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Anticancer mechanisms of cannabinoids

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ABSTRACT

In addition to the well-known palliative effects of cannabinoids on some cancer-associated symptoms, a large body of evidence shows that these molecules can decrease tumour growth in animal models of cancer. They do so by modulating key cell signalling pathways involved in the control of cancer cell proliferation and survival. In addition, cannabinoids inhibit angiogenesis and decrease metastasis in various tumour types in laboratory animals. In this review, we discuss the current understanding of cannabinoids as antitumour agents, focusing on recent discoveries about their molecular mechanisms of action, including resistance mechanisms and opportunities for their use in combination therapy. Those observations have already contributed to the foundation for the development of the first clinical studies that will analyze the safety and potential clinical benefit of cannabinoids as anticancer agents.

Key Words Cannabinoids, apoptosis, autophagy, cell proliferation, angiogenesis, cell signalling, combination therapy

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INTRODUCTION

Preparations of *Cannabis sativa* L. (marijuana) have been used for many centuries both medicinally and recreationally. However, the chemical structures of their unique active components—the cannabinoids—were not elucidated until the 1960s. Three decades later, the first solid clues to cannabinoid molecular action were established, which led to an impressive expansion of basic cannabinoid research and a renaissance in the study of the therapeutic effects of cannabinoids in various fields, including oncology. Today, it is widely accepted that, of the approximately 108 cannabinoids produced by *C. sativa*, Δ^9-tetrahydrocannabinol (THC) is the most relevant because of its high potency and abundance in plant preparations[1,2].

Tetrahydrocannabinol exerts a wide variety of biologic effects by mimicking endogenous substances—the endocannabinoids anandamide[3] and 2-arachidonoylglycerol[4,5]—that engage specific cell-surface cannabinoid receptors[6]. So far, two major cannabinoid-specific receptors—CB1 and CB2—have been cloned from mammalian tissues and characterized[7,8]. In addition, other receptors such as the transient receptor potential cation channel subfamily V, member 1, and the orphan G protein–coupled receptor 55 have been proposed to act as endocannabinoid receptors[6]. Most of the effects produced by cannabinoids in the nervous system and in non-neural tissues rely on CB1 receptor activation. In contrast, the CB2 receptor was initially described to be present in the immune system[6], but was more recently shown to also be expressed in cells from other origins[9,10]. Notably, expression of the CB1 and CB2 receptors has been found in many types of cancer cells, but not necessarily correlating with the expression of those receptors in the tissue of origin[9,11,12].

The endocannabinoids, together with their receptors and the proteins responsible for their synthesis, transport, and degradation, constitute the endocannabinoid system. Aside from its pivotal neuromodulatory activity[13], the endocannabinoid system exerts other regulatory functions in the body such as control of cardiovascular tone, energy metabolism, immunity, and reproduction[14,15]. This miscellaneous activity makes the pharmacologic manipulation of the endocannabinoid system a promising strategy for the management of many diseases. Specifically, cannabinoids are well known to exert palliative effects in cancer patients[14,15]. Their best-established use is the inhibition of chemotherapy-induced nausea and vomiting[15,16]. Today, capsules of THC (Marinol: AbbVie, North Chicago, IL, U.S.A.) and its synthetic analogue nabilone (Cesamet: Meda Pharmaceuticals, Somerset, NJ, U.S.A.) are approved for that purpose. Cannabinoids also inhibit pain, and thus a standardized cannabis extract (Sativex: GW Pharmaceuticals, Salisbury, U.K.) has already been approved in Canada and is currently the subject of large-scale phase III clinical trials for managing cancer-associated pain. Another potential palliative effect of cannabinoids in oncology, supported by phase III clinical trials, includes appetite stimulation and attenuation of wasting. In that respect, Marinol can currently be prescribed for anorexia associated with weight loss in AIDS patients.

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The therapeutic potential of cannabinoids in oncology might not be restricted to their aforementioned palliative actions. Thus, numerous studies have provided evidence that THC and other cannabinoids exhibit antitumour effects in a wide array of animal models of cancer[12,16,17]. Moreover, those observations led to the development of several clinical studies to investigate the antitumour activity of THC in humans (see "Clinical Antitumour Effects of Cannabinoids" later in this article). Nonetheless, a few studies have shown that, under certain conditions, cannabinoid treatment can stimulate cancer cell proliferation *in vitro*[18,19] and interfere with the tumour-suppressor role of the immune system[20,21]. Likewise, reports about the role of the endocannabinoid system in cancer (tumour-suppressor or oncogenic) are conflicting[22].

Reports concerning the biologic role of the endocannabinoid system in cancer physiopathology are sparse. Although some exceptions that might be tumour-type-specific are known, cannabinoid receptors and their endogenous ligands are both generally upregulated in tumour tissue compared with non-tumour tissue[16,22–24]. Additionally, various studies have associated the expression levels of cannabinoid receptors, endocannabinoids, or endocannabinoid-metabolizing enzymes with tumour aggressiveness[22,25,26], which suggests that the endocannabinoid system might be overactivated in cancer and hence pro-tumourigenic[22]. In support of that hypothesis, reports showed that genetic ablation of CB1 and CB2 receptors in mouse models of cancer reduces ultraviolet light–induced skin carcinogenesis[27], that overexpression of CB2 receptor enhances the predisposition to leukemia after leukemia viral infection[28], and that CB2 promotes HER2 (human epidermal growth factor receptor 2) pro-oncogenic signalling in breast cancer[29].

Conversely, and in line with the evidence supporting the hypothesis that pharmacologic activation of cannabinoid receptors decreases tumour growth[12,16], upregulation of endocannabinoid-degrading enzymes has been observed in aggressive human tumours and cancer cell lines[25,26], indicating that endocannabinoid signalling can also have a tumour-suppressive role. In support of that observation, deletion of CB1 receptors was noted to accelerate intestinal tumour growth in a genetic mouse model of colon cancer[30], increased endocannabinoid levels were observed to diminish azoxymethane-induced precancerous lesions in the mouse colon[31], and reduction in the expression of the endocannabinoid-degrading enzyme monoacylglycerol lipase was seen to decrease tumour growth in xenografted mice[25].

Further studies—including analyses of the activation of the precise signalling mechanisms involved in the regulation of cannabinoid-induced cell death, and of cell proliferation upon genetic or pharmacologic manipulation of the endocannabinoid system—are therefore needed to clarify the contextual determinants that result in the system acting as either a guardian or an inducer of tumourigenesis or tumour progression. The present review summarizes such observations and provides an integrated view of the molecular mechanisms responsible for cannabinoid antitumour activity. It also discusses the experimental evidence supporting the existence of mechanisms of resistance to the cell death–promoting actions of THC in certain types of cancer cells, the strategies that could possibly be used to overcome such resistance, and the preclinical data supporting the potential usefulness of the combined administration of cannabinoids and other drugs in anticancer therapies.

PRECLINICAL ANTITUMOUR ACTIVITY

Since the late 1990s, a large body of evidence has accumulated demonstrating that various cannabinoids exert antitumour effects in a wide variety of experimental models of cancer, ranging from cancer cell lines in culture to genetically-engineered mice (reviewed by Velasco *et al.*[17]). Multiple cannabinoids have shown this activity, including THC; the endocannabinoids 2-arachidonoylglycerol and anandamide; and various synthetic cannabinoid receptor agonists that have either comparable affinity for the CB1 and CB2 receptors (for example, WIN 55,212-2 or HU-210), a higher affinity for CB1 (for example, methanandamide), or a higher affinity for CB2 (for example, JWH-133). Those findings strongly support that, aside from the role played by the endogenous cannabinoid system in cancer, pharmacologic stimulation of CB receptors is, in most cases, antitumourigenic. Nonetheless, a few reports have proposed a tumour-promoting effect of cannabinoids[18–21]. Those apparently conflicting observations are discussed in the next subsection.

Mechanisms of Antitumour Effects

Cannabinoids impair tumour progression at various levels. Their most prevalent effect is the induction of cancer cell death by apoptosis and the inhibition of cancer cell proliferation. At least one of those actions has been demonstrated in almost all cancer cell types tested[17]. In addition, *in vivo* experiments have shown that cannabinoids impair tumour angiogenesis and block invasion and metastasis.

Induction of Cancer Cell Death

A significant amount of the research conducted so far on the mechanism of cannabinoid antitumour activity has focussed on glioma cells. Initial studies showed that THC and other cannabinoids induce the apoptotic death of glioma cells by CB1- and CB2-dependent stimulation of the *de novo* synthesis of the pro-apoptotic sphingolipid ceramide[23,32–34]. Further studies based on the analysis of the gene expression profile of THC-sensitive and -resistant glioma cells yielded further insight into the specific signalling events downstream of ceramide that are activated in cancer cells by cannabinoids[35]. Thus, it was found that treatment with THC results in enhanced expression of the stress-regulated protein p8 (NUPR1), a transcriptional regulator that has been implicated in the control of tumourigenesis and tumour progression[36], together with several of its downstream targets, such as the endoplasmic reticulum (ER) stress–related transcription factors ATF4 and CHOP, and the pseudokinase tribbles homologue 3 (TRIB3)[35]. This THC-triggered stimulation of the p8-regulated pathway (Figure 1) enhances the inhibitory interaction of TRIB3 with a pro-survival kinase, AKT[37,38], which leads to inhibition of the mammalian target of rapamycin complex 1 (mTORC1)

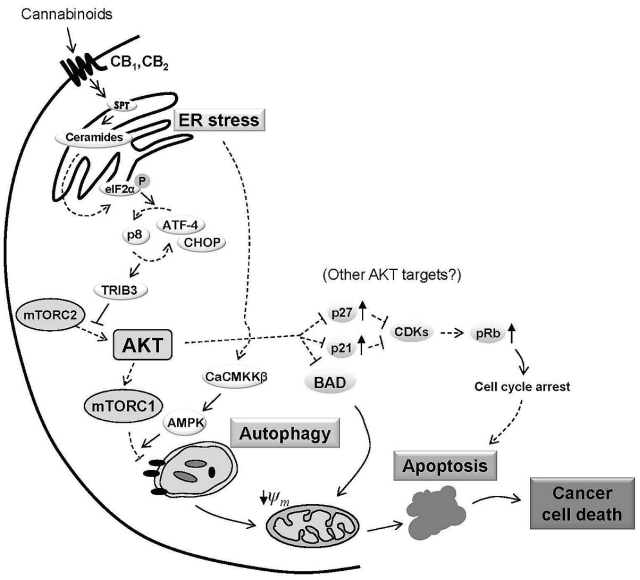

FIGURE 1 Cannabinoid-induced apoptosis relies on the stimulation of endoplasmic reticular (ER) stress and autophagy. Here, the mechanism of cannabinoid-induced apoptosis in glioma, pancreatic, and hepatocellular carcinoma cells is depicted. This signalling route could constitute the main mechanism of cannabinoid-induced cell death, with some variations inherent to different types of cancer cells. CB1 = cannabinoid CB1 receptor; CB2 = cannabinoid CB2 receptor; SPT = serine palmitoyltransferase; eIF2α = eukaryotic translation initiation factor 2α; P inscribed in a circle = protein phosphorylation upon treatment with Δ^9-tetrahydrocannabinol (THC); ATF-4 = activating transcription factor 4; CHOP = C/EBP homologous protein; AKT = protein kinase B; TRIB3 = tribbles-homologue 3; mTORC2 = mammalian target of rapamycin complex 2; CDKs = cyclin-dependent kinases; pRb = retinoblastoma protein; CaCMKKβ = calcium/calmodulin–dependent protein kinase kinase 2β; AMPK = AMP-activated protein kinase.

and the subsequent stimulation of autophagy-mediated cell death[37].

Autophagy is an essential cellular process participating in a number of physiologic functions within the cell[39,40]. During autophagy, organelles and other cytoplasmic components are engulfed within double-membrane vesicles called "autophagosomes." The maturation of those vesicles involves their fusion with lysosomes, which leads in turn to the degradation of the autophagosome components by lysosomal enzymes[40]. Autophagy is primarily a cytoprotective mechanism, although its activation can also lead to cell death[40–42].

Cannabinoids induce autophagy in various types of cancer cells in culture, and pharmacologic or genetic inhibition of autophagy prevents cannabinoid antitumour action in various animal models of cancer (Figure 1), thus demonstrating that autophagy is important for cannabinoid antineoplastic activity[37,43,44]. Moreover, autophagy blockade prevents cannabinoid-induced apoptosis and cell death, whereas apoptosis blockade prevents cannabinoid-induced cell death, but not autophagy[37,43,44]. Those observations indicate that autophagy is upstream of apoptosis in the mechanism of cannabinoid-induced cell death (Figure 1).

The importance of this pathway is highlighted by the ability of various chemical and genetic manipulations to block cannabinoid-induced cell death. In hepatocellular carcinoma cells, the cannabinoid-evoked and ER stress–dependent activation of calcium/calmodulin-dependent protein kinase kinase 2β and AMP-activated protein kinase leads, together with the p8–TRIB3 pathway, to autophagy and apoptosis[43]. The cannabinoid-evoked inhibition of AKT was able to promote cycle arrest in breast cancer and melanoma cells, as well as apoptosis through additional mechanisms, including decreased phosphorylation of the pro-apoptotic protein BCL2-associated agonist of cell death[45] and activation of the cyclin-dependent kinase inhibitory proteins p21 and p27[24,46,47], leading to the subsequent decreased phosphorylation of the retinoblastoma protein, which would thus arrest the cell cycle.

The direct participation of the p8/TRIB3–mediated autophagy pathway in the antitumour action of cannabinoids has been clearly demonstrated in glioma cells, pancreatic and hepatic cancer cells, and melanoma cells[35,37,43,44,48,49]. At least part of that signalling route has also been found to be upregulated with cannabinoid treatment in other types of cancer cells, an observation which suggests that—with some variations—this pathway could be a general mechanism by which activation of CB1 and CB2 receptors promotes cancer cell death.

Additional mechanisms might nonetheless cooperate with the p8/TRIB3–mediated autophagy pathway to evoke cancer cell death (Figure 1). For example, in hepatocellular carcinoma cells, THC and the CB2 receptor agonist JWH-015 can trigger an ER stress–dependent activation of AMP-activated protein kinase that cooperates with the TRIB3-mediated inhibition of the AKT–MTORC1 axis in the stimulation of autophagy-mediated cell death[44]. In melanoma[46], breast carcinoma[24,50], and prostate carcinoma[51] cells, cannabinoids can induce cell-cycle arrest in concert with apoptosis[24,46,51]. Notably, cannabinoid antiproliferative action—at least in melanoma[46] and breast cancer[24] cells—also relies on AKT inhibition.

Likewise, the effect of cannabinoids in hormone-dependent tumours might rely, at least in part, on the ability to interfere with the activation of growth factor receptors[12,16]. Some of those and other mechanisms[52] might participate, together with the autophagy-mediated cell death pathway, in the cytotoxic action of cannabinoids in various types of cancer cells. However, further investigation is required to clarify the issue.

Research conducted during the last few years has shed light on the intracellular signalling mechanisms underlying cannabinoid anticancer action. However, a number of important observations—in particular, those related to the role played by cannabinoid receptors in the triggering of the signals—remain to be clarified. For example, in contrast to the death-promoting action of cannabinoids on cancer cells, the viability of normal (non-transformed) cells is unaffected or, under certain conditions, even enhanced by cannabinoid challenge[33–35,37,53]. For example, THC treatment of astrocytes (a cell type that expresses functional CB1 receptors) does not trigger the activation of ER stress, upregulation of the p8 pathway, inhibition of the AKT–MTORC1 axis, or stimulation of autophagy and

apoptosis, even when concentrations of THC higher than those that promote glioma cell death are used[35,37]. Similar results were obtained for primary embryonic fibroblasts[35,41] and other types of non-transformed cells expressing functional cannabinoid receptors in comparison with their transformed counterparts[24,46,54,55]. Thus, stimulation of cannabinoid receptors seems to be coupled with the activation of different signalling mechanisms in transformed and non-transformed cells. The precise molecular reasons for this variation in behaviour remain as an important open question in the cannabinoid field. Another intriguing observation is that, in some types of cancer cells (such as glioma cells), pharmacologic blockade of either CB1 or CB2 prevents cannabinoid-induced cell death with similar efficacy[33,56], and yet in other types of cancer cells (pancreatic[48], breast[24], or hepatic[43] carcinoma cells, for example), antagonists of CB2 but not of CB1 inhibit cannabinoid antitumour actions. The reason that cannabinoids produce their antitumour actions through one or the other of these receptor types depending on the type of cancer cell has yet to be established.

Some cannabinoid receptor agonists promote cancer cell death more efficiently than other agonists that exhibit similar or even higher affinity for the CB1 or CB2 receptors. For example, THC promotes cancer cell death in a CB1- or CB2-dependent manner (or both) at lower concentrations than does the synthetic cannabinoid receptor agonist WIN 55,212-2, although the latter agent shows significantly higher affinity for CB1 and CB2 in binding assays[6].

Further work aimed at investigating, for example, CB receptor homo- or hetero-oligomerization in response to various cannabinoid agonists, their associations with specific domains in the plasma membrane such as lipid rafts, changes in the subcellular location of CB receptors, and the selective coupling to various G proteins and other signalling proteins, will be essential to answer the foregoing questions and to precisely define the role played by each cannabinoid receptor type as an anticancer signalling platform.

Notably, cannabidiol (CBD), a phytocannabinoid with a low affinity for cannabinoid receptors[15], and other marijuana-derived cannabinoids[57] have also been proposed to promote the apoptotic death of cancer cells acting independently of the CB1 and CB2 receptors. The mechanism by which CBD produces this effect has not as yet been completely clarified, but it seems to rely—at least in part—on its ability to enhance the production of reactive oxygen species in cancer cells[58,59]. It has also been proposed that CBD might activate TRPV2 receptors to promote glioma cell death[60].

Inhibition of Angiogenesis, Invasion, and Metastasis

In cancer cells, cannabinoids block the activation of the vascular endothelial growth factor (VEGF) pathway, an inducer of angiogenesis. Specifically, various elements of the cascade, such as the main ligand (VEGF) and the active forms of its main receptors (VEGFR1 and VEGFR2), are downregulated with cannabinoid treatment of skin carcinomas[54], gliomas[32,61], and thyroid carcinomas[62]. In vascular endothelial cells, cannabinoid receptor activation inhibits proliferation and migration, and induces apoptosis[61,63]. Those and perhaps other cannabinoid-evoked actions result in a normalized tumour vasculature—that is, smaller and fewer vessels that are more differentiated and less leaky.

Likewise, CB1 or CB2 receptor agonists (or both) reduce the formation of distant tumour masses in animal models of both induced and spontaneous metastasis, and inhibit adhesion, migration, and invasiveness of glioma[64], breast[65,66], lung[67,68], and cervical[68] cancer cells in culture. Those effects depend, at least in part, on the modulation of extracellular proteases (such as matrix metalloproteinase 2)[64] and their inhibitors (such as tissue inhibitor of matrix metalloproteinases 1)[68].

Notably, pharmacologic inhibition of ceramide biosynthesis abrogates the antitumour and antiangiogenic effect of CB1 or CB2 receptor agonists (or both) in glioma xenografts, and decreases VEGF production by glioma cells *in vitro* and *in vivo*[32]. Likewise, inhibition of matrix metalloproteinase 2 expression and glioma cell invasion is prevented by blocking ceramide biosynthesis and by knocking down p8 expression[64]. Although further research is still necessary to precisely define the molecular mechanisms responsible for those actions of cannabinoids, the observations indicate that the ceramide/p8–regulated pathway plays a general role in the antitumour activity of cannabinoids targeting CB1 and CB2.

It is worth noting that CBD, by acting independently of the CB1 and CB2 receptors, produces a remarkable antitumour effect—including reduction of invasiveness and metastasis—in various animal models of cancer. This effect of CBD seems to rely, at least in part, on the downregulation of the helix-loop-helix transcription factor inhibitor of DNA binding 1[69,70].

Regulation of Antitumour Immunity

Notably, stimulation of cannabinoid receptors can lead to important changes in the processes that regulate antitumour immunity. Thus, for example, treatment of mice with THC triggers a shift (from Th1 to Th2) of cytokine profile[20,71–73] and induces mobilization of myeloid-derived suppressor cells[74], two events that play a critical role in the suppression of antitumour immunity. In agreement with that notion, stimulation of CB2 has been proposed in some reports to enhance tumourigenesis by interfering with tumour surveillance by the immune system[20,21].

By contrast, cannabinoids can also enhance immune system–mediated tumour surveillance in some contexts: the antitumour action of WIN 55,212-2 (a mixed CB1 or CB2 agonist) or JWH-133 (a CB2-selective agonist) was more pronounced in melanoma xenografts generated in immunocompetent mice than in those generated in immunodeficient mice[46], a finding which also indicates that, at least in this model, stimulation of CB2 inhibits tumour growth primarily through direct effects on cancer cells rather than necessarily by interfering with the normal antitumour function of the immune system. In line with that idea, treatment of immunocompetent rats with very high doses of THC (50 mg/kg daily 5 times per week) for 2 years lowered the incidence of several types of tumours and enhanced the overall survival of the animals[55]. Those observations might be related to the ability of THC to reduce inflammation[75,76], an effect that might prevent certain types of cancer[76,77].

For cannabinoid use to be clinically successful, antitumour effects will have to overcome immunosuppressive (potentially tumour-promoting) effects. Additional studies should clarify the issue. For example, it could be conceivable to study the effect of cannabinoid administration on the generation and progression of tumours with varying sensitivity to cannabinoids and generated in immunocompetent or immunodeficient mice in which the expression of CB1 or CB2 receptors (or both) in cells from the immune system has been genetically manipulated.

RESISTANCE MECHANISMS

Numerous studies have contributed to an appreciation of the heterogeneity of cancer, whereby each subtype of cancer—and even each individual tumour—exhibits a series of molecular characteristics that determines its behaviour and, in particular, its responsiveness to various anticancer drugs. In agreement with that line of reasoning, a recent report investigated the molecular features associated with the resistance of a collection of human glioma cell lines and primary cultures to cannabinoid antitumour action[56]. The study showed that, although the apoptotic effect of THC on glioma cells relied on the stimulation of cannabinoid receptors and activation of the p8-mediated autophagy pathway, the differences in the sensitivity to THC-induced cell death correlated with enhanced expression of a particular set of genes in the THC-resistant glioma cells rather than with the presence of different expression levels of CB1 or CB2 receptors[56]. Interestingly, upregulation of one of those genes, midkine (*MDK*), which encodes a growth factor that was previously associated with increased malignancy and resistance to anticancer therapies in several types of tumours[77,78], correlates with lower overall survival in patients with glioblastoma[56]. Moreover, MDK plays a direct role in the resistance to THC action through stimulation of anaplastic lymphoma kinase (ALK[79]). Thus, the stimulation of ALK by MDK inhibits the THC-evoked autophagy-mediated cell-death pathway. Further research should clarify whether that mechanism could also be responsible for the resistance to other therapies of cancer cells expressing high levels of MDK. Interestingly, *in vivo* silencing of *MDK* or pharmacologic inhibition of ALK in a mouse xenograft model abolishes the resistance to THC treatment of established tumours derived from cannabinoid-resistant glioma cells[56].

Taken together, the foregoing findings support the idea that stimulation of the MDK–ALK axis promotes resistance to THC antitumour action in gliomas and could help to set a foundation for the potential clinical use of THC in combination with inhibitors of the MDK–ALK axis (Figure 2). Glioblastoma is highly resistant to current anticancer therapies[80–82]. Specifically, resistance of glioma cells to cannabinoid-induced cell death relies, at least in part, on enhanced expression of MDK and the subsequent activation of ALK[56]. Likewise, enhanced expression of the heparin-bound epidermal growth factor receptor (EGFR) ligand amphiregulin can promote resistance to THC antitumour action by stimulation of extracellular signal-regulated kinase (ERK)[83]. The combination of THC with pharmacologic inhibitors of ALK (or genetic inhibition of *MDK*) enhances cannabinoid action in resistant tumours, which provides a rationale for the design of targeted therapies capable of increasing cannabinoid antineoplastic activity[56]. Combinations of cannabinoids with classical chemotherapeutic drugs such as the alkylating agent temozolomide (the benchmark agent for the management of glioblastoma[80,84]) have been shown to produce a strong anticancer action in animal models[85]. Combining cannabinoids and temozolomide is thus a very attractive possibility for clinical studies aimed at investigating cannabinoid antitumour effects in glioblastoma. Other potentially interesting strategies to enhance cannabinoid anticancer action (still requiring additional experimental support from data obtained using preclinical models) could be to combine cannabinoids with ER stress or autophagy inducers (or both) or with inhibitors of the MTORC1 axis.

In line with that idea, ALK inhibitors have started to be used in clinical trials for the management of non-small-cell lung cancer and other types of tumours[86,87]. Future research should clarify whether this mechanism of resistance to cannabinoid action operates in other types of tumours. In agreement with that possibility, *MDK* silencing enhanced the sensitivity of cannabinoid-resistant pancreatic cancer cells to THC-induced cell death[56].

The release by cancer cells of other growth factors has also been implicated in the mechanism of resistance to cannabinoid antitumour action. Thus, increased expression of amphiregulin is associated with enhanced resistance to THC antitumour action in glioma xenografts[83]. Notably illustrating how the dose of cannabinoids could be crucial for optimal therapeutic effect, low (submicromolar) concentrations of THC or other synthetic cannabinoid agonists enhance the proliferation of several cancer cell lines *in vitro*. That effect relies on activation of the protease ADAM17, the shedding of heparin-bound EGFR ligands including amphiregulin and the subsequent stimulation of the ERK and AKT pathways[19]. In line with that idea, a recent report showed that treatment with the synthetic cannabinoid CP 55,940 increases the proliferation of murine glioma cells engineered to express CB1 or CB2 receptors only when those receptors are coupled to AKT activation[18]. Although a pro-tumourigenic effect has not been observed for the growth of tumour xenografts generated with glioma cells and treated with low doses of THC[85], increased expression of amphiregulin promotes resistance to THC antitumour action through a mechanism that involves the EGFR-dependent stimulation of ERK and the subsequent inhibition of p8 and TRB3 expression. Likewise, pharmacologic inhibition of EGFR, ERK[83], or AKT enhances the cell-death-promoting action of THC in glioma cultures (unpublished observations by the authors), which suggests that targeting EGFR and the AKT and ERK pathways could enhance the antitumour effect of cannabinoids.

CANNABINOID-BASED COMBINATION THERAPIES

The use of combinational anticancer therapies has a number of theoretical advantages over single-agent strategies, because they allow for the simultaneous targeting of tumour growth, progression, and spread at various

levels. In line with that idea, recent observations suggest that the combined administration of cannabinoids with other anticancer drugs acts synergistically to reduce tumour growth. For example, the administration of THC and temozolomide exerts strong antitumour action in glioma xenografts, an effect that is also evident in temozolomide-resistant tumours[85]. A similar effect was observed when THC and CBD were combined with radiotherapy in animal models of glioma. Interestingly, no toxicity was observed in mice treated with combinations of THC and temozolomide[85]. Because most patients with glioblastoma undergo temozolomide treatment, the foregoing findings indicate that the combined administration of temozolomide and cannabinoids could be therapeutically exploited for the management of glioblastoma (Figure 2) and perhaps other tumour types such as melanoma[44].

Likewise, another study recently showed that the combined administration of gemcitabine (the benchmark agent for the treatment of pancreatic cancer) and various cannabinoid agonists synergistically reduced the viability of pancreatic cancer cells[88]. Other reports indicated that anandamide and HU-210 might also enhance the anticancer activity of paclitaxel[89] and 5-fluorouracil[90] respectively.

An additional approach has been to combine THC with CBD, a phytocannabinoid that reduces (although to a lower extent than THC) the growth of several types of tumour xenografts through a still poorly-defined mechanism[59,91,92]. Combined administration of THC and CBD enhances the anticancer activity of THC and reduces the dose of THC needed to induce its tumour growth-inhibiting activity[85,93]. Moreover, the combination of THC and CBD together with temozolomide produces a striking reduction in the growth of glioma xenografts even when low doses of THC are used[85]. Likewise, the combination of THC, CBD, and radiotherapy also produced clear anticancer activity in an orthotopic model of glioma[94]. Notably, CBD was also shown to alleviate some of the undesired effects of THC administration such as convulsions, discoordination, and psychotic events, thus improving the tolerability of cannabis-based medicines[15]. As mentioned earlier, *C. sativa* produces approximately 108 different cannabinoids, and apart from CBD, some of the other cannabinoids present in marijuana might attenuate the psychoactive side effects of THC or even produce other therapeutic benefits[15]. Thus, we think that clinical studies aimed at analyzing the efficacy of cannabinoids as antitumour agents should be based on the use both of pure substances, such as THC and CBD, and of cannabis extracts containing controlled amounts of THC, CBD, and other cannabinoids.

CLINICAL ANTITUMOUR EFFECTS OF CANNABINOIDS

The clinical approval of cannabinoids is largely restricted to palliative uses in various diseases, but since the emergence of promising preclinical data, the antitumour effects of cannabinoids are beginning to be clinically assessed.

In a pilot phase I clinical study, 9 patients with actively-growing recurrent glioblastoma for whom standard therapy had previously failed underwent intracranial THC administration[11]. Under those conditions, cannabinoid delivery was safe and could be achieved without significant

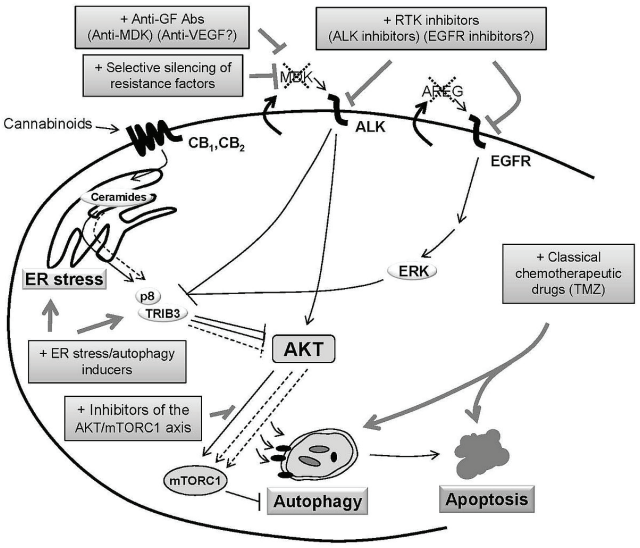

FIGURE 2 Possible strategies for optimizing cannabinoid-based therapies against gliomas. GF = growth factors; Abs = antibodies; MDK = growth factor midkine; VEGF = vascular endothelial growth factor; RTK = receptor tyrosine kinase; ALK = anaplastic lymphoma receptor tyrosine kinase; EGFR = epidermal growth factor receptor; CB1 = cannabinoid CB1 receptor; CB2 = cannabinoid CB2 receptor; AREG = amphiregulin; ER stress = endoplasmic reticular stress; ERK = extracellular signal-regulated kinase; TMZ = temozolomide; TRIB3 = tribbles-homologue 3; AKT = protein kinase B; mTORC1 = mammalian target of rapamycin complex 1.

unwanted effects. In addition, although no statistically significant conclusions can be extracted from a cohort of 9 patients, the results obtained in the study suggest that some patients responded—at least partially—to THC treatment in terms of a decreased tumour growth rate as evaluated by magnetic resonance imaging[11]. Importantly, analyses of samples obtained from 2 study patients before and after THC administration indicated that the molecular mechanism of cannabinoid antitumour action—namely, p8 and TRIB3 upregulation[35,37], mTORC1 inhibition[37], stimulation of autophagy and apoptosis[11,35,37], inhibition of cell proliferation[11], decreased VEGF signalling[32], and matrix metalloproteinase 2 downregulation[64] (delineated here earlier)—also operates *in vivo*.

Those findings were encouraging and reinforced interest in the potential use of cannabinoids in cancer therapies. However, they also highlighted the need for further research aimed at optimizing the use of cannabinoids in terms of patient selection, combination with other anticancer agents, and use of other routes of administration.

Administration of endocannabinoids or inhibitors of endocannabinoid-degrading enzymes has been shown to reduce the growth of various tumour xenograft types[95,96] and could therefore be a reasonable strategy for targeting cannabinoid receptors for anticancer purposes. However, as discussed here earlier, the role of the endocannabinoid system, including the endocannabinoid-degrading enzymes, in the control of tumour generation and progression is not well understood. Because enhancing endocannabinoid tone only has mild antitumour effects

in mice and because no inhibitor of endocannabinoid degradation has yet been approved for use in humans, clinical studies aimed at analyzing the efficacy of cannabinoids as antitumour agents should be based on the use of plant-derived or synthetic agonists of cannabinoid receptors rather than on endocannabinoids or inhibitors of endocannabinoid degradation.

The long-known therapeutic properties of *C. sativa*—including amelioration of symptoms associated with cancer and its chemotherapy—have led to the authorization of the medical use of the plant and its extracts in several countries. As already mentioned, *C. sativa* produces approximately 108 different cannabinoids, including THC and CBD. Some of the other cannabinoids present in marijuana might contribute to the attenuation of THC's psychoactive side effects or even to the production of other therapeutic benefits[15]. However, pure drugs are more prone to standardization than complex molecular cocktails. Thus, it would be ideal for studies aiming to investigate the anticancer actions of cannabinoids in patients to be performed comparatively with both pure substances and cannabis extracts containing controlled amounts of THC, CBD, and other cannabinoids.

The most widely used route of administration for recreational and self-medicating marijuana is smoking. Although THC and other phytocannabinoids are rapidly absorbed by inhalation, smoking is an unattractive clinical option. Preclinical work in animal models has typically used peri-tumoural administration of cannabinoids. Likewise, in the only clinical trial in which a cannabinoid was assayed as an antitumour agent, THC was administered locally (intracranial delivery to patients with glioblastoma multiforme)[11]. Nevertheless, this route of administration has many obvious limitations. Currently available cannabis-based medicines are administered as capsules or using an oromucosal spray[15]. Preclinical animal models have yielded data indicating that systemic (oral or intraperitoneal) administration of cannabinoids effectively decreases tumour growth (GV, CS, and MG. Unpublished observations), and so it seems reasonable that future clinical studies with the goal of determining the efficacy of cannabinoids as antitumour agents use oral or oromucosal routes of administration.

Two currently ongoing clinical trials could shed some light on these issues. One of the studies is a phase I/II trial aimed at evaluating the combined effect of Sativex (an oromucosal cannabis extract whose main active components are THC and CBD in a *c.* 1:1 ratio) and temozolomide in patients with recurrent glioblastoma multiforme (https://clinicaltrials.gov/ct2/show/NCT01812603 and https://clinicaltrials.gov/ct2/show/NCT01812616). The other is a phase II trial aimed at evaluating the effect of CBD as single treatment in patients with solid tumours (https://clinicaltrials.gov/ct2/show/NCT02255292). Hopefully, in the near future, new clinical trials will start, helping to determine whether cannabinoids can be used, for other than their palliative effects, in the treatment of cancer patients

CONCLUSIONS AND FUTURE DIRECTIONS

It is widely believed that strategies aimed at reducing mortality from cancer should consist of targeted therapies capable of providing the most efficacious and selective treatment for each individual tumour and patient. Thus, the major focus of anticancer drug development has progressively moved from nonspecific chemotherapies to molecularly-targeted inhibitors. However, despite the huge amount of preclinical literature on how these rationally designed compounds work, their use in clinical practice is still limited.

How do cannabinoid-based medicines fit into this ongoing scenario? Consider glioma, the type of cancer in which the most detailed cannabinoid research has been conducted to date. As discussed here, engagement of a molecular target (the CB receptors) by a family of selective drugs (THC and other cannabinoid agonists) inhibits tumour growth in animal models through a well-established mechanism of action that also seems to operate in human patients. Moreover, cannabinoids potentiate the antitumour efficacy of temozolomide and ALK inhibitors in mice harbouring gliomas. Those findings provide preclinical proof-of-concept that "cannabinoid sensitizers" could improve the clinical efficacy of classical cytotoxic drugs in glioblastoma (Figure 2) and perhaps other highly malignant tumours such as pancreatic cancer, melanoma, and hepatocellular carcinoma. However, further research is required to define the precise molecular cross-talk between cannabinoids and chemotherapeutic drugs and to optimize the pharmacology of preclinical cannabinoid-based combination therapies.

With respect to patient stratification, the particular individuals that are potentially responsive to cannabinoid administration should be unequivocally determined. To that end, high-throughput approaches should be implemented to find cannabinoid therapy–associated biomarkers in tumour biopsies or, ideally, in easily acquired fluids containing circulating cancer cells or enhanced levels of resistance factors that might have been released by cancer cells. Such biomarkers would conceivably relate to cannabinoid pharmacodynamics—namely, expression and activity of cannabinoid receptors and their downstream cell-death-inducing effectors. The approach would be analogous to the biochemical evaluation of estrogen and ErbB2 receptors, which respectively predict benefit from endocrine therapies and trastuzumab in breast cancer. Predictive markers to define the sensitivity of a particular tumour to cannabinoid-based therapies could also include the status of growth factors, such as MDK in gliomas, and their receptors and signalling partners.

To summarize, cannabinoids induce tumour cell death and inhibit tumour angiogenesis and invasion in animal models of cancer, and there are indications that they act similarly in patients with glioblastoma. Given that cannabinoids show an acceptable safety profile, clinical trials testing them as single drugs or, ideally, in combination therapies in glioblastoma and other types of cancer are both warranted and urgently needed.

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CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare the following interests: GW Pharmaceuticals and Cellmid fund part of the research conducted by our laboratory. Likewise, a portion of the data obtained by the authors concerning the antitumoural action of cannabinoids is included in three patent applications presented by GW Pharmaceuticals.

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