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Review

Phytocannabinoids in Triple Negative Breast Cancer Treatment: Current Knowledge and Future Insights

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Abstract. *Triple negative breast cancer (TNBC) represents an aggressive subtype of breast cancer, which is deficient in estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression. Thus, TNBC cells are unable to respond to the conventional hormonal therapies, making chemotherapy the only therapeutic choice. Patients with TNBC develop metastasis and recurrence over time and have reduced survival compared to patients with other subtypes of breast cancer. Therefore, there is a need for innovative therapies. Data emerged from pre-clinical studies, highlighted various antitumor activities of plant-derived Cannabis sativa and synthetic cannabinoids (CBs), including delta-9-tetrahydrocannabinol (THC) and non-psychoactive cannabidiol (CBD). On the contrary, some studies indicated that CBs might also promote tumor progression. At present, clinical studies on the effects of CBs from Cannabis sativa in cancer patients are few. In the present study, we reviewed known and possible interactions between cannabinoids and TNBC therapies.*

Triple negative breast cancer (TNBC) represents an aggressive subtype of breast cancer, considering its high proliferation index and high rate of local and distant recurrence. Moreover, since TNBC tumors are negative for estrogen receptor (ER), progesterone receptor (PR) or human epidermal growth factor receptor 2 (HER2), they cannot be treated with targeted therapies, such as anti-hormonal or anti-HER2 drugs. Conventional therapies, such as neo-adjuvant chemotherapy, represent the only therapeutic choice; however, there is a high percentage of treated patients, which develop metastases, short recurrence after treatment, and a lower survival rate (1-8). Recently the use of immunotherapy was approved for metastatic PD-L1 positive TNBC (9). Thus, a new schedule for TNBC treatment is required. *Cannabis sativa* plant is also termed marijuana, and since 1990, its derived compounds, the cannabinoids (CBs), have been widely tested for the treatment of several pathologies (*e.g.*, pain, sleep disorders, emesis, depression, cancer) (10-14). Particularly, the FDA already approved the use of CB-based medicines tested in these studies, for clinical application in some countries. For instance, two delta-9-tetrahydrocannabinol (THC) synthetic analogs, nabilone (Cesamet®), and dronabinol (Marinol®), are officially used to overcome the undesirable effects (*e.g.*, nausea, vomit) induced by chemotherapy (15, 16). Nabiximols (Sativex®), a 1:1 mixture of THC and cannabidiol (CBD), is used in multiple sclerosis treatment (17), while CBD oil (Epidiolex®) is administered in pediatric patients suffering from epilepsy (18). Moreover, different types of CBs, have been tested for cancer treatments. Some studies demonstrated that CBs, particularly endocannabinoids (ECs), possess a dual behavior on tumor proliferation, which is strictly related to the concentration used (19). The main cannabinoids are represented by phytocannabinoids, of which the most studied are the THC, with psychotropic activity, and the non-psychoactive CBD. CBD and THC are formed by the decarboxylation at high temperatures, of their acidic precursors, respectively, cannabidiolic acid (CBDA) and Δ9-tetrahydrocannabinolic acid (THCA). Other minor phytocannabinoids with relevant pharmacological features are cannabigerol (CBG), cannabinol (CBN), and cannabidivarin (CBDV) (20). Three types of phenotypes of the *C. Sativa* plant have been identified: i) phenotype I with a higher concentration of THC, ii) phenotype II with variable concentrations of THC and a higher concentration of CBD, iii) phenotype III with a

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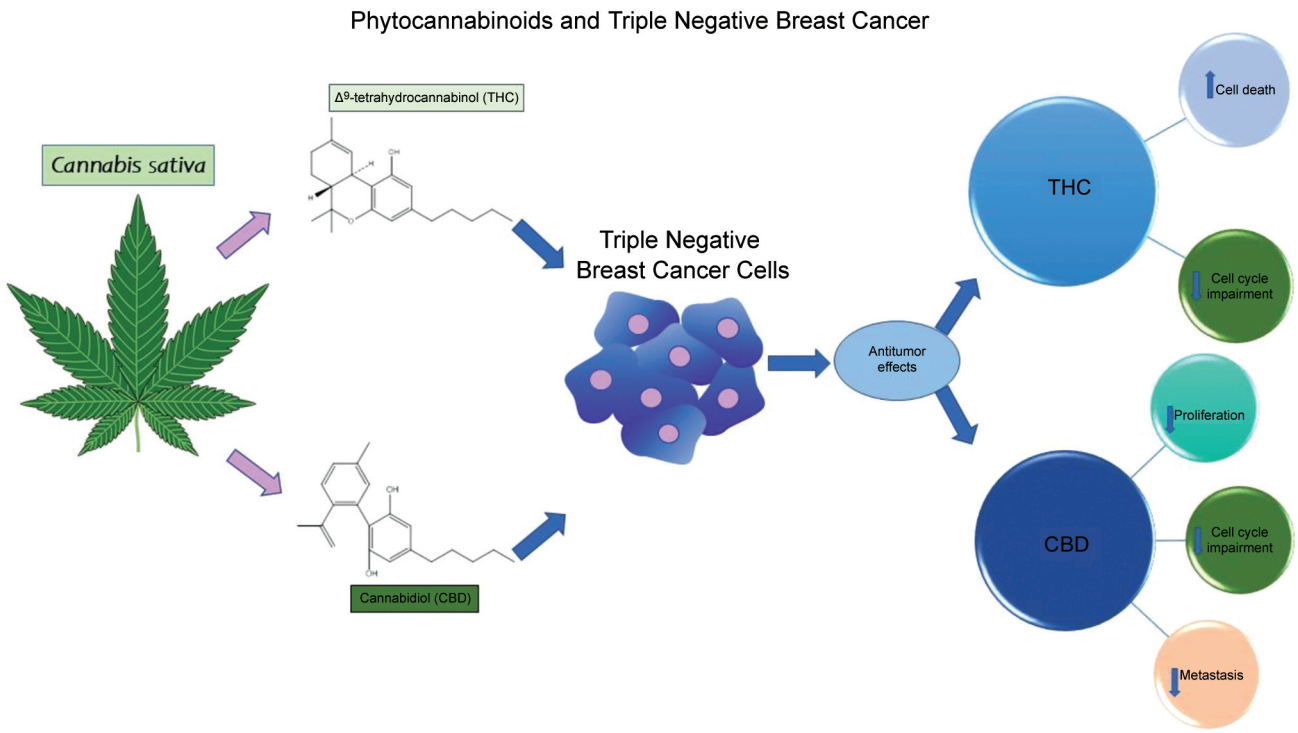

Figure 1. *The antitumor effects of cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) in triple negative breast cancer (TNBC) treatment.*

higher concentration of CBD (21). Other types of cannabinoids are endogenous cannabinoids, endocannabinoids, and synthetic cannabinoids, which have shown a high anticancer potential, but also many side effects (10). CBs interact with specific receptors, termed cannabinoid receptors (CB-R), which exert many functions, and particularly, their antitumor activity in several tumors (including breast cancer), through the modulation of different signaling pathways (22). To date, two receptors have been isolated and identified namely CB1-R and CB2-R. CB1-R is expressed in the central nervous system and possesses many biological functions, whereas CB2-R is expressed in peripheral cells and the immune system and is able to modulate immune cell migration and cytokine release (23, 24). Both receptors have different expression profiles in various types of cancer (25). Here, we revise the published studies on the effects of phytocannabinoids in TNBC and highlight their potential use in clinical practice.

Phytocannabinoids: Chemical Structure and Biological Effects

Delta-9-tetrahydrocannabinol (THC). THC is a psychotropic component of *C. sativa* with known structure (Figure 1) (23, 24). Regarding the absorption kinetics of THC (which depend on the exposure route, as in other CBs), when THC is inhaled, it spreads rapidly in the blood, reaching peak concentrations within a few minutes. Subsequently, when its concentration decreases, the formation of psychoactive metabolites is arrested. THC has low oral viability, its principal active metabolite featured by psychoactive activity is 11-hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC). This compound is then degraded into inactive forms, which are then excreted in the urine over a period varying between hours and days (25). Side effects (*e.g.*, conjunctivitis and impairment of attention and memory) associated with THC toxicity may occur in both pediatric patients and adults after inhalation (2-3 mg) or ingestion (5-20 mg) of THC. In addition, treatment with THC at high doses can cause more severe symptoms (*e.g.*, panic, delirium, *etc.*) in both pediatric patients and adults. Finally, children treated chronically with THC, developed neurological abnormalities and more severe cognitive deficits (26, 27). THC acts as a partial agonist of CB1-R and CB2-R. Thus, its psychotropic effects (*e.g.*, euphoria and dysphoric reactions) are determined by a complex process mediated by CB1-R. Specifically, THC, by activating CB1-R in the central nervous system, inhibits the function of gamma-aminobutyric acid/glutamatergic neurotransmission system and the release of dopamine (28). In addition, a preclinical study conducted on cannabinoid-induced tetrad mouse models, showed that THC by activating CB1-R, provoked analgesia, hypolocomotion, hypothermia, and catalepsy (29). Moreover, activation of

CB2-R and PPARs by THC exerts anti-inflammatory, anti-proliferative, and neuroprotective effects (30, 31). Interaction of THC with other receptors has been reported by De Meijer *et al*. (14). As reported above, the activation of CB-R induced by CBs, is linked to their antitumor effects. Specifically, the activation of CB2-R induced by THC arrests the cell cycle at the G2/M phase and alters the expression of cell division control 2 (Cdc2) (32, 33). Moreover, CB2-R activation, promotes apoptosis and inhibits the proliferation of cancer cells, through the activation of different transcription factors (34). Moreover, CB2-R activation also induces peroxisome proliferator-activated receptor gamma (PPARγ)-regulated pathways in carcinoma cells, thus conferring THC anti-inflammatories properties (35). Finally, THC antagonizes the tumor-promoting effects of the G protein-coupled receptor (GPR), by acting on the single receptor and CB2-R-GPR55 heterodimers. These latter enhance tumor growth by interfering with extracellular signal-regulated kinases (ERK)-1/2 and cyclic adenosine monophosphate (cAMP) signaling pathways (36).

Cannabidiol (CBD). CBD is a non-psychotropic component of *C. Sativa* (Figure 1) (37, 38) with pharmacokinetic properties depending on the route of administration. Typically, CBD is excreted into the urine, in its glucuronidated form or intacted, as the hydroxylated 7-carbonyl CBD derivatives, which have displayed anti-inflammatory properties in animal studies. Specifically, preclinical studies conducted by testing CBD on different models of inflammation, demonstrated that the 7-carbonyl metabolites, block oxidative stress by inhibiting the formation of reactive oxygen species (ROS) and by reducing the formation of nitric oxide (NO) (39, 40). It has been shown that CBD modulates negatively CB1-R and CB2-R activities (41, 42). Studies have also reported that many pharmacological effects linked to CBD, are provoked by its binding to other GPRs (*e.g*., GPR18, GPR55) and other receptors (12, 43, 44). CBD counteracts some THC-induced effects (*e.g*., tachycardia, anxiety, *etc*.), probably due to its low affinity for CB-R (45). Several pre-clinical and clinical studies have highlighted many biological effects (*e.g*., anti-inflammatory, neuroprotective, antioxidative, antiemetic, and analgesic effects) associated with CBD, varying according to its concentration and the study models adopted (46, 47).

Minor phytocannabinoids. Similarly, to THC and CBD, other phytocannabinoids (*e.g.,* cannabinol, cannabigerol, cannabidivarin, and cannabichromene) exert anti-inflammatory effects due to activation of CB-R and other receptors. They have a variable and low concentration in *C. sativa*, but they are highly concentrated in specific cultivated types (48). Cannabinol (CBN) is highly concentrated in aging plants. Presumably, CBN arises as a THC metabolite during the storage of harvested plants (49). CBN binds to CB1-R and CB2-R agonists and has a higher affinity to CB2-R than to CB1-R. Cannabigerol (CBG), is a partial agonist for CB1-R and CB2-R, has no-psychotropic effects, and can inhibit the activation of CB1-R. Moreover, a putative use of CBG in the treatment of neurological disorders has been suggested (49, 50). Moreover, cannabichromene (CBC) has psychotropic properties, stimulates bone growth, and has anticonvulsive effects. Its use can be suggested for the treatment of hypothermia hypomotility, and catalepsy (51). Tetrahydrocannabivarin (THCV) acts as a partial agonist of CB-R in *in vitro* conditions. In *in vivo* studies, THCV has been shown to act as a CB1-R antagonist or agonist in a dose-dependent manner. Furthermore, THCV has antimicrobial and anti-convulsive properties and may prevent obesity (52, 53).

Phytocannabinoids in Triple-negative Breast Cancer: An Update on Preclinical Studies

Data from experimental studies highlight the antitumor effects of phytocannabinoids in TNBC through the regulation of different molecular pathways (Table I and Figure 1). TNBC cells are more aggressive and thus more sensitive to CB treatment (54). Most of these studies were conducted by using CBD. Ligresti *et al*. (55) and Bisogno *et al*. (56), demonstrated that CBD inhibited the proliferation of MDA-MB-231 cells, by inducing apoptosis *via* two possible mechanisms of action: i) the activation (direct or indirect) of vanilloid transient receptor potential vanilloid type-1 (TRPV1) and the involvement of CB2-R, ii) the increase in intracellular Ca^{2+} levels and ROS caused by unknown CBD targets. Similar findings were described by Shrivastava *et al*. (57). They demonstrated that CBD causes the death of TNBC cancer cells by inhibiting protein kinase B (Akt) and mechanistic target of rapamycin (mTOR) signaling, inducing endoplasmic reticulum (ER) stress, and promoting ROS generation. Moreover, CBD mediates a balance between mitochondria-mediated apoptosis and autophagy in MDA-MB-231 breast cancer cells. Sultan *et al*. (58), demonstrated that CBD treatment stimulates an interaction between mTOR, PPARγ, and cyclin D1, thus promoting MDA-MB-231 cell apoptosis. Interestingly, Elbaz *et al*. (59) demonstrated that CBD modulates the tumor microenvironment (*e.g*., reduced proliferation, invasion, and impaired cell migration), by inhibiting the epidermal growth factor (EGF)/epidermal growth factor receptor (EGFR) signaling in several TNBC cell lines (4T1.2, SUM159, and SPC2). Specifically, CBD inhibits EGF-induced activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), that possesses a tumor pro-survival effect causing resistance to chemotherapy. Furthermore, the authors demonstrated that CBD arrests matrix metalloproteinases (MMPs) secretion, thus inhibiting the effects of EGF induced on the

Table I. *Antitumor effects of phytocannabinoids in triple negative breast cancer (TNBC): evidence from preclinical studies.*

Model	Phyto-CB	Antitumoral effects	Mechanism	References
MDA-MB-231 cells	CBD	Cell proliferation inhibition; induction of apoptosis	CB1-R, CB2-R, TRPV1 and unknown target; increased ROS production	55, 56
MDA-MB-231 cells	CBD	Cell proliferation inhibition; induction of apoptosis; autophagy	AKT/mTOR inhibition, endoplasmic reticulum stress, increased ROS production	57
MDA-MB-231 and T-47D cells	CBD	Induction of apoptosis	Inhibition of mTOR, up-regulation of PPARγ, and cyclin D1	58
4T1.2, SUM159, and SPC2 cells	CBD	Cell proliferation inhibition; cell migration and invasion impairment	Inhibition of EGF)/(EGFR) signaling, down-regulation of NF-κB, down-regulation of MMPs	59
MDA-MB-231 and 4T1 cells	CBD	Cell proliferation inhibition	Down-regulation of ID-1 *via* ERK signaling	60
MDA-MB-231	CBD	Cell proliferation inhibition	Increased ROS production	61
Orthotopic xenograft generated from 4T1 cells in syngeneic BALB/c mice and heterotopic xenograft generated from MDA-MB-231 cells in athymic mice	CBD	Cell proliferation inhibition	Down-regulation of ID-1	57, 61
MDA-MB-231 cells	CBDA	Cell migration inhibition	Modulation of the activity and the expression of COX-2 *via* CB1-R and CB2-R	63-65
MDA-MB-231 cells	CBDA	Cell growth and migration inhibition	Inhibition of cAMP-(PKA) through the activation of the small GTPase, and RhoA, *via* CB1-R and CB2-R	65
MDA-MB-231 cells	CBDA	Invasiveness reduction	Down-regulation of ID-1 and SHARP-1	66
MDA-MB-231 cells	CBDA	Cell growth inhibition	*Via* CB2-R	55
MDA-MB-231 and MDA-MD-468 cells	THC	Cell proliferation inhibition	Down-regulation of ID-1	61
MDA-MB-231 cells	THC	Cell growth inhibition	Activation of CB2-R	55
MDA-MB-231, MDA-MB468 cells	THC	Induction the apoptosis, cell cycle arrest thought inhibition of G2-M transition	Down-regulation of Cdc2 *via* CB2-R	67
MDA-MB-231 and 4T1 cells, orthotopic xenograft generated from 4T1 cells in syngeneic mice	THC	Enhancement of cell growth and metastasis	Increased production of IL-4 and IL-10, suppression of Th1 response and Th2-associated cytokine secretion *via* CB1-R and CB2-R	68
MDA-MB-231 cells	THCA	Cell growth inhibition	CB2-R	55
MDA-MB-231 and MDA-MB-468 cells	CBN	Cell proliferation and invasiveness inhibition	Down-regulation of ID-1	61
MDA-MB-231 and MDA-MB-468 cells	CBG	Cell proliferation and invasiveness inhibition	Down-regulation of ID-1	61
MDA-MB-231 cells	CBG	Cell growth inhibition	*via* CB2-R	55
MDA-MB-231 cells	CBC	Cell growth inhibition	*via* CB2-R	55

Phyto-CB: Phytocannabinoid; CB1-R: cannabinoid receptor 1; CB2-R: cannabinoid receptor 2: CBD: cannabidiol; CBDA: cannabidiolic acid; THC: delta-9-tetrahydrocannabinol; THCA: Δ9-tetrahydrocannabinolic acid; CBN: cannabinol; CBG: cannabigerol; CBC: cannabichromene; TRPV1: vanilloid transient receptor potential vanilloid type-1; ROS: reactive oxygen species; AKT: protein kinase B; mTOR: mechanistic target of rapamycin; ER: endoplasmic reticulum; PPARγ: peroxisome proliferator-activated receptor gamma; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; MMP: metalloproteinase; ID-1: inhibitor of DNA binding 1; ERK: extracellular signal-regulated kinase; COX-2: cyclooxygenase 2; cAMP: cyclic adenosine monophosphate; PKA: protein kinase A; GTP: guanosine diphosphate; RhoA: Ras homolog family member A; SHARP: split-and hairy-related protein; Cdc2: cell division control 2; IL-4: interleukin-4; IL-10: interleukin-10; Th1: T helper 1; Th2: T helper 2.

cytoskeleton. McAllister *et al*., in two distinct studies (60, 61), highlighted an inhibitory role of CBD in TNBC proliferation by interfering with cell cycle progression and by promoting apoptosis. Specifically, the authors showed that CBD decreases the expression of inhibitor of DNA Binding 1 (ID-1) in metastatic breast cancer cells (*e.g*., MDA-MB231,

the 4T1) leading to the inhibition of tumor aggressiveness through ERK. Moreover, it was reported that CBD, in MDA-MB231 cells, increases ROS levels, which in turn down-regulates the activity of ID-1, potentiating apoptotic effects. Additionally, the inhibitory effects of CBD on cell proliferation were also verified by performing experiments on different mouse models of TNBC (57). In both cases, CBD reduced tumor growth, but over time resistance to CBD developed. Similarly, as accurately reported by Almeida *et al*. (62), CBD inhibits mTOR and cyclin D1 expression, thus inducing apoptosis in ER+ breast cells. Moreover, CBD enhances apoptosis in ER+ breast tumors, by activating TRPV1 through ER stress. Altogether these studies showed that CBD displays significant antitumor properties, particularly in TNBC, where it acts as a promising therapeutic agent. Several experiments were also conducted with CBDA, the precursors of CBD in MDA-MB-231 cells (63-65). Data emerged from these studies, demonstrated that CBDA inhibits cell migration *via* CR-1 and CR-2, by modulating Cyclooxygenase (COX)-2 activity and expression (63-65). Moreover, Takeda *et al*. (65), showed that CBDA inhibits MDA-MB-231 cell growth and migration, by playing an inhibitory effect on cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) through the activation of the small guanosine diphosphate (GTP)ase, Ras homolog family member A (RhoA). Additionally, CBDA can attenuate the invasiveness of MDA-MB-231 cells by down-regulating ID-1 and split-and hairy-related protein (SHARP)-1 (66). Finally, the inhibitory role of CBDA on MDA-MB-231 cell growth *via* CB2-R, was reported by Ligresti *et al*. (55). Fewer studies on TNBC cells were conducted with THC, while consistent results were obtained on HER2+ and ER+ breast tumors (62). Specifically, as reported by Mc Allister *et al*. (60), THC possesses anti-proliferative activity on MDA-MB-231 and MDA-MD-468 cells and reduces the invasiveness *via* ID-1. Furthermore, THC and its precursor THCH, inhibit MDA-MB-231 cell growth *via* CB2-R (55). Interestingly, Caffarell *et al*. (67), showed that THC inhibits the progression of cell cycle thought the inhibition of G2-M transition *via* down-regulation of cell division cycle 2 (Cdc2) and thus inducing the apoptosis of breast cancer cells (*e.g*., MDA-MB-231, MDA-MB468). Different data were described by McKallip *et al*. (68). Authors demonstrated that THC enhances the growth of breast cancer and the formation of metastasis by increasing the production of Interleukin (IL)-4 and IL-10, by suppressing the cell-mediated T helper (Th) 1 response, and by potentiating the Th2-associated cytokine secretion in MDA-MB-231 and 4T1 cells, and in mouse mammary carcinoma 4T1. Pre-clinical studies were also performed with minor phytocannabinoids, CBN, CBG, and CBC highlighting interesting results in breast cancer cells. In MDA-MB-231 and MDA-MB-468 cells, CNB and CBG inhibited cell proliferation and reduced the invasiveness of cells, probably by reducing ID-1 expression (60). Moreover, CBG and CBC inhibited MDA-MB-231 cell growth by activating CB2-R (55). Altogether, the preclinical studies highlighted the antitumor effects of phytocannabinoids on TNBC models, by regulating several signaling pathways involved in cell proliferation and by enhancing apoptosis and autophagy through the actions on CB-R. Further studies will be necessary to unravel the molecular mechanisms underlying the antitumor effects of phytocannabinoids.

Phytocannabinoids in Patients With Breast Cancer: An Update on Clinical Studies

In contrast to the pre-clinical studies mentioned above, few clinical studies on the antitumor effects of CBs have been conducted in breast cancer patients, probably due to unknown underlying molecular mechanisms (62). Several clinical studies (69) have evaluated the safety and efficacy of CBs and their preparation alone or combined with chemotherapeutic agents in cancer patients. Data emerged from these studies demonstrated that CBs, being well-tolerated, can modulate pain, thus acting as palliative substances, and ameliorating the undesirable effects induced by chemotherapy (70). Moreover, clinical studies demonstrated that dronabinol (Marinol®) and nabilone (Cesamet®), two THC synthetic analogs, are less effective in relieving cancer-related but overcome the side effects (*e.g*., nausea, vomit) induced by chemotherapy (15, 16). Important clinical aspects concerning chemotherapy have been reported by Fraguas-Sanchez *et al*. (71) on chemotherapy with paclitaxel and doxorubicin treatment in breast cancer and by Ward *et al*. (72) on the inhibition of paclitaxel-induced neuropathic pain by cannabidiol.

Concluding Remarks and Future Perspectives

Pre-clinical studies proved shreds of evidence that CBD and THC, possess antitumor and anti-inflammatory activities on TNBC, by acting on specific molecular signaling pathways *via* action through CB-R (highly expressed in breast cancer cells). Taken thus, phytocannabinoids can be viewed as promising agents for inhibiting TNBC progression, which has scarce therapeutic options and is featured by inauspicious prognosis and low survival rates. Some preclinical studies, mainly conducted with THC in TNBC, showed that phytocannabinoids can also act as pro-tumoral agents. This dual behavior depends on the CBs concentration tested, since lower concentrations promote cancer cell proliferation, while higher concentrations inhibit cancer cell growth thus enhancing cancer cell death. Thus, this issue should be considered to develop therapies with satisfactory results. Despite the abundant pre-clinical studies, few clinical trials on the antitumor roles of CBs have been conducted in patients with cancer, probably due to the underlying molecular mechanism not yet known. Some clinical

studies have been performed to evaluate the safety and the effectiveness of CBs in several cancer types, including breast cancer, but more additional studies would be necessary to understand the potential of these molecules in TNBC and other breast cancer subtypes. Overall, apart from the need for other studies aimed to dissect the molecular pathways underlying the antitumor CBs' properties, phytocannabinoids should be considered as potential agents for inhibiting TNBC progression.

Conflicts of Interest

The Authors have no conflicts of interest to disclose in relation to this study.

Authors' Contributions

Conceptualization, S. Bimonte, M. Cascella and G. Palma; A. Cuomo; writing—original draft preparation; S. Bimonte and M. Cascella: writing—review and editing. All Authors have read and agreed to the published version of the manuscript.

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