

# Critical appraisal of the potential use of cannabinoids in cancer management

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**Abstract:** Cannabinoids have been attracting a great deal of interest as potential anticancer agents. Originally derived from the plant *Cannabis sativa*, there are now a number of endo-, phyto- and synthetic cannabinoids available. This review summarizes the key literature to date around the actions, antitumor activity, and mechanisms of action for this broad range of compounds. Cannabinoids are largely defined by an ability to activate the cannabinoid receptors – CB<sub>1</sub> or CB<sub>2</sub>. The action of the cannabinoids is very dependent on the exact ligand tested, the dose, and the duration of exposure. Some cannabinoids, synthetic or plant-derived, show potential as therapeutic agents, and evidence across a range of cancers and evidence in vitro and in vivo is starting to be accumulated. Studies have now been conducted in a wide range of cell lines, including glioma, breast, prostate, endothelial, liver, and lung. This work is complemented by an increasing body of evidence from in vivo models. However, many of these results remain contradictory, an issue that is not currently able to be resolved through current knowledge of mechanisms of action. While there is a developing understanding of potential mechanisms of action, with the extracellular signal-regulated kinase pathway emerging as a critical signaling juncture in combination with an important role for ceramide and lipid signaling, the relative importance of each pathway is yet to be determined. The interplay between the intracellular pathways of autophagy versus apoptosis is a recent development that is discussed. Overall, there is still a great deal of conflicting evidence around the future utility of the cannabinoids, natural or synthetic, as therapeutic agents.

**Keywords:** cancer, cannabinoid, endocannabinoid, tetrahydrocannabinol, JWH-133, WIN-55,212-2

## Introduction

The cannabinoids are a class of over 60 compounds derived from the plant *Cannabis sativa*, as well as the synthetic or endogenous versions of these compounds.<sup>1</sup> Cannabis has been used as a medicinal and recreational drug for many centuries, but its psychoactive properties have led to legal regulations around access and use in most countries.<sup>2</sup> Despite this, scientific research into both natural and synthetic cannabinoids has continued. Studies are now being conducted on the potential efficacy of cannabinoids, both natural and synthetic, as anticancer agents and their possible mechanisms of action.

The first cannabinoid to be intensively studied was *trans*- $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) which was first isolated in the 1960s.<sup>3</sup> While several other active compounds, notably  $\Delta^8$ -THC, cannabiol, cannabidiol, and cannabicyclol, were able to be isolated, it was not until 1992 that an analogous endogenous ligand – anandamide

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(AEA) – was identified (Table 1).<sup>4</sup> This discovery was closely followed by the identification of the endogenous ligands 2-arachidonoyl glycerol (2-AG), 2-arachidonoyl glyceryl ether (2-AGE), *O*-arachidonoyl ethanolamine (virodhamine), and *N*-arachidonoyl dopamine (NADA).<sup>5</sup> As the names of these compounds suggest, they are derivatives of the parent compound arachidonic acid, which is an important lipid-signaling molecule and a key component of the inflammatory pathway.<sup>6</sup> The endocannabinoids mimic the actions of  $\Delta^9$ -THC in mouse behavioral tests, interfere with learning and memory, activate the hypothalamic–pituitary–adrenal axis, decrease hypothalamus prolactin secretion, decrease intraocular pressure, cause hypotension and bradycardia, and modulate the immune system.<sup>7,8</sup>

The first cannabinoid receptor was discovered in 1988<sup>9</sup> and then cloned in 1990.<sup>10</sup> This was followed in 1993 with the discovery of a second form of the receptor, which shares 44% amino acid identity and a distinct yet similar binding profile for cannabinoid compounds.<sup>11</sup> This development led to the current terminology of CB<sub>1</sub>, for the original receptor form, and CB<sub>2</sub>. CB<sub>1</sub> receptors are found throughout the brain, spleen, eye, testis, and uterus,<sup>10–12</sup> whereas CB<sub>2</sub> receptors are associated with the cells and organs of the immune system as well as tumor cells.<sup>11,13,14</sup> Both receptors are part of the G-protein-coupled receptor (GPR) superfamily. In general, cannabinoid agonists do not show a great deal of selectivity between CB<sub>1</sub> and CB<sub>2</sub> binding; however, newly developed synthetic antagonists are now available that allow the experimental delineation of CB<sub>1</sub> versus CB<sub>2</sub> effects. On a general level, CB<sub>1</sub> binding is responsible for the psychoactive properties of the cannabinoid agonists, and CB<sub>2</sub> binding mediates immune effects.<sup>5</sup> This is discussed in more detail in later sections. The first cannabinoid-receptor antagonist was released in 1994 – SR141716 (rimonabant).<sup>15</sup>

Originally designed as a treatment for obesity, it has seen extensive use as a pharmacological tool in assessing agonist actions through the CB<sub>1</sub> receptor; however, the classification of this compound as a pure antagonist is likely to be misleading, and its more recent classification as a selective CB<sub>1</sub>-receptor inverse agonist is more accurate. Following the discovery of SR141716, a range of antagonists and inverse agonists has been developed, including SR144528, which is an inverse agonist at the CB<sub>2</sub> receptor<sup>16–18</sup> (Table 1).

In the 20 years since the discovery of the CB<sub>2</sub>-receptor isoform, there have been a number of anomalous results that suggest that cannabinoid agonists exert actions beyond those mediated by CB<sub>1</sub> or CB<sub>2</sub>.<sup>19</sup> This has led to the suggestion that there may be more isoforms of the CB receptor still to be identified; possible candidates include GPR119, GPR55, and GPR18.<sup>20</sup> Of the three, GPR55 has the largest body of evidence, suggesting it should be renamed as a cannabinoid receptor, but low-sequence homology with CB<sub>1</sub> and CB<sub>2</sub> along with conflicting results in agonist-binding studies means that it has not yet been fully reclassified.<sup>20,21</sup> Both GPR119 and GPR18 show evidence of cannabinoid binding, but the results are not sufficiently robust to rename either protein as a cannabinoid-receptor isoform.<sup>5,20,21</sup> The transient receptor potential cation channel subfamily V member 1 (TRPV1) receptor has also been proposed as a possible cannabinoid isoform. The endogenous cannabinoids AEA and NADA are potent TRPV1 agonists, and a raft of evidence has established the *in vivo* activity of these compounds unique to TRPV1 signaling.<sup>22</sup> However, TRPV1 is an ionotropic receptor rather than a GPR, which means it does not meet one of the five criteria established for classification as a cannabinoid receptor. The ruling body, the International Union of Basic and Clinical Pharmacology, is seeking further evidence before renaming TRPV1 as a possible ionotropic cannabinoid CB<sub>3</sub> receptor.<sup>20</sup>

**Table 1** A selection of cannabinoid receptor ligands and their specificities

Ligand	Source	Action	Specificity	K <sub>i</sub> CB <sub>1</sub> (nM)	K <sub>i</sub> CB <sub>2</sub> (nM)
$\Delta^9$ -THC	Plant-derived	Nonspecific agonist	CB <sub>1</sub> > CB <sub>2</sub>	5–80	3–75
Cannabidiol	Plant-derived	Low-to-no receptor affinity			
Anandamide (AEA)	Endogenous	Nonspecific agonist	CB <sub>1</sub> » CB <sub>2</sub>	61–543	279–1,940
2-arachidonoylglycerol (2-AG)	Endogenous	Nonspecific agonist	CB <sub>1</sub> > CB <sub>2</sub>	58–472	145–1,400
R-(+)-Met-anandamide	Synthetic	Nonspecific agonist	CB <sub>1</sub> » CB <sub>2</sub>	18–28	815–868
WIN-55,212-2	Synthetic	Nonspecific agonist	CB <sub>1</sub> = CB <sub>2</sub>	2–123	0.3–16
HU-210	Synthetic	Nonspecific agonist	CB <sub>1</sub> = CB <sub>2</sub>	0.06–0.7	0.2–0.52
JWH-133	Synthetic	Selective agonist	CB <sub>2</sub>	677	3.4
SR141716	Synthetic	Selective antagonist	CB <sub>1</sub>	1.8	514
SR144528	Synthetic	Selective antagonist	CB <sub>2</sub>	50–10,000	0.3–6

**Notes:** K<sub>i</sub> values are reported based on reported values for the *in vitro* displacement of [<sup>3</sup>H]CP 55,940 (CB<sub>1</sub>)- or [<sup>3</sup>H]HU 243 (CB<sub>2</sub>)-binding sites.

**Abbreviation:** THC, tetrahydrocannabinol.

## Physiological functions of cannabinoids

### Synthesis

The endocannabinoids (AEA and 2-AG) are generally accepted to be synthesized on demand following receipt of an intracellular signal, such as cellular depolarization or  $\text{Ca}^{2+}$ -dependent receptor stimulation. However, there is also mounting evidence to suggest that AEA may be stored to some degree within the adiposome cellular compartment.<sup>23</sup> AEA is synthesized from lipid precursors via enzymatic hydrolysis of *N*-acyl-phosphatidylethanolamines (NAPEs). This reaction is catalyzed by phospholipase D (PLD), which shows selectivity for this reaction.<sup>24,25</sup> PLD activity is regulated by cellular depolarization, by activation of the NMDA, and by the metabotropic classes of glutamate receptor.<sup>24</sup> The NAPE precursor is derived from the transfer of arachidonic acid from phosphatidylcholine to phosphatidylethanolamine, a reaction catalyzed by an *N*-acetyltransferase isoform. This transfer is modulated by the presence of  $\text{Ca}^{2+}$  and cyclic adenosine monophosphate (cAMP).<sup>24</sup>

The synthesis of 2-AG may theoretically proceed through several putative pathways, but consensus favors hydrolysis of membrane lipids through PLC to produce 1,2-diacylglycerol.<sup>26</sup> 1,2-diacylglycerol can then be converted through the action of diacylglycerol lipase to the 2-AG molecule. Although 2-AG concentrations are linked to membrane depolarization, similar to AEA, the underlying control mechanisms are thought to be different, as intracellular AEA concentrations cannot be directly linked to 2-AG concentrations.<sup>24,26</sup>

### Degradation

Once synthesized, the endocannabinoids act locally, either by interacting with plasma-bound cannabinoid receptors on the cell where they were produced or on directly neighboring cells. Signaling is terminated by the uptake of the endocannabinoid by a regulated mechanism facilitated by the AEA membrane transporter.<sup>25</sup> Following uptake, the cannabinoid is hydrolyzed by the fatty acid amide hydrolase and the monoacylglyceride hydrolase. Fatty acid amide hydrolase is a non-specific enzyme which catalyzes the hydrolysis of numerous fatty acid molecules and as such is widely distributed around the body.<sup>27</sup> Monoacylglyceride hydrolase, in contrast, is limited in its distribution to the nerve terminals of specific brain neurons.<sup>28</sup>

### Intracellular actions

Both forms of the traditional cannabinoid receptor,  $\text{CB}_1$  and  $\text{CB}_2$ , are G-protein-linked. This means their activation results

in inhibition of adenylate cyclase which is blocked by pertussis toxin. This causes a decrease in cellular cAMP, activation of the p38 mitogen-activated protein kinase (MAPK) pathway, and/or activation of ion channels. Evidence suggests that different conformational forms of GPRs allows downstream activities to be specifically modulated based on the individual ligand that binds and activates the receptor (Table 2).<sup>29</sup> This specificity of action has been observed for the  $\text{CB}_1$  receptor whereby the antagonist WIN-55,212-2 activated different  $\text{G}_{i/o}$  subtypes with differing sensitivities<sup>25,26,30,31</sup> (Table 2).

### Systemic actions of cannabinoids

The most obvious action of the cannabinoids is in the brain. Receptors and synthesis and degradation enzymes of the cannabinoids are found distributed throughout the central nervous system.<sup>32</sup> Ingestion of cannabinoids results in mood alterations, sedation, increased appetite, hallucinations, and impairment of memory, coordination, and executive function.<sup>33</sup> At higher concentrations, cannabinoids produce analgesia.<sup>34</sup> The majority of these effects are purported to be mediated through alterations of signaling through the glutamatergic neurons or  $\gamma$ -aminobutyric acid (GABA) neurons located in different areas of the brain.<sup>35</sup> Cannabinoid action in the motor cortex, basal ganglia, or cerebellum leads to impaired motor performance and ataxia.<sup>36,37</sup> The extreme effect of catalepsy is thought to be mediated by action at the globus pallidus, striatal spiny neurons, and cortical glutamate neurons.<sup>38,39</sup> The analgesic properties of

**Table 2** Intracellular action of cannabinoids

Second messenger	Direct effect	Cellular result
$\text{G}_{i/o}$	Inhibits adenylate cyclase	Decreased cAMP, inhibition of phosphokinase A (PKA)
$\text{G}_s$	Stimulates adenylate cyclase	Increase cAMP, activation of PKA
$\text{G}_q$	Modulation of $\text{Ca}^{2+}$ channel	Changes in intracellular $\text{Ca}^{2+}$
$\text{G}_{i/o}$	Activation of G-protein-coupled inwardly rectifying potassium channels (GIRKs)	Increase in intracellular K
$\text{G}_{i/o}$	Modulation of ERK1/2	Modulation of p38 MAPK and JNK pathways thereby regulating cellular proliferation, differentiation, movement and death

**Notes:** Data from.<sup>19,20,25</sup>

**Abbreviations:** ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; JNK, c-Jun NH(2)-terminal protein kinase; cAMP, cyclic adenosine monophosphate.

the cannabinoids are linked to action on GABA neurons in the periaqueductal grey and rostroventral medulla regions and the ventral posterolateral nucleus of the thalamus.<sup>40</sup> CB<sub>1</sub> receptors are also present in the spinal cord and on peripheral nociceptors, where they can mediate neuropathic pain and inflammation.<sup>41–43</sup> However, recently there has been a degree of controversy around the specificity of antibodies used in several cannabinoid-receptor immunolocalization studies.<sup>44</sup> Therefore, it is possible that the results regarding the exact distribution of the receptor in anatomical substructures are likely to be challenged and refined over the coming years.

The role of cannabinoids as modulators of the immune system is becoming more obvious. CB<sub>1</sub> receptors are located on T lymphocytes<sup>45</sup> whereas CB<sub>2</sub> receptors, historically associated with immune function, are located in human B cells, natural killer cells, monocytes, polymorphonuclear neutrophils, and T cells.<sup>46</sup> In general, cannabinoid agonists are associated with decreased immune function and decreased release of inflammatory mediators, such as interleukin (IL)-1, IL-2, IL-6, and IL-12.<sup>47</sup> However, this effect is complicated by duration of exposure and dose.<sup>46,48</sup>

## Role of cannabinoids in cancer

Two therapeutic avenues exist for the development of cannabinoids as anticancer agents. As antiemetic and analgesic compounds, this class of compounds has been explored in terms of palliative care. More recently, cannabinoid agonists and antagonists have been screened for potential direct antitumorigenic properties.

### Palliative care

Cannabinoids can play an important role in the palliation of pain, nausea, vomiting, and appetite for cancer patients; however, this is beyond the scope of this review. The palliative uses of cannabinoids have been reviewed elsewhere.<sup>49–51</sup>

### Antitumorigenic properties

Cannabinoids are not yet approved for the treatment of tumor progression, although their antitumorigenic effects have been known for over 30 years.<sup>52</sup> Cannabinol and  $\Delta^8$ -THC inhibited tumor growth in a mouse model of Lewis lung adenocarcinoma after 20 days of treatment, whereas cannabidiol or  $\Delta^9$ -THC failed to show any effect.<sup>52</sup> Since this pioneering study, a vast range of cancer cell and tumor models have been used to evaluate the possible efficacy and mechanisms of cannabinoid antitumor activity. This work is supported by findings that the endocannabinoid system may be altered during disease states. Significant levels of the cannabinoid

receptor are found in prostate, breast, leukemia, melanoma, and thyroid cell lines, as well as colorectal and hepatocellular carcinoma tissue.<sup>53–58</sup> Of particular significance is the fact that in prostate cancer cell lines, there is evidence that the expression of both CB<sub>1</sub> and CB<sub>2</sub> is elevated compared to normal prostate cells.<sup>53</sup> Similarly, in lymphoma and breast cancer tissue, as well as some derived cell lines, CB<sub>1</sub> and CB<sub>2</sub> are overexpressed.<sup>59,60</sup> The degree of increased expression correlates with tumor aggression and progression, an effect also reported in human astrocytes.<sup>54,61</sup> In contrast, McKallip et al found that Michigan Cancer Foundation (MCF)-7 and MDA-MB-231 breast cancer cell lines showed very low levels of CB<sub>1</sub> expression, and CB<sub>2</sub> was below detectable levels, as determined by reverse-transcription polymerase chain reaction.<sup>62</sup> One reason for the reported discrepancies may be inaccuracies in reporting the subcellular localization of the receptor. It is probable that extensive trafficking between the plasma membrane and internal compartments, such as lysosomes, occurs, and that this is altered by exposure to cannabinoid agonists<sup>59,63–69</sup> (Table 3).

## Anticancer actions of specific compounds

### Endo- and phytocannabinoids ( $\Delta^9$ -THC, AEA, cannabidiol)

The primary active constituent of cannabis,  $\Delta^9$ -THC, has been investigated in a number of in vitro- and in vivo-based systems. Overall, some efficacy has been recorded in breast, prostate, glioma, lymphoma, and pancreatic cancer cell lines. In contrast, there are reports of pro-cancerous activity in breast, bronchial, hepatoma, and lung cell lines (Table 3).

Results are not clear-cut as to whether  $\Delta^9$ -THC causes pro- or antiproliferative effects in breast cancer cells. A study of  $\Delta^9$ -THC in MCF-7 and MDA-MB-231 cells ( $\leq 5 \mu\text{M}$ ) reported proliferation in response to cannabinoid treatment.<sup>62</sup> This finding is supported by the work of Takeda et al, who also documented a proliferative response to  $\Delta^9$ -THC in MCF-7 cells.<sup>70</sup> In contrast, McAllister et al reported decreased proliferation in MDA-MB-231 and MDA-MB-468 cells, while studies in EVSA-T cells also showed  $\Delta^9$ -THC inhibited cell growth.<sup>71</sup> In MCF-7 cells, von Bueren et al reported that  $\Delta^9$ -THC did not induce cell proliferation at concentrations up to 1 mM, but did inhibit 17 $\beta$ -estradiol (100 nM)-induced proliferation at concentrations above 1  $\mu\text{M}$ .<sup>72</sup> In mouse models of breast cancer, contradictory results have also been reported. In a xenotransplant model of 4T1 paw cells in BALB/c mice, an increase in tumor size was recorded following  $\Delta^9$ -THC (25 mg/kg, intraperitoneally, 21 days).<sup>62</sup> In contrast, in MMTV-neu mice that showed

**Table 3** Cannabinoid action in various cancer cell lines

Cell type/line	Source	Cannabinoid	Effect
ND	Bronchial epithelium	THC	Increased proliferation
ND	Endothelial	THC	Increased proliferation
NCI-H292	Lung	THC	Increased proliferation
Hepa	Hepatoma	THC	Increased proliferation
A549	Lung	THC	Increased proliferation
A549, H460, H358	Lung	CBD	Decreased invasion
LNCaP	Prostate	R-(+)-Met	Increased proliferation
PC-3, LNCaP	Prostate	AEA, R-(+)-Met, THC	Decreased proliferation
LNCaP	Prostate	WIN-55,212-2	Decreased proliferation
MCF-7	Breast	AEA, 2-AG, HU-210	Decreased proliferation
MCF-7, MDA-MB-231, MDA-MB-436	Breast	THC, Met-F-AEA, WIN-55,212-2, JWH-133	Decreased proliferation
4T1	Mouse mammary	THC	Decreased proliferation
TSA-E1	Mouse breast cancer	Met-F-AEA	Decreased proliferation
U87-MG, U373	Glioma	CBD	Decreased proliferation
C6	Rat glioma	THC, JWH-122, WIN-55,212-2	Decreased proliferation
U251, SF-126	Glioblastoma	THC	Decreased proliferation
GBM	Glioblastoma	THC, WIN-55,212-2	Decreased proliferation
	Human astrocytoma	JWH-133	Decreased proliferation
KiMol	K-ras-transformed FRTL-5 thyroid	Met-F-AEA	Decreased proliferation
EL-4	Thymoma/lymphoma	HU-210	Decreased proliferation
PDV-C57	Mouse skin carcinoma	JWH-133, WIN-55,212-2	Decreased proliferation
HUVEC	Umbilical vein	JWH-133	Decreased proliferation

**Notes:** Data from.<sup>55-58</sup>

**Abbreviations:** ND, not described; THC, tetrahydrocannabinol; CBD, cannabidiol; MCF, Michigan Cancer Foundation; R-(+)-Met, R-(+)-methanandamide; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; Met-F-AEA, Met-fluoro-anandamide; GBM, glioblastoma multiforme; HUVEC, human umbilical vein endothelial cell.

spontaneous development of mammary tumors,  $\Delta^9$ -THC (0.5 mg/animal/day, peritumorally, 90 days) decreased the size of mammary tumors, the occurrence of new spontaneous tumors, and the degree of metastasis to the lungs.<sup>73</sup> These results indicate that the role of compounds such as  $\Delta^9$ -THC is not well understood, and its effects are possibly regulated by a range of factors that are yet to be determined.

In prostate cancer cell lines,  $\Delta^9$ -THC and 2-AG increased cellular proliferation, whereas AEA had the opposite effect. Sánchez et al documented increased proliferation of LNCaP and PC3 cells following treatment with  $\Delta^9$ -THC (50 nM).<sup>74,75</sup> In the same cell lines, AEA decreased proliferation. Mimeault et al evaluated AEA in PC3, LNCaP, and DU145 cell lines, and showed a significant decrease in cell viability at concentrations above 2  $\mu$ M.<sup>76</sup> Similarly, Nithipatikom et al documented a decrease in PC-3 cellular proliferation at AEA concentrations above 1  $\mu$ M, whereas 2-AG at similar concentrations caused an increase in cellular growth.<sup>77</sup>

In glioma cell lines, almost all studies show that cannabinoids decrease cell proliferation.  $\Delta^9$ -THC has been investigated in C6, SF126, U87-MG, U251, SF188, and U373-MG cell lines at concentrations of 1–2  $\mu$ M. Results showed a consistent decrease in cell viability independent of cell line.<sup>78,79</sup> In U87 and U373 cells, cannabidiol inhibited cell proliferation, but the half-maximal inhibitory concentration

(IC<sub>50</sub>) value for this effect was unusually high at 25  $\mu$ M.<sup>80</sup> Both AEA and 2-AG also demonstrated antiproliferative effects in the C6 cell line with IC<sub>50</sub> values of 1.6 and 1.8  $\mu$ M, respectively.<sup>81</sup>

$\Delta^9$ -THC shows inhibitory effects in a range of pancreatic cell lines; however, this effect appears to be biphasic, with evidence of proliferation at concentrations under 1  $\mu$ M and inhibition at concentrations above 2  $\mu$ M. This biphasic trend was most obvious in the Panc1 and Capan2 cell lines and less obvious in MiaPaCa2 and BxPc3 cells.<sup>82</sup> When MiaPaCa2 cells were implanted subcutaneously into nude mice and left to form tumors,  $\Delta^9$ -THC (15 mg/kg/day, 15 days) caused a significant reduction in tumor growth.<sup>82</sup>

Both  $\Delta^9$ -THC and AEA (or the stable equivalent Met-AEA) inhibited the growth of lymphoma cell lines, although this effect was related to the serum content of the cell media used. In serum-free media,  $\Delta^9$ -THC showed significant inhibition of growth in EL-4, LSA, and P815 cells at concentrations above 3  $\mu$ M. However, with the inclusion of 5% calf serum in the media, no significant effect was observed until concentrations of 10  $\mu$ M were reached.<sup>83</sup> Herrera et al reported a decrease in the viability of Jurkat cells at concentrations of 1.5  $\mu$ M and above when using heat-inactivated fetal bovine serum, though this study did not assess cell viability in a completely media-free system.<sup>84</sup> However, several other

studies have reported a decrease in the cell viability of Jurket cells following exposure to  $\Delta^9$ -THC.<sup>83,85-88</sup> In mantle-cell lymphoma, two studies reported an effect of AEA or the stable equivalent, Met-AEA, with the effective concentration ranging from 5 to 10  $\mu\text{M}$  for each compound.<sup>89,90</sup>

In contrast to the inhibitory effects documented above, the natural cannabinoids have also shown to increase cell proliferation, although normally these results have been documented in studies investigating smoke mixtures that include natural cannabinoid compounds. One study using pure  $\Delta^9$ -THC at concentrations between 0.1 and 0.3  $\mu\text{M}$  showed increased proliferation of NCI-H292 lung carcinoma cells.<sup>91</sup> In the lungs of habitual marijuana smokers, significant increases in the proliferation marker Ki67 were observed along with changes in the expression of the epidermal growth-factor receptor, the human epidermal growth-factor receptor 2/neu receptor, p53, and DNA ploidy.<sup>92</sup> In hepatoma cells,  $\Delta^9$ -THC (2  $\mu\text{g}/\text{mL}$ ) induced the drug metabolizing enzyme cytochrome P450 1 A1, which is linked to the development of tobacco-related cancers.<sup>93</sup> This induction effect was seen with both a marijuana-derived tar mixture and pure extracts of  $\Delta^9$ -THC. No effects on cellular proliferation were reported.<sup>93</sup> Epidemiological studies around the development of cancer in cannabis smokers have been similarly inconclusive as to whether natural cannabinoids are pro- or anticarcinogenic.<sup>94-96</sup>

### Synthetic cannabinoids (WIN-55,212-2, and JWH-133)

WIN-55,212-2 is a nonselective agonist of  $\text{CB}_1$  and  $\text{CB}_2$  that exhibits anticancer effects in prostate, glioblastoma, glioma, breast, lymphoma, and melanoma cell lines.<sup>53,79,97,98</sup> In the LNCaP prostate cancer cell line, WIN-55,212-2 dose-dependently decreased cell viability, with an overall  $\text{IC}_{50}$  value of 6  $\mu\text{M}$ .<sup>53</sup> Similar to malignant glioma cells, the levels of both  $\text{CB}_1$  and  $\text{CB}_2$  were elevated in this cell line, as well as in DU145, PC3, and CWR22Rv1 lines.<sup>53</sup> The inhibition of prostate cancer growth can be mediated by both the  $\text{CB}_1$ - and  $\text{CB}_2$ -receptor isoforms. When WIN-55,212-2 (7.5  $\mu\text{M}$ ) was coadministered with either SR141716 or SR144528 (2  $\mu\text{M}$ ), the cell growth recovered.<sup>53</sup> In a study of cell invasion, WIN-55,212-2 at 1 nM caused a 40% reduction in the invasion measure of PC3 cells.<sup>97</sup> In contrast, in DU-145 cells no reduction was seen until 100 nM (causing a 20% reduction), and no effect was observed in LNCaP cells. In breast cancer cells (MDA-MB-231 and MDA-MB-468) and an MDA-MB-231 tumor-implant model, WIN-55,212-2 inhibited cell and tumor growth (in vitro  $\text{IC}_{50} \leq 10 \mu\text{M}$ , in vivo

5 mg/kg/day, intratumorally for 4 weeks).<sup>54</sup> One factor of critical importance in all these studies is the dose of agonist administered. The cannabinoid receptor shows ligand-binding affinity for WIN-55,212-2 within the nanomolar range (3 and 16 nM for human  $\text{CB}_1$  and  $\text{CB}_2$ , respectively).<sup>18</sup> Therefore, it can be suggested that at doses above 4  $\mu\text{M}$ , any observed effects may not be cannabinoid receptor-mediated. This warrants further investigation, particularly in the prostate and breast cancer cell lines previously discussed.

WIN-55,212-2 (1.25  $\mu\text{M}$ ) inhibited cell growth by greater than 50% in the SF126, U87-MG, U251, U373-MG, and SF-188 glioblastoma cell lines, while in C6 glioma cell lines 15  $\mu\text{M}$  was required to generate a similar effect.<sup>79,98</sup> In vivo, regression of C6 cell tumors was observed following 8 days of WIN-55,212-2 administration (50  $\mu\text{g}/\text{day}$  intratumorally).<sup>61</sup>

In vivo, the growth of melanoma xenografts was decreased in WIN-55,212-2-treated mice. Blázquez et al demonstrated decreased cell viability in the melanoma-derived B16 and A375 cell lines and a significant reduction in tumor volume in B16-implanted mice following 8 days of WIN-55,212-2 treatment (50  $\mu\text{g}/\text{day}$ , peritumorally).<sup>56</sup> Similar results were seen in a PDV.C57 implantation model of melanoma, with tumor shrinkage observed following 11 days of WIN-55,212-2 treatment (1.5  $\mu\text{g}/\text{day}$ , peritumorally by continuous-flow pump).<sup>99</sup>

Similarly, WIN-55,212-2 has been shown to have efficacy in mantle-cell lymphoma tumors and cell lines. In Rec-1, JeKo, and JVM-2 cell lines, WIN-55,212-2 induced cell death and cleavage of caspase 3 at doses above 5  $\mu\text{M}$ .<sup>89,100</sup> This effect was blocked by pre-treatment with either a  $\text{CB}_1$  or  $\text{CB}_2$  inhibitor (SR141716A or S144528).<sup>100</sup> In tumors derived from mantle-cell lymphoma patient samples, similar cellular toxicity was observed, with  $\text{IC}_{50}$  values ranging between 1.47 and 4.81  $\mu\text{M}$ , depending on the individual patient.<sup>60</sup> Interestingly, this decrease in cell viability did not correlate with the cleavage of caspase 3, suggesting that the mechanism of action is not via apoptosis.<sup>60</sup>

JWH-133 is a selective agonist for the  $\text{CB}_2$  receptor; therefore, it has been investigated as a possible cancer therapeutic that may lack psychoactive side effects. The first study to demonstrate the cytotoxicity of JWH-133 in vitro was conducted in glioma cells.<sup>61</sup>  $\text{CB}_2$  is expressed at low levels in microglial cells under normal conditions; however, overexpression is correlated with the development of malignancy.<sup>101,102</sup> In glioma cells, JWH-133 reduced cell viability by 50% in vitro, while in vivo studies showed a 71% decrease in tumor growth after 8 days (Rag<sup>-/-</sup> mice,

40 µg/day intratumorally). This effect on tumor growth was inhibited by the CB<sub>2</sub> antagonist SR144528, but not the CB<sub>1</sub>-specific antagonist SR141716.<sup>61</sup> These results are supported by studies in glioma (C6 cells) and astrocytoma xenografts, where the overall vascularization of tumors was reduced by 88% and 21% respectively (50 µg/day intratumorally, 8 or 25 days for gliomas and astrocytes).<sup>103</sup>

In a skin-tumor model, JWH-133 (83 µg/day for 11 days via continuous-flow pump) caused a 60% decrease in tumor size in PDV.C57 cell xenografts.<sup>99</sup> In melanoma xenografts, JWH-133 decreased tumor volume by 75% over an 8-day period (50 µg/day), with tumors displaying decreased vascularity and increased numbers of apoptotic cells.<sup>56</sup>

In breast cancer cell lines, JWH-133 decreases cell proliferation and induces apoptosis. In the breast MDA-MB-231 cell line, a 58% reduction in growth and migration was observed at 10 µM.<sup>54</sup> A similar effect was seen in the MDA-MB-468 breast cancer cell line. These results were confirmed in vivo, with a 46% reduction in MDA-MB-231-implanted tumor growth over an 8-week period (5 mg/kg/day intraperitoneally), an effect that was blocked by the simultaneous administration of SR144528.<sup>54</sup> In MMTV-neu mice, a model of Erb2-driven metastatic breast cancer, JWH-133 at 50 µg/day (peritumorally twice a week for 90 days) exhibited a range of anticancer effects, including decreased tumor size, inhibition of new tumor development, decreased tumor cell proliferation, inhibition of angiogenesis, and decreased lung metastasis.<sup>73</sup> These results were comparable to findings with the mixed agonist Δ<sup>9</sup>-THC, suggesting an important role for the CB<sub>2</sub> receptor in the Δ<sup>9</sup>-THC mediated effect.

## Overview of potential mechanisms Effects on tumor growth and development

Cannabinoids affect a range of pathways that regulate cell division and viability; however, the knowledge in this area remains incomplete. For example, it is still difficult to explain the myriad of results around cell survival that have been reported in the literature. This is confounded by a lack of understanding of the possible receptors involved and ongoing doubt over their definitive localization. In addition, the actual mechanisms of cell death remain controversial, with some authors maintaining that autophagy precedes apoptosis and others suggesting that apoptosis is stimulated directly. Current evidence suggests that the type and stage of the cancer is likely to be important, with hormone-dependent cancers possibly reacting differently to cannabinoid exposure than gliomas (the most studied cancer type in terms of cannabinoid action).

Endogenous cannabinoids regulate the de novo synthesis of ceramides, lipid-based components of the cell membrane that perform both structural and signaling functions. It is becoming increasingly obvious that ceramide functions as a physiological signaling molecule, particularly with regard to the control of apoptosis, but also growth arrest, differentiation, cell migration, and adhesion.<sup>104</sup> As such, the role and regulation of ceramide signaling is attracting increasing attention, and ceramide now has an accepted role in the development of some cancers.<sup>105</sup> Activation of either CB<sub>1</sub> or CB<sub>2</sub> in glioma cells is associated with an increase in ceramide levels leading to the activation of the extracellular signal-regulated kinase (ERK) pathway via Raf-1 activation and p38 MAPK activation.<sup>14,106</sup> Both these pathways ultimately cause apoptosis through caspase activation and/or cell-cycle arrest.<sup>14</sup> In breast cancer cells, the CB<sub>1</sub> antagonist SR141716 inhibited cell proliferation through the effects of ERK1/2 colocalized inside membrane lipid rafts/caveolae.<sup>59</sup> Such rafts play a critical role in the growth and metastasis of breast tumors.<sup>107,108</sup> A final component of the ERK pathway, p53, plays a crucial role in switching between cell-cycle arrest and apoptosis.<sup>109</sup> In cultured cortical neurons, Δ<sup>9</sup>-THC activated p53 via the CB<sub>1</sub> receptor, thereby activating the apoptotic cascade involving B-cell lymphoma (Bcl)-2 and Bcl-2-associated X protein, suggesting that the cannabinoid pathway ultimately causes cellular death via apoptosis.<sup>110</sup>

It is also likely, at least in some cell types, that autophagy precedes the apoptotic cascade. Autophagy has been reported in glioma, pancreatic, breast, and hepatocellular carcinoma cells, with additional reports that WIN-55,212-2 causes autophagy in mantle-cell lymphoma.<sup>111,112</sup> As yet, there is no evidence for autophagy in rhabdomyosarcoma, leukemia, prostate, or melanoma cell lines.<sup>111</sup> Autophagy is linked to endoplasmic reticulum stress, and follows similar pathways to the aforementioned ERK-mediated mechanism. The upregulation of ceramide appears to be the likely link between cannabinoid exposure and these endoplasmic reticulum effects. CB<sub>1</sub> and CB<sub>2</sub> activation induces serine palmitoyltransferase, the rate-limiting step of de novo ceramide synthesis.<sup>113</sup> Ceramide activates nuclear protein 1 (previously p8) through the action of ER-associated eIF2α which triggers a signaling cascade through tribbles homologue 3, AKT, and mammalian target of rapamycin complex 1 to cause autophagy.<sup>114</sup> This process involves the encapsulation of key organelles in double-membrane vesicles for breakdown and recycling. There has been debate as to whether this process is cytoprotective or cytotoxic, but it appears it can be both. In the case of cannabinoid treatment, apoptosis through mitochondrial disruption

is observed.<sup>114,115</sup> Alternatively, signaling via the putative GPR55 receptor in ovarian and prostate cells activates ERK and AKT signaling pathways, which alters intracellular calcium signaling, thereby affecting cellular proliferation.<sup>116</sup>

In addition to regulation through the ceramide pathway, cannabinoids exhibit a direct effect on cAMP levels through the regulation of adenylate cyclase, downregulation of protein kinase A, and a decrease in gene transcription.<sup>14,68,117</sup> In hormone-responsive cancer cells, this leads to decreases in the expression of breast cancer-associated antigen 1, prostate-specific antigen, and the androgen receptor in breast and prostate cells, respectively.<sup>25,53</sup> The downregulation of protein synthesis also results in a decrease in the expression of the high-affinity nerve-growth factor tyrosine-kinase receptor A and the prolactin receptor, thereby decreasing cell sensitivity to key growth promoters.<sup>69</sup> In addition to cell-level effects through protein kinase A, cannabinoids regulate the action of hormones through the hypothalamic–pituitary–gonadal axis. For example, exposing rats to  $\Delta^9$ -THC (1 mg/kg, intravenously) or WIN-55,212-2 (0.5 mg/kg, intravenously) resulted in a decrease in the secretion of luteinizing hormone within 30 minutes.<sup>118</sup> This effect was blocked by the preadministration of the CB<sub>1</sub> antagonist SR141716.<sup>118</sup> Similar results have been reported for prolactin release, while levels of adrenocorticotropic hormone have been shown to increase following  $\Delta^9$ -THC.<sup>119,120</sup> Normal anterior pituitary tissue expresses cannabinoid receptors, which indicates that the cannabinoids may be able to exert modulatory actions directly at the level of the pituitary gland.<sup>121</sup> This further complicates the results that may occur in *in vivo* investigations of hormone-responsive tumors. However, the decrease in luteinizing hormone may suggest a concomitant decrease in steroid-hormone production that may be protective, especially in breast cancer.<sup>122</sup>

In glioma cells, there is evidence that cannabinoids, specifically cannabidiol, activate apoptosis independently of cannabinoid-receptor binding. The mechanism for this effect is likely to involve the induction of oxidative stress through the generation of reactive oxygen species. This concept is supported by the observation that the antiproliferative effect was not blocked by a CB<sub>2</sub> antagonist, but was inhibited by tocopherol, a potent antioxidant. This effect was not observed in noncancerous primary glial cells.<sup>123</sup>

## Effects on invasion and metastasis

Cannabinoids affect a wide range of markers associated with the invasion and metastasis of cancers, including markers of migration, adhesion, invasion, and metastasis itself.<sup>124</sup> For example, studies on migration have shown that AEA,

2-AG, cannabidiol, HU-210, JWH-133, Met-fluoro-AEA (Met-F-AEA),  $\Delta^9$ -THC, and WIN-55,212-2 all decreased migration, or markers of migration, in a wide range of cell lines. Joseph et al reported that the adrenalin-induced migration of SW480 and MDA-MB-468 cells was inhibited following exposure to AEA or JWH-133 at 40 and 10 nM, respectively.<sup>125</sup> A similar effect was reportedly observed following treatment with HU-210, although detail was not provided.<sup>125</sup> In T lymphocytes, only JWH-133 (10 nM) was able to reduce stromal cell-derived factor 1-induced migration, with AEA (10 nM) showing no effect.<sup>125</sup> These results have been corroborated in cervical cells exposed to 2-AG or WIN-55,212-2, with both compounds reducing scratch closure in monolayers of SW756 cells.<sup>126</sup> Cannabidiol ( $\geq 3 \mu\text{M}$ ) inhibited the migration of U87 glioma cells in a Boyden chamber assay, but interestingly this effect was not blocked by the cannabinoid receptor antagonists SR141716 (CB<sub>1</sub>) or SR144528 (CB<sub>2</sub>).<sup>127</sup> This suggests that some, if not all, the migration effects observed in cell systems may not be mediated by the cannabinoid receptor.

The adhesion of cells within the extracellular matrix is an important component of maintaining correct multicellular structure, with dysfunction of this process associated with metastasis. In a model of metastatic spreading using MDA-MB-231 cells, Met-F-AEA (0.5 mg/kg every 72 hours for 21 days) significantly reduced the number and size of metastatic nodes, an effect antagonized by SR141716.<sup>128</sup> The authors were able to demonstrate that the effect on metastasis was linked to the phosphorylation of two tyrosine-kinase proteins involved in migration and adhesion: focal adhesion kinase (FAK) and Src.<sup>128</sup> This contradicts previous results in neuroblastoma cells, where HU-210 at 10 nM caused phosphorylation of the FAK-related nonkinase but not FAK itself.<sup>129</sup> However, WIN-55,212-2 (20  $\mu\text{M}$ ) also modulated a range of adhesion proteins, including intercellular adhesion molecule 1, IL-1, vascular cell-adhesion protein 1, and IL-8.<sup>130</sup> The authors conclude that this inhibition of the IL-1 pathway is mediated by effects of WIN-55,212-2 on nuclear factor  $\kappa\text{B}$  transactivation. This inhibition is likely to mediate a range of downstream effects, including anti-inflammatory and anticancerous actions.<sup>130</sup>

Tissue inhibitors of matrix metalloproteinases (TIMPs) may be a key mechanism by which cannabinoids inhibit tissue invasion. Decreased TIMP expression is highly correlated with cancer invasiveness, and the expression of TIMP-1 is a potent suppressor of tumor growth and angiogenesis.<sup>131,132</sup> However, the effect of cannabinoids on TIMP expression levels is controversial. Several studies have documented



an increase in TIMP-1 levels following cannabinoid administration. In lung and cervical cell lines (A549 and HeLa, C33A, respectively),  $\Delta^9$ -THC at 0.01  $\mu$ M increased the expression of TIMP-1, which correlated with a decrease in cell invasion.<sup>133</sup> This effect was blocked by the preadministration of the specific inverse agonists AM-251 ( $CB_1$ ) or AM-630 ( $CB_2$ ).<sup>133</sup> However,  $\Delta^9$ -THC decreased TIMP-1 expression in C6.9 and C6.4 glioma cell lines, a result that was confirmed in biopsies from patients with recurrent glioblastoma multiforme tumors undergoing a clinical trial of THC efficacy.<sup>134</sup> JWH-133 also downregulated TIMP-1 expression in glioma cells.<sup>134</sup> This suggests that the effects of cannabinoids on invasion may prove to be cancer- and cannabinoid-specific.

In terms of migration, cannabidiol, Met-F-AEA,  $\Delta^9$ -THC, and WIN-55,212-2 have all proven to have direct effects on migration markers. In breast cancer cells, cannabidiol decreased lung metastasis of MDA-MB-231 cells,<sup>135</sup> while Met-F-AEA inhibited the migration of this cell line on type IV collagen.<sup>128</sup> This effect was also seen in the TSA-E1 murine breast cancer cell line and was antagonized by the administration of SR141716.<sup>128</sup> Similarly,  $\Delta^9$ -THC (1–20  $\mu$ M) inhibited the epidermal growth factor-induced growth, chemotaxis, and chemoinvasion of the lung cancer cell lines A549 and SW1573.<sup>136</sup> Finally, WIN-55,212-2 (50  $\mu$ g/day, daily for 8 days) reduced the metastasis of B16 melanoma cells to the lung and liver in a nude mouse implantation model.<sup>56</sup>

All these results suggest that overall the cannabinoids affect multiple cellular signaling pathways, which means they have the potential to decrease cancer development, growth, and metastasis. However, there are likely to be both cancer- and cannabinoid-specific elements to these effects. The final role of the cannabinoid receptors ( $CB_1$  and  $CB_2$ ) versus novel receptors (eg, GPR55) is also likely to be of ongoing importance.

### Effects on angiogenesis

Angiogenesis is critical for tumor development, and many anticancer agents are selected for their antiangiogenic properties. In vivo models show tumors from cannabinoid-treated animals have a decreased number of sprouting blood vessels, reduced vascular networks, and small, undifferentiated intratumoral blood vessels.<sup>103,137,138</sup> Cannabinoids may produce a dual attack on the development of tumor blood vessels, through the inhibition of proangiogenic regulators, such as vascular endothelial growth factor (VEGF), and through a direct effect on endothelial cells.<sup>103</sup>

A number of experiments have determined that the levels of the major vascularization factors, including VEGF,

are downregulated following administration of  $\Delta^9$ -THC, Met-F-AEA, WIN-55,212-2, and JWH-133.<sup>103</sup>  $\Delta^9$ -THC decreased VEGF levels in lung cancer cell lines (A549 and SW1573), and this effect correlated with a decrease in vascularization of A549 xenoplatation tumors in severe combined immunodeficient mice.<sup>136</sup> Met-F-AEA decreased the production of VEGF and the expression of its receptor, VEGFR-1, in K-ras-transformed thyroid cells.<sup>138</sup> WIN-55,212-2 and JWH-133 both showed inhibitory effects on VEGF and related markers of angiogenesis in skin carcinoma tumors (implanted PDV.C57 cells).<sup>99</sup> JWH-133 downregulated VEGF in subcutaneously implanted glioma cells, and caused a concomitant decrease in the associated compounds connective tissue growth factor, heme oxygenase 1, Id-3, midkine, and Tie-1.<sup>139</sup> Overall, cannabinoids appear to have consistent effects on the vascularization pathway, causing a decrease in tumor vascularization in in vivo models.

The endothelial cell lines human umbilical vein endothelial cells (HUVEC) and ECV304 showed direct susceptibility to WIN-55,212-2, with exposure to concentrations above 25 nM inducing cell death.<sup>103</sup> This effect was repeatable on exposure to other cannabinoids, including HU-210 (25 nM),  $\Delta^9$ -THC (1  $\mu$ M), or JWH-133 (25 nM).<sup>103</sup> Similarly, the administration of synthetic cannabinoid analogues (LYR-7 or LYR-8) both decreased HUVEC viability at concentrations of 5  $\mu$ M and above.<sup>140</sup> However, this effect was not blocked by the preincubation of cells with the specific inhibitors AM281 ( $CB_1$ ) or AM630 ( $CB_2$ ).<sup>140</sup> Therefore, the cannabinoids show significant potential as antiangiogenic agents, and this may prove key to their success as a clinical therapy, but the role of the cannabinoid receptors in this response is still to be fully elucidated.

### The future of cannabinoid compounds in cancer treatment

Overall, the cannabinoids may show future promise in the treatment of cancer, but there are many significant hurdles to be overcome. There is much still to be learned about the action of the cannabinoids and the endocannabinoid system. The current disagreements in the literature suggest gaps remain in the knowledge base around the normal signaling pathways used by endocannabinoids, the physiological systems that are involved, and the range of effects that these compounds cause. Future research will help clarify the actions of the cannabinoids, and particularly the endocannabinoid signaling pathway, which will be critical in the ongoing development of these compounds.

It is a distinct possibility that the cannabinoids may have a place in the future treatment of cancer. Several reports have shown that the synthetic cannabinoids in particular have the potential to show sufficient specificity and efficacy to be precursors to clinical treatments. However, at this point in time, the results from studies are lacking sufficient depth of understanding to allow this transition to occur. The contradictory nature of reports around the efficacy of compounds highlights our lack of detailed understanding of mechanisms of action. The resolution of the conflicting evidence around cannabinoid action will continue to be a research priority in the near future, and it is expected that developing a more robust understanding of the mechanisms of action underlying cannabinoid action will facilitate the acceptance of cannabinoid use in a clinical setting.

## Disclosure

The authors declare they have no conflict of interest, financial or otherwise, regarding this work.

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