CANNABINOIDS: POTENTIAL ANTICANCER AGENTS

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Cannabinoids — the active components of *Cannabis sativa* and their derivatives — exert palliative effects in cancer patients by preventing nausea, vomiting and pain and by stimulating appetite. In addition, these compounds have been shown to inhibit the growth of tumour cells in culture and animal models by modulating key cell-signalling pathways. Cannabinoids are usually well tolerated, and do not produce the generalized toxic effects of conventional chemotherapies. So, could cannabinoids be used to develop new anticancer therapies?

CANNABINOIDS
Compounds with tetrahydrocannabinol (THC)-like structures and/or THC-like pharmacological properties. Many compounds with a THC-like structure are present in cannabis, but not all of them have THC-like pharmacological properties. In addition, some natural or synthetic compounds have THC-like pharmacological properties but not THC-like structure.

CANNABIMIMETIC
Tetrahydrocannabinol (THC)-like in pharmacological terms. A compound is usually accepted as cannabimimetic if it produces four characteristic THC effects in an *in vivo* assay known as the 'mouse tetrad model': hypomotility, hypothermia, analgesia and a sustained immobility of posture (catalepsy).

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Preparations from *Cannabis sativa* have been used for many centuries both medicinally and recreationally. However, the chemical structure of their unique active components — the CANNABINOIDS — was not elucidated until the early 1960s. As they are highly hydrophobic, cannabinoids were initially believed to mediate their actions by inserting directly into biomembranes. This scenario changed markedly in the early 1990s, when specific cannabinoid receptors were cloned and their endogenous ligands were characterized, therefore providing a mechanistic basis for cannabinoid action. This led not only to an impressive expansion of basic cannabinoid research, but also to a renaissance in the study of the therapeutic effects of cannabinoids, which now constitutes a widely debated issue with ample scientific, clinical and social relevance. The scientific community has gained substantial knowledge of the palliative and antitumour actions of cannabinoids during the past few years. However, further basic research and more exhaustive clinical trials are still required before cannabinoids can be routinely used in cancer therapy.

Cannabinoids and their receptors

The hemp plant *Cannabis sativa* produces ~60 unique compounds known as cannabinoids. Although the pharmacology of most of the cannabinoids is unknown, it is widely accepted that Δ^9-tetrahydrocannabinol (THC) is the most important, owing to its high potency and abundance in cannabis[1]. Other relevant plant-derived cannabinoids include Δ^8-THC, which is almost as active as Δ^9-THC but less abundant; cannabinol, which is produced in large amounts but is a weak CANNABIMIMETIC agent; and CANNABIDIOL, which is abundant but has no cannabimimetic activity. THC exerts a wide variety of biological effects by mimicking endogenous substances — the endocannabinoids anandamide and 2-arachidonoylglycerol — that activate specific cannabinoid receptors (BOX 1).

So far, two cannabinoid-specific receptors — CB_1 and CB_2 — have been cloned and characterized from mammalian tissues[2]. Both the central effects and many of the peripheral effects of cannabinoids depend on CB_1-receptor activation. Expression of this receptor is abundant in the brain, particularly in discrete areas that are involved in the control of motor activity (basal ganglia and cerebellum), memory and cognition (cortex and hippocampus), emotion (amygdala), sensory perception (thalamus), and autonomic and endocrine functions (hypothalamus, pons and medulla), but the CB_1 receptor is also expressed in peripheral nerve terminals and various extraneural sites such as the testis, eye, vascular endothelium and spleen. By contrast, the CB_2 receptor is almost exclusively expressed in the immune system, both by cells, including B and T lymphocytes and macrophages, and by tissues, including the spleen, tonsils and lymph nodes[2–4].

Other than the endocannabinoids, there are three main structural classes of cannabinoid-agonist ligands. These are the 'classical' cannabinoid analogues of THC, the 'non-classical' bicyclic and tricyclic cannabinoid analogues of THC, and the aminoalkylindoles. All have

Summary

- Cannabinoids, the active components of *Cannabis sativa* and their derivatives, act in the organism by mimicking endogenous substances, the endocannabinoids, that activate specific cannabinoid receptors. Cannabinoids exert palliative effects in patients with cancer and inhibit tumour growth in laboratory animals.
- The best-established palliative effect of cannabinoids in cancer patients is the inhibition of chemotherapy-induced nausea and vomiting. Today, capsules of Δ^9-tetrahydrocannabinol (dronabinol (Marinol)) and its synthetic analogue nabilone (Cesamet) are approved for this purpose.
- Other potential palliative effects of cannabinoids in cancer patients — supported by Phase III clinical trials — include appetite stimulation and pain inhibition. In relation to the former, dronabinol is now prescribed for anorexia associated with weight loss in patients with AIDS.
- Cannabinoids inhibit tumour growth in laboratory animals. They do so by modulating key cell-signalling pathways, thereby inducing direct growth arrest and death of tumour cells, as well as by inhibiting tumour angiogenesis and metastasis.
- Cannabinoids are selective antitumour compounds, as they can kill tumour cells without affecting their non-transformed counterparts. It is probable that cannabinoid receptors regulate cell-survival and cell-death pathways differently in tumour and non-tumour cells.
- Cannabinoids have favourable drug-safety profiles and do not produce the generalized toxic effects of conventional chemotherapies. The use of cannabinoids in medicine, however, is limited by their psychoactive effects, and so cannabinoid-based therapies that are devoid of unwanted side effects are being designed.
- Further basic and preclinical research on cannabinoid anticancer properties is required. It would be desirable that clinical trials could accompany these laboratory studies to allow us to use these compounds in the treatment of cancer.

been subjected to comprehensive structure–activity relationship studies, which, by selectively modifying the chemical structure of cannabinoid molecules, have led to the generation of various types of potent synthetic cannabinoid-receptor agonists. Selective cannabinoid-receptor antagonists such as the diarylpyrazoles (prototypical compounds developed by Sanofi: for example, SR141716 for CB_1 and SR144528 for CB_2) have also been developed[2,5]. All of these compounds have been excellent pharmacological tools that have been used to achieve a detailed knowledge of cannabinoid action, and might serve as templates for the design of clinically useful drugs.

Palliative effects of cannabinoids

Cannabinoids have been known to exert palliative effects in oncology since the early 1970s, and for this reason they are given to patients — although quite restrictedly — in the clinic. The molecular basis of the established and potential palliative applications of cannabinoids are still being dissected.

Inhibition of nausea and emesis. Prolonged nausea and emesis/vomiting is a devastating side effect that regularly accompanies the administration of cancer chemotherapeutic drugs. This unwanted effect can be so severe that some patients stop their treatments despite the persistence of malignant cancer. When nausea and vomiting are frequent, antiemetic drugs are routinely given before and after chemotherapy.

Cannabinoids are antiemetic in animal models of vomiting[6]. As the CB_1 receptor is present in cholinergic nerve terminals of the MYENTERIC AND SUBMUCOSAL PLEXUS of the stomach, duodenum and colon, it is probable that cannabinoid-induced inhibition of digestive-tract motility is caused by blockade of acetylcholine release in these areas[6]. There is also evidence that cannabinoids act on CB_1 receptors that are localized in the dorsal–vagal complex of the brainstem — the region of the brain that controls the vomiting reflex — and that endocannabinoids and their inactivating enzymes are present in the gastrointestinal tract and might have a physiological role in the control of emesis[6,7].

One of the earliest studied, and so far the best established, therapeutic benefits of cannabinoids in humans is the treatment of nausea and vomiting. A great number of clinical trials with THC, synthetic cannabinoids and cannabis smoking in the 1970s and 1980s showed that the antiemetic potency of cannabinoids was at least equivalent to that of the antiemetics widely used at that time, such as the dopamine D_2-receptor antagonists prochlorperazine, domperidone and metoclopramide[8–10]. In addition, most of the patients tested had a clear preference for cannabinoids as antiemetics. META-ANALYSIS indicates that an optimal balance of efficacy and unwanted effects was achieved with relatively modest doses of THC (~5.0 mg/day), and that the dose could be increased during chemotherapy cycles[8–10]. Today, capsules of THC (dronabinol (Marinol)) and its classical synthetic analogue LY109514 (nabilone (Cesamet)) are approved to treat nausea and emesis associated with cancer chemotherapy (TABLE 1). Nabilone also inhibits nausea and vomiting associated with radiation therapy and anaesthesia after abdominal surgery. However, the effect of nabilone in these treatments is moderate[8–10].

Although it is clear that cannabinoids serve as antiemetic agents in cancer therapy, several questions remain to be answered[9]. Cannabinoids should be compared alone and in combination with modern antiemetics, such as the selective serotonin 5-HT_3-receptor antagonist ondansetron and the selective substance P/neurokinin-1-receptor antagonist aprepitant, which have fewer associated side effects than the antiemetics that were used when the original cannabinoid trials were carried out. Of interest, cannabinoids are relatively effective in preventing nausea and emesis in patients during the delayed phase of chemotherapy-induced emesis, which usually occurs 24 hours or more after chemotherapy and is poorly controlled in about half of the patients receiving 5-HT_3-receptor antagonists[6,7]. The reason for this distinct behaviour of cannabinoids and 5-HT_3-receptor antagonists is unknown, but might be because of the different pathophysiological bases of acute and delayed emesis. In addition, it is worth noting that cannabinoids can block 5-HT_3 receptors[11]. Further studies will be required to establish which patients and what types of cancer chemotherapy are suited to cannabinoid use for the prevention of nausea and emesis.

CANNABIDIOL
A non-psychoactive cannabinoid present in cannabis that inhibits convulsions, anxiety, vomiting and inflammation; it is now in Phase III clinical trials in combination with tetrahydrocannabinol for the treatment of multiple-sclerosis-associated muscle disorders.

MYENTERIC AND SUBMUCOSAL PLEXUS
A network of sympathetic and parasympathetic nerve fibres and neuron cell bodies that are tucked in among the interstices of the smooth-muscle layer surrounding the digestive mucosa (myenteric plexus) or just underneath the digestive mucosa (submucosal plexus) and that coordinately control gastrointestinal contractions.

META-ANALYSIS
Statistical analysis of a large collection of results from individual studies for the purpose of integrating their findings.

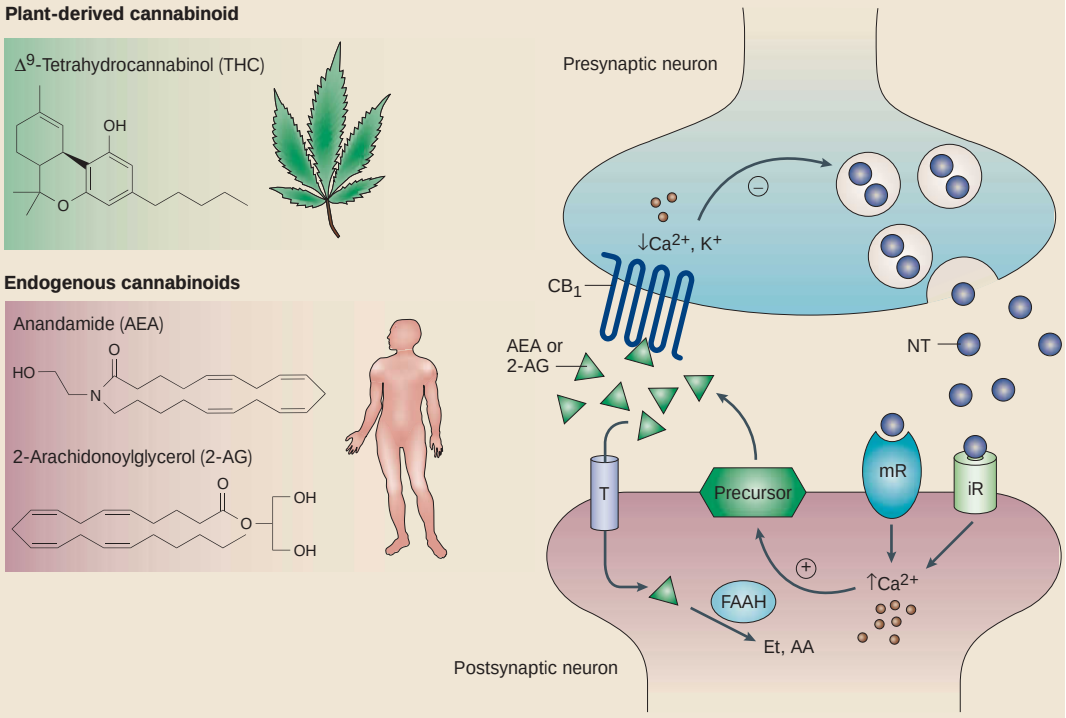
Appetite stimulation. More than half of the patients with advanced cancer experience lack of appetite and/or weight loss, and they consistently rank anorexia as one of the most troublesome symptoms. Anorexia might ultimately lead to massive weight loss — cachexia — which is an important risk factor for morbidity and mortality in cancer. About one-third of cancer patients lose more than 5% of their original body weight, and cachexia is estimated to account for ~20% of cancer deaths[12].

Many studies have reported that THC and other cannabinoids have a stimulatory effect on appetite and increase food intake in animals. These effects are particularly seen when cannabinoids are administered at low to moderate doses, which do not produce marked side effects[13]. The endogenous cannabinoid system might serve as a physiological regulator of feeding behaviour. For example, endocannabinoids and CB_1 receptors are present in the hypothalamus, the area of the brain that controls food intake; hypothalamic endocannabinoid levels are reduced by leptin, one of the main anorexic hormones; and blockade of tonic endocannabinoid signalling with the CB_1 antagonist

Box 1 | The endogenous cannabinoid system

Plant-derived cannabinoids such as Δ^9-tetrahydrocannabinol (THC), as well as their synthetic analogues, act in the organism by activating specific cell-surface receptors that are normally engaged by a family of endogenous ligands — the endocannabinoids (see figure). The first endocannabinoid discovered was named anandamide (AEA), from the sanscrit ananda, 'internal bliss', and with reference to its chemical structure — arachidonoylethanolamide, the amide of arachidonic acid (AA) and ethanolamine (Et)[100]. A second arachidonic-acid derivative (2-arachidonoylglycerol (2-AG)) that binds to cannabinoid receptors was subsequently described[101,102]. These endocannabinoid ligands, together with their receptors[103,104] and specific processes of synthesis[105,106], uptake[107] and degradation[108], constitute the endogenous cannabinoid system.

A well-established function of the endogenous cannabinoid system is its role in brain neuromodulation. Postsynaptic neurons synthesize membrane-bound endocannabinoid precursors and cleave them to release active endocannabinoids following an increase of cytosolic free Ca^{2+} concentrations: for example, after binding of neurotransmitters (NTs) to their IONOTROPIC (iR) or METABOTROPIC (mR) receptors[109]. Endocannabinoids subsequently act as retrograde messengers by binding to presynaptic CB_1 cannabinoid receptors, which are coupled to the inhibition of voltage-sensitive Ca^{2+} channels and the activation of K^+ channels[110]. This blunts membrane depolarization and exocytosis, thereby inhibiting the release of NTs such as glutamate, dopamine and γ-aminobutyric acid (GABA) and affecting, in turn, processes such as learning, movement and memory, respectively[111]. Endocannabinoid neuromodulatory signalling is terminated by an unidentified membrane-transport system[107] (T) and a family of intracellular degradative enzymes, the best characterized of which is fatty acid amide hydrolase (FAAH), which degrades AEA to AA and Et[108]. The endogenous cannabinoid system might also exert modulatory functions outside the brain, both in the peripheral nervous system and in extraneural sites, controlling processes such as peripheral pain, vascular tone, INTRAOCULAR PRESSURE and immune function.



IONOTROPIC RECEPTORS
Channel-like receptors that are opened by agonist binding and through which ions such as Na^+, K^+ and/or Ca^{2+} can pass. Ionotropic glutamate receptors are usually divided into three groups: *N*-methyl-D-aspartic acid (NMDA) receptors, kainate receptors and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors.

METABOTROPIC RECEPTORS
Seven-transmembrane (heptahelical) receptors that couple to heterotrimeric G proteins, thereby modulating pathways such as cyclic AMP–protein kinase A (via G_s or G_i), diacylglycerol–protein kinase C (via G_q) and inositol 1,4,5-trisphosphate–Ca^{2+} (via G_q). At least eight subtypes of glutamate metabotropic receptors are known.

INTRAOCULAR PRESSURE
Pressure inside the eye. When it increases — for example, in glaucoma — damage to the optic nerve of the eye can result in blindness. Cannabinoids decrease intraocular pressure.

Table 1 | Palliative effects of THC and nabilone in cancer therapy

Palliative effect on cancer therapy	Cannabinoid	Stage in clinical trials	References
Inhibition of nausea and emesis	THC, nabilone	Dronabinol and nabilone approved for cancer chemotherapy	6–10
Appetite stimulation	THC	Phase III with THC for cancer anorexia (however, dronabinol is approved for AIDS wasting syndrome)	8,10,13,18,19
Analgesia	THC	Phase III with THC for cancer pain	21,22,24–26
Inhibition of muscle weakness	THC, nabilone	Phase I/II with THC and nabilone for cancer depression and anxiety	8,10
Mood effects (sedation, antidepression, hypnosis)	THC (± cannabidiol)	Not for cancer, but Phase III with THC for multiple sclerosis muscle-debilitating symptoms	7,28

THC, Δ^9-tetrahydrocannabinol.

rimonabant (SR141716) — now in Phase III clinical trials for the treatment of obesity — inhibits appetite and induces weight loss[13,14]. Although the usual view is that cannabinoids centrally control appetite — as they are expressed in the brain — CB_1 receptors present in nerve terminals[15] and adipocytes[16,17] might also participate in the regulation of feeding behaviour.

Considerable anecdotal information from cannabis smokers and, more importantly, a series of clinical trials support the appetite-stimulating properties of THC[8,10,13]. In particular, the appetite-stimulating (orexigenic) action of THC has been repeatedly observed in AIDS patients, and so dronabinol is prescribed for anorexia associated with weight loss in AIDS patients (TABLE 1), at a dosage range of 2.5–5.0 mg/day[8,10]. In cancer patients, at least three Phase II clinical trials have established a relation between increased appetite and the prevention of body weight loss following THC treatment[10,18], and a recent Phase III trial has confirmed the appetite-stimulating effect of oral THC at 5.0 mg/day in advanced cancer[19].

Further research should elucidate the clinical relevance of cannabinoids for cancer anorexia. For example, the efficacy:safety ratio of different regimens of cannabinoid administration should be evaluated in comparison with the progesterone derivative megestrol acetate, the most extensively used agent for treating cancer anorexia[19]. Moreover, cachexia is caused not only by depression of food intake, but also by increased energy wasting[12]. In this respect, it is interesting that the CB_1 antagonist rimonabant not only suppresses appetite, but also enhances energy expenditure, indicating that CB_1 activation could be involved in energy preservation[16,17].

Pain inhibition. Pain has a negative impact on the quality of life of cancer patients. Almost half of all patients with cancer experience moderate to severe pain, and it increases in patients with metastatic or advanced-stage cancer. Chronic cancer pain usually has a NOCICEPTIVE component, which originates from inflammatory reactions around the sites of injury, and a neuropathic component, which results from damage to the nervous system. So, the pharmacological management of chronic pain should target peripheral nerves, the spinal cord and the brain[20].

Cannabinoids inhibit pain in animal models of acute and chronic HYPERALGESIA, ALLODYNIA and spontaneous pain, caused by heat, mechanical pressure, abdominal stretching, nerve injury and formalin injection[21,22]. There is sufficient evidence that cannabinoids produce antinociception by activating CB_1 receptors in the brain (thalamus, periaqueductal grey matter and rostral ventromedial medulla), the spinal cord (dorsal horn) and nerve terminals (dorsal root ganglia and peripheral terminals of primary-afferent neurons), and that endocannabinoids function naturally to suppress pain by inhibiting nociceptive neurotransmission[21,22]. In addition, peripheral CB_2 and/or CB_2-like receptors might mediate local analgesia, possibly by inhibiting the release of various mediators of pain and inflammation[21,23], which could be important in the management of cancer pain[20].

A meta-analysis of the clinical trials on cannabinoid analgesia is not feasible because of the dearth and heterogeneity of the trials carried out so far[24]. Nonetheless, there are some human data to support the effectiveness of cannabinoids in alleviating pain associated with cancer (TABLE 1), the effects of surgery, phantom limbs, multiple sclerosis, spinal-cord injury and migraine[21,22]. In particular, four Phase III clinical trials on cancer pain have been carried out, one with THC and the other three with two first-generation synthetic cannabinoid derivatives that are not used at present owing to their low potency and specificity. The general conclusion is that cannabinoids have similar analgesic potency to codeine — a moderate opioid analgesic[24,25].

Further clinical trials on cannabinoids in the treatment of cancer pain — including terminal care — seem justified[24,26] and, in fact, are now in progress. An adjunctive role for cannabinoids in analgesia seems the most likely[21,22] and, in this respect, it would be interesting to exploit the synergistic interactions that occur between cannabinoid and opioid antinociception observed in animal models[21,27].

Psychological effects. Studies in animal models indicate that cannabinoids — at least at low doses — exert anti-anxiety effects, and there is considerable anecdotal information about the effects of cannabis use on mood-related disorders[4,10]. However, only a few small trials with cannabinoids have systematically evaluated the mood

NOCICEPTIVE
A stimulus that causes pain or a reaction that is caused by pain.

HYPERALGESIA
An increased sensitivity and lowered threshold to a stimulus — such as burn of the skin — that is normally painful.

ALLODYNIA
Pain caused by a stimulus — such as touch, pressure and warmth — that does not normally provoke pain.

Table 2 | Tumours that are sensitive to cannabinoid-induced growth inhibition

Tumour type	Experimental system	Effect	Receptor	References
Lung carcinoma	*In vivo* (mouse); *in vitro*	Decreased tumour size; cell-growth inhibition	N.D.	29
Glioma	*In vivo* (mouse, rat); *in vitro*	Decreased tumour size; apoptosis	CB_1, CB_2	50,51,53,85
Thyroid epithelioma	*In vivo* (mouse); *in vitro*	Decreased tumour size; cell-cycle arrest	CB_1	60
Lymphoma/leukaemia	*In vivo* (mouse); *in vitro*	Decreased tumour size; apoptosis	CB_2	96
Skin carcinoma	*In vivo* (mouse); *in vitro*	Decreased tumour size; apoptosis	CB_1, CB_2	61
Uterus carcinoma	*In vitro*	Cell-growth inhibition	N.D.	97,98
Breast carcinoma	*In vitro*	Cell-cycle arrest	CB_1	57–59
Prostate carcinoma	*In vitro*	Apoptosis	CB_1?	54,59,99
Neuroblastoma	*In vitro*	Apoptosis	VR_1	51,73

N.D., not determined; VR_1, type 1 vanilloid receptor.

state of cancer patients. THC and nabilone might lead to several positive psychological effects, including a reduction in depression and anxiety, which could result in improved sleep[8,10] (TABLE 1). These potentially positive effects, which can influence the medical benefits, need to be objectively evaluated with further clinical trials.

Inhibition of muscle weakness. Muscle weakness occurs in several chronic and debilitating neurological conditions such as multiple sclerosis and spinal-cord injury, and might also affect patients with cancer who have developed paraneoplastic syndromes such as SENSORY-MOTOR PERIPHERAL NEUROPATHIES and other MYASTHENIC syndromes. Increasing amounts of laboratory research and anecdotal information from cannabis users have led to Phase III clinical trials in which THC alone or in combination with other cannabinoids is being tested for treatment of spasticity and other muscle-debilitating symptoms of multiple sclerosis[7,28] (TABLE 1). The potential applicability of cannabinoids to cancer-related muscle weakness is, as yet, unknown.

Antitumour effects of cannabinoids

Inhibition of tumour-cell growth. The antiproliferative properties of cannabis compounds were first reported almost 30 years ago by Munson *et al.*[29], who showed that THC inhibits lung-adenocarcinoma cell growth *in vitro* and after oral administration in mice. Although these observations were promising, further studies in this area were not carried out until the late 1990s. Several plant-derived (for example, THC and cannabidiol), synthetic (for example, WIN-55, 212-2 and HU-210) and endogenous cannabinoids (for example, anandamide and 2-arachidonoylglycerol) are now known to exert antiproliferative actions on a wide spectrum of tumour cells in culture[30] (TABLE 2). More importantly, cannabinoid administration to nude mice slows the growth of various tumour xenografts, including lung carcinomas, gliomas, thyroid epitheliomas, skin carcinomas and lymphomas. The requirement of CB_1 and/or CB_2 receptors for this antitumour effect (TABLE 2) has been shown by various biochemical and pharmacological approaches, in particular by determining cannabinoid-receptor expression and by using selective cannabinoid-receptor agonists and antagonists. In one study, endocannabinoids were suggested to exert their apoptotic effect by binding to the type 1 vanilloid receptor (VR_1), a non-selective cation channel targeted by capsaicin, the active component of hot chilli peppers (TABLE 2). However, the precise role of this receptor in cannabinoid signalling is still unclear[2].

Possible mechanisms of antitumour action. Cannabinoids affect various cellular pathways by binding and activating their specific G-protein-coupled cannabinoid receptors. They inhibit the adenylyl cyclase–cyclic AMP (cAMP)–protein kinase A pathway and modulate the activity of Ca^{2+} and K^+ channels[2], which inhibits neurotransmitter release (BOX 1). Cannabinoids have also been found to modulate several signalling pathways that are more directly involved in the control of cell fate[30]: they stimulate mitogen-activated protein kinases (MAPKs) — the extracellular-signal-regulated kinase[31,32] (ERK) and the stress-activated kinases JUN amino-terminal kinase (JNK) and p38 MAPK[33–35] — which have prominent roles in the control of cell growth and differentiation[36] (FIG. 1). Cannabinoid-induced MAPK stimulation has been observed in primary neural cells, neural cell lines, lymphoid cells, vascular endothelial cells and Chinese hamster ovary cells that were transfected with cannabinoid-receptor complementary DNAs. By contrast, cannabinoids have been found to attenuate ERK in a neuronal-like cell line *in vitro*[37]. Cannabinoid receptors are also coupled to stimulation of the phosphatidylinositol 3-kinase (PI3K)–AKT survival pathway[38–40]. Activated AKT can phosphorylate and inhibit nuclear translocation of FORKHEAD TRANSCRIPTION FACTORS[41], thereby preventing the expression of pro-apoptotic proteins. Similar to ERK, the negative coupling of cannabinoid receptors to AKT has also been reported[42]. A role for PI3K as an upstream component of cannabinoid-induced ERK activation is seen in some systems[43,44] but not in others[45].

SENSORY-MOTOR PERIPHERAL NEUROPATHIES
Diseases or abnormalities of the peripheral nervous system that affect senses and movement.

MYASTHENIC
Abnormal muscle weakness or fatigue.

FORKHEAD TRANSCRIPTION FACTORS
A family of proteins that regulate the expression of genes that are involved in the control of cell survival, death, growth, differentiation and stress responses. Their activity is tightly controlled by AKT, so that phosphorylated forkhead transcription factor FOXO is retained in the cytoplasm and remains transcriptionally inactive.

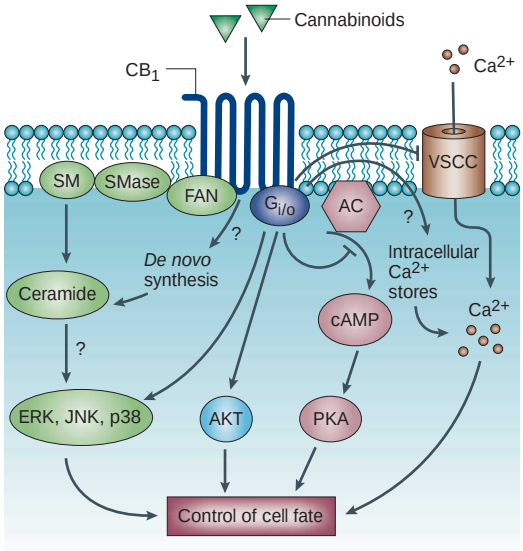


Figure 1 | **Signalling pathways involved in the control of cell fate by cannabinoids.** Cannabinoids exert their effects by binding to specific G-protein-coupled receptors. The cannabinoid receptor CB_1 signals several different cellular pathways. These include inhibition of the adenylyl cyclase (AC)–cyclic AMP–protein kinase A (PKA) pathway; modulation of ion conductances, by inhibition of voltage-sensitive Ca^{2+} channels (VSCC) and activation of Ca^{2+} release from intracellular stores; activation of mitogen-activated protein kinase cascades (extracellular-signal-regulated kinase (ERK), JUN amino-terminal kinase (JNK) and p38); activation of the phosphatidylinositol 3-kinase (PI3K)–AKT pathway; and ceramide generation, both acutely through FAN–sphingomyelinase (factor associated with neutral sphingomyelinase activation–SMase) and sustainedly through *de novo* synthesis. The crosstalk between the different pathways has been omitted for simplification.

Cannabinoids can modulate sphingolipid-metabolizing pathways by inducing sphingomyelin breakdown and acutely increasing the levels of ceramide[46] — a lipid second messenger that can induce apoptosis and cell-cycle arrest[47,48]. This effect is cannabinoid-receptor dependent but G-protein independent, and seems to involve the adaptor protein FAN (factor associated with neutral sphingomyelinase activation)[49]. Cannabinoid-receptor activation can also generate a sustained peak of ceramide accumulation through enhanced *de novo* synthesis[42,50].

Other targets for cannabinoids that might be involved in the control of cell fate include the transcription factor NF-κB and nitric-oxide synthase (NOS). However, the effects of cannabinoids on these two proteins are variable, ranging from activation to inhibition, and the underlying mechanisms of cannabinoid action remain obscure[2].

Cannabinoids might exert their antitumour effects by several different mechanisms, including direct induction of transformed-cell death, direct inhibition of transformed-cell growth and inhibition of tumour angiogenesis and metastasis (TABLE 3).

Cannabinoid-induced apoptosis can be exemplified by glioma cells[51], in which apoptotic death depends on sustained ceramide generation[50]. The increased ceramide levels observed in glioma cells after cannabinoid challenge would lead to prolonged activation of the RAF1–MEK–ERK signalling cascade[50] and AKT inhibition[42]. It is generally accepted that ERK activation leads to cell proliferation; however, the relation between ERK activation and cell fate is complex and depends on many factors, one of which is the duration of the stimulus, as prolonged ERK activation can mediate cell-cycle arrest and cell death. Following cannabinoid-receptor activation, two peaks of ceramide generation are observed in glioma cells that have different kinetics (minute- versus day-range), magnitude (twofold versus fourfold), mechanistic origin (sphingomyelin hydrolysis versus *de novo* ceramide synthesis) and function (metabolic regulation versus induction of apoptosis)[52] (FIG. 2a). The apoptotic action of cannabinoids on glioma cells clearly depends on the second peak of ceramide generation and ERK activation[42,50,53]. Pharmacological inhibition of *de novo* ceramide synthesis also prevents cannabinoid-induced death of prostate tumour cells[54]. The involvement of oxidative stress[55] and stress-activated protein kinases[50,56] in cannabinoid-induced apoptosis can not be ruled out.

CB_1-receptor activation in breast carcinoma cells blocks the cell cycle at the G1–S transition[57], and this has been ascribed to the inhibition of adenylyl cyclase and the cAMP–protein kinase-A pathway. Protein kinase A phosphorylates and inhibits RAF1, so cannabinoids prevent the inhibition of RAF1 and induce prolonged activation of the RAF1–MEK–ERK signalling cascade[58]. These signalling events mediate the antiproliferative action of cannabinoids on breast carcinoma cells by reducing the expression of two specific receptors, the high-molecular-weight (100 kDa) form of the prolactin receptor and the high-affinity neurotrophin TRK receptor[58,59]. CB_1-receptor activation also induces cell-cycle arrest at the G1–S transition in thyroid epithelioma cells that are transformed with the *KRAS* oncogene both *in vitro* and *in vivo*[60]. The mechanism of cannabinoid action on the cell cycle remains to be established.

Inhibition of growth-factor-receptor signalling following cannabinoid-receptor activation has also been observed in PHEOCHROMOCYTOMA[37], skin carcinoma[61] and prostate carcinoma[54] cells, and could therefore constitute a general mechanism of cannabinoid antiproliferative action. However, its consequences on ERK activity are not obvious: for example, in pheochromocytoma cells, cannabinoids inhibit ERK[37], whereas in breast carcinoma cells, cannabinoids activate ERK[58].

To grow beyond minimal size, tumours must generate a new vascular supply (angiogenesis) for purposes of cell nutrition, gas exchange and waste disposal — therefore, blocking the angiogenic process constitutes one of the most promising antitumour approaches now available[62]. Immunohistochemical and functional analyses in mouse models of glioma[63] and skin carcinoma[61] have shown that administration of cannabinoids turns the vascular hyperplasia that is characteristic of actively growing tumours into a pattern of blood vessels that is characterized by small, differentiated and impermeable

PHEOCHROMOCYTOMA
A relatively severe tumour of adrenal-gland chromaffin cells that causes excess release of adrenaline and noradrenaline and is therefore characterized by hypertension and tachycardia.

Table 3 | Possible mechanisms of cannabinoid antitumour action

Process	Possible mechanisms	References
Induction of apoptosis	Ceramide accumulation by *de novo* synthesis; sustained ERK activation and AKT inhibition	42,50,53
Cell-cycle arrest	Adenylyl cyclase inhibition and sustained ERK activation? Inhibition of growth-factor-receptor signalling	57–59
Inhibition of angiogenesis and metastasis	Decreased expression of pro-angiogenic factors and matrix metalloproteinases; inhibition of vascular-endothelial-cell migration and survival?	61,63,64

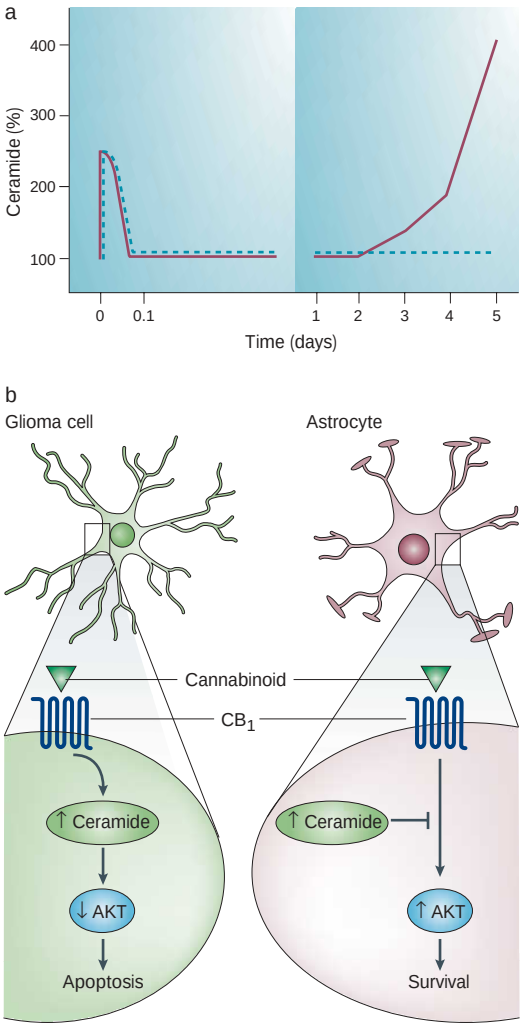


Figure 2 | **Differential cannabinoid signalling in transformed versus non-transformed glial cells.** **a** | In glioma cells, cannabinoids can induce two peaks of ceramide (solid line). The short-term peak occurs through sphingomyelin hydrolysis and is not related to apoptosis. The long-term peak occurs by *de novo* ceramide synthesis, is involved in apoptosis, and does not occur in normal astrocytes or in glioma-cell clones that are resistant to cannabinoid-induced apoptosis (dashed line). **b** | In glioma cells, cannabinoid-induced ceramide accumulation inhibits AKT and induces apoptosis, whereas in normal astrocytes cannabinoids activate AKT and prevent ceramide-induced AKT inhibition, thereby promoting survival.

capillaries. This is associated with a reduced expression of vascular endothelial growth factor (VEGF) and other pro-angiogenic cytokines[61,63,64], as well as of VEGF receptors (C. Blázquez and M.G., unpublished observations). In addition, activation of cannabinoid receptors in vascular endothelial cells inhibited cell migration and survival, which might contribute to impaired tumour vascularization[63]. Administration of cannabinoids to tumour-bearing mice also decreased the activity and expression of matrix metalloproteinase 2 — a proteolytic enzyme that allows tissue breakdown and remodelling during angiogenesis and metastasis[63]. This might explain at least in part why cannabinoid-induced inhibition of tumour metastasis was observed in mice injected with lung carcinoma cells[64].

Selectivity of antiproliferative action. Antitumour compounds should selectively affect tumour cells. It seems that cannabinoids can do this, as they kill tumour cells but do not affect their non-transformed counterparts and might even protect them from cell death. The best characterized example is that of glial cells. Cannabinoids induce apoptosis of glioma cells in culture and induce regression of gliomas in mice and rats (TABLE 2). By contrast, cannabinoids protect normal glial cells of astroglial[65] and oligodendroglial[66] lineages from apoptosis. This protective effect is mediated by the CB_1 receptor and the PI3K–AKT survival pathway. Cannabinoid-induced apoptosis of glioma cells is mediated by ceramide generation[42,50]; however, cannabinoids attenuate ceramide-induced apoptosis of normal astrocytes both *in vitro* and *in vivo*[65].

The molecular basis of this 'ying–yang' behaviour is not yet completely understood, but could result from the differential capacity of tumour and non-tumour cells to synthesize ceramide in response to cannabinoids[52]. As mentioned above, after cannabinoid-receptor activation two peaks of ceramide generation are observed in glioma cells, the second of which is due to enhanced *de novo* ceramide synthesis and triggers apoptosis. However, this second peak does not occur in normal astrocytes or in glioma-cell clones that are refractory to cannabinoid-induced apoptosis, despite the expression of functional cannabinoid receptors[50,52] (FIG. 2a). Of interest, this resistance of primary astrocytes to cannabinoid-induced *de novo* ceramide synthesis and apoptosis is specific, as exposure of these cells to other stimuli such as uptake of the fatty acid palmitate[67] or serum deprivation (A. Carracedo, M.G. & G. Velasco, unpublished observations) does induce apoptosis through *de novo* ceramide synthesis. It is therefore conceivable that cannabinoid receptors regulate cell survival and cell death differently in transformed and non-transformed cells. In glioma cells, cannabinoids inhibit AKT through ceramide[42], whereas in primary astrocytes cannabinoids activate AKT and abrogate ceramide-induced AKT inhibition[65] (FIG. 2b).

The possibility that the 'ying–yang' action of cannabinoids depends on different patterns of cannabinoid-receptor expression and/or on the coupling of cannabinoid receptors to different types of G protein

Box 2 | Potential adverse effects of cannabinoids

The administration of cannabinoids to humans and laboratory animals exerts psychoactive effects[7,81,82]. In humans, cannabinoids induce a unique mixture of depressing and stimulatory effects in the central nervous system that can be divided into four groups: affective (euphoria and easy laughter), sensory (alterations in temporal and spatial perception and disorientation), somatic (drowsiness, dizziness and motor discoordination) and cognitive (confusion, memory lapses and difficulties in concentration). Owing to the ubiquitous distribution of cannabinoid receptors, cannabinoids might affect not only the brain, but also almost every body system; for example, the cardiovascular (tachycardia), respiratory (bronchodilatation), musculoskeletal (muscle relaxation) and gastrointestinal (decreased motility) systems[7,81,82].

The central and peripheral effects of cannabinoids are variable and sometimes pronounced in those smoking cannabis for recreational purposes, but are not necessarily apparent in a controlled clinical setting. In fact, dronabinol (Marinol) and nabilone (Cesamet) are usually innocuous when administered as antiemetics to patients with cancer[10,82]. Moreover, tolerance to the unwanted effects of cannabinoids develops rapidly in humans and laboratory animals[81,82]. For example, the most frequently reported adverse psychoactive effects of dronabinol during clinical trials occurred in 33% of patients. This value decreased to 25% reporting minor psychoactivity after 2 weeks and 4% after 6 weeks of treatment. The possibility that tolerance also develops to therapeutically sought effects has not been substantiated. Cannabinoid tolerance is mainly attributed to PHARMACODYNAMIC changes, such as a decrease in the number of total and functionally coupled cannabinoid receptors on the cell surface, with a possible minor PHARMACOKINETIC component caused by increased cannabinoid biotransformation and excretion[7,81,82].

Some people consider cannabinoids as addictive drugs. A withdrawal syndrome, which consists of irritability, insomnia, restlessness and a sudden, temporary sensation of heat — 'hot flashes' — has been occasionally observed in chronic cannabis smokers after abrupt cessation of drug use. However, this occurs rarely, and symptoms are mild and usually dissipate after a few days[7,81,82]. Similarly, after chronic tetrahydrocannabinol (THC) treatment, no somatic signs of spontaneous withdrawal appear in different animal species, even at extremely high doses[112]. Animal models of cannabinoid dependence have been obtained only after administration of an antagonist of cannabinoid receptor CB_1 together with the cessation of chronic administration of high doses of THC to precipitate somatic manifestations of withdrawal[112]. In the clinical context, long-term surveys of dronabinol administration at prescription doses have shown no sign of dependence[82,113]. The low-addictive capacity of THC is usually ascribed to its pharmacokinetic properties (BOX 3) as, unlike commonly used drugs, cannabinoids are stored in adipose tissue and excreted at a low rate. So, cessation of THC intake is not accompanied by rapid decreases in drug plasma concentration[82].

can not be ruled out. However, this seems unlikely. On the one hand, glioma cell clones that are resistant to cannabinoid-induced apoptosis express similar amounts of CB_1 and CB_2 receptors, compared with cannabinoid-sensitive clones[50]; this is further supported by pharmacological studies using selective cannabinoid-receptor antagonists[50]. On the other hand, although activation of G_s proteins by the CB_1 receptor has been reported[68], increasing evidence indicates that cannabinoid receptors have a clear preference for coupling to $G_{i/o}$ proteins[2,69,70].

Other reported examples of cannabinoid selectivity towards tumour cells include thyroid epithelioma[60] and skin carcinoma[61] cells. In addition, though perhaps mechanistically unrelated, cannabinoids protect neurons from death in various models of toxic damage[7,71,72], whereas neuroblastoma cells are sensitive to cannabinoid-induced death[51,73]. A possible exception to this cannabinoid selectivity might be immune cells, although this can depend on experimental conditions — mostly stimulus strength[74]. For example, cannabinoids at high concentrations induce apoptosis of non-transformed monocytes, macrophages and lymphocytes[75,76], which might contribute to impaired host antitumour responses by inhibiting the production of antitumour cytokines such as interferon-γ and interleukin-12 (REF. 77). By contrast, low cannabinoid doses enhance lymphocyte[78] and myeloid-cell growth[79]. In any event, the issue of immunosuppression needs to be explicitly investigated in any trial of cannabinoids in cancer patients[80], although long-term surveys of HIV-positive patients have shown no link between dronabinol use or cannabis smoking and average T-cell counts or progression to AIDS[8,10].

PHARMACODYNAMICS
Mechanisms by which drugs affect their target sites in the body to produce their desired therapeutic effects and their adverse side effects.

PHARMACOKINETICS
Time course of drug and metabolite levels in different fluids, tissues and excreta of the body, and of the mathematical relationships required to develop models to interpret such data.

Towards the clinical application

Side effects and how to circumvent them. Cannabinoids have a favourable drug safety profile[8,81,82]. Acute fatal cases due to cannabis use in humans have not been substantiated, and median lethal doses of THC in animals have been extrapolated to several grams per kilogram of body weight[82]. Cannabinoids are usually well tolerated in animal studies and do not produce the generalized toxic effects of most conventional chemotherapeutic agents. For example, in a 2-year administration of high oral doses of THC to rats and mice, no marked histopathological alterations in the brain and other organs were found. Moreover, THC treatment tended to increase survival and lower the incidence of primary tumours[83]. Similarly, long-term epidemiological surveys, although scarce and difficult to design and interpret, usually show that neither patients under prolonged medical cannabinoid treatment nor regular cannabis smokers have marked alterations in a wide array of physiological, neurological and blood tests[8,10,82].

The use of cannabinoids in medicine, however, is severely limited by their psychoactive effects (BOX 2). Although these adverse effects are within the range of

Box 3 | Cannabinoid pharmacokinetics

The route of administration affects the time course and intensity of the drug effects. At present, clinical use of cannabinoids is limited to oral administration of dronabinol and nabilone. However, absorption by this route is slow and erratic; cannabinoids might be degraded by the acid of the stomach; rates of FIRST-PASS METABOLISM in the liver vary greatly between individuals; and patients sometimes have more than one plasma peak, which makes it more difficult to control the drug effects[82].

Anecdotal reports indicate that in certain patients cannabis is more effective and might have fewer psychological effects when smoked than when taken orally. However, cannabis smoke contains the same chemical carcinogens that are found in tobacco, making it potentially harmful in long-term use and difficult to investigate in clinical trials[80]. A safer alternative for inhaled administration of cannabinoids has been recently produced by GW Pharmaceuticals and Bayer AG. This is a medicinal cannabis extract known as Sativex, which contains tetrahydrocannabinol (THC) and cannabidiol, that is administered by spraying into the mouth and is now in clinical trials for pain and the debilitating symptoms of multiple sclerosis.

Other routes of cannabinoid administration tested so far in humans include intravenous (THC and dexanabinol in saline/ethanol/adjuvant), rectal (THC-hemisuccinate suppositories) and sublingual administration (THC- and cannabidiol-rich cannabis extracts)[82]. These three routes circumvent the aforementioned problems of oral administration by producing single, rapid and high drug-plasma peaks.

Owing to its high hydrophobicity, absorbed THC binds to lipoproteins and albumin in plasma and is mainly retained in adipose tissue — the main long-term THC storage site. THC is only slowly released back into the bloodstream and other body tissues, so that full elimination from the body is slow (half-life 1–3 days). THC metabolism occurs mainly by hepatic cytochrome P450 isoenzymes. The process yields 11-hydroxy-THC and many other metabolites resulting from hydroxylation, oxidation, conjugation and other chemical modifications that are cleared from the body by excretion.

those accepted for other medications, especially in cancer treatment, and tend to disappear with tolerance following continuous use (BOX 2), it is obvious that cannabinoid-based therapies devoid of side effects would be desirable.

As the unwanted psychotropic effects of cannabinoids are mediated largely or entirely by CB_1 receptors in the brain, a first possibility would be to use cannabinoids that target CB_2 receptors. Selective CB_2-receptor activation in mice induces regression of gliomas[53] and skin carcinomas[61] and can also inhibit pain[84] in the absence of overt signs of psychoactivity. Certain cannabinoids that act through non-cannabinoid receptors — and are therefore devoid of psychoactivity — would also be useful in cancer therapy. These include cannabidiol, which inhibits glioma-cell growth *in vitro*[85,86], DEXANABINOL, of which the effect on tumour-cell growth has not yet been tested[71,87], and AJULEMIC ACID, which inhibits glioma-cell growth *in vitro* and *in vivo*[88] — the pharmacological properties of ajulemic acid are, however, controversial[88,89]. Alternatively, the design of cannabinoids that do not cross the blood–brain barrier might exert antitumour, pain-killing and appetite-stimulating effects without causing psychoactivity. Another strategy would be to manipulate the effects of endocannabinoids. Achieving high endocannabinoid levels in the location of the tumour by selectively inhibiting endocannabinoid degradation has proved successful in animal models, as drugs that block anandamide breakdown exert antitumour effects with little psychoactivity[90].

FIRST-PASS METABOLISM
Pre-systemic metabolism of a drug that limits its exposure to the body. For example, chemical or enzymatic breakdown of a drug in the gastrointestinal lumen or in the stomach, intestine or liver cells can greatly reduce the amount of drug that ends up in the bloodstream.

DEXANABINOL
(HU-211). A non-psychoactive synthetic derivative of tetrahydrocannabinol that blocks ionotropic glutamate receptors and has antioxidant and anti-inflammatory properties; it is now in Phase III clinical trials for the management of brain trauma.

AJULEMIC ACID
(CT3). A synthetic derivative of the tetrahydrocannabinol metabolite 11-carboxy-THC that inhibits pain and inflammation; it is entering Phase II clinical trials for the treatment of pain and spasticity in multiple sclerosis.

Cannabinoids are poorly soluble in water, which determines their pharmacokinetic behaviour, in particular their poor bioavailability when given orally, and has been one of the difficulties in formulating preparations of pure compounds for medicinal use and for finding appropriate routes of delivery (BOX 3). In the case of a possible application in cancer therapy, it is conceivable that administration of a low dose of cannabinoid directly to the target site would increase effectiveness and reduce adverse side effects. So, using water-soluble cannabinoids — such as O-1057 — might help to overcome some of the pharmacokinetic peculiarities of cannabinoids[5].

Combined therapies. Cannabinoids should also be tested in combination with other chemotherapeutic drugs or radiotherapy to establish whether they can enhance present drug treatments. So far, only two such studies have been carried out. In one study, γ-radiation was found to increase cannabinoid-induced leukaemic cell death[91]. However, in the second study synergism was not observed between cannabinoids and tamoxifen during the induction of glioma-cell death[85]. In any event, compounds that induce cell death through ceramide have proved useful in combined therapies[92]. For example, fenretinide (N-(4-hydroxyphenyl)retinamide) kills various types of tumour cell by enhancing ceramide synthesis, and this effect shows potent synergism with that of other compounds that raise intracellular ceramide levels[93]. So, the usefulness of cannabinoids in combination therapy is still unclear.

A pilot clinical trial. Glioblastoma multiforme, or grade IV astrocytoma, is the most frequent class of malignant primary brain tumour and is one of the most malignant forms of cancer. As a consequence, survival after diagnosis is normally just 6–8 months[94,95]. Present therapeutic strategies for the treatment of glioblastoma multiforme and other malignant brain tumours are usually inefficient and in most cases just palliative, and include surgery and radiotherapy. Some chemotherapeutic agents, such as temozolomide, carmustin, carboplatin and thalidomide have been tested and the most recent strategies for glioblastoma multiforme treatment are focused on gene therapy, but no trial carried out so far has been successful[94,95]. It is therefore essential to develop new therapeutic strategies for the management of glioblastoma multiforme, which will probably require a combination of therapies to obtain significant clinical results.

The Spanish Ministry of Health has recently approved a Phase I/II clinical trial, carried out in collaboration with the Tenerife University Hospital and my laboratory, aimed at investigating the effect of local administration of THC — as a single agent — on the growth of recurrent glioblastoma multiforme. This will be the first human study in which THC is administered intracranially through an infusion cannula connected to a subcutaneous reservoir. The clinical trial has just started, and it will be some time before the results can be determined. In the meantime, it is desirable that other trials —

on this and other types of tumours — are initiated to determine how cannabinoids can be used, other than for their palliative effects, to treat patients with cancer.

Implications and future directions

One must be cautious when envisaging the potential clinical use of new anticancer therapies. Despite the huge amount of literature on how tumour cells work, there has been no parallel advance in the clinical practice of chemotherapy, and many compounds that inhibit tumour-cell growth in culture and in laboratory animals turn out to be disappointingly ineffective and/or toxic when tested in patients. Regarding effectiveness, cannabinoids exert notable antitumour activity in animal models of cancer, but their possible antitumour effect in humans has not been established. Regarding toxicity, cannabinoids not only show a good safety profile but also have palliative effects in patients with cancer, indicating that clinical trials with cannabinoids in cancer therapy are feasible.

As with many other antitumour agents, further research on cannabinoids is required and the precise mechanism of cannabinoid antitumour action needs to be clarified in more detail. If we can better understand the intracellular signalling pathways that are involved in cannabinoid antitumour action, determine which intercellular factors and processes (for example, angiogenesis and metastasis) are modulated by cannabinoids in tumours and which tumours are sensitive or resistant to cannabinoids and why, we will be one step closer to understanding how these compounds can be used in a clinical setting. Preclinical studies in animal models should also be carried out to optimize administration routes, delivery schedules, new ligands and adjuvants for potential cannabinoid therapies. As cannabinoids are relatively safe compounds, it would be desirable that clinical trials using cannabinoids as a single drug or in combined anticancer therapies could accompany these laboratory studies to allow us to use these compounds in the treatment of cancer.

1. Gaoni, Y. & Mechoulam, R. Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* **86**, 1646–1647 (1964).
2. Howlett, A. C. *et al.* International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol. Rev.* **54**, 161–202 (2002).
A comprehensive update on cannabinoid receptors and their biochemistry and pharmacology.
3. Herkenham, M. *et al.* Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J. Neurosci.* **11**, 563–583 (1991).
4. Porter, A. C. & Felder, C. C. The endocannabinoid nervous system. Unique opportunities for therapeutic intervention. *Pharmacol. Ther.* **90**, 45–60 (2001).
5. Pertwee, R. G. Cannabinoid receptor ligands: clinical and neuropharmacological considerations, relevant to future drug discovery and development. *Expert Opin. Investig. Drugs* **9**, 1553–1571 (2000).
6. Di Carlo, G. & Izzo, A. A. Cannabinoids for gastrointestinal diseases: potential therapeutic applications. *Expert Opin. Investig. Drugs* **12**, 39–49 (2003).
7. Croxford, J. L. Therapeutic potential of cannabinoids in CNS disease. *CNS Drugs* **17**, 179–202 (2003).
8. Robson, P. Therapeutic aspects of cannabis and cannabinoids. *Br. J. Psychiatry* **178**, 107–115 (2001).
9. Tramer, M. R. *et al.* Cannabinoids for control of chemotherapy induced nausea and vomiting: quantitative systematic review. *BMJ* **323**, 16–21 (2001).
A detailed review on the best palliative action of cannabinoids in cancer therapy that has been established so far.
10. Walsh, D., Nelson, K. A. & Mahmoud, F. A. Established and potential therapeutic applications of cannabinoids in oncology. *Support Care Cancer* **11**, 137–143 (2003).
11. Barann, M. *et al.* Direct inhibition by cannabinoids of human 5-HT3A receptors: probable involvement of an allosteric modulatory site. *Br. J. Pharmacol.* **137**, 589–596 (2002).
12. Tisdale, M. J. Cachexia in cancer patients. *Nature Rev. Cancer* **2**, 862–871 (2002).
13. Berry, E. M. & Mechoulam, R. Tetrahydrocannabinol and endocannabinoids in feeding and appetite. *Pharmacol. Ther.* **95**, 185–190 (2002).
An authoritative review on the physiological role and therapeutic potential of cannabinoids in appetite stimulation.
14. Cota, D. *et al.* Endogenous cannabinoid system as a modulator of food intake. *Int. J. Obes. Relat. Metab. Disord.* **27**, 289–301 (2003).
15. Gomez, R. *et al.* A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. *J. Neurosci.* **22**, 9612–9617 (2002).
16. Bensaid, M. *et al.* The cannabinoid CB_1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Mol. Pharmacol.* **63**, 908–914 (2003).
17. Cota, D. *et al.* The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J. Clin. Invest.* **112**, 423–431 (2003).
18. Nelson, K., Walsh, D., Deeter, P. & Sheehan, F. A phase II study of δ-9-tetrahydrocannabinol for appetite stimulation in cancer-associated anorexia. *J. Palliat. Care* **10**, 14–18 (1994).
19. Jatoi, A. *et al.* Dronabinol versus megestrol acetate versus combination therapy for cancer-associated anorexia: a North Central Cancer Treatment Group study. *J. Clin. Oncol.* **20**, 567–573 (2002).
20. Mantyh, P. W., Clohisy, D. R., Koltzenburg, M. & Hunt, S. P. Molecular mechanisms of cancer pain. *Nature Rev. Cancer* **2**, 201–209 (2002).
21. Pertwee, R. G. Cannabinoid receptors and pain. *Prog. Neurobiol.* **63**, 569–611 (2001).
22. Walker, J. & Huang, S. Cannabinoid analgesia. *Pharmacol. Ther.* **95**, 127–135 (2002).
23. Calignano, A., LaRana, G., Giuffrida, A. & Piomelli, D. Control of pain initiation by endogenous cannabinoids. *Nature* **394**, 277–281 (1998).
24. Campbell, F. A. *et al.* Are cannabinoids an effective and safe treatment option in the management of pain? A qualitative systematic review. *BMJ* **323**, 13–16 (2001).
A lively discussion on the possible therapeutic value of cannabinoids as analgesic agents.
25. Noyes, R. Jr, Brunk, S. F., Avery, D. A. H. & Canter, A. C. The analgesic properties of delta-9-tetrahydrocannabinol and codeine. *Clin. Pharmacol. Ther.* **18**, 84–89 (1975).
26. Iversen, L. & Chapman, V. Cannabinoids: a real prospect for pain relief. *Curr. Opin. Pharmacol.* **2**, 50–55 (2002).
27. Manzanares, J. *et al.* Pharmacological and biochemical interactions between opioids and cannabinoids. *Trends Pharmacol. Sci.* **20**, 287–294 (1999).
28. Baker, D. & Pryce, G. The therapeutic potential of cannabis in multiple sclerosis. *Expert Opin. Investig. Drugs* **12**, 561–567 (2003).
29. Munson, A. E., Harris, L. S., Friedman, M. A., Dewey, W. L. & Carchman, R. A. Antineoplastic activity of cannabinoids. *J. Natl Cancer Inst.* **55**, 597–602 (1975).
The seminal demonstration that THC inhibits tumour-cell growth in culture and in mice.
30. Guzman, M., Sanchez, C. & Galve-Roperh, I. Cannabinoids and cell fate. *Pharmacol. Ther.* **95**, 175–184 (2002).
31. Bouaboula, M. *et al.* Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem. J.* **312**, 637–641 (1995).
32. Bouaboula, M. *et al.* Signaling pathway associated with stimulation of CB2 peripheral cannabinoid receptor. Involvement of both mitogen-activated protein kinase and induction of Krox-24 expression. *Eur. J. Biochem.* **237**, 704–711 (1996).
33. Liu, J. *et al.* Functional CB_1 cannabinoid receptors in human vascular endothelial cells. *Biochem. J.* **346**, 835–840 (2000).
34. Rueda, D., Galve-Roperh, I., Haro, A. & Guzman, M. The CB_1 cannabinoid receptor is coupled to the activation of c-Jun *N*-terminal kinase. *Mol. Pharmacol.* **58**, 814–820 (2000).
35. Derkinderen, P., Ledent, C., Parmentier, M. & Girault, J. A. Cannabinoids activate p38 mitogen-activated protein kinases through CB1 receptors in hippocampus. *J. Neurochem.* **77**, 957–960 (2001).
36. Chang, L. & Karin, M. Mammalian MAP kinase signalling cascades. *Nature* **410**, 37–40 (2001).
37. Rueda, D., Navarro, B., Martinez-Serrano, A., Guzman, M. & Galve-Roperh, I. The endocannabinoid anandamide inhibits neuronal progenitor cell differentiation through attenuation of the Rap1/B-Raf/ERK pathway. *J. Biol. Chem.* **277**, 46645–46650 (2002).
38. Gomez del Pulgar, T., Velasco, G. & Guzman, M. The CB_1 cannabinoid receptor is coupled to the activation of protein kinase B/Akt. *Biochem. J.* **347**, 369–373 (2000).
39. Sanchez, M. G., Ruiz-Llorente, L., Sanchez, A. M. & Diaz-Laviada, I. Activation of phosphoinositide 3-kinase/PKB pathway by CB_1 and CB_2 cannabinoid receptors expressed in prostate PC-3 cells. Involvement in Raf-1 stimulation and NGF induction. *Cell. Signal.* **15**, 851–859 (2003).
40. Vivanco, I. & Sawyers, C. L. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nature Rev. Cancer* **2**, 489–501 (2002).
41. Samson, M. T. *et al.* Differential roles of CB1 and CB2 cannabinoid receptors in mast cells. *J. Immunol.* **170**, 4953–4962 (2003).
42. Gomez del Pulgar, T., Velasco, G., Sanchez, C., Haro, A. & Guzman, M. *De novo*-synthesized ceramide is involved in cannabinoid-induced apoptosis. *Biochem. J.* **363**, 183–188 (2002).
43. Bouaboula, M. *et al.* A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. *J. Biol. Chem.* **272**, 22330–22339 (1997).
44. Galve-Roperh, I., Rueda, D., Gomez Del Pulgar, T., Velasco, G. & Guzman, M. Mechanism of extracellular signal-regulated kinase activation by the CB_1 cannabinoid receptor. *Mol. Pharmacol.* **62**, 1385–1392 (2002).
45. Derkinderen, P. *et al.* Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. *J. Neurosci.* **23**, 2371–2382 (2003).
46. Sanchez, C., Galve-Roperh, I., Rueda, D. & Guzman, M. Involvement of sphingomyelin hydrolysis and the mitogen-activated protein kinase cascade in the Δ^9–tetrahydrocannabinol-induced stimulation of glucose metabolism in primary astrocytes. *Mol. Pharmacol.* **54**, 834–843 (1998).
47. Hannun, Y. A. & Obeid, L. M. The Ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind. *J. Biol. Chem.* **277**, 25847–25850 (2002).
48. Kolesnick, R. The therapeutic potential of modulating the ceramide/sphingomyelin pathway. *J. Clin. Invest.* **110**, 3–8 (2002).

49. Sanchez, C. *et al.* The CB_1 cannabinoid receptor of astrocytes is coupled to sphingomyelin hydrolysis through the adaptor protein fan. *Mol. Pharmacol.* **59**, 955–959 (2001).
50. Galve-Roperh, I. *et al.* Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. *Nature Med.* **6**, 313–319 (2000).
The first identification of a signalling mechanism for the apoptotic action of cannabinoids on tumour cells.
51. Sanchez, C., Galve-Roperh, I., Canova, C., Brachet, P. & Guzman, M. Δ^9–Tetrahydrocannabinol induces apoptosis in C6 glioma cells. *FEBS Lett.* **436**, 6–10 (1998).
52. Guzman, M., Galve-Roperh, I. & Sanchez, C. Ceramide: a new second messenger of cannabinoid action. *Trends Pharmacol. Sci.* **22**, 19–22 (2001).
53. Sanchez, C. *et al.* Inhibition of glioma growth *in vivo* by selective activation of the CB_2 cannabinoid receptor. *Cancer Res.* **61**, 5784–5789 (2001).
54. Mimeault, M., Pommery, N., Wattez, N., Bailly, C. & Henichart, J. P. Anti-proliferative and apoptotic effects of anandamide in human prostatic cancer cell lines: implication of epidermal growth factor receptor down-regulation and ceramide production. *Prostate* **56**, 1–12 (2003).
55. Sarker, K. P., Obara, S., Nakata, M., Kitajima, I. & Maruyama, I. Anandamide induces apoptosis of PC-12 cells: involvement of superoxide and caspase-3. *FEBS Lett.* **472**, 39–44 (2000).
56. Sarker, K. P. *et al.* ASK1-p38 MAPK/JNK signaling cascade mediates anandamide-induced PC12 cell death. *J. Neurochem.* **85**, 50–61 (2003).
57. De Petrocellis, L. *et al.* The endogenous cannabinoid anandamide inhibits human breast cancer cell proliferation. *Proc. Natl Acad. Sci. USA* **95**, 8375–8380 (1998).
58. Melck, D. *et al.* Involvement of the cAMP/protein kinase A pathway and of mitogen-activated protein kinase in the anti-proliferative effects of anandamide in human breast cancer cells. *FEBS Lett.* **463**, 235–240 (1999).
59. Melck, D. *et al.* Suppression of nerve growth factor trk receptors and prolactin receptors by endocannabinoids leads to inhibition of human breast and prostate cancer cell proliferation. *Endocrinology* **141**, 118–126 (2000).
60. Bifulco, M. *et al.* Control by the endogenous cannabinoid system of *ras* oncogene-dependent tumor growth. *FASEB J.* **15**, 2745–2747 (2001).
61. Casanova, M. L. *et al.* Inhibition of skin tumor growth and angiogenesis *in vivo* by activation of cannabinoid receptors. *J. Clin. Invest.* **111**, 43–50 (2003).
62. Kerbel, R. & Folkman, J. Clinical translation of angiogenesis inhibitors. *Nature Rev. Cancer* **2**, 727–739 (2002).
63. Blazquez, C. *et al.* Inhibition of tumor angiogenesis by cannabinoids. *FASEB J.* **17**, 529–531 (2003).
The first paper showing that cannabinoid administration to mice impairs tumour angiogenesis.
64. Portella, G. *et al.* Inhibitory effects of cannabinoid CB_1 receptor stimulation on tumor growth and metastatic spreading: actions on signals involved in angiogenesis and metastasis. *FASEB J.* 3 Jul 2003 (doi:10.1096/fj.02-1129fje).
65. Gomez Del Pulgar, T., De Ceballos, M. L., Guzman, M. & Velasco, G. Cannabinoids protect astrocytes from ceramide-induced apoptosis through the phosphatidylinositol 3-kinase/protein kinase B pathway. *J. Biol. Chem.* **277**, 36527–36533 (2002).
66. Molina-Holgado, E. *et al.* Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. *J. Neurosci.* **22**, 9742–9753 (2002).
67. Blazquez, C., Galve-Roperh, I. & Guzman, M. *De novo*-synthesized ceramide signals apoptosis in astrocytes via extracellular signal-regulated kinase. *FASEB J.* **14**, 2315–2322 (2000).
68. Glass, M. & Felder, C. C. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *J. Neurosci.* **17**, 5327–5333 (1997).
69. Vasquez, C. & Lewis, D. L. The CB1 cannabinoid receptor can sequester G-proteins, making them unavailable to couple to other receptors. *J. Neurosci.* **19**, 9271–9280 (1999).
70. Mukhopadhyay, S., McIntosh, H. H., Houston, D. B. & Howlett, A. C. The CB_1 cannabinoid receptor juxtamembrane C-terminal peptide confers activation to specific G proteins in brain. *Mol. Pharmacol.* **57**, 162–170 (2000).
71. Mechoulam, R., Panikashvili, D. & Shohami, E. Cannabinoids and brain injury: therapeutic implications. *Trends Mol. Med.* **8**, 58–61 (2002).
72. van der Stelt, M. *et al.* Acute neuronal injury, excitotoxicity, and the endocannabinoid system. *Mol. Neurobiol.* **26**, 317–346 (2002).
73. Maccarrone, M., Lorenzon, T., Bari, M., Melino, G. & Finazzi-Agro, A. Anandamide induces apoptosis in human cells via vanilloid receptors. Evidence for a protective role of cannabinoid receptors. *J. Biol. Chem.* **275**, 31938–31945 (2000).
74. Guzman, M., Sanchez, C. & Galve-Roperh, I. Control of the cell survival/death decision by cannabinoids. *J. Mol. Med.* **78**, 613–625 (2001).
75. Schwarz, H., Blanco, F. J. & Lotz, M. Anadamide, an endogenous cannabinoid receptor agonist inhibits lymphocyte proliferation and induces apoptosis. *J. Neuroimmunol.* **55**, 107–115 (1994).
76. Zhu, W., Friedman, H. & Klein, T. W. Δ^9-tetrahydrocannabinol induces apoptosis in macrophages and lymphocytes: involvement of Bcl-2 and caspase-1. *J. Pharmacol. Exp. Ther.* **286**, 1103–1109 (1998).
77. Zhu, L. X. *et al.* Δ-9-tetrahydrocannabinol inhibits antitumor immunity by a CB2 receptor-mediated, cytokine-dependent pathway. *J. Immunol.* **165**, 373–380 (2000).
78. Derocq, J. M., Segui, M., Marchand, J., Le Fur, G. & Casellas, P. Cannabinoids enhance human B-cell growth at low nanomolar concentrations. *FEBS Lett.* **369**, 177–182 (1995).
79. Valk, P. *et al.* Anandamide, a natural ligand for the peripheral cannabinoid receptor is a novel synergistic growth factor for hematopoietic cells. *Blood* **90**, 1448–1457 (1997).
80. Tashkin, D. R., Baldwin, G. C., Sarafian, T., Dubinett, S. & Roth, M. D. Respiratory and immunologic consequences of marijuana smoking. *J. Clin. Pharmacol.* **42**, 71S–81S (2002).
81. Adams, I. B. & Martin, B. R. Cannabis: pharmacology and toxicology in animals and humans. *Addiction* **91**, 1585–1614 (1996).
82. Grotenhermen, F. Pharmacokinetics and pharmacodynamics of cannabinoids. *Clin. Pharmacokinet.* **42**, 327–360 (2003).
83. Chan, P. C., Sills, R. C., Braun, A. G., Haseman, J. K. & Bucher, J. R. Toxicity and carcinogenicity of Δ^9–tetrahydrocannabinol in Fischer rats and B6C3F1 mice. *Fund. Appl. Toxicol.* **30**, 109–117 (1996).
84. Malan, T. P. *et al.* CB_2 cannabinoid receptor agonists: pain relief without psychoactive effects? *Curr. Opin. Pharmacol.* **3**, 62–67 (2003).
85. Jacobsson, S. O., Rongard, E., Stridh, M., Tiger, G. & Fowler, C. J. Serum-dependent effects of tamoxifen and cannabinoids upon C6 glioma cell viability. *Biochem. Pharmacol.* **60**, 1807–1813 (2000).
86. Mechoulam, R., Parker, L. A. & Gallily, R. Cannabidiol: an overview of some pharmacological aspects. *J. Clin. Pharmacol.* **42**, 11S–19S (2002).
87. Pop, E. Dexanabinol Pharmos. *Curr. Opin. Investig. Drugs* **1**, 494–503 (2000).
88. Recht, L. D. *et al.* Antitumor effects of ajulemic acid (CT3), a synthetic non-psychoactive cannabinoid. *Biochem. Pharmacol.* **62**, 755–763 (2001).
89. Rhee, M. H. *et al.* Cannabinol derivatives: binding to cannabinoid receptors and inhibition of adenylylcyclase. *J. Med. Chem.* **40**, 3228–3233 (1997).
90. Bifulco, M. & Di Marzo, V. Targeting the endocannabinoid system in cancer therapy: a call for further research. *Nature Med.* **8**, 547–550 (2002).
An enjoyable commentary about the possible antitumour action of the endogenous cannabinoid system.
91. Gallily, R. *et al.* γ-Irradiation enhances apoptosis induced by cannabidiol, a non-psychotropic cannabinoid, in cultured HL–60 myeloblastic leukemia cells. *Leukemia Lymphoma* **44**, 1767–1773 (2003).
92. Radin, N. S. Killing tumours by ceramide-induced apoptosis: a critique of available drugs. *Biochem. J.* **371**, 243–256 (2003).
93. Maurer, B. J., Melton, L., Billups, C., Cabot, M. C. & Reynolds, C. P. Synergistic cytotoxicity in solid tumor cell lines between N-(4- hydroxyphenyl)retinamide and modulators of ceramide metabolism. *J. Natl Cancer Inst.* **92**, 1897–1909 (2000).
94. Maher, E. A. *et al.* Malignant glioma: genetics and biology of a grave matter. *Genes Dev.* **15**, 1311–1333 (2001).
95. Louis, D. N., Pomeroy, S. L. & Cairncross, J. G. Focus on central nervous system neoplasia. *Cancer Cell* **1**, 125–128 (2002).
96. McKallip, R. J. *et al.* Targeting CB2 cannabinoid receptors as a novel therapy to treat malignant lymphoblastic disease. *Blood* **100**, 627–634 (2002).
97. Mon, M. J., Jansing, R. L., Doggett, S., Stein, J. L. & Stein, G. S. Influence of Δ^9–tetrahydrocannabinol on cell proliferation and macromolecular biosynthesis in human cells. *Biochem. Pharmacol.* **27**, 1759–1765 (1978).
98. Blevins, R. D. & Smith, D. P. Effects of Δ-9-tetrahydrocannabinol on cultured HeLa cell growth and development. *Growth* **44**, 133–138 (1980).
99. Ruiz, L., Miguel, A. & Diaz-Laviada, I. Δ^9–tetrahydrocannabinol induces apoptosis in human prostate PC-3 cells via a receptor-independent mechanism. *FEBS Lett.* **458**, 400–404 (1999).
100. Devane, W. *et al.* Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949 (1992).
The discovery of anandamide, the first endogenous ligand of cannabinoid receptors.
101. Mechoulam, R. *et al.* Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **50**, 83–90 (1995).
102. Sugiura, T. *et al.* 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* **215**, 89–97 (1995).
103. Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C. & Bonner, T. I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**, 561–564 (1990).
The molecular characterization of CB_1 — the first specific cannabinoid receptor.
104. Munro, S., Thomas, K. L. & Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365**, 61–65 (1993).
105. Di Marzo, V. *et al.* Formation and inactivation of endogenous cannabinoid anandamide. *Nature* **372**, 686–691 (1994).
The discovery of the currently accepted molecular mechanism of endocannabinoid biosynthesis.
106. Stella, N., Schweitzer, P. & Piomelli, D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388**, 773–778 (1997).
107. Beltramo, M. *et al.* Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* **277**, 1094–1097 (1997).
108. Cravatt, B. F. *et al.* Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**, 83–87 (1996).
109. Piomelli, D., Giuffrida, A., Calignano, A. & Rodríguez de Fonseca, F. The endocannabinoid system as a target for therapeutic drugs. *Trends Pharmacol. Sci.* **21**, 218–224 (2000).
110. Wilson, R. I. & Nicoll, R. A. Endogenous cannabinoids mediate retrograde signaling at hippocampal synapses. *Nature* **410**, 588–592 (2001).
111. Schlicker, E. & Kathmann, M. Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol. Sci.* **22**, 565–572 (2001).
112. Maldonado, R. & Rodriguez de Fonseca, F. Cannabinoid addiction: behavioral models and neural correlates. *J. Neurosci.* **22**, 3326–3331 (2002).
113. Calhoun, S. R., Galloway, G. P. & Smith, D. E. Abuse potential of dronabinol (Marinol). *J. Psychoactive Drugs* **30**, 187–196 (1998).

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