

toxicity raised by immunotherapy has caused inflammatory conditions in various organs of the patients. The significant contribution to obstructing the inhibitory pathways that terminate the immune response against the tumors is a notable function of immune checkpoint inhibitors. The disruption of inherent regulators for immunity is exhibited by inhibitors employed for the sake of anti-tumor effects (Zhou et al., 2019).

In regard to an analytical model, adverse events mediated by anti-tumor immune response posed genotoxicity in approximately 66% of the patients who were given treatments by PD-L1 therapy.

Immune-related adverse events are mediated by the activation of T cells. This activation notably produces the inflammatory cytokines along with autoantibodies generated by B cells. The genotoxicity stimulated in different organs of patients largely varies depending upon the type of therapeutic approaches employed. For example, anti-CTLA-4 therapy gives rise to colitis, and hypophysitis and anti-PD-1 therapy mediates pneumonitis and thyroiditis.

The cross-reaction build-up by tumoral antigenicity stimulates immune-related adverse events. Both normal and cancerous cells contain the T-cell antigens, which is proved by a study on treating non-small lung cancer using PD-1 therapy that mediated dermatologic toxicity that pinpointed the T-cell's presence in skin tumor cells. (Murakami et al., 2020).

Immune-related adverse events (IRAEs) tend to be more prevalent when immune checkpoint inhibitors (ICIs) are used in conjunction with cytotoxic chemotherapy. Despite this, the overall rates of IRAEs remain comparable between patients receiving combination therapy and those undergoing ICI monotherapy. Breast cancer patients when subjected to ICI and nabpaclitaxel are more prone to pneumonitis than the case of monotherapy (Fernandes et al., 2019).

Types of IRAEs

Acute IRAEs are those events that occur amid immune checkpoint inhibitor treatments, while the events manifested after completion of treatments are delayed IRAEs. Chronic IRAEs are supposedly mediated 12 weeks apart from the termination of ICI therapy. Examples of IRAEs with a high chance of chronicity are neurotoxicity, arthritis, and xerostomia.

The reasons behind varying susceptibility to immune-related adverse events (IRAEs) among individuals remain unclear, but it has been suggested that genetic factors could play a role.

Patients with autoimmune disorders are more vulnerable to immune-related adverse events. A study revealed that inheritance of autoimmune disorder led to rheumatic IRAEs in 10% of patients. The composition of microbiota and genetic changes may add to the risk of acquiring IRAEs. Nevertheless, the precise contribution of genetics to the susceptibility of developing IRAEs remains inadequately understood (Virassamy et al., 2018).

Therapy Type	Combination Treatments	Description
Chemotherapy	Anthracyclines, Taxanes, Albumin-PTX, Doxorubicin, Cisplatin, Eribulin	These chemotherapy drugs are combined with ICIs to enhance clinical responses in early-stage and metastatic TNBC.
Radiotherapy	Low-dose RT + Nivolumab RT + Pembrolizumab	These two combinations enhances anti-tumor efficacy in TNBC.
Target Therapy	Olaparib + Durvalumab	Combining Olaparib, a PARP inhibitor, with Durvalumab (an anti-PD-L1 therapy) shows favorable outcomes in TNBC.
Combination 1	Ipatasertib/Cobimetinib + Atezolizumab + Paclitaxel/Nab-Paclitaxel	Combining these drugs and ICIs with chemotherapy drugs like Paclitaxel enhances TNBC management.
Combination 2	Entinostat + Atezolizumab	This combination of a histone deacetylase inhibitor (Entinostat) and anti-PD-L1 therapy shows efficacy in TNBC.

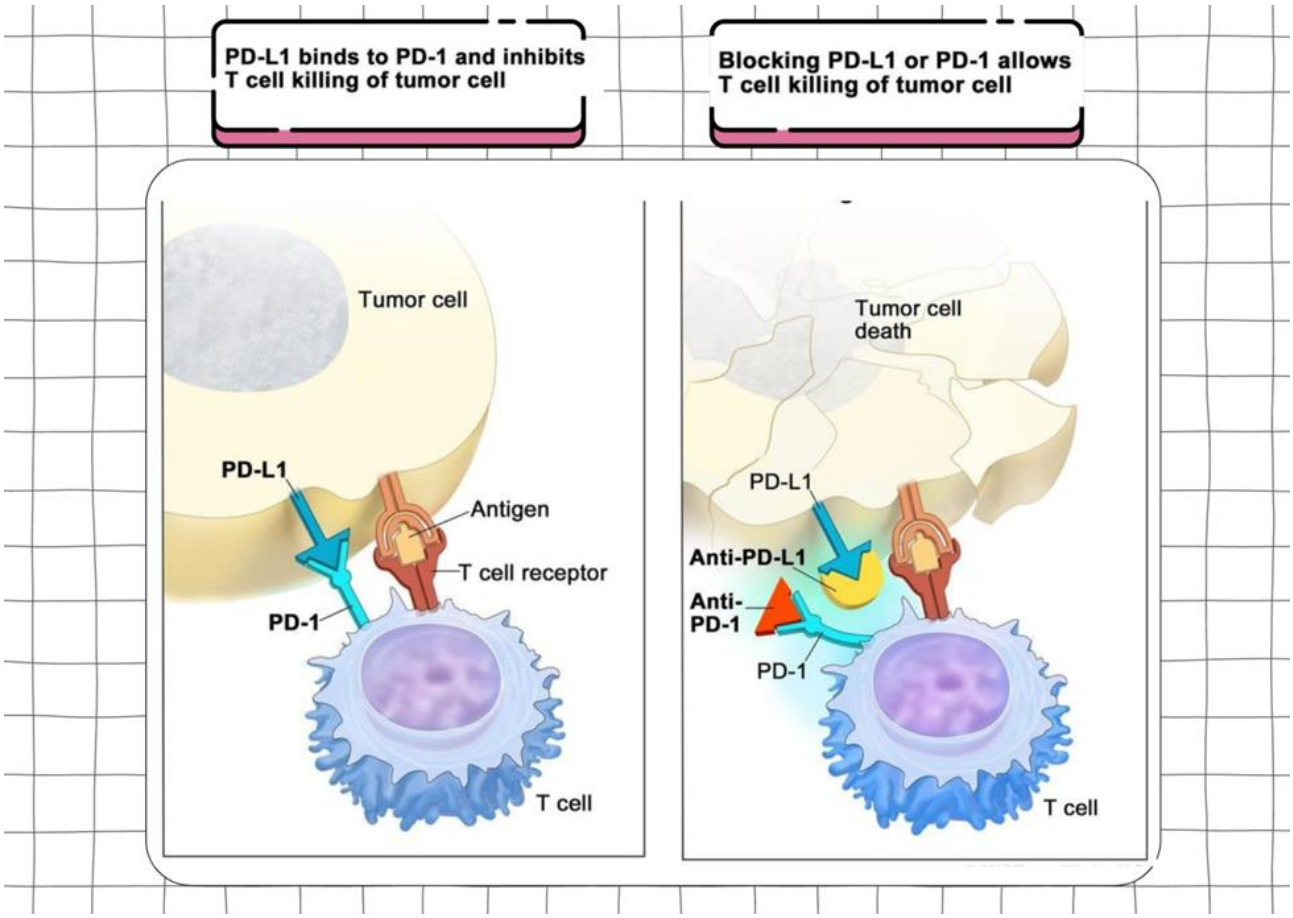
ICIs may lead to endocrine toxicities encompassing conditions such as hypothyroidism, hyperthyroidism, thyroiditis, and insulin-dependent diabetes mellitus. The specificity of the agent and type of endocrinopathy influence the period of endocrine IRAEs. Hormone replacement therapy is permanently employed to target permanent endocrine toxicity. Other IRAEs can be treated and resolved. (Shah et al., 2018)

Immune checkpoint blockade (ICB) may lead to toxicities as a result of an exaggerated immune response against normal tissues. In severe cases, oral corticoids, high-dose steroids, and additional immunosuppressants are used for suppression of the immune system to manipulate IRAEs. For cases refractory to steroids, such as severe IRAEs unresponsive within 48–72 hours, additional immunosuppressants or plasmapheresis, guided by a disease-specific specialist, can be initiated.

Combination 3	Ladiratuzumab Vedotin + Pembrolizumab	A combination that improves TNBC treatment outcomes by pairing an antibody-drug conjugate with Pembrolizumab.
Combination 4	Trastuzumab Deruxtecan + Durvalumab	Combining these drugs, which target HER2-positive cells, with Durvalumab offers promising results in TNBC

Introduction to Biomarkers for immunotherapy in TNBC

Over time, progress in cancer immunotherapy has made the prognosis of many patients with a range of malignancies much easier. Treatment and its response are currently under observation. Predictive biomarkers are being researched to increase ICI efficiency. This is important to reduce toxic side effects and financial strain caused by the treatments (De Angelis et al., 2022). To develop the predictive biomarkers, TNBC heterogeneity is taken into consideration.



Some TNBC subtypes are better candidates for strategies of immunotherapy as demonstrated by using predictive markers. It has been shown that PD-1/PD-L1 ICI was not predictive for response when it was used as a neoadjuvant treatment in early TNBC. However, the response was predicted when it was used in combination with chemotherapy in the patients having metastatic TNBC (Cortes et al., 2020) PD-L1 expression was also affected by the tumor microenvironment

as it changes with regards to the stage of disease and organs associated. Approved agnostic biomarkers include Microsatellite instability (MSI) and high tumor mutational burden (TMB-H). These are used in association with ICI to treat solid tumors (Prasad and Addeo, 2020). TMB is widely known as a surrogate for the neoantigen and T cell activation biomarker (Litchfield et al., 2017).

But TMB-H and MSI are both uncommon markers in TNBC, therefore few TNBC patients are considered suitable for ICI therapy. Studies that covered past responses and future potential were evaluated comprising of patients who were treated with ICIs in combination with chemotherapy or in monotherapy. Among the subtypes, the IM and BLIA are significant due to increased immune gene expression and immune checkpoints that are potential targets so they are linked to improved prognosis (Venet et al., 2018)

There are only two available biomarkers for customized therapy in TNBC including PD-L1 IHC staining for immunotherapy and germline BRCA1/2 mutations for PARP inhibitors. Current studies are looking into TNBC subtypes as biomarkers for subgroup analysis (Liu et al., 2021). Other limitations include the absence of a consensus between different classification systems and that no one has been validated in a metastatic setting. PD-L1 IHC was related to a greater response rate to ICI monotherapy in phase 1-2 trials in metastatic TNBC. Patients with PD-L1-positive tumors were included in these studies (Loi et al., 2019). The phase 3 IMpassion 130 and KEYNOTE 355 trials evaluated a combination of an anti-PD-L1 ICI and chemotherapy as the forefront option of therapy. This depicted the predictive value of PD-L1 IHC and its relation to better outcomes in metastatic TNBC (Molinero et al., 2021).

PD-L1 is not considered the best biomarker. Response to ICI treatment is also noticed in patients with tumors that are negative for PD-L1 and all patients with PD-L1 tumors that are positive derive benefit from immunotherapy. This difference in result can be due to tumor heterogeneity or inconsistency in PD-L1 assessment methods (Zerdes, et al., 2021). Another reason for different results can be attributed to the dynamic expression of PD-L1 as depicted by the change in PD-L1 status after neoadjuvant chemotherapy (Bhalla, et al., 2021).

The non-uniformity in the PD-L1 predictive value between early and metastatic settings can also be attributed to the ability of cancer cells to perform immunoediting processes when evolving from a primary tumor to a metastatic lesion and the ongoing development of TME more conducive to immune invasion. In that case, the combination of a greater immunosuppressive TME and the appearance of less immunogenic tumor in the metastatic lesions might make ICI response prediction more dependent on biomarkers such as PD-L1 expression (Thompson et al., 2016). Hence, the PD-L1 biomarker has numerous flaws to overcome after testing. It should be limited to metastatic TNBC for now.

The distribution of CD274 amplification in the context of the 4-type molecular TNBC classification was investigated. They found a generally low prevalence with the M subtype showing the highest incidence and the BL2 subtype following a little less. Notably, M tumors exhibited increased methylation in the CD274 gene promoter, leading to reduced expression of PD-L1 on the cell surface. The study suggested that neoadjuvant chemotherapy might contribute to the selection of CD274 amplification in TNBC, resulting in elevated PD-L1 expression. The SAFIR02 BREAST IMMUNO phase 2 trial supported these findings, indicating that CD274 gain or amplification predicted the benefit of durvalumab in metastatic breast cancer. The assessment was performed using comparative genomic hybridization arrays (CGH arrays) (Schwarz et al., 2016).

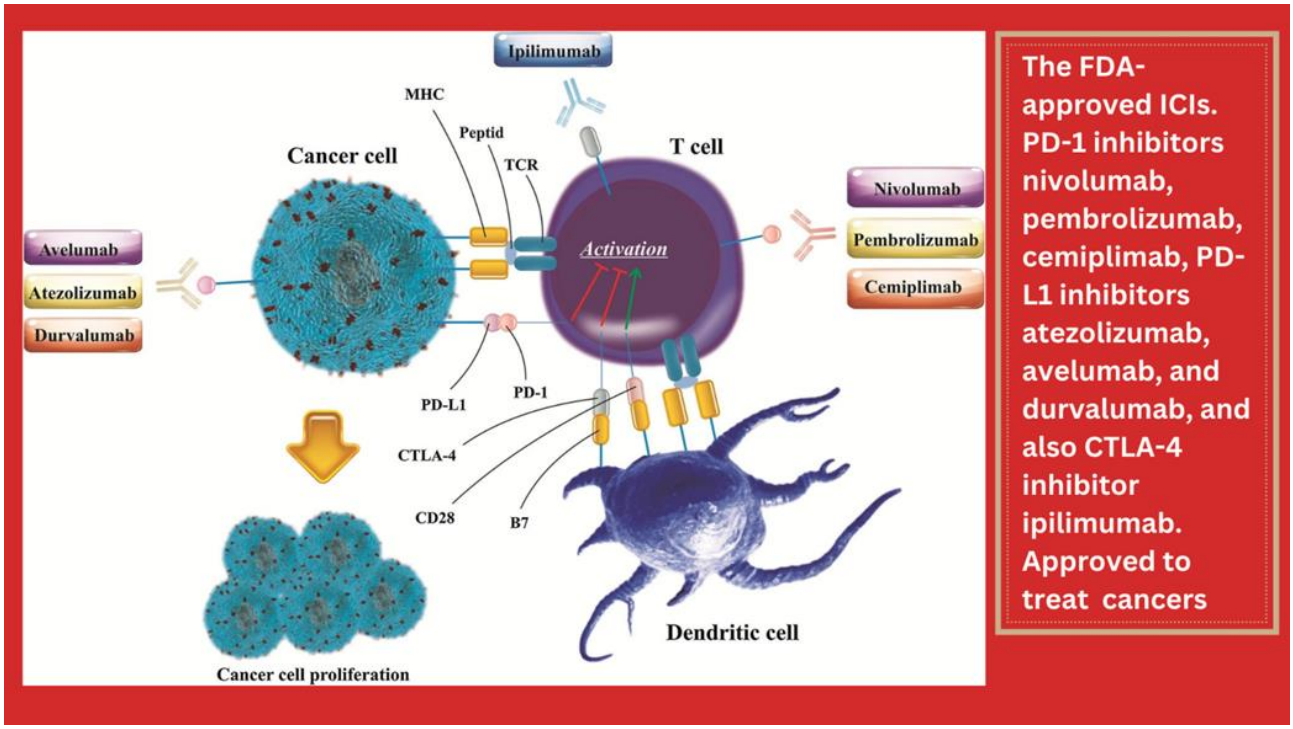
Immune Checkpoint Blockade

Traditional therapies such as surgery, radiation, and especially chemotherapy are proving to be ineffective options. If all subtypes are to be targeted then the latter option is insufficient and condensed chemotherapy is a better alternative. It still poses an economic challenge since it requires growth factors. Additionally, chemotherapeutic drug response is difficult to tackle (Dewanjee et al., 2022). Hormone therapy is also not a promising non-targeted treatment option due to its harmful effects (Masoud and Pagès, 2017). Various subtype targets are handled using a targeted therapy approach. Despite this, the efficacy of target tissues needs to be critically checked in clinical conditions. Likewise, immunotherapy is considered a possible therapy for TNBC. Modifying the immune system is being looked into as a treatment approach. (Fortis et al., 2021). HER2+ tumor gene expression in TNBC showed influence due to immune factors in a microarray-based study (De Caluwé et al., 2023). The immunotherapeutic approach varies from patient to patient on the basis of subtype. Thus, prognostic biomarkers enable customized therapies. The most effective of these is Programmed death-ligand 1 (PD-L1) in TNBC. The tumor mutational burden (TMB) is a designated marker for foreignness and immunogenicity (Hubbard-Lucey et al., 2019). Tumor-infiltrating lymphocytes (TILs), interferon Y (IFN-Y), programmed cell ligand-1 (PD-L1), and human leukocyte antigen-I (HLA-I) are also considered as predictive markers.

For specific subtypes of cancer, the effector TILs in trace amounts in TME form a barrier for therapy based on T cells. Hence, this kind of approach seems promising. Inducing hyperthermia for the TME offers a direct approach to killing tumor cells (Toraya-Brown and Fiering, 2014). This aids in exposing cancer cells to natural killer (NK) cells and CD8+ cells in human leukocyte antigen-I (HLA-I) in a manner dependent on the polypeptide. Anti-estrogenic factors could instigate the immunotherapeutic drug action because estrogen is responsible for suppressing HLA-I. Hence, HLA-I expression has an important role in immunotherapeutic drugs.

Both T-cell activation and tolerance are included in immune checkpoints. In usual conditions, these are crucial for maintaining homeostasis. Immune escape of tumor antigens can occur from immune inhibitory signals from tumors. PD-1/PD-L1 axis as well as cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) act as inhibitory signals to suppress the

immune response of T-cells (Stefanovic et al., 2017). A key role is played by CTLA-4 to ensure that T-cell response can be prevented from reinitiating. Whereas, PD-1 plays a greater role afterward by abstracting T-cell activity in the immunological setting of TME. CTLA-4 binds to those markers present on dendritic cells such as CD86 and CD80 thereby, reducing the effect of immune reaction caused by T cells. On the flip side, PD-L1 activating PD-1 causes inhibition of immune activity by T-cells, activation of T-cell death, pro-inflammatory cytokine production suppression, and antigen tolerance induction. Immune checkpoints are inhibited by blocking the CTLA-4 or PD-1/PD-L1 axis could as a result lower tumor cell immune escaping and present a potential immunotherapeutic approach. (Miao et al., 2021). Some immune checkpoints are being developed and others such as AntiPD-1, anti-PD-L1, or anti-CTLA-4 monoclonal antibodies are currently used in clinical practice. Certain types of Anti-PD-1 antibodies such as pembrolizumab, atezolizumab, and anti-CTLA-4 including tremelimumab and ipilimumab have shown potential for therapy and are approved for treating certain malignant tumors solid in nature.



PD/PD-1 Blockers as Immunotherapeutic Agents

The immunotherapeutic significance of the PD1/PDL1-based pathway has grown, making it a crucial immune checkpoint. The targeting mechanism of PD1 and PDL1 provides ample information regarding immunotherapy-based combinations and problem diagnosis. Inhibitors like specific antibodies blocking PD1 or PDL1 exhibit clinical efficacy, enhancing T-cell responses and mediating antitumor activity across various tumors.

CD28 family contains PD1 protein specified as a checkpoint inhibitor. The activation factors for regulating the function of this protein are the stimulus received after antigen recognition and cytokines synthesis (Lawani et al., 2013).

Situated advantageously, it can control T cell activity within dendritic cells and (APCs) Along with the elimination of tumor cells by T cells, the recognition of PD1 protein by tumor cells triggers an upregulation of PDL1 protein. Apoptosis is mediated by the attachment of PD1 to PDL1 in T cells (Taube et al., 2016).

The binding of PD1 to PDL1 hampers T cell-mediated immune surveillance, causing a lack of immune response and potential T cell apoptosis. Additionally, tumor infiltration is retarded and results in reduced cytokine levels, including tumor necrosis factor. This paves the way for cancer cells to evade immune reactions (Sage et al., 2010).

For complete T cell activation, a stimulatory signal requires the binding of CD80, and CD40 on APC surfaces to ligands on T lymphocytes, such as CD28, and CD40 ligands. Simultaneously, an inhibitory signal deactivates T lymphocytes post-activation, safeguarding against excessive immune reactions and cell damage (Yano et al., 2019).

CTLA4 Blockers as Immunotherapeutic Agents

T cells are known to contain a molecule called CTLA4 having a crucial role immune checkpoint inhibitory molecule. Its principal role involves modulating early-stage T-cell activation intensity. CTLA4 shares ligands, CD80 (B7.1) and CD86 (B7.2), with the co-stimulatory receptor CD28] (Bakker et al., 1996; Lo and Abdel-Motal, 2017).

T cells are activated by the CTLA4 expression on its surface as these molecules overpower the + co-stimulatory signals released from the CD28 molecule. The reduction in T cell proliferation and IL-2 production is seen because CTLA4, CD80/CD86 interact, and negative signals dominate (Taylor et al., 2009).

Foxp3 enhances and stabilizes CTLA-4 expression. It is evident in typical post-TCR stimulation of T-cells. Moreover,

CTLA-4 initiates reverse signaling via B7, inducing indoleamine-2,3-dioxygenase (IDO). This prompts the catabolism of tryptophan, inhibiting T-cell proliferation (Ribas, 2010).

Anti-CTLA-4 therapy involves the enhancement of co-stimulation such as using irradiated tumor cells expressing GM-CSF. Preclinical studies show that CTLA-4 blockade strengthens therapeutic immunity against cancer. Currently, the use of antibodies like ipilimumab and tremelimumab greatly enhances the anti-tumor effect, and malignant diseases are targeted effectively (Chen et al., 2018).

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