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RECENT ADVANCEMENTS AND FUTURE TREATMENTS FOR TRIPLE NEGATIVE BREAST CANCER

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ABSTRACT

Triple-negative breast cancer (TNBC) is a subtype of breast cancer that lacks the expression of estrogen, progesterone, and HER2 receptors, and is associated with poor prognosis and high risk of metastasis. This paper reviews the current advances in the molecularly targeted therapies for TNBC, focusing on the role of the immune system and natural medications. It discusses the evidence for the efficacy of immune checkpoint inhibitors and poly (ADP-ribose) polymerase inhibitors, as well as the potential of natural drug extracts such as paclitaxel and curcumin. It also explores the challenges and opportunities for developing more effective and personalized treatments for TNBC patients.

KEYWORDS: triple-negative breast cancer, targeted therapy, immune system, natural medications.

INTRODUCTION

According to the American Cancer Society, breast cancer (BC) is now the second most common cause of cancer-related deaths among women, and its prevalence is rising each.[1,2] HER-2 overexpression, luminal A and luminal B, and triple-negative breast cancer (TNBC) subtypes are the key characteristics of breast cancer (BC) based on the expression of biomarkers such as progesterone, estrogen, and Ki67.[3] TNBC is a distinct subtype of BC that accounts for 15% to 20% of BC. It is characterized by the absence of HER2, progesterone, and estrogen receptor expression on the cell surface.[4,5] Gene expression profiling revealed that TNBC belonged to the basal-like BC subtype.[6] In contrast to other BC subtypes, TNBC typically affects young females. additionally is associated with increased malignancy and mortality.[7,8] Patients with metastatic triple-negative breast cancer (TNBC)

have a median overall survival of only 18 months, and the disease is linked to an extremely high risk of metastasis.[9] With advances in the understanding of pathways connected to TNBC, surgical procedures, radiation treatment, chemotherapy, and neoadjuvant therapy have improved patient survival rates. The prognosis of patients is still greatly threatened by the adverse effects and rising medication resistance connected to various treatment approaches. As a result of these conventional approaches' inability to satisfy the therapeutic needs of treatment, research into more potent treatment strategies to slow the advancement of TNBC has taken precedence.

Significant research conducted in the past ten years has demonstrated that the immune system plays a major role in determining how TNBC develops. In both adjuvant and neoadjuvant situations of TNBC, the presence of tumor-infiltrating lymphocytes (TILs), as determined by immunohistochemical labeling, is widely acknowledged as a predictor of excellent prognosis.[10,13] Furthermore, a more thorough analysis of immune infiltrates can identify TNBC patients who have a better prognosis after neoadjuvant chemotherapy, such as those with a high ratio of CD8+/FOXP3+ or a large number of cytotoxic (CD8+) TILs.[14] The expression of immune evasion molecules, such as programmed death-ligand 1 (PD-L1), in the tumor microenvironment has also been demonstrated to affect TNBC prognosis in addition to the presence of TILs.[15,18] These findings support the evaluation of immunotherapeutic strategies in TNBC patients, together with the introduction of novel therapy drugs that target immune checkpoint molecules, such as anti-PD-1 and anti-PD-L1 monoclonal antibodies.

Multi-target and multi-path intervention are features of natural medications.[19] Certain natural drug extracts with broad pharmacological effects and little adverse effects, such paclitaxel[20] and curcumin[21], have demonstrated good efficacy in TNBC thus far.

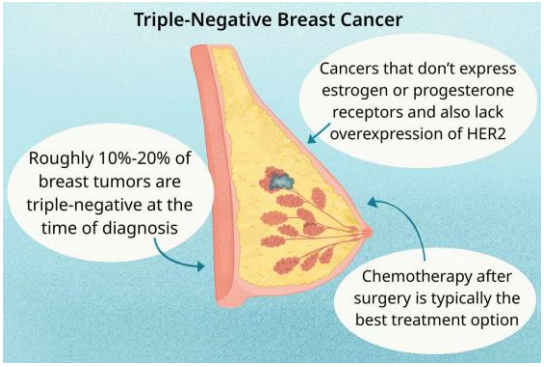

Figure 1: Triple Negative Breast Cancer.

Encouraging clinical outcomes from molecularly focused treatments for triple negative breast cancer (TNBC), such as immune checkpoint inhibition and poly (ADP-ribose) polymerase inhibition. TNBC researchers are also actively pursuing the potential beneficial targeted therapy approaches of inhibiting angiogenesis, epigenetic changes, cell cycle regulation, and signaling kinases (serine/threonine or tyrosine type). TNBC's targeted treatment approaches were investigated in preclinical and clinical research.

Inhibition of Poly (ADP-ribose) Polymerases in TNBC

A class of proteins known as poly (ADP-ribose) polymerases (PARPs) is involved in the repair of DNA damage as well as several other cellular functions. In humans, 17 PARP members have been found thus far.[22] Of the family members, PARP1 has the best character and accounts for 85–90% of the total PARP activity. Single-strand breaks (SSBs) cause it to become active, which in turn catalyzes the creation of poly (ADP-ribose) chains, which act as a platform and signal for the recruitment of additional DNA repair proteins. Double-strand breaks are created when SSBs cannot be repaired due to a PARP shortage or inhibition (DSBs).

The repair of double-strand breaks (DSBs) in cells that express the BRCA1 and BRCA2 breast cancer susceptibility gene products is possible through a mechanism known as homologous recombination (HR). As a result of coupled PARP depletion, BRCA-mutated cancers are more susceptible to PARP inhibition.

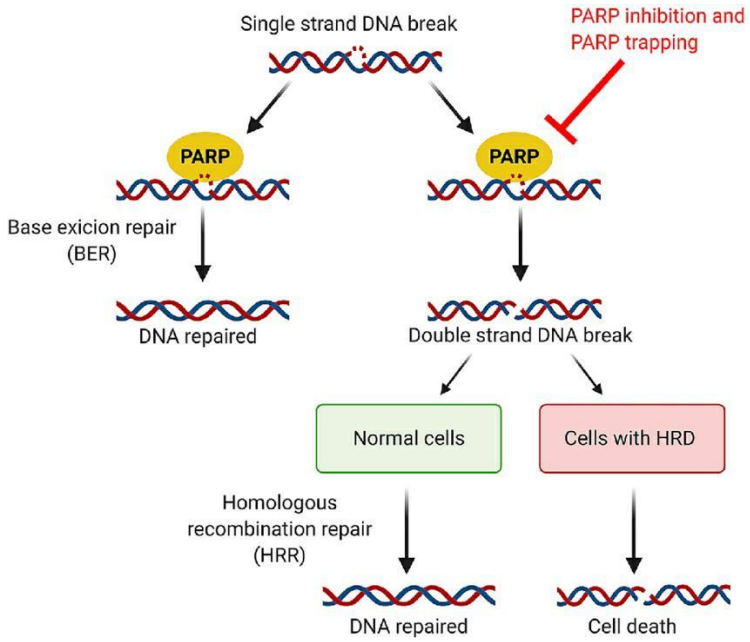

Figure 2: Inhibition of poly- polymerase in TNBC.

As well as HR repair, a phenomenon known as "synthetic lethality".[23–24] When PARP inhibitors (PARPi's) are present, cells with BRCA abnormalities are unable to repair DNA damage and ultimately perish, but cells with functional BRCAs are able to effectively repair DNA damage and continue to live (figure 2). BRCA1 expression is either negligible or absent in up to 80% of ER/PR-negative breast tumors.[26] Despite the fact that germline mutations in BRCA1/2 are usually rare, they can carry a lifetime risk of up to 85% for breast cancer development, with 90% of these tumors being triple-negative.[26] Thus, in BRCA-mutated cancers, a synthetic lethality method based on PARP inhibition may be used to treat TNBC.

PARP Inhibitors and Clinical Trials in TNBC

Synthetic lethality brought on by BRCA and PARPI deficiencies. When cells are exposed to PARPi's (olaparib, rucaparib, niraparib, and talazoparib), single strand breaks (SSBs) transform into double strand breaks (DSBs). While homologous recombination can mend these breaks, cells with complete BRCA function may be able to survive, while cells with faulty BRCA are unable to repair double strand breaks (DSBs). The term "synthetic lethality" describes this phenomena.

Homologous Recombination Deficiency (HRD) as the Predictive Biomarker for PARP Inhibitors

Homologous recombination deficiency (HRD), which is caused by DSB repair defects such as BRCA1/2 and other HR-related gene mutations, can result in genomic instability and increased susceptibility to PARPi’s. Consequently, the response of PARPi’s could be predicted by assessing HRD status, which includes but is not limited to BRCA1/2 mutations.[27,28,29]

Inhibition of Immune Checkpoints in TNBC

Immunotherapy against cancer has garnered a lot of attention lately, especially immune checkpoint-based immunotherapy. This is evidenced by the fact that Tasuku Honjo of Kyoto University and James P. Allison of the University of Texas MD Anderson Cancer Center were awarded the 2018 Nobel Prize in Physiology or Medicine for their groundbreaking work in identifying immune checkpoint molecules, such as cytotoxic T lymphocyte-associated protein 4 (CTLA4), programmed cell death-1 (PD1), and programmed death-ligand 1 (PDL1).

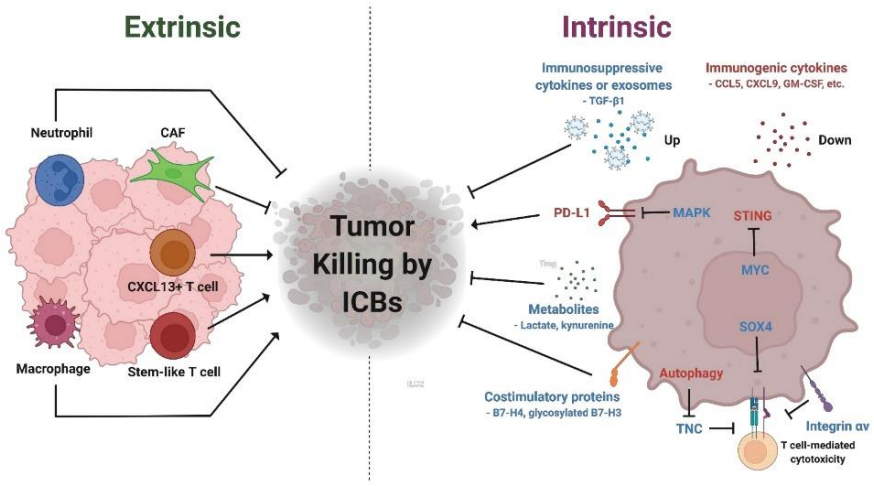

Figure 3: Immune checkpoint Inhibitor.

Immune Checkpoint Inhibitors and Clinical Trials in TNBC

TNBC immune checkpoint blockage. The antigens on the surface of cancer cells known as major histocompatibility complexes (MHCs) are recognized by the cytotoxic T lymphocyte (CTL) through the TCR. When PD1 and its ligand PDL1 attach to the surface of CTLs, the CTL's activity is suppressed, ultimately resulting in cell death. CTLs express CTLA4, an additional inhibitory immunological checkpoint molecule. These immune checkpoint proteins are inhibited by antibodies (anti-CTLA4/ipilimumab, anti-PD1/pembrolizumab and nivolumab, anti-PD1/atezolizumab and durvalumab) in order to activate CTLs again and destroy cancer cells.

PDL1 Expression, Tumor Mutation Burden (TMB), and Immune Infiltration as Predictive Biomarkers of Immune Checkpoint Inhibitors

High expression of PDL1 has been linked in clinical studies to immune checkpoint inhibitor effectiveness in metastatic triple negative breast cancer. PDL1 may therefore be a promising predictive biomarker of immunotherapy response. There are two companion diagnostics for PDL1 expression that are based on antibodies. Patients can be chosen for pembrolizumab treatment utilizing the PDL1 IHC 22C3 pharmDx (Agilent Technologies), with a combined positive score (CPS) cutoff of 10. Using a cutoff immune cell (IC) score of 1%, Roche Diagnostics' Ventana PDL1 (SP142) assay is approved for use in metastatic TNBC patients receiving atezolizumab treatment.[30,31]

Application of Antibody-Drug Conjugates in TNBC

Complexly constructed therapies known as antibody-drug conjugates (ADCs) are made up of cytotoxic drugs that bind to the antibody through a linker and monoclonal antibodies that precisely identify tumor-associated antigens. ADCs are ingested by endocytosis and have the ability to target cells accurately. After that, they break down to produce cytotoxic chemicals, which ultimately cause cell death. By reducing the amount of normal tissues that are exposed to the cytotoxic chemicals, this targeted therapeutic delivery strategy may be able to lessen off-target toxicity.[33]

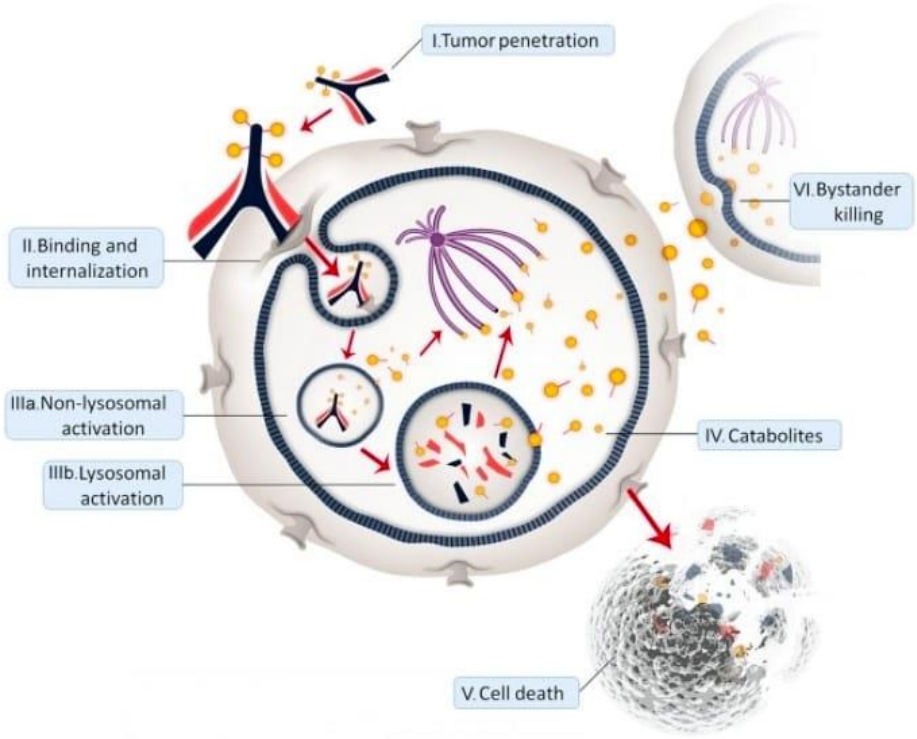

Figure 4: mechanism of Antibody-Drug Conjugates.

The component of sacituzumab govitecan is an antibody that binds to trophoblast cell-surface antigen 2 (Trop2) and combines with SN-38, an inhibitor of topoisomerase I, via a cleavable CL2A linker. Sacituzumab govitecan was tested in a phase III ASCENT study (NCT02574455) against single-agent chemotherapy for patients with metastatic TNBC who had relapsed or were refractory. PFS and OS were significantly extended, and the pCR rate rose in the sacituzumab govitecan group.[32] Sacituzumab govitecan has recently been approved for metastatic TNBC patients who have received two prior lines of therapy based on the therapeutic effect.

Inhibition of Signaling Pathways in TNBC

Certain signaling pathways, like EGFR and its downstream PI3K/Akt/mTOR pathway (Figure 5), are highly active in cancer cells and may hasten the start and progression of tumors. For TNBC patients, blocking these signaling pathways may therefore be a useful treatment approach.

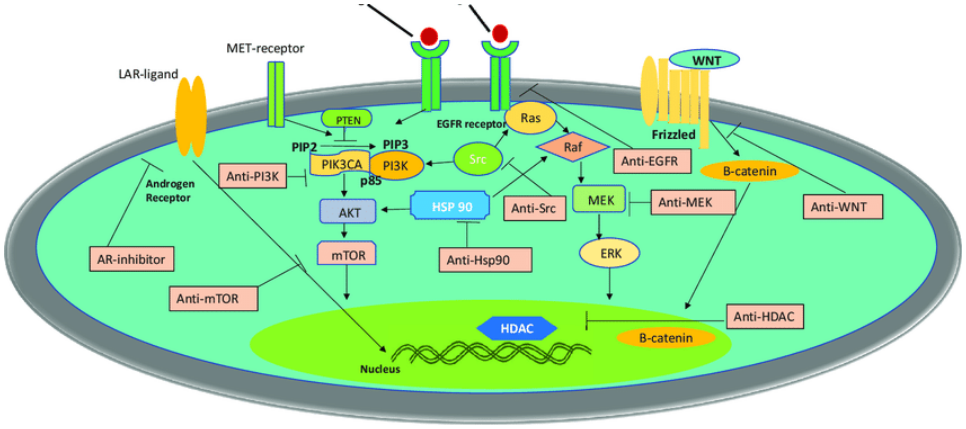

Figure 5: Inhibition of signalling pathway in TNBC.

EGFR Inhibition

suppression of EGFR and downstream signaling pathways in tumor necrosis. The ligands EGF and TGFa (transforming growth factor a) have the ability to activate the epidermal growth factor receptor (EGFR). Following activation, it can form homo- or hetero-dimers with all HER family members, thereby initiating a variety of downstream signaling pathways, including PI3K/Akt/mTOR, Ras/Raf/MEK/ERK, and PLCY/PKC. By inhibiting signal transduction, EGFR inhibitors (cetuximab, TKIs, panitumumab, and erlotinib), PI3K inhibitors (BKM120), mTORC1 inhibitors (rapamycin), and Akt inhibitors (ipatasertib) may be able to prevent tumor development and progression.

PI3K/Akt/mTOR Inhibition

Phosphoinositide 3-kinase (PI3K) is a lipid kinase that catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate (PIP2) to inositol 1,4,5-trisphosphate (IP3) after being activated by receptor tyrosine kinases (RTKs). IP3 recruits phosphoinositide-dependent kinase 1 (PDK1) and Akt, which are both found close to the plasma membrane. Then, PDK1 phosphorylates Akt at Thr308, partially activating it. When mTORC2 phosphorylates Akt at Ser473, Akt is fully activated.[34] PTEN and INPP4B adversely control the PI3K/Akt/mTOR signaling pathway, which is essential for cell growth, proliferation, angiogenesis, and metabolism.[35,36]

Among TNBC, the PI3K/Akt/mTOR pathway is a significant oncogenic driver. In TNBC, there are 23.7% activation mutations in PIK3CA, the gene encoding PI3K's catalytic subunit.[37] TNBC treatment appears to be promising when the PI3K/Akt/mTOR signaling pathway is inhibited.

Inhibition of Angiogenesis in TNBC

Without blood channels, solid tumors could not spread to other organs or develop larger than necessary.[38] Therefore, preventing tumor angiogenesis may stop the flow of oxygen and nutrients into the intertumoral space, stopping tumor growth. Angiogenesis has been shown to be significantly influenced by vascular endothelial growth factor (VEGF) and its receptor, VEGFR.[39] Rapid tumor growth and the possibility of metastasis are caused by the stimulation of cellular pathways by VEGF signaling, which encourages the production of intertumoral blood vessels.

Solid tumors couldn't grow beyond a certain size or metastasize to another organ without blood vessels.[38] Thus, blocking tumor angiogenesis could cut off intertumoral oxygen and nutritional supply and arrest tumor growth.

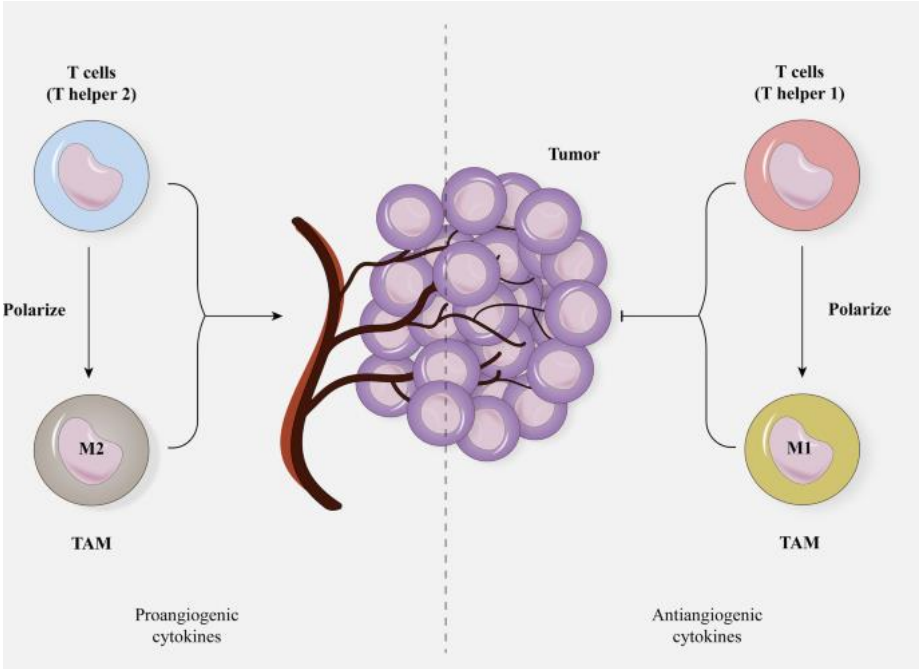

Figure 6: Inhibition of Angiogenesis in TNBC.

Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) have been demonstrated to be major contributors to angiogenesis.[39] The VEGF signaling stimulates

cellular pathways that promote the formation of intertumoral blood vessels, leading to rapid tumor growth and metastatic potential.VEGF is highly expressed in TNBC and a higher VEGF content is significantly correlated with shorter relapse-free survival (RFS) as well as OS.[40] Bevacizumab is a humanized antibody binding to VEGF-A, the prototype VEGF family member, which prevents VEGF from interacting with its receptor, VEGFR.

Inhibition of Epigenetic Modifications in TNBC

Epigenetic modifications often specify stably heritable changes in phenotype resulting from changes in a chromosome without alterations in the DNA sequence.[41] With decades of research, epigenetic modifications have emerged as fundamental players in cancer development and progression, which mainly include DNA modifications (such as DNA methylation) and histone modifications (such as histone deacetylation) (Figure).[42] DNA methylation recruits proteins involved in gene repression or inhibits the binding of transcription factors to DNA to regulate gene expression.[43] Histone modifications could influence chromatin compaction and accessibility through many ways, including acetylation, phosphorylation, ubiquitinylation, and sumoylation.[44] Additionally, epigenetic modifications are being developed as clinical biomarkers for diagnostic, prognostic, and therapeutic applications in tumors.[45,46] Therefore, inhibiting DNA methylation and histone deacetylation may be a probable targeted therapeutic strategy.

DNMT (DNA methyltransferases) Inhibition

The process of adding a methyl group to the cytosine ring's 5′ position in CpG dinucleotides is known as DNA methylation. Promoter hypermethylation may be a key factor in the progression of primary breast cancer by inhibiting tumor suppressor genes, like BRCA1, in tumors.[47,48] According to a study based on the examination of numerous cases of breast cancer, methylation of BRCA1 is a major factor in the development of breast tumors and is aberrantly methylated in sporadic tumors. Furthermore, there is a negative correlation between ER and PR expression and BRCA1 methylation.

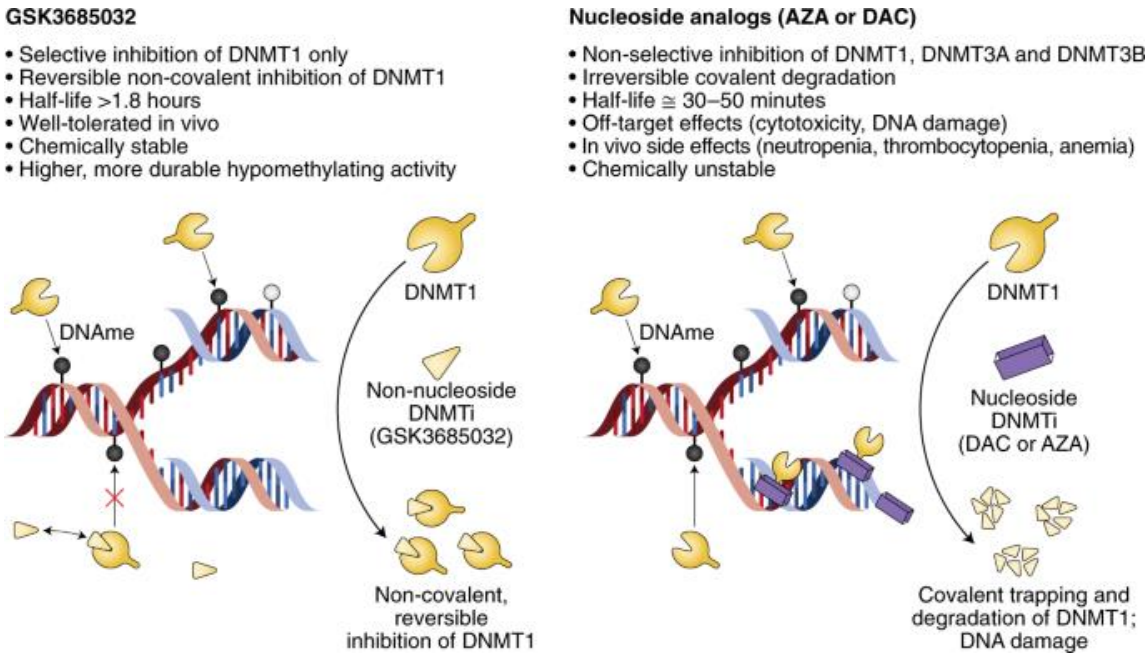

Figure 7: DNMT inhibition.

DNA methyltransferases (DNMTs) are the enzymes that start DNA methylation. DNMT1, DNMT2, DNMT3A, and DNMT3B are the enzymes that make up the DNMT family; in humans, DNMT1 is the essential maintenance methyltransferase.[49] When DNMT1 was compared to other subtypes, it was strongly expressed in TNBC. In breast cancer, DNMT1 expression was inversely correlated with OS.[50] In TNBC cells carrying wild-type BRCA1, a preclinical investigation demonstrated that PARPi's with DNMT inhibitors (DNMTi's, 5-azacytidine/AZA, decitabine/DAC) enhanced PARPi efficacy and resulted in further tumor suppression compared with each medication alone.[51] DNMTi's have been approved by the US FDA to treat various cancers, such as myeloid malignancies, and may be promising drugs for the treatment of TNBC, despite the fact that the trial was only preclinical.

HDAC (histone deacetylase) Inhibition

An enzyme called histone deacetylase (HDAC) deacetylates histone proteins. Histone deacetylation causes chromatin condensation, which in turn suppresses transcription and suppresses the expression of genes. Tumor cell invasion, migration, proliferation, and angiogenesis are linked to the negative regulation of the tumor suppressor gene. HDAC inhibitors (HDACi's), on the other hand, have the ability to reverse the inhibition of gene expression by chromatin relaxation and histone hyperacetylation. More precisely, HDACi's may prevent angiogenesis, cell migration, and invasion while also inducing death in tumor cells.

In a preclinical investigation, scientists discovered that the HDACi entinostat (ENT) enhanced the expression of aromatase and estrogen receptor-α (ERα) in breast cancer cells, thereby restoring the cells' susceptibility to the aromatase inhibitor letrozole in both in vitro and in vivo settings. These findings showed that ER-negative and endocrine therapy-resistant breast cancer might be treated with a combination of aromatase inhibitors and histone deacetylase inhibitors.[52] According to Sulaiman et al., TNBC had higher levels of mTORC1 and HDAC expression than luminal breast cancer. In TNBC cells, co-inhibition of mTORC1 and HDAC with rapamycin with valproic acid consistently increased the expression of the estrogen receptor 1 (ESR1) gene.[53] HDAC inhibitors decrease the percentage of CD4Foxp3+ T cells and raise PDL1 and HLA-DR expression in TNBC. Tumor shrinkage, cell apoptosis, and TIL infiltration were all aided by PD1 and CTLA4 inhibition. Consequently, in TNBC, HDAC inhibition by HDACi's may enhance the tumor-suppressive effects of immunotherapy.[54] Another study has shown that by controlling the expression of homologous recombination repair (HRR)-related genes and impeding DNA repair, the HDACi suberoylanilide hydroxamic acid (SAHA) may increase the anti-tumor effects of the PARPi olaparib in TNBC cells.[55]

Inhibition of Cell Cycle in TNBC

Four orderly phases, known as G1 (resting stage), S (DNA synthesis), G2 (protein synthesis), and M (mitosis), comprise the cell cycle (Figure). Several checkpoints stop the cell cycle to allow cells to appropriately repair errors during DNA synthesis and chromosome segregation, ensuring the integrity of the cell cycle.[56] When cyclins are generated and removed during the cell cycle, cyclin-dependent kinases (CDKs) are triggered and aid in the advancement of the cell cycle.[57] Dysregulated CDKs in tumors frequently cause unplanned proliferation.

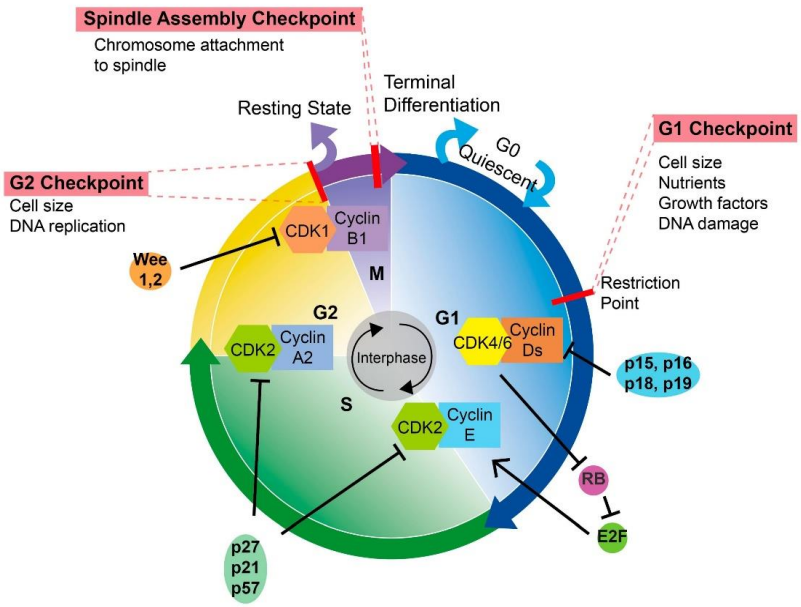

Figure 8: Inhibition of cell cycle in TNBC.

It is commonly recognized that CDK4/6 inhibitors are essential in halting the growth of cancer cells because they stop the cell cycle during the G1 to S transition by causing the dephosphorylation of the retinoblastoma tumor suppressor protein (Rb).[58] The FDA has approved palbociclib, ribociclib, and abemaciclib as CDK4/6 inhibitors for the treatment of HR-positive or HER2-negative breast cancer as of right now.[59,62] However, because loss of Rb frequently occurs, the therapeutic impact of CDK4/6 inhibitors in TNBC is low. Numerous studies have shown that combining inhibition or therapy with other molecules, such as immune checkpoint blocking, PI3K inhibition, AR inhibition, or chemotherapy, may be able to help TNBC patients overcome their medication resistance.[65] Dual inhibition of CDK4/6 and PI3K exhibited a synergistic impact in a preclinical research and could cause immunogenic cell death in TNBC cells.[63] Rb-positive TNBC cells may be more sensitive to paclitaxel after receiving palbociclib pretreatment.[64] There are ongoing Phase I/II clinical trials investigating the safety and effectiveness of CDK4/6 inhibition in TNBC, either in combination with or apart from other therapies (anti-androgen medicine, anti-PDL1 antibody, and chemotherapeutic medications).[65]

TTK protein kinase inhibitors are another family of drugs that specifically target the cell cycle. The spindle assembly checkpoint (SAC), which maintains the stability and integrity of the genome during mitosis, is under the direction of TTK, specifically monopolar spindle 1 (MPS1).[66] Because TNBC expresses many mitotic checkpoint molecules, TTK inhibitors

may be able to stop TNBC from growing and proliferating.[67] Half-maximal inhibitory concentration (IC50) values for MPS1/TTK inhibitors ranged from 0.05 to 1.0 μM in a preclinical experiment that showed the, Inhibitors' anti-proliferative effects in basal BC cell lines.[68] In various xenograft models of human TNBC, Anderhub et al. Demonstrated that the combination of paclitaxel with MPS1 inhibitor BOS172722 results in significant in vivo efficacy, displaying significant tumor shrinkage compared with either medication alone.[69]

Maintaining the numerical integrity of the centriole and centrosome depends on Polo-like kinase 4 (PLK4), a regulator of centriole duplication. PLK4 inhibitors would increase genomic instability and aneuploidy, ultimately resulting in the death of cancer cells.[70] In comparison to control or single-agent treatment, an in vitroexperimental study demonstrated that a novel PLK4 inhibitor, CFI-400945, in conjunction with radiation, demonstrated a synergistic anti-cancer effect in TNBC cell lines and patient-derived organoids and significantly increased tumor endpoint survival in xenograft models in vivo.[71] Nonetheless, centrosome amplification (CA), which raises the risk of breast cancer, is invariably associated with PLK4 overactivation.[72] It is necessary to conduct additional preclinical research to fully understand the molecular mechanisms of action of this combination, as well as any possible clinical uses. This will provide the theoretical groundwork for PLK4 to be a viable target in triple negative breast cancer.

Beyond this, ATR, CHK1, WEE1, and TRAIL may be other TNBC targets. ATR or CHK1 inhibitor has been shown in preclinical studies to hinder TNBC cell viability and postpone radiation-induced DNA repair.[73,74] Contrarily, WEE1 inhibition may be able to eliminate TNBC cells' cisplatin resistance[75], while TRAIL receptor agonist may induce apoptosis in TNBC cells that expressed vimentin and Axl.[76]

Phytochemicals from medicinal plants to treat TNBC

Since ancient times, phytochemicals have been utilized to treat cancer. A number of traditional medical systems have used plant extracts to treat cancer. Green tea polyphenols, resveratrol, and curcumin are among the phytochemicals that have been shown in numerous recent studies to be beneficial in the treatment of TNBC. It has been demonstrated that these phytochemicals target specific TNBC signaling pathways and inhibit the growth of cancer cells. Many phytochemicals have been shown to target multiple common cancer hallmarks, and many of these phytochemicals exert their anticancer actions through the same molecular mechanisms.

Synthetic 13-arylalkyl derivatives were produced as a result of berberine's anticancer properties, which are attributed to the isoquinoline plant source. Berberine inhibits the transcriptional activity of κ-catenin and increases the production of E-cadherin via targeting the Wnt/κ-catenin signaling pathway. Up to 20 μM, berberine has relatively minimal cytotoxicity against normal cells. Compared to the natural chemical, several synthetic compounds derived from berberine exhibit greater activity.[77] Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is another phytochemical that inhibits Wnt signaling. This substance can be found in the bark and roots of many different plants that are known to have therapeutic benefits. These consist of Japanese knotweed, buckthorn, and rhubarb. This anthraquinone has the ability to suppress Wnt signaling in colorectal cancer cells, specifically SW480 and SW620.[78]

Citrus fruits include phytochemicals called naringin, naringenin, tangeretin, nobiletin, hesperidin, and hesperetin, some of which have been demonstrated to have anti-metastasis properties. These effects have been seen in xenografted mice models of cancer as well as in a variety of cancer cell lines. These substances specifically target NOTCH, TGF/SMAD, and Wnt signalling.[79]

Together with resveratrol and curcumin, other phytochemicals that show promise in inhibiting cancer in TNBC cells include capsaicin, which is found in chili peppers, quercetin, which is found in onions, and epigallocatechin-3-0-gallate (EGCG), which is found in green tea.[80,83] These phytochemicals have been shown to influence the immune system, inhibit angiogenesis, and encourage apoptosis in TNBC cells.

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