

CANNABIDIOL AS POTENTIAL ANTICANCER DRUG

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Summary

Over the past years, several lines of evidence support an antitumorigenic effect of cannabinoids including Δ^9 -tetrahydrocannabinol (Δ^9 -THC), synthetic agonists, endocannabinoids and endocannabinoid transport or degradation inhibitors. Indeed, cannabinoids possess anti-proliferative and pro-apoptotic effects and they are known to interfere with tumour neovascularization, cancer cell migration, adhesion, invasion and metastasization. However, the clinical use of Δ^9 -THC and additional cannabinoid agonists is often limited by their unwanted psychoactive side effects, and for this reason interest in non-psychoactive cannabinoid compounds with structural affinity for Δ^9 -THC, such as cannabidiol (CBD), has substantially increased in recent years. The present review will focus on the efficacy of CBD in the modulation of different steps of tumourigenesis in several types of cancer and highlights the importance of exploring CBD/CBD analogues as alternative therapeutic agents.

The endocannabinoid system: a brief overview

The endocannabinoid system (eCB) is a recently discovered signalling system comprising the cannabinoid CB₁ and CB₂ receptors, their intrinsic lipid ligands, endocannabinoids (eCBs), such as the N-arachidonylethanolamide (anandamide, AEA) and the 2-arachidonoylglycerol (2AG), and the associated enzymatic machinery (transporters, biosynthetic and degradative enzymes).

The cannabinoid CB₁ and CB₂ receptors are both G protein-coupled receptors: CB₁ receptors are highly distributed in the central nervous system (CNS), with low to moderate expression in periphery, whereas CB₂ receptors are high in the immune system, with much lower and more restricted distribution in the CNS [1, 2].

Endogenous ligands for the cannabinoid receptors were discovered soon after their characterization.

The two major known endogenous ligands are anandamide (AEA) and 2-arachidonoylglycerol (2AG) [3-6]. Both are arachidonic acid derivatives produced from phospholipid precursors through

activity-dependent activation of specific phospholipase enzymes [7]. Later on, a number of other eCB ligands have been discovered, including N-arachidonoyldopamine, N-arachidonoylglycerolether and O-arachidonylethanolamine [8].

AEA and 2-AG do not share the same biosynthetic or metabolic pathways. Different pathways can produce AEA from the phospholipid precursor N-arachidonoyl-phosphatidylethanolamine, the most important being a direct conversion catalysed by an N-acyl-phosphatidylethanolamine-selective phosphodiesterase. 2AG is mainly synthesized through activation of phospholipase C and subsequent production of diacylglycerol, which is converted to 2AG by diacylglycerol lipase. After its re-uptake, AEA is hydrolysed by the enzyme fatty acid amide hydrolase (FAAH), producing arachidonic acid and ethanolamine, while 2AG is primarily metabolized by monoacylglycerol lipase, leading to the formation of arachidonic acid and glycerol [9]. Apart from their binding to CB₁ and CB₂ receptors, eCBs may bind to other receptors: for example, AEA may intracellularly activate the potential vanilloid receptor type 1 (TRPV1) [10]. Moreover, other putative cannabinoid receptors are the 'orphan' G protein-coupled receptor, GPR55 [11], and the peroxisome proliferator-activated receptor, PPAR [12, 13]. However, CB₁ and CB₂ receptors are certainly the most known targets for AEA and 2AG, which activate them with different affinity: AEA has the highest affinity in both cases, whereas 2AG has the highest efficacy in both cases [14].

Physiological or pathological stimuli induce synthesis and release of endocannabinoids, which can subsequently activate cannabinoid receptors. Therefore eCBs are synthesized and released 'on demand' through the cleavage of membrane phospholipid precursors.

Multiple sclerosis and spinal cord injury, neuropathic pain, cancer, atherosclerosis, stroke, myocardial infarction, hypertension, glaucoma, obesity/metabolic syndrome and osteoporosis are some of the diseases in which alterations in the eCB system have been demonstrated, thus paving the way for new therapeutic strategies aimed at restoring normal eCB system functionality [15].

Accepted Article

Currently, the term ‘cannabinoid’ refers to more than 100 terpenophenols derived from *Cannabis sativa* [16], as well as to synthetic compounds that directly or indirectly interact with cannabinoid receptors. Δ^9 -THC is the most psychoactive component of the plant *Cannabis sativa*, and its biological actions as well as the ones of synthetic cannabinoid compounds (synthetic compounds active on cannabinoid receptors) are primarily mediated by CB₁ and CB₂ receptors. Cannabinoids are further classified into phytocannabinoids (subclassified in different categories according to their chemical structures, such as Δ^9 -THC, Δ^8 -THC, cannabinal, CBD and cannabicyclol), synthetic compounds active on cannabinoid receptors (i.e., JWH133, WIN55212-2, SR141716) and endocannabinoids (i.e., AEA and 2-AG) which are produced endogenously (Fig. 1).

Cannabinoids in the treatment of cancer

Cannabinoids are currently used in cancer patients to palliate wasting, emesis and pain that often accompany cancer. A significant advancement in cannabinoid use in cancer treatment came from the discovery of a potential utility of these compounds for targeting and killing cancer cells. In 1975 Munson et al. [17] demonstrated that the administration of Δ^9 -THC, Δ^8 -THC and cannabinal inhibited the growth of Lewis lung adenocarcinoma cells *in vitro* as well as *in vivo* after oral administration in mice. The interest in anticarcinogenic properties of cannabinoids was even renewed after the discovery of the eCB system and the cloning of the specific cannabinoid receptors. Since then, several cannabinoids have been shown to exert anti-proliferative, and pro-apoptotic effects in various cancer types (lung, glioma, thyroid, lymphoma, skin, pancreas, uterus, breast, prostate and colorectal carcinoma) both *in vitro* and *in vivo* [18–26]. Moreover, other antitumorogenic mechanisms of cannabinoids are currently emerging, showing their ability to interfere with tumour neovascularisation, cancer cell migration, adhesion, invasion and metastasization [27].

However, the clinical use of Δ^9 -THC and additional synthetic agonists is often limited by their unwanted psychoactive side effects, and for this reason interest in non-psychoactive phytocannabinoids, such as CBD, has substantially increased in recent years. Interestingly CBD has no psychotropic activity and, although its very low affinity for both CB₁ and CB₂ receptors, it has been recently reported to act with unexpectedly high potency *in vitro* as antagonist of CB₁ receptors in the mouse vas deferens [28] and brain [29] tissues. Additionally, CBD displays inverse agonism at human CB₂ receptors [29]. Moreover, other putative CBD's molecular targets are TRPV, 5-HT_{1A}, GPR55 and PPAR γ receptors (see Fig. 2). Interestingly CBD does not have any psychotropic activity. Besides its beneficial effects in the treatment of pain and spasticity and other CNS pathologies, several reports demonstrated that CBD possesses antiproliferative, pro-apoptotic effects and inhibits cancer cell migration, adhesion and invasion.

This review will focus on the most recent evidence regarding the efficacy of CBD in the modulation of tumorigenesis in several types of cancer. The data available so far are summarized in Table 1 and are discussed in detail in the following paragraphs.

CBD and breast cancer

In 2006 Ligresti et al. [30] demonstrated for the first time that CBD potently and selectively inhibited the growth of different breast tumour cell lines (MCF7, MDA-MB-231), with an IC₅₀ of about 6 μ M, and exhibited significantly lower potency in non-cancer cells. CBD and CBD-rich extracts (containing approximately 70% CBD together with lesser amounts of other cannabinoids) also inhibited the growth of xenografts, obtained by *s.c.* injection into athymic mice of human MDA-MB-231 cells, and reduced infiltration of lung metastases derived from intrapaw injection of breast carcinoma cells. Among the possible cellular and molecular mechanisms underlying these effects, CBD seemed to involve direct TRPV1 activation and/or CB₂ indirect activation (via FAAH), as well as induction of oxidative stress. Later on, McAllister's group [31] demonstrated

that, besides proliferation, CBD also interfered with two other crucial steps of breast cancer cell progression: invasion and metastasization. Among the three different groups of cannabinoid compounds tested (phytocannabinoids with affinity for CB₁ and CB₂ receptors; phytocannabinoids with no appreciable affinity for CB₁ and CB₂ receptors; synthetic compounds with affinity for CB₁ and CB₂ receptors), CBD was shown to be one of the most effective inhibitors of human breast cancer cell proliferation, being equipotent to Δ^9 -THC and CP55940 in inhibiting respectively MDA-MB-231 and MDA-MB-436 cell growth, and resulting as the most potent inhibitor of the MDA-MB-231 cell migration. Interestingly, CBD regulated the expression of key genes involved in the control of cell proliferation and invasion through the downregulation of Id-1 expression, an inhibitor of basic helix-loop-helix transcription factors, whose overexpression in breast cancer cells is responsible for proliferation, migration and invasion. Therefore, the ability of CBD to significantly decrease Id-1 expression in breast cancer cells was associated with its efficacy in reducing tumour aggressiveness.

Four years later, the same group [32] demonstrated that the observed effect of CBD on Id-1 expression was mediated by the upregulation of the extracellular signal-regulated kinase phosphorylation (pERK). Indeed, the ERK inhibitor, U0126, partially reverted CBD-induced inhibition of proliferation and invasion as well as its effect on Id-1 expression. Besides ERK upregulation, also the production of reactive oxygen species (ROS) was shown to mediate CBD-induced effects on cell proliferation and Id-1 expression, since the use of a ROS scavenger (tocopherol) reversed the aforementioned CBD effects. Moreover, these authors demonstrated that CBD was effective in reducing the primary tumour mass and the size and number of metastatic foci *in vivo*.

Finally, the excellent paper of Shrivastava et al. [33] adds fresh light on the cellular mechanism through which CBD induces cell death in breast cancer cells. These authors showed that CBD induced a concentration-dependent cell death of both estrogen receptor-positive and estrogen

receptor-negative breast cancer cells with a mechanism independent of CB₁, CB₂ and TRPV1 receptor activation. Interestingly, the effective concentrations of CBD in tumour cells have little effect on non tumorigenic, mammary cells. Moreover, electron microscopy revealed morphologies consistent with the coexistence of autophagy and apoptosis, these events being promoted by the induction of endoplasmic reticulum (ER) stress and the inhibition of Akt/mTOR/4EBP1 signalling. In addition, CBD-driven increase in ROS production is fundamental to induce both apoptosis and autophagy. Examining further the cellular mechanism involved in CBD-induced cell death, they found that CBD reduced mitochondrial membrane potential, triggered the translocation of the Beclin2 Interacting Protein (Bid) to the mitochondria and the release of cytochrome C to the cytosol and, ultimately, the activation of the intrinsic apoptotic pathway.

Finally, the relationship between CBD-induced apoptosis and autophagic cell death was explored by blocking each form of cell death with specific inhibitors. Caspase inhibition reduced CBD-induced apoptosis and the expression of protein markers in breast cancer cells, whereas the inhibition of autophagy enhanced apoptosis as a compensatory/alternative mechanism of cell death. The apoptosis:autophagy ratio in CBD-induced cell death seemed to be mediated via Beclin1, a key signalling molecule in the autophagic process. CBD treatment induced the cleavage of Beclin1 and the subsequent translocation of the cleavage product to the mitochondria where it may induce apoptosis through the enhancement of cytochrome C release [34, 35].

As a whole this work highlights the presence of a complex balance between autophagy and mitochondria-mediated apoptosis in CBD-induced breast cancer cell death and strengthens the idea that CBD can be considered as an alternative agent for breast cancer therapy. Figure 3 shows a schematic representation of the signalling pathways associated with the effect of CBD in breast cancer cell proliferation and invasion.

CBD and glioma

CBD possesses anti-tumoural properties also in gliomas, tumours of glial origin characterized by a high morphological and genetic heterogeneity and considered one of the most devastating neoplasms, showing high proliferative rate, aggressive invasiveness and insensitivity to radio- and chemotherapy.

After the seminal paper of Jacobsson et al. (2000) [36] demonstrating a serum-dependent effect of CBD upon C6 murine glioma cells proliferation, Massi et al. in 2004 [37] reported that CBD was effective in inhibiting U87-MG and U373 human glioma cell proliferation *in vitro* through the induction of apoptosis. Interestingly, CBD did not affect viability of non-transformed primary glial cells [38]. When tumour xenografts were generated in immune-deficient mice, *in vivo* intratumoural treatment with CBD significantly reduced tumour growth [37].

The anti-proliferative effect of CBD was cannabinoid and vanilloid receptors independent; the CB₂ receptor antagonist SR144528 reverted CBD effect, but in a weak and transient manner [37]. More important, this paper demonstrated for the first time that the anti-tumour effect of CBD involved the induction of oxidative stress, through increased early production of ROS, depletion of intracellular glutathione and increased GSH-associated enzymatic activity. Accordingly, CBD anti-proliferative effect was reversed by the anti-oxidant, tocopherol. Importantly, CBD did not induce ROS production in non-transformed primary glial cells [38].

Among the cellular events involved in glioma cell death, CBD produced a time-dependent release of cytochrome C and activation of caspase-8, -9 and -3, suggesting the involvement of both the intrinsic and extrinsic apoptotic pathways [38]. Marcu et al. [39] later confirmed the efficacy of CBD in inhibiting the growth of multiple glioblastoma cell lines in a more potent way than Δ^9 -THC.

Interestingly, combined treatment of Δ^9 -THC with CBD demonstrated that CBD enhanced Δ^9 -THC inhibitory effect on glioblastoma cell growth, but not on invasiveness [39]. In line with this, more recently Torres et al. [40] confirmed that combined treatment with CBD and Δ^9 -THC greatly reduced several human glioma cell viability enhancing both autophagy and apoptosis and triggering

caspase-3 activation. Moreover, combined administration of submaximal doses of CBD and Δ^9 -THC reduced the growth of U87-MG cell-derived tumour xenografts in nude mice at a higher extent than the treatment with the individual compounds, suggesting the potential use of a combinatory therapy which would reduce the amount of the psychoactive Δ^9 -THC.

The synergistic effect of combined therapy implied *in vitro* cell cycle arrest, ROS induction and apoptosis through sustained activation of caspases-3, -7 and -9, as well as specific modulation of the extracellular signal-regulated kinase, ERK [39]. These effects were not observed with either compound individually, indicating them as a prerogative of combination treatment. Differently from Marcu's data [39], our recent results [41] showed that, both in U87-MG and in Δ^9 -THC-resistant T98G human glioma cell lines, CBD *per se* strongly down-regulates two signalling molecules crucial in tumour cell proliferation such as ERK and PI3K/Akt. In addition it inhibited the hypoxia-inducible transcription factor HIF-1 α , one of the most critical stimuli for cell survival, motility and tumour angiogenesis. Thus, inhibition of these three molecules appears as part of the multiple molecular targets for CBD anti-neoplastic activity [41].

Further biochemical analysis of glioma tumour tissues excised from nude mice treated *in vivo* with CBD indicated a significant decrease of activity and content of 5-LOX, as well as a marked stimulation of FAAH and a decrease of AEA content [42].

Besides cell growth, CBD reduced glioma cell migration [43] and invasiveness in a Boyden chamber test [39], at concentrations lower than those required to inhibit cell proliferation. CBD anti-migratory effect was not antagonized by the selective cannabinoid receptor antagonists or by pretreatment with pertussis toxin, indicating no involvement of classical cannabinoid receptors and/or G_{i/o} protein-coupled receptors.

As a whole, CBD seems to counteract glioma cell proliferation and invasion through multiple mechanisms, as summarized in Figure 4.

CBD and leukaemia/lymphoma

Gallily et al. [44] provided first evidence of a possible exploitation of CBD in the treatment of lymphoblastic diseases. They demonstrated that CBD treatment induced apoptosis, through caspase-3 activation in human acute myeloid leukaemia HL-60 cell line, whereas had no effect on human monocytes from normal individuals.

Later on, McKallip et al. [45], using the murine EL-4 lymphoma cell line and the human Jurkat and Molt-4 leukaemia cell lines, demonstrated that CBD exposure led to a significant CB₂ receptor-mediated decrease in the number of viable cells as well as to the induction of apoptosis, both *in vitro* and *in vivo*.

In Jurkat cells, CBD exposure resulted in the activation of caspase-8, -9, and -3, the cleavage of poly(ADPribose) polymerase, and the decrease in full-length Bid, suggesting a possible cross-talk between the intrinsic and extrinsic apoptotic pathways. Moreover, exposure to CBD led to the loss of mitochondrial membrane potential and subsequent release of cytochrome C. As in other tumour cells, CBD-induced apoptosis required an increase of ROS production. Finally, CBD decreased the levels of phospho-p38 mitogen-activated protein kinase [45], and this effect was blocked by treatment with a CB₂-selective antagonist or ROS scavenger. In addition, CBD treatment caused a significant reduction in tumour burden and increased the level of apoptotic tumours in EL-4-bearing mice [45].

Together, the results suggest that CBD, acting through CB₂ receptors and ROS production, may represent a novel and highly selective treatment for leukaemia. Moreover, previous evidence indicated that human leukaemias and lymphomas expressed significantly higher levels of CB₂ receptors compared to other tumour cell lines, suggesting that tumours of immune origin may be highly sensitive to the CB₂-mediated effects of CBD [46].

CBD and lung cancer

Given the poor response of lung cancer to available therapy and their aggressive biological nature, a series of targets and new therapeutic strategies for their treatment are currently being investigated [47-50].

Recently, Ramer et al. [51-53] investigated the effect of CBD on the invasive properties of A549 cells, a line of human lung carcinoma cells expressing both CB₁ and CB₂, as well as TRPV1 receptors. Using Matrigel invasion assays, they found a CBD-driven impaired invasion of A549 cells that was reversed by CB₁ and CB₂ receptors as well as TRPV1 antagonists.

CBD treatment concomitantly upregulated tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) and the CBD-elicited decrease in tumour cell invasiveness was reversed by knocking down TIMP-1 expression through a siRNA approach. These results suggest a causal link between TIMP-1 upregulation and CBD anti-invasive action. CBD was also shown to induce p38 and ERK phosphorylation as upstream mechanisms for TIMP-1 induction and subsequent decreased invasiveness. Interestingly all these cellular events were blocked by cannabinoids or TRPV1 receptor antagonists.

The significant inhibition of A549 cell invasion following CBD treatment was also accompanied by the downregulation of another important factor involved in the regulation of cell spreading, the plasminogen activator inhibitor PAI-1 [52]. CB₁ and CB₂ as well as TRPV1 receptor antagonists suppressed the observed effect of CBD on PAI-1 secretion and cell invasion. Recombinant human PAI-1 and PAI-1 siRNA led to a concentration-dependent up- and down-regulation of invasiveness, respectively, suggesting a crucial role of PAI-1 in A549 invasiveness.

Additionally, *in vivo* studies in thymic aplastic nude mice revealed a significant inhibition of A549 lung metastases following CBD treatment [51] and a significant downregulation of PAI-1 protein was demonstrated in A549 xenografts of CBD-treated rats [52].

It is worth to note that CBD decreased invasiveness in a range of therapeutically relevant concentrations (0.01 to 0.05µM), since the peak plasma concentrations of CBD in healthy

volunteers following administration of Sativex™ (1:1 ratio of Δ^9 -THC and CBD) was reported to be between 0.01 μ M to 0.05 μ M [54].

Together, these findings provide a novel mechanism underlying CBD anti-invasive action on human lung cancer cells and imply its use as a therapeutic option for the treatment of highly invasive cancers.

CBD and endocrine tumours

Thyroid cancer is the most common endocrine malignancy, and a work by Ligresti et al. [30] demonstrated that CBD exerted anti-proliferative effects on rat thyroid KiMol cells, transformed with the v-K-ras oncogene. This effect of CBD was associated with a cell cycle block at the G1/S phase transition, as well as the induction of apoptosis.

Later on, Lee et al. [55] demonstrated that CBD markedly induced apoptosis in both murine thymocytes and EL-4 thymoma cells, with CBD-mediated apoptosis occurring earlier in EL-4 cells than in thymocytes. The cellular events triggered by CBD were similar in both T cell, ROS generation playing a pivotal role. The presence of N-acetyl-L-cysteine (NAC), a precursor of glutathione, markedly attenuated the induction of apoptosis, and restored the diminished levels of cellular thiols.

The observation that CBD induced oxidative stress in thymocytes, EL-4 cells and splenocytes [56] substantiates the notion that, unlike monocytes, T cells both primary and immortalized are all sensitive and respond similarly to CBD, with a central role of ROS generation.

CBD and colon cancer

Colon cancer is a major cause of morbidity and mortality in Western countries. A recent paper from Izzo's group [57] demonstrated the chemopreventive effect of CBD in a preclinical animal model of colon cancer based on azoxymethane (AOM) administration in mice. AOM treatment was

associated with Aberrant Crypt Foci (ACF), polyps, and tumour formation, as well as with the upregulation of phospho-Akt, iNOS and COX-2 and the downregulation of caspase-3. CBD was effective in reducing ACF, polyps and tumours and counteracted AOM-induced phospho-Akt and caspase-3 changes. *In vitro* studies, supported the beneficial effect of CBD. Indeed, in colorectal carcinoma cell lines, CBD protected DNA from oxidative damage, increased endocannabinoid levels and reduced cell proliferation in a CB₁-, TRPV1- and PPAR γ -antagonist sensitive manner. In the light of its safety records, these results suggest that CBD might be worthy of clinical consideration in colon cancer prevention.

CBD and angiogenesis

Angiogenesis consists in the formation of new blood vessels from pre-existing ones and represents another promising therapeutic target for cancer therapy. Collectively, cannabinoids have been demonstrated to act as anti-angiogenic factors by disposing tumour cells to decrease the production of pro-angiogenic factors and/or by direct modulation of endothelial cells [58].

Surprisingly, so far no study investigated the effect of CBD on angiogenesis. Our data currently under publication [59] demonstrated that CBD potently inhibited HUVE cells proliferation, migration and invasion through the induction of endothelial cell cyostasis without triggering apoptosis. Interestingly, CBD also affected endothelial cell differentiation into tubular capillaries as well as the outgrowth of capillary-like structures from HUVEC spheroids *in vitro*. In addition, the anti-angiogenic properties of CBD were demonstrated also *in vivo*, using a matrigel sponge model. These effects were associated with down-modulation of several molecules associated with angiogenesis, including MMP2, MMP9, uPA, Endothelin-1, PDGF-AA and CXCL16.

Collectively, these preliminary data demonstrate that, besides its well known pro-apoptotic anti-proliferative and anti-invasive actions, CBD may also exert anti-angiogenic effects, thus further strengthening its potential application in cancer therapy.

Conclusion and future directions

Collectively, the non-psychoactive plant-derived cannabinoid CBD exhibits pro-apoptotic and anti-proliferative actions in different types of tumours and may also exert anti-migratory, anti-invasive, anti-metastatic and perhaps anti-angiogenic properties. On these bases, evidence is emerging to suggest that CBD is a potent inhibitor of both cancer growth and spreading.

Interestingly, the anticancer effect of this compound seems to be selective for cancer cells, at least *in vitro*, since it does not affect normal cell lines. The efficacy of CBD is linked to its ability to target multiple cellular pathways that control tumorigenesis through the modulation of different intracellular signalling depending on the cancer type considered. The most common effect of CBD is the increase in ROS production that seems to be determinant for triggering its beneficial action in all the considered cancer cell types. The role of cannabinoid/vanilloid receptors in mediating CBD effects is more controversial. In some cases (lung, leukaemia, colon) a clear contribution of these receptors has been demonstrated through the use of specific antagonists, but in other cancer types (glioma and breast) their relevance appears only marginal or absent. Besides the *in vitro* data, the efficacy of CBD in reducing tumour growth and, in some case, metastasization was confirmed in experimental animal models. However, the potential clinical application of CBD for cancer therapy needs some consideration. Its low toxicity is certainly a good starting point. CBD behaves as a non toxic compound; indeed oral administration of CBD 700mg/day for 6 weeks did not show any overt toxicity in humans [60] suggesting its possible exploitation for prolonged treatment. The route of administration appears more problematic since CBD oral absorption is slow and unpredictable. However, 6 weeks of oral CBD treatment 10mg/kg/day provoked a mean plasma level of CBD between 6 and 11ng/ml (about 0.036 μ M) [61] that did not differ significantly over the 6 weeks of administration. Interestingly, this range of concentration was demonstrated to be active in inhibiting lung cancer cell invasion [52, 53] thus suggesting that in some cases the oral route could be the

appropriate choice. Additionally, experimental data showed that combined treatment with CBD and Δ^9 -THC could be more effective in reducing cancer cell proliferation, suggesting that the co-administration may represent a better choice for cancer therapy. Accordingly, oromucosal treatment with SativexTM 10mg (a formulation including 1:1 ratio of CBD and Δ^9 -THC recently approved for multiple sclerosis) provoked CBD plasma level of approximately 0.01 μ M and up to 0.05 μ M in humans, a concentration range effective in reducing lung cell invasion *in vitro*. Thus, the results obtained with SativexTM suggest that the use of different associations of phytocannabinoids in a variable proportion might lead to a better outcome without pharmacokinetic interaction [62]. Moreover, oromucosal administration may represent a first choice in presence of nausea and vomiting. Finally, the use of CBD/Sativex can be suggested in combination with classical chemotherapeutic agents to check for the presence of a synergistic effect that might potentially allow clinical chemotherapeutic dose reduction, thereby reducing toxicity while maintaining efficacy. In the light of its safety records and considering that CBD is already currently used in patients with multiple sclerosis, the findings here summarized suggest that CBD might be worthy of clinical consideration for cancer therapy.

Nomenclature

The drug/molecular target nomenclature conforms to the BJP's Guide to Receptors and Channels [63].

References

- 1 Howlett AC. The cannabinoid receptors. *Prostaglandins Other Lipid Mediat* 2002; 68-69: 619-31.
- 2 Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS, Marnett LJ, Di Marzo V, Pittman QJ, Patel KD, Sharkey KA. Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 2005; 310: 329-32.
- 3 Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 1992; 258: 1946-9.
- 4 Stella N, Schweitzer P, Piomelli D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 1997; 388: 773-8.
- 5 Sugiura T, Kishimoto S, Oka S, Gokoh M. Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. *Prog Lipid Res* 2006; 45: 405-46.
- 6 Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J, Vogel Z. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* 1995 50: 83-90.
- 7 Piomelli D. The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci* 2003; 4: 873-84.
- 8 De Petrocellis L, Di Marzo V. An introduction to the endocannabinoid system: from the early to the latest concepts. *Best Pract Res Clin Endocrinol Metab* 2009; 23: 1-15.
- 9 Di Marzo V, Petrosino S. Endocannabinoids and the regulation of their levels in health and disease. *Curr Opin Lipidol* 2007; 18: 129-40.

- 10** Ross RA. Anandamide and vanilloid TRPV1 receptors. *Br J Pharmacol* 2003; 140: 790-801.
- 11** Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson NO, Leonova J, Elebring T, Nilsson K, Drmota T, Greasley PJ. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 2007; 152: 1092-101.
- 12** O'Sullivan SE. Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. *Br J Pharmacol* 2007; 152: 576-82.
- 13** Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross RA. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB₁ and CB₂. *Pharmacol Rev* 2010 62: 588-631.
- 14** McPartland JM, Norris RW, Kilpatrick CW. Coevolution between cannabinoid receptors and endocannabinoid ligands. *Gene* 2007; 397: 126-35.
- 15** Pacher P, Bátkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev* 2006; 58: 389-462.
- 16** Appendino G, Chianese G, Tagliatalata-Scafati O. Cannabinoids: occurrence and medicinal chemistry. *Curr Med Chem* 2011; 18: 1085-1099.
- 17** Munson AE, Harris LS, Friedman MA, Dewey WL, Carchman RA. Antineoplastic activity of cannabinoids. *J Natl Cancer Inst* 1975; 55: 597-602.
- 18** Galve-Roperh I, Sánchez C, Cortés ML, Gómez del Pulgar T, Izquierdo M, Guzmán M. Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. *Nat Med*; 2000 6: 313-9.
- 19** Sánchez C, de Ceballos ML, Gomez del Pulgar T, Rueda D, Corbacho C, Velasco G, Galve-Roperh I, Huffman JW, Ramón y Cajal S, Guzmán M. Inhibition of glioma growth in vivo by selective activation of the CB(2) cannabinoid receptor. *Cancer Res* 2001; 61: 5784-9.

- 20 Casanova ML, Blázquez C, Martínez-Palacio J, Villanueva C, Fernández-Aceñero MJ, Huffman JW, Jorcano JL, Guzmán M. Inhibition of skin tumour growth and angiogenesis in vivo by activation of cannabinoid receptors. *J Clin Invest* 2003; 111: 43–50.
- 21 Blázquez C, Carracedo A, Barrado L, Real PJ, Fernández-Luna JL, Velasco G, Malumbres M, Guzmán M. Cannabinoid receptors as novel targets for the treatment of melanoma, *FASEB J* 2006; 20: 2633–5.
- 22 Carracedo A, Gironella M, Lorente M, Garcia S, Guzmán M, Velasco G, Iovanna JL. Cannabinoids induce apoptosis of pancreatic tumor cells via endoplasmic reticulum stress-related genes. *Cancer Res* 2006; 66: 6748–55.
- 23 Cianchi F, Papucci L, Schiavone N, Lulli M, Magnelli L, Vinci MC, Messerini L, Manera C, Ronconi E, Romagnani P, Donnini M, Perigli G, Trallori G, Tanganelli E, Capaccioli S, Masini E. Cannabinoid receptor activation induces apoptosis through tumor necrosis factor alpha-mediated ceramide de novo synthesis in colon cancer cells. *Clin Cancer Res* 2008; 14: 7691–700.
- 24 Bifulco M, Di Marzo V. Targeting the endocannabinoid system in cancer therapy: a call for further research. *Nat Med* 2002; 8: 547–50.
- 25 Bifulco M, Laezza C, Pisanti S, Gazerro P. Cannabinoids and cancer: pros and cons of an antitumour strategy. *Br J Pharmacol* 2006; 148: 123–35.
- 26 Bifulco M, Malfitano AM, Pisanti S, Laezza C. Endocannabinoids in endocrine and related tumours. *Endocr Relat Cancer* 2008; 15: 391–408.
- 27 Freimuth N, Ramer R, Hinz B. Antitumorigenic effects of cannabinoids beyond apoptosis. *J Pharmacol Exp Ther* 2010; 332: 336–44.
- 28 Pertwee RG, Ross RA, Craib SJ, Thomas A. (-)-Cannabidiol antagonizes cannabinoid receptor agonists and noradrenaline in the mouse vas deferens. *Eur J Pharmacol* 2002 456: 99–106.

- 29** Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG. Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *Br J Pharmacol* 2007 150: 613-23.
- 30** Ligresti A, Moriello AS, Starowicz K, Matias I, Pisanti S, De Petrocellis L, Laezza C, Portella G, Bifulco M, Di Marzo V. Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *J Pharmacol Exp Ther* 2006; 318: 1375-87.
- 31** McAllister SD, Christian RT, Horowitz MP, Garcia A, Desprez PY. Cannabidiol as a novel inhibitor of Id-1 gene expression in aggressive breast cancer cells. *Mol Cancer Ther* 2007; 6: 2921-7.
- 32** McAllister SD, Murase R, Christian RT, Lau D, Zielinski AJ, Allison J, Almanza C, Pakdel A, Lee J, Limbad C, Liu Y, Debs RJ, Moore DH, Desprez PY. Pathways mediating the effects of cannabidiol on the reduction of breast cancer cell proliferation, invasion, and metastasis. *Breast Cancer Res Treat* 2011; 129: 37-47.
- 33** Shrivastava A, Kuzontkoski PM, Groopman JE, Prasad A. Cannabidiol induces programmed cell death in breast cancer cells by coordinating the cross-talk between apoptosis and autophagy. *Mol Cancer Ther* 2011; 10: 1161-72.
- 34** Cho DH, Jo YK, Hwang JJ, Lee YM, Roh SA, Kim JC. Caspase-mediated cleavage of ATG6/Beclin-1 links apoptosis to autophagy in HeLa cells. *Cancer Lett* 2009; 274: 95-100.
- 35** Wirawan E, Vande Walle L, Kersse K, Cornelis S, Claerhout S, Vanoverberghe I, Roelandt R, De Rycke R, Verspurten J, Declercq W, Agostinis P, Vanden Berghe T, Lippens S, Vandenabeele P. Caspase-mediated cleavage of Beclin-1 inactivates Beclin-1-induced autophagy and enhances apoptosis by promoting the release of proapoptotic factors from mitochondria. *Cell Death Dis* 2010; 1:e18.
- 36** Jacobsson SO, Rongård E, Stridh M, Tiger G, Fowler CJ. Serum-dependent effects of tamoxifen and cannabinoids upon C6 glioma cell viability. *Biochem Pharmacol* 2000 60: 1807-13.

- 37 Massi P, Vaccani A, Ceruti S, Colombo A, Abbracchio MP, Parolaro D. Antitumor effects of cannabidiol, a nonpsychoactive cannabinoid, on human glioma cell lines. *J Pharmacol Exp Ther* 2004; 308: 838-45.
- 38 Massi P, Vaccani A, Bianchessi S, Costa B, Macchi P, Parolaro D. The non-psychoactive cannabidiol triggers caspase activation and oxidative stress in human glioma cells. *Cell Mol Life Sci* 2006; 63: 2057-66.
- 39 Marcu JP, Christian RT, Lau D, Zielinski AJ, Horowitz MP, Lee J, Pakdel A, Allison J, Limbad C, Moore DH, Yount GL, Desprez PY, McAllister SD. Cannabidiol enhances the inhibitory effects of delta9-tetrahydrocannabinol on human glioblastoma cell proliferation and survival. *Mol Cancer Ther* 2010; 9: 180-9.
- 40 Torres S, Lorente M, Rodríguez-Fornés F, Hernández-Tiedra S, Salazar M, García-Taboada E, Barcia J, Guzmán M, Velasco G. A combined preclinical therapy of cannabinoids and temozolomide against glioma. *Mol Cancer Ther* 2011; 10: 90-103.
- 41 Massi P, Solinas M, Valenti M, Cinquina V, Bolognini D, Gariboldi M, Monti E, Parolaro D. Cannabidiol, a non-psychoactive cannabinoid compound, inhibits cell invasion and prosurvival intracellular pathways in U87-MG human glioma cells. Submitted to *European Journal of Pharmacology* (2011).
- 42 Massi P, Valenti M, Vaccani A, Gasperi V, Perletti G, Marras E, Fezza F, Maccarrone M, Parolaro D. 5-Lipoxygenase and anandamide hydrolase (FAAH) mediate the antitumor activity of cannabidiol, a non-psychoactive cannabinoid. *J Neurochem* 2008;104: 1091-100.
- 43 Vaccani A, Massi P, Colombo A, Rubino T, Parolaro D. Cannabidiol inhibits human glioma cell migration through a cannabinoid receptor-independent mechanism. *Br J Pharmacol* 2005; 144: 1032-6.

- 44 Gallily R, Even-Chena T, Katzavian G, Lehmann D, Dagan A, Mechoulam R. Gamma-irradiation enhances apoptosis induced by cannabidiol, a non-psychotropic cannabinoid, in cultured HL-60 myeloblastic leukemia cells. *Leuk Lymphoma* 2003; 44: 1767-73.
- 45 McKallip RJ, Jia W, Schlomer J, Warren JW, Nagarkatti PS, Nagarkatti M. Cannabidiol-induced apoptosis in human leukemia cells: A novel role of cannabidiol in the regulation of p22phox and Nox4 expression. *Mol Pharmacol* 2006; 70: 897-908.
- 46 McKallip RJ, Lombard C, Fisher M, Martin BR, Ryu S, Grant S, Nagarkatti PS, Nagarkatti M. Targeting CB2 cannabinoid receptors as a novel therapy to treat malignant lymphoblastic disease. *Blood* 2002; 100: 627-34.
- 47 Li R, Wang H, Bekele BN, Yin Z, Caraway NP, Katz RL, Stass SA, Jiang F. Identification of putative oncogenes in lung adenocarcinoma by a comprehensive functional genomic approach. *Oncogene* 2006; 25: 2628–35.
- 48 Adjei AA. Novel combinations based on epidermal growth factor receptor inhibition. *Clin Cancer Res* 2006; 12: 4446s–50s.
- 49 Erler JT, Bennewith KL, Nicolau M, Dornhöfer N, Kong C, Le QT, Chi JT, Jeffrey SS, Giaccia AJ. Lysyl oxidase is essential for hypoxia-induced metastasis. *Nature* 2006; 440: 1222–6.
- 50 Molina JR, Adjei AA, Jett JR. Advances in chemotherapy of non-small cell lung cancer. *Chest* 2006; 130: 1211–9.
- 51 Ramer R, Merkord J, Rohde H, Hinz B. Cannabidiol inhibits cancer cell invasion via upregulation of tissue inhibitor of matrix metalloproteinases-1. *Biochem Pharmacol* 2010; 79: 955-66.
- 52 Ramer R, Rohde A, Merkord J, Rohde H, Hinz B. Decrease of plasminogen activator inhibitor-1 may contribute to the anti-invasive action of cannabidiol on human lung cancer cells. *Pharm Res* 2010; 27: 2162-74.

- 53** Ramer R, Hinz B. Inhibition of cancer cell invasion by cannabinoids via increased expression of tissue inhibitor of matrix metalloproteinases-1. *J Natl Cancer Inst* 2008; 100: 59–69.
- 54** Sativex Product Monograph. Salisbury, Wiltshire U.K.: GW Pharma Ltd.; SP4 0JQ Submission Control No: 091289; April 2003.
- 55** Lee CY, Wey SP, Liao MH, Hsu WL, Wu HY, Jan TR. A comparative study on cannabidiol-induced apoptosis in murine thymocytes and EL-4 thymoma cells. *Int Immunopharmacol* 2008; 8: 732-40.
- 56** Wu HY, Chu RM, Wang CC, Lee CY, Lin SH, Jan TR. Cannabidiol-induced apoptosis in primary lymphocytes is associated with oxidative stress-dependent activation of caspase-8. *Toxicol Appl Pharmacol* 2008; 226: 260-70.
- 57** Aviello G, Romano B, Borrelli F, Capasso R, Gallo L, Piscitelli F, Di Marzo V, Izzo AA. Chemopreventive effect of the non-psychoactive phytocannabinoid cannabidiol on experimental colon cancer. *J Mol Med (Berl)* 2012. [Epub ahead of print].
- 58** Freimuth N, Ramer R, Hinz B. Antitumorigenic effects of cannabinoids beyond apoptosis. *J Pharmacol Exp Ther* 2010; 332: 336-44.
- 59** Solinas M, Massi P, Cantelmo AR, Cattaneo MG, Cammarota R, Cinquina V, Valenti M, Vicentini L, Noonan D, Albin A, Parolaro D. Cannabidiol inhibits angiogenesis by multiple mechanisms. Submitted to *British Journal of Pharmacology* (2012).
- 60** Consroe P, Laguna J, Allender J, Snider S, Stern L, Sandyk R, Kennedy K, Schram K. Controlled clinical trial of cannabidiol in Huntington's disease. *Pharmacol Biochem Behav* 1991 40: 701-8.
- 61** Consroe P, Kennedy K, Schram K. Assay of plasma cannabidiol by capillary gas chromatography/ion trap mass spectroscopy following high-dose repeated daily oral administration in humans. *Pharmacol Biochem Behav* 1991 40: 517-22.

62 Karschner EL, Darwin WD, Goodwin RS, Wright S, Huestis MA. Plasma cannabinoid pharmacokinetics following controlled oral delta9-tetrahydrocannabinol and oromucosal cannabis extract administration. *Clin Chem* 2011 57: 66-75.

63 Alexander SPH, Mathie A, Peters JA. *Guide to Receptors and Channels (GRAC)*, 5th edition. *Br J Pharmacol* 2011 164 (Suppl. 1): S1-S324.

Table 1 - Effects of cannabidiol on different types of cancer

Cancer	<i>In vitro</i> effect	Receptor involvement	ROS production	Molecular cell signalling	Autophagy	Apoptosis	<i>In vivo</i> effect	Reference
Breast	↓ proliferation	CB ₂ ; TRPV1	↑	NC	NC	+	↓ xenografts growth ↓ lung metastases	[26]
	↓ viability	non-CB ₁ ; non-CB ₂ ; non-TRPV1	↑	↓ pAkt; ↑ cytochrome C Bid translocation	+	+	NC	[29]
	↓ invasion	NC	↑	↓ Id-1; ↑ pERK	NC	NC	↓ tumour growth ↓ size and number of metastases	[27, 28]
Glioma	↓ proliferation	non-CB ₁ ; partial-CB ₂ ; non-TRPV1	↑	↓ pERK; ↓ pAkt; ↓ HIF-1 α ↑ cytochrome C caspase activation	NC	+	↓ tumour growth	[32, 33, 36, 37]
	↓ proliferation and invasiveness	NC	NC	NC	NC	NC	NC	[34]
	↓ migration	non-CB ₁ ; non-CB ₂ ; non-TRPV1	NC	Ptx insensitive	NC	NC	NC	[38]
Leukaemia	↓ viability	NC	NC	caspase-3 activation	NC	+	NC	[39]
	↓ viability	CB ₂	↑	↓ p-p38 caspase activation; ↓ Bid ↑ cytochrome C	NC	+	↓ tumour burden ↑ tumour cell apoptosis	[40]
Lung	↓ invasion	CB ₁ ; CB ₂ ; TRPV1	NC	↑ p-p38; ↑ p-ERK; ↑ TIMP-1 ↓ PAI-1	NC	NC	↓ lung metastases ↓ PAI-1 in xenografts	[46, 47]
Thyroid	cytostatic effect	NC	NC	NC	NC	+	NC	[26]
Thymoma	↓ viability	NC	↑	NC	NC	+	NC	[50]
Colon	↓ proliferation	CB ₁ ; TRPV1; PPAR γ	NC	↓ Akt; ↑ 2AG ↑ caspase-3	NC	+	↓ ACF, polyps and tumours	[52]

NC = not checked

↑ increase; ↓ decrease

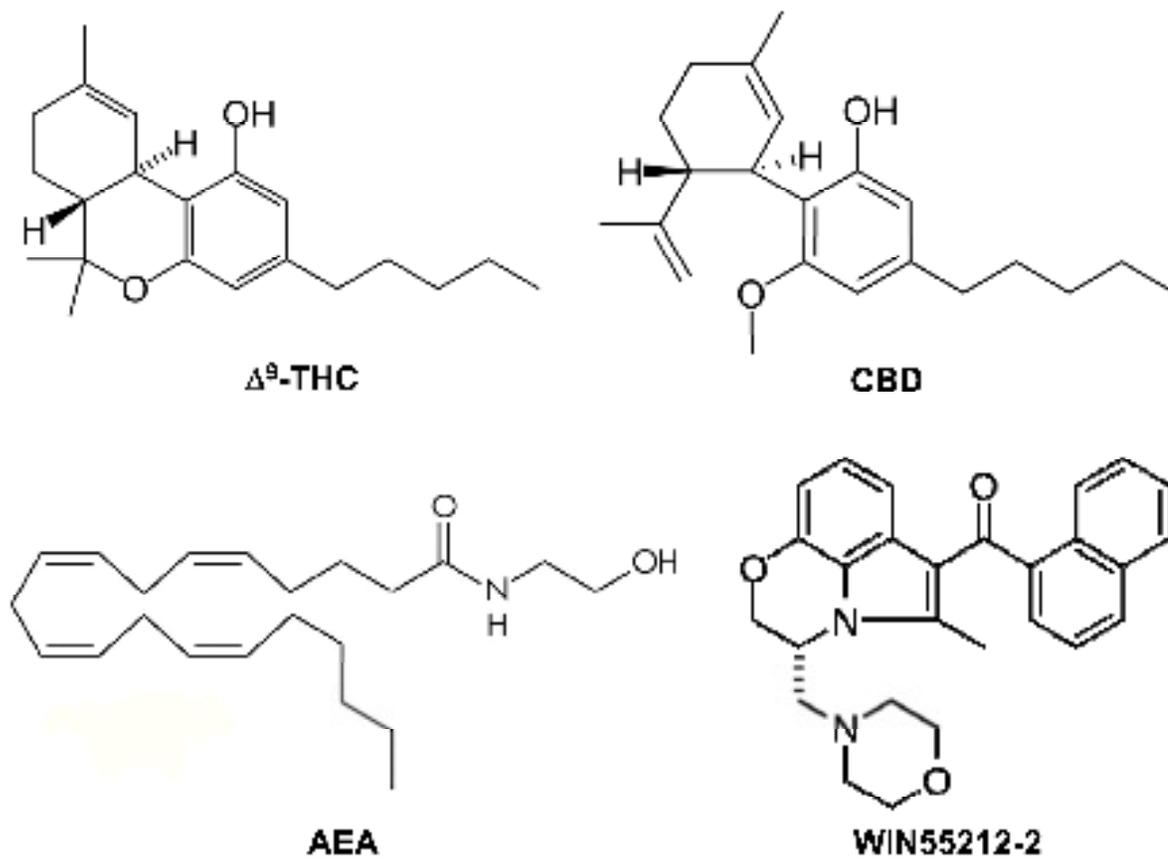


Figure 1 - Chemical structures of Δ^9 -tetrahydrocannabinol (Δ^9 -THC), cannabidiol (CBD), anandamide (AEA) and WIN55212-2

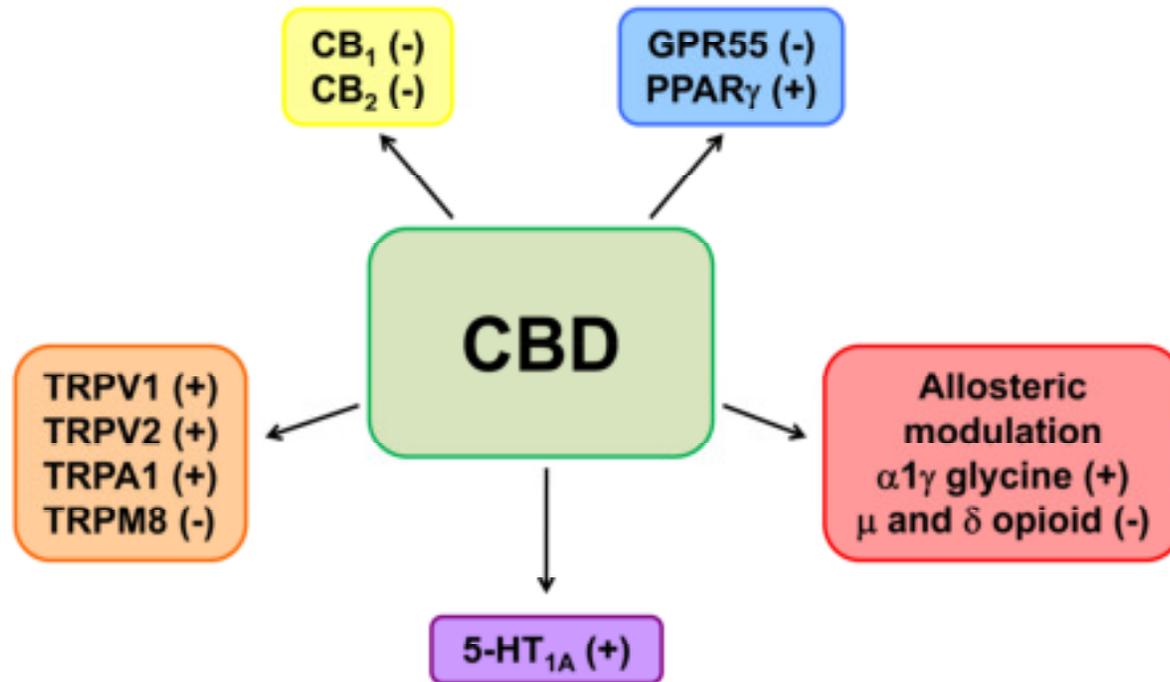


Figure 2 – Some of the potential biological targets of CBD

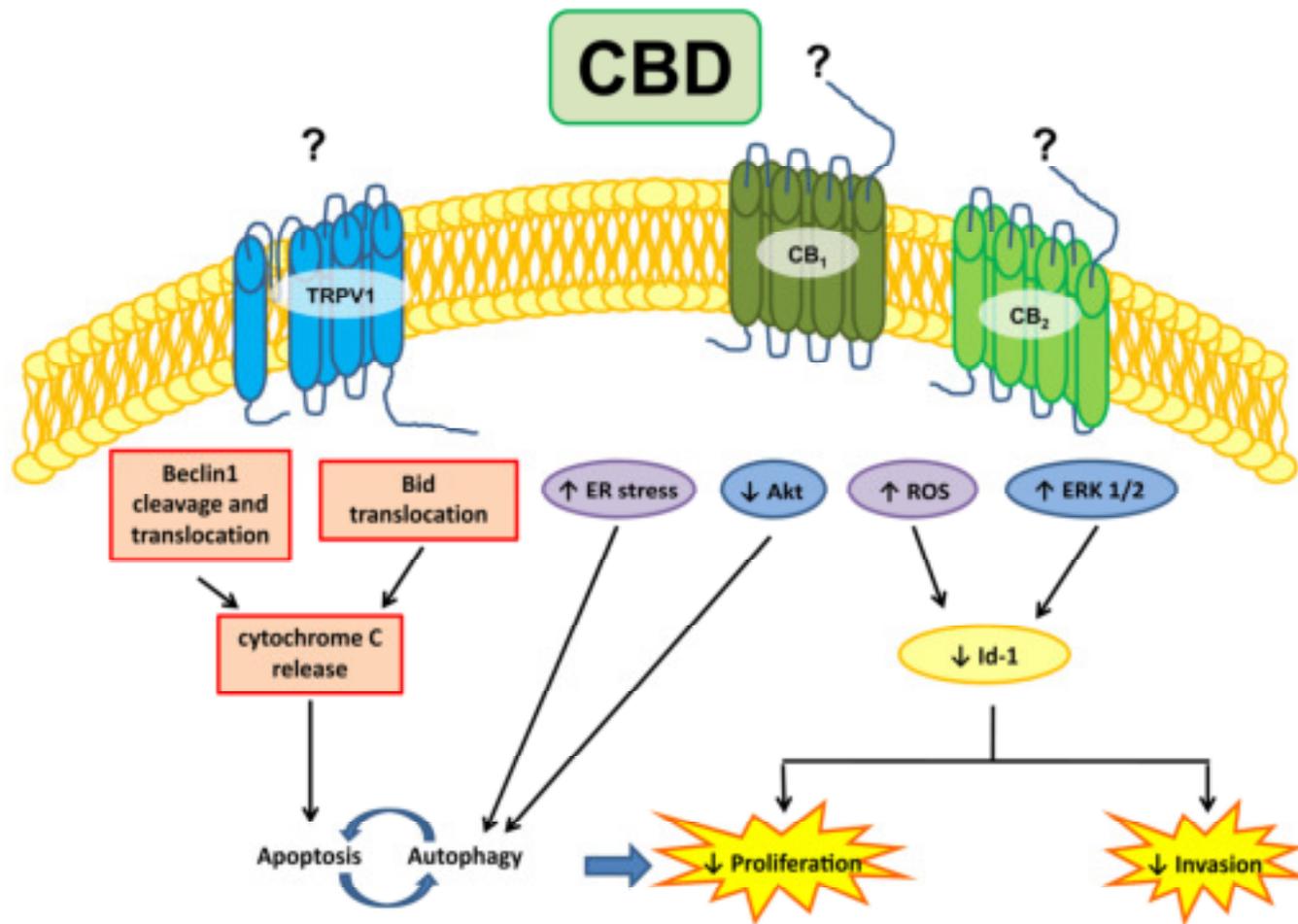


Figure 3 – Schematic representation of the signalling pathways associated with CBD effects on breast cancer

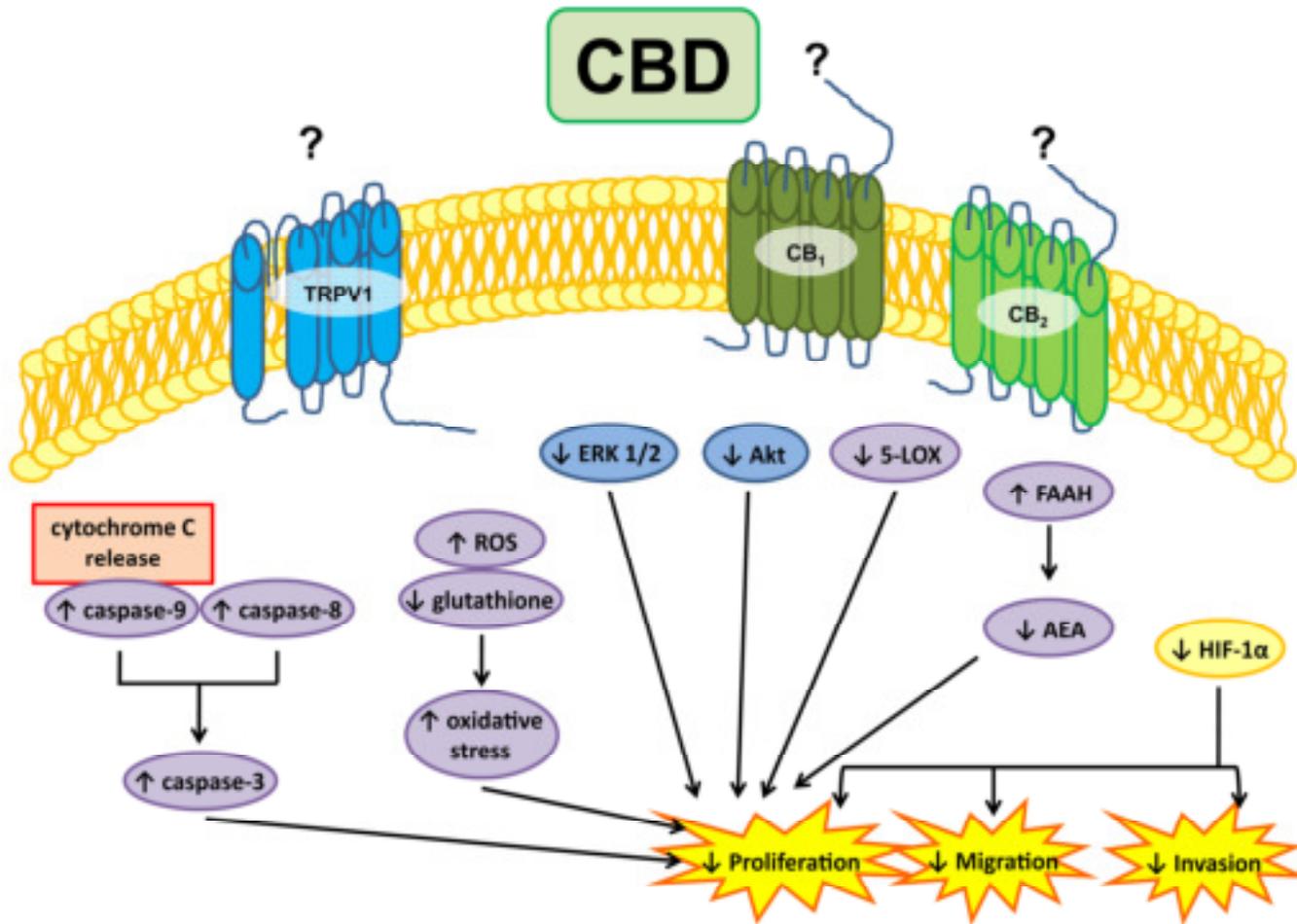


Figure 4 – Schematic representation of the signalling pathways associated with CBD effects on glioma