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Use of cannabinoid receptor agonists in cancer therapy as palliative and curative agents

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Cannabinoids (the active components of *Cannabis sativa*) and their derivatives have received renewed interest in recent years due to their diverse pharmacological activities. In particular, cannabinoids offer potential applications as anti-tumour drugs, based on the ability of some members of this class of compounds to limit cell proliferation and to induce tumour-selective cell death. Although synthetic cannabinoids may have pro-tumour effects in vivo due to their immunosuppressive properties, predominantly inhibitory effects on tumour growth and migration, angiogenesis, metastasis, and also inflammation have been described. Emerging evidence suggests that agonists of cannabinoid receptors expressed by tumour cells may offer a novel strategy to treat cancer. In this chapter we review the more recent results generating interest in the field of cannabinoids and cancer, and provide novel suggestions for the development, exploration and use of cannabinoid agonists for cancer therapy, not only as palliative but also as curative drugs.

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An overview of the endocannabinoid system

The therapeutic and psychotropic actions of the plant *Cannabis sativa* were first described about 4000 years ago in India, a long time before the discovery of the endocannabinoid system.¹ By the 19th century cannabis extracts had gained widespread use for medicinal purposes until 1937, when concerns about the dangers of abuse led to the banning of marijuana for further medicinal use in the United States. However, over the last 40 years the isolation and characterization of the psychoactive component of *C. sativa*, Δ^9 -tetrahydrocannabinol (Δ^9 -THC) represented a challenging research task, awakening renewed interest in its use for pharmacotherapy.² To date, about 60 different plant terpenophenols more or less structurally related to THC have been isolated and defined as cannabinoids. They include cannabidiol (CBD), cannabinol, cannabigerol, and cannabichromene. The majority of these lack psychoactivity. The chemical and biological characterization of these principles encouraged the synthesis of novel analogous compounds, similar to phytocannabinoids or with different chemical structures, such as classic and non-classic cannabinoids and aminoalkylindoles.³ Finally, studies on cannabinoids have led to the discovery of the endogenous arachidonic acid derivatives now known as endocannabinoids.

Endocannabinoids are lipid molecules, ubiquitously expressed, containing long-chain polyunsaturated fatty acids, amides, esters and ethers; the first-characterized compounds were anandamide (AEA) and 2-arachidonoylglycerol (2-AG). More recently, several other bioactive lipid mediators have been described: 2-arachidonoyl-glycerol-ether (noladin ether), o-arachidonoyl-ethanolamine (virodhamine), *N*-arachidonoyl-dopamine, oleamide, and the 'endocannabinoid-like' *N*-palmitoylethanolamine (PEA), *N*-oleoylethanolamine (OEA) and *N*-stearoylethanolamine (SEA).^{4,5}

Physiological or pathological stimuli induce the synthesis and immediate release of endocannabinoids which can subsequently activate cannabinoid receptors, either after previous release into the extracellular space or directly moving within the cell membrane. It seems clear that the regulation of endocannabinoid signalling is tightly controlled by their synthesis, release, uptake and degradation, and all the enzymes involved in these pathways are potential targets for pharmacological intervention in a wide range of diseases where a lack of balance in the endocannabinoid system has been reported. Mood and anxiety disorders, movement disorders such as Parkinson's and Huntington's disease, neuropathic pain, multiple sclerosis and spinal cord injury, cancer, atherosclerosis, myocardial infarction, stroke, hypertension, glaucoma, obesity/metabolic syndrome and osteoporosis are just some of the diseases in which an altered endocannabinoid system plays an interesting role for pharmacological intervention.⁶

Endocannabinoids, as well as phytocannabinoids and their synthetic analogues, show different selectivities for the two-cannabinoid receptor types (CB1 and CB2) that have so far been cloned from mammalian tissues. Both the CB1 and CB2 genes encode a seven-transmembrane-domain protein belonging to the $G_{i/o}$ protein-coupled receptor family.⁷ The receptors display different patterns of expression; CB1 receptor is preferentially expressed in the central nervous system and in several peripheral organs, whereas CB2 receptor is the predominant form expressed in immune cells and is unrelated to cannabinoid psychoactive effects. Different structural classes of cannabinoid receptor agonists have the unique ability to activate different signalling cascades which, in turn, influence agonist efficacy. Among these pathways, inhibition of adenylate cyclase⁸ stimulation of mitogen-activated protein kinase (MAPK)⁹ and phosphatidylinositol-3-kinase pathway¹⁰ and, in the case of CB1, modulation of ion channels have been reported.¹¹

Current thoughts about the endocannabinoid system in cancer

Preclinical data

Modulating the activity of proteins and nuclear factors involved in cell proliferation, differentiation and apoptosis, the endocannabinoid signalling system controls, among the other effects, cell survival, death and neoplastic transformation.^{12,13} Recent studies have therefore proposed that the endocannabinoid system could be an attractive anticancer target, and the cannabinoid agonists might directly inhibit tumour growth in vitro and in vivo. Several cannabinoids – including Δ^9 -THC and

cannabidiol, synthetic cannabinoid agonists (HU210, JWH133, WIN-55,212-2), endocannabinoids (anandamide, its congeners and 2-AG), and endocannabinoid transport or degradation inhibitors (VDM-11 and AA-5-HT) – have been shown to inhibit tumour growth and progression of several types of cancers, including glioma, glioblastoma multiforme, breast, prostate and thyroid cancer, colon carcinoma, leukaemia and lymphoid tumours (see Table 1).¹³ The proposed mechanisms are complex and different, depending on the tumour type, and may involve induction of apoptosis in tumour cells, anti-proliferative action through the suppression of mitogenic signals, and anti-metastatic effect through inhibition of neo-angiogenesis and tumour cell migration.^{13,14} Moreover, the effects, depending on the specific type of agonist and target tissue, have been reported to be CB1, CB2 or TRPV1 receptor-dependent or receptor-independent (e.g. cyclooxygenase, lipid rafts), all leading to activate different downstream signalling pathways.

Anti-proliferative effects occur possibly through inhibition of proliferative pathways such as adenylyl cyclase and cAMP/protein kinase A pathway¹⁶, cell cycle blockade with induction of the cyclin-dependent kinase inhibitor p27^{kip1}³⁴, decrease in epidermal growth factor receptor (EGF-R) expression and/or attenuation of EGF-R tyrosine kinase activity^{41,24}, decrease in the activity and/or expression of nerve growth factor, prolactin or vascular endothelial growth factor tyrosine kinase receptors.^{15,17,34} The pro-apoptotic effect of cannabinoids in tumour cells is complex and may involve increased synthesis of the pro-apoptotic sphingolipid ceramide²⁸, ceramide-dependent up-regulation of the stress protein p8 and several downstream stress-related genes expressed in the endoplasmic reticulum (ATF-4, CHOP, and TRB3)⁴⁸, prolonged activation of the Raf-1/extracellular signal-regulated kinase cascade²⁸, and inhibition of Akt^{28,30}, c-Jun NH₂-terminal kinase and p38 mitogen-activated protein kinase.^{28,32} More importantly, systemic or local treatment with cannabinoids inhibited the growth of various types of tumours or tumour cell xenografts in vivo, including lung carcinoma⁴⁶, glioma^{28,29}, thyroid epithelioma³³, lymphoma³⁷, and skin carcinoma⁴⁵ in mice. Inhibition of the formation of colon polyps in the genetic model of Apc^{+/-} mice and further of precancerous aberrant crypt foci induced by the potent carcinogen azoxymethane in mice have also been reported recently.^{43,44} Based on the in vivo efficacy, it has been suggested that anti-tumour efficacy of cannabinoid-related drugs could be partially ascribed to the inhibition of tumour metastatic spreading and neo-angiogenesis in several models.^{34,41,49} Cannabinoid agonists also directly inhibited angiogenesis induced by basic fibroblast growth factor (bFGF) in vitro and in vivo in a CB1-dependent manner⁵⁰, and reduced the invasiveness of different cancer cell lines through the increased expression of tissue inhibitor of metalloproteinases TIMP-1.⁵¹ In addition to cannabinoid agonists, inhibitors of endocannabinoid transport or degradation (VDM-11 and AA-5-HT) have been shown to inhibit tumour growth and progression in numerous types of cancer, enhancing the levels of endocannabinoids in the cells.^{41,43}

Cannabinoids, immune suppression, and potential increased cancer risk

In the light of the available literature, potential tumour-promoting effects of cannabinoid agonists have been taken into account and analysed. Hart et al⁴⁷ reported pro-proliferative effects of cannabinoids in different cancer cell lines at submicromolar doses, very low if compared to both the anti-proliferative and pro-apoptotic doses widely reported (in the micromolar range) and to the concentration achieved in vivo for most anticancer drugs during a chemotherapy protocol.⁴⁷ However, the same study documented that at micromolar concentrations cannabinoids induced cancer-cell apoptosis, in agreement with previous reports. These results highlight a likely bimodal action of cannabinoid agonists on cancer cell growth, with low concentrations being pro-proliferative and high concentrations having anti-proliferative effects. More appropriate is the concern about the immunosuppressive properties of cannabinoids in vivo through CB2 receptor stimulation in immune cells and the risk of the anti-tumour immune response inhibition. Indeed cannabinoids have been shown to modulate a variety of immune-cell functions in humans and animals, and more recently have been shown to modulate T-helper-cell development, chemotaxis, and tumour development.⁵² Many of these effects occur through cannabinoid receptor CB2 signalling mechanisms and the modulation of cytokines and other gene products. THC has been reported to exert immunosuppressive effects in vitro and in vivo on macrophages, NK cells and T lymphocytes. Endocannabinoid synthetic analogues

Table 1

Effect of cannabinoid agonists in cancer treatment.

Tumour (cell type/animal model)	Cannabinoid (concentration or dose)	Mechanism of action	Reference
Breast cancer:			
Human breast cancer cell lines (MCF-7; EFM-19; T47D)	AEA (2–10 μ M) 2-AG (2–10 μ M) HU210 (\geq 4 μ M)	Inhibition of the mitogen-induced stimulation of the G0/G1–S phase	15
	AEA (\geq 2 μ M) 2-AG, HU210 (\geq 1 μ M)	Inhibition of NGF-induced proliferation Inhibition adenylyl cyclase; down-regulation PRLr TRK	16,17
Human breast cancer cell line (MDA-MB-231)	AEA (10 μ M)	S phase arrest; induction Chk1 intra-S phase checkpoint	18
Human breast cancer cell line (MDA-MB-231)	AEA (10 μ M and 0.5 mg/kg/dose)	Inhibition of adhesion and migration	19
Mouse breast cancer cell line (TSA-E1)		In vivo, reduction of number and dimension of metastatic nodes	
Human breast cancer cell lines (MCF-7; MDA-MB-231)	THC (\leq 5 μ M)	Increased tumour growth and metastasis	20
Mouse mammary carcinoma (4T1)		In vivo, decreased anti-tumour immune response	
Human breast cancer cell lines (MCF-7; T47D; MDA-MB- 231; MDA-MB-468)	THC (\geq 12 μ M)	G2/M phase transition blockade through Cdc2 and apoptosis induction	21
Human breast cancer cell lines (MCF-7; MDA-MB-231)	Cannabidiol (8–12 μ M)	Inhibition of proliferation; apoptosis induction	22
Human breast cancer cell lines (MCF-7; MDA-MB-231; T47D)	Rimonabant (0.1 μ M and 0.7 mg/kg/dose)	Inhibition of proliferation; G1 arrest In vivo, growth inhibition of breast xenografts tumours	23
Prostate cancer:			
Androgen-independent prostate cancer cells (PC3, DU145)	AEA, R-(+)-MET (\geq 2 μ M)	Inhibition of mitogen-induced proliferation, G1 arrest	17,24,25
	THC (1 μ M)	Apoptosis	
Androgen-dependent prostate cancer cells (LNCaP)	AEA, R-(+)-MET (\geq 2 μ M)	Inhibition of mitogen-induced proliferation, G1 arrest	24
Androgen-dependent prostate cancer cells (LNCaP)	WIN-55,212-2 (\geq 2.5 μ M)	Dose- and time-dependent induction of apoptosis; decreased expression of AR and PSA	26
Androgen-dependent prostate cancer cells (LNCaP)	R-(+)-MET (0.1–0.2 μ M)	Increased proliferation and AR expression	27
Glioma and brain cancers:			
Rat glioma cell line (C6)	THC (1 μ M)	Apoptosis via ceramide de-novo synthesis In vivo, regression of C6-derived glioma	28–30
	JWH133, WIN-55,212-2 (0.1 μ M)	Apoptosis via ceramide de-novo synthesis	
	WIN-55,212-2 (15 μ M)	Apoptosis via activation of caspase cascade	
Human astrocytoma (grade IV)	JWH-133 (50 μ g/d)	In vivo, inhibited growth of tumours induced in deficient mice	29
Human glioblastoma multiforme cell line (GBM)	THC (1 μ M) WIN-55,212-2	Decreased proliferation and increased cell death	31
Human neuroglioma cells	R-(+)-MET (1–10 μ M)	Apoptosis induction	32
Thyroid cancer:			
K-ras-transformed FRTL-5 thyroid cells (KiMol)	Met-F-AEA (0.5 mg/kg/dose)	In vivo, inhibited growth of tumours induced in nude mice	33
Thyroid tumour xenografts	Met-F-AEA (0.5 mg/kg/dose)	In vivo, inhibited growth of thyroid tumour xenografts induced in athymic mice	34
Experimental lung metastases		In vivo, inhibited development of lung metastases	
Thyroid tumour xenografts	Met-F-AEA (0.5 mg/kg/d); VDM-11, AA-5HT (5 mg/kg/d); rimonabant (0.7 mg/kg/dose)	In vivo, inhibited growth of thyroid tumour xenografts induced in athymic mice	35

Table 1 (continued)

Tumour (cell type/animal model)	Cannabinoid (concentration or dose)	Mechanism of action	Reference
Haematological malignancies:			
Lymphoma U937 cells	AEA (10 μ M)	Apoptosis induction via TRPV1	36
Lymphoma cell lines	THC (10 μ M), HU210 (5 μ M)	Apoptosis induction via CB2	37
Mantle-cell lymphoma cell lines	AEA, WIN-55,212-2, rimonabant (1–10 μ M)	Growth inhibition; apoptosis induction	38,39
Human leukaemia cells	THC (1–5 μ M); JWH-133	Apoptosis via CB2	40
C6 glioma cells	THC (14–25 μ M); CBD (6–20 μ M)	Growth inhibition; apoptosis induction	22
Colorectal cancer:			
Colorectal carcinoma cells (DLD-1, CaCo-2)	AEA (2.5 μ M), 2AG (1 μ M), HU210 (0.1 μ M), VDM-11, AA-5-HT (10 μ M)	Inhibition of proliferation	41
Colorectal carcinoma cells (HT29)	AEA (25 μ M)	Cell death via COX-2	42
Azoxymethane-induced aberrant crypt foci (ACF) in the mouse colon	AA-5-HT (5 mg/kg), HU210 (0.1 mg/kg)	Inhibition of ACF formation; caspase-3 activation	43
Intestinal tumor (Apc ^{+/-} mice)	Methanandamide (10 mg/kg), AM251 (10 mg/kg)	Inhibition of colon polyps Stimulation of colon polyps	44
Other cancers:			
Mouse skin carcinoma cells (PDV-C57)	JWH-133, WIN-55,212-2 (1.58 μ g)	In vivo, inhibited growth of tumours induced in nude mice	45
Lung carcinoma	THC 100 mg/kg	Growth inhibition	46
Lung cancer cells (NCI-H292)	THC (0.1–0.3 μ M)	Increased proliferation	47
Glioblastoma cell line (U373-MG)			
Pancreatic tumour cells (Panc1; MiaPaCa2)	THC (2 μ M and 15 mg/kg/d)	Apoptosis induction through ceramide In vivo, inhibited growth of xenografts and intrapancreatic tumours	48

AEA, anandamide; NGF, nerve growth factor; PRLr, prolactin receptor; THC, tetrahydrocannabinol; MET, methanandamide; AR, androgen receptor; PSA, prostate-specific antigen; TRPV1, transient receptor potential vanilloid type 1; CBD, cannabidiol; COX-2, cyclooxygenase 2.

suppress T-cell proliferation, inhibit interferon γ (IFN- γ) production, and shift the balance of T-helper-1 (Th1)/T-helper-2 (Th2) cytokines.⁵³

Although most of the studies propose an anti-tumour efficacy of cannabinoid agonists, a few studies suggest a potential increased cancer risk following cannabinoid exposure due to a biasing toward Th2 immunity. The immune response to tumours is primarily mediated by Th1 response. Skewing of the immune response from the cell-mediated Th1 response to the humoral-mediated Th2 response may lead to a positive environment for tumour growth. Increased levels of these cytokines and a shift toward the Th2 immune response have been associated with breast and lung cancers, directly correlated with suppression of the immune response.⁵⁴ T cells secreting type-2 cytokines, including IL-10, inhibit cell-mediated immunity and anti-tumour responses. In contrast, T cells producing type-1 cytokines, including IL-2 and IFN- γ , are potent activators of cell-mediated immunity. Regulation of cytokine production profiles allows a controlled balance between stimulation and suppression of cell-mediated responses. THC and other cannabinoid agonists may exert their immunosuppressive effects through disruption of these homeostatic mechanisms by inhibiting the production of type-1 cytokines and promoting type-2 cytokine production by lymphocytes.⁵⁵ Host immunity plays an important role in limiting tumour growth.⁵⁶ In murine lung cancer models, biasing toward Th2 immunity was reported⁵⁷, showing that THC promotes tumourigenicity and limits immunogenicity in vivo by up-regulating the immune inhibitory cytokines interleukin 10 (IL-10) and

tumour growth factor β (TGF- β). Furthermore, lymphocytes from THC-treated mice transferred the effect to normal mice, resulting in accelerated tumour growth similar to that observed in the 7THC-treated mice. These effects were CB2-receptor-dependent. A similar CB2-dependent shift to Th2 cytokines was demonstrated in response to THC in activated peripheral-blood T-cell cultures.⁵⁸ A possible key could be the relative levels of CB receptors expressed by tumour cells. When these levels are high, both tumour and immune cells are targeted by cannabinoid agonists, and the consequent effect is the inhibition of tumour growth. In contrast, tumour cells expressing low or undetectable levels of CB receptors are resistant to the anti-proliferative effects and immunosuppression through CB2 prevails. A study demonstrated that exposure to THC led to increased tumour growth and metastasis of the mouse mammary carcinoma 4T1, which expresses low to undetectable levels of cannabinoid receptors CB1 and CB2; these effects were due to inhibition of the specific anti-tumour immune response in vivo. Furthermore, exposure to THC led to increased production of Th2-associated cytokines, IL-4 and IL-10. Such findings suggest that cannabinoid agonists used either recreationally or medicinally may increase the susceptibility to and/or incidence of breast cancer as well as other cancers that do not express cannabinoid receptors and are resistant to THC-induced apoptosis.³⁷

Relevance of cannabinoid receptor levels in cancer

Cannabinoid receptor levels seem to be a fundamental element for the growth-inhibitory effects of cannabinoid agonists. It has been reported that the expression of CB1 receptor was regulated in opposite ways in normal and malignant cells. This pattern of expression seems to be a common mechanism for the general protection of normal cells from the pro-apoptotic and anti-proliferative effects of cannabinoid agonists.¹³ THC-induced apoptosis in several human cancer cell lines, but showed less efficacy in non-transformed cell counterparts that might be protected from cell death.^{25,28,31,45} Therefore, a relevant issue seems to be the evaluation of cannabinoid receptor expression in tumour versus normal tissues in order to achieve a significant anti-tumour effect with cannabinoid agonists without immunosuppression, and also for the prognostic value that CB receptor levels could have alone or in association with other recognized prognostic markers.

To date there have been few studies in this field. Analyses of astrocytomas demonstrated that 70% of the tumours express CB1 and/or CB2, and the extent of CB2 expression was directly related to tumour malignancy.²⁹ In hepatocellular carcinoma, over-expression of CB1 and CB2 receptors correlated with improved prognosis.⁵⁹ Increased expression of CB1 has been reported in mantle-cell lymphoma⁶⁰ and of CB2 in breast cancer, where a correlation among CB2 expression, histological grade of tumour and other markers of prognostic and predictive value – such as oestrogen receptor, progesterone receptor, and ERBB2/HER-2 oncogene – has been observed.²¹ In gliomas a higher expression of CB2 compared to CB1 was reported and was related to tumour grade.⁶¹ Interestingly, it has recently been reported that CB1 expression was silenced in human colorectal cancer due to promoter methylation.⁴⁴ Regulation of CB receptors by factors naturally expressed in the tumour microenvironment is intriguing, and must be understood if we are to understand their role and biological relevance during carcinogenesis and tumour progression. Studies at the promoter level of CB receptor genes could therefore be very informative. Moreover, the trafficking and recycling of CB receptors and their sublocalization and compartmentalization (e.g. lipid rafts/caveolae, organuli) in tumour cells compared with normal cells could be useful.

Anti-inflammatory activity of cannabinoid agonists and cancer prevention

The link between inflammation and cancer was noticed 150 years ago by Virchow, who indicated that cancers frequently occur at sites of chronic inflammation.⁶² Recently it has turned out that acute inflammation contributes to the regression of cancer.⁶³ However, accumulated epidemiological studies support the idea that chronic inflammatory diseases are frequently associated with an increased risk of cancer.^{62–64} It has been realized that the development of cancer from inflammation might be a process driven by inflammatory cells as well as a variety of mediators, including cytokines, chemokines, and enzymes, which altogether establish an inflammatory microenvironment.⁶⁴ As discussed above, although this host response may suppress tumours, it may also facilitate cancer development via multiple signalling pathways.⁶⁵

Studies examining the effect of cannabinoid-based drugs on immunity have shown that many cellular and cytokine mechanisms are suppressed by these agents, leading to the hypothesis that these drugs may be of value in the management of chronic inflammatory diseases. Evidence of the role of cannabimimetic compounds – such as anandamide (AEA), 2-arachidonoylglycerol (2-AG) and palmitoylethanolamide (PEA) – in the control of inflammation and proliferation of tumour cells has been described. AEA was found to enhance the release of the anti-inflammatory cytokine IL-6 from astrocytes infected with Theiler's murine encephalomyelitis virus.⁶⁶ The anti-inflammatory effects of PEA have been also described.⁶⁷ In particular, reduction of substance-P-induced mast-cell degranulation and extravasation, passive cutaneous anaphylaxis-induced extravasation, formalin and dextran-induced oedema, and carrageenan-induced oedema thermal hyperalgesia were found, and the last effect being described also for AEA.⁶⁸ A synthetic agonist at CB1 and CB2, HU-210, exerts an anti-inflammatory effect. It was shown that this compound abolished abdominal pain associated with pancreatitis and also reduced inflammation and decreased tissue pathology in mice without producing central, adverse effects.⁶⁹ Furthermore, treatment with HU-210 or genetic ablation of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) resulted in protection against 2,4-dinitrobenzene sulphonic-acid-induced colitis, thus suggesting that the endocannabinoid system could be a promising therapeutic target for the treatment of intestinal disease characterized by excessive inflammatory responses.⁷⁰

Along with anti-inflammatory effects, anti-tumour properties of endocannabinoids have been reported; in particular, an inhibitory effect of AEA on migration of both colon carcinoma cells and T lymphocytes has been described, thus suggesting relevance as a tool to prevent metastasis formation without depreciatory effects on the immune system of cancer patients.⁷¹ An exception is UVB-induced inflammation. Very recent work showed that the CB1/2 receptors are required in the induction of the proinflammatory-cascade-dependent skin tumour development in response to UVB. It was shown that the absence of the CB1/2 receptors in mice results in a dramatic resistance to UVB-induced inflammation and a marked decrease in UVB-induced skin carcinogenesis.⁷²

Human data

Although the use of cannabinoids-related drugs for medicinal purposes could be limited by concerns about their psychotropic effects, they have shown a fair safety profile, especially with respect to current chemotherapeutics which all display toxic adverse effects.

Despite the overall collected evidence on the therapeutic potential of cannabinoids and related drugs in several types of cancer, only a single pilot clinical study has been performed so far, and the results have been published recently.⁷³ This phase-I/II clinical trial was approved by the Spanish Ministry of Health in 2002 and was aimed at evaluating the safety profile of THC administration and its anti-tumour activity in a cohort of nine terminally ill patients affected by recurrent glioblastoma multiforme, an aggressive primary brain tumour with poor prognosis (6–12 months survival) and no efficacious treatment. The first goal of the study was to confirm the safety of intracranial administration of THC and the absence of significant psychotropic effects at the used regimen. Moreover, the study was reassuring about the possibility that cannabinoids could have tumour-promoting effects, since THC administration did not induce tumour growth or decrease patient survival. THC decreased tumour-cell proliferation, and also induced apoptosis; however, it had only a slight impact on the overall median survival of the cohort (24 weeks). This pioneer study suffers some limitations due to its design. The results are somewhat encouraging, and open the way to new studies with different characteristics. It will be interesting in the future to perform other clinical trials aimed at evaluating the efficacy of cannabinoid agonists (not limited to THC) in cancer treatment in different types of tumours. To optimize the results, the protocols should involve large cohorts of patients, and combinatorial studies with commonly used chemotherapeutic drugs could be interesting.

CB1 antagonism by rimonabant: unexpected anti-tumour efficacy

The role of the CB1–endocannabinoid axis in physiopathology is reflected in the ongoing development of high-affinity CB1 antagonists and inverse agonists, in addition to cannabinoid agonists, as

therapeutic drugs. The first highly selective CB1 receptor antagonist was discovered by Sanofi-aventis and was the diarylpyrazole [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride], so-called rimonabant (SR141716, Acomplia™), which showed a number of beneficial pharmacological effects in several pathological situations: e.g. obesity, metabolic syndrome, diabetes, nicotine dependence, and a plethora of unexpected biological effects in vitro and in vivo.⁷⁴ It is noteworthy that most of the CB1 receptor antagonists developed to date, including rimonabant, have inverse agonist properties, so their effects do not necessarily reflect reversal of the tonic action of the endocannabinoids.⁷⁵ In some experiments, rimonabant has been found to produce effects that are opposite in direction from those produced by cannabinoid receptor agonists. Indeed, the availability of rimonabant on the pharmaceutical market as a drug for obesity and metabolic syndrome management has raised the alarming question of the potential tumour-promoting effect of cannabinoid antagonism. Rimonabant counteracts the anti-tumour effects of anandamide-related compounds and other cannabinoid agonists in thyroid, breast and prostate cancers.^{19,26,34} Unexpectedly, rimonabant per se showed a potential anti-tumour action in thyroid, mantle-cell lymphoma and breast tumours both in vitro and in vivo.^{23,33,38} Furthermore, rimonabant displayed anti-proliferative properties in preadipocyte and hepatic myofibroblasts in vitro and in vivo.^{76,77} At least in breast cancer cells, the ability of rimonabant to early compartmentalize CB1 receptors in lipid rafts and segregate effector key signalling proteins (G proteins, p21ras, ERK), physically sequestering them from RTKs, could represent a possible explanation for its anti-proliferative activity. It appears clear that either cannabinoid agonists or the antagonist/inverse agonist rimonabant targeting CB1 signalling display a comparable inhibitory efficacy on cancer growth, even though through a different mechanism of action. This is unsurprising, since there are multiple oncogenic cascades and potential tumour-suppressing targets. Further studies will be necessary to ensure the efficacy of rimonabant and other CB1 receptor antagonists/inverse agonists as anticancer drugs. Positive results in this field could start the development of selective antagonists unable to pass the blood–brain barrier in order to avoid the psychiatric effects, above all major depression, which represent the major side-effects of rimonabant.

Cannabinoids in cancer therapy as palliative agents

The potential application of cannabinoid agonists as anticancer agents is still at the preclinical level. Meanwhile the cannabinoids are emerging as valuable adjunctive agents for optimizing the management of multiple symptoms of cancer and the treatment of therapy-related side-effects. Indeed, while much about the pathophysiological mechanisms of the endocannabinoid system remains unknown, available data support a broad spectrum of palliative properties, including appetite stimulation, inhibition of nausea and emesis associated with chemotherapy or radiotherapy, pain relief, mood amelioration, and relief from insomnia.⁷⁸ The commercially available cannabinoids target ubiquitous cannabinoid receptors in the central (CB1) and peripheral (CB1 and CB2) nervous system.⁷⁹

In the United States, two FDA-approved medicinal cannabis products are available: Marinol, a synthetic form of THC (the most active ingredient in cannabis) and Cesamet, a synthetic THC analogue. Both are currently approved for chemotherapy-induced nausea and vomiting in patient who have failed to respond adequately to conventional anti-emetic compounds. Dronabinol is also approved for the treatment of anorexia associated with AIDS. A third medicinal cannabis product, Sativex (a combination of THC and CBD) is already approved and marketed in Canada as adjunctive treatment for the symptomatic relief of neuropathic pain in multiple sclerosis.⁷⁹ Health Canada has approved Sativex under its Notice of Compliance with conditions (NOC/c) policy. It is also available on a named-patient basis in the United Kingdom and the Catalunya Autonomous Region of Spain. In the USA, the lead indication for Sativex is cancer pain in patients who have not been adequately relieved by opioid medications, and in 2007 the first USA phase-III cancer pain trial with this drug started (Table 2).

Recognized role

Inhibition of chemotherapy-induced nausea and emesis

Two of the most prevalent side-effects of cancer and its treatment are chemotherapy-induced nausea and vomiting (CINV); approximately one half of cancer patients will experience nausea or

Table 2

Available cannabinoid-containing drugs.

Cannabinoid	Source	Registered name and official status	Indications	Route of administration and formulation	Onset and duration of action
Dronabinol	Synthetic Δ^9 -THC	Marinol (Solvay Pharmaceuticals) FDA approval (2003)	Anorexia/weight loss (AIDS patients) Nausea and vomiting (cancer patients)	Oral Capsule formulated with sesame oil	30–60 min, 4–6 h
Nabilone	Synthetic Δ^9 -THC analogue	Cesamet (Valeant Pharmaceuticals) FDA approval (2006)	Nausea and vomiting (cancer patients)	Oral Crystalline powder capsule	60–90 min, 8–12 h
THC & CBD	Isolated from <i>Cannabis sativa</i>	Sativex (GW Pharmaceuticals) Approval with conditions in Canada (2005) Limited availability in Spain and UK	Symptomatic relief of neuropathic pain (multiple sclerosis patients)	Sublingual Oro-mucosal spray	15–40 min, 2–4 h

vomiting during the disease. Control of CINV is mediated by multiple neurotransmitters, including serotonin, dopamine, histamine, endorphins, acetylcholine, γ -aminobutyric acid and cannabinoids.⁸⁰ Cannabinoids are used for patients who have nausea and vomiting that are not responsive to standard anti-emetic therapy. Cannabinoids not only interact with CB receptors but also with the dopaminergic, serotonin, monoaminergic, noradrenergic and opioid system, important pathways involved in emesis.⁸¹ There is evidence that cannabinoids act on CB1 receptors in the dorsal–vagal complex of the brainstem region controlling 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.⁸² Clinical studies and case reports (the main ones listed in Table 3) have confirmed that natural and synthetic THC are more effective than placebo. Cannabinoids are unlikely to be used as first-line treatment for nausea and vomiting, but they may be used as adjuvant treatment to enhance the effects of existing anti-emetic medications.

Emerging roles

Pain inhibition

Pain has a negative impact on the life quality in cancer patients. Almost half of all patients with cancer experience moderate to severe pain, and this increases in patients with metastatic or advanced stages of cancer. This burden negatively impacts on their life quality, functional status and life expectancy. Cancer pain is often treated with opioid drugs (e.g. codeine, morphine, and/or their synthetic analogues); however, these drugs have dose-limiting side-effects.⁹³ The use of cannabinoids to treat cancer pain may provide a novel therapeutic approach. Several studies have shown that systemic administration of cannabinoids produces anti-nociception and attenuates hyperalgesia and allodynia in animal models of acute and chronic pain.⁹⁴ Potenziari et al demonstrated that tumour-evoked hyperalgesia was dose-dependently attenuated by local administration of non-selective cannabinoid receptor agonist WIN-55,212-2 into the tumour-bearing hind paw in a model of rodent cancer pain.⁹⁵ Cannabinoids produce anti-nociception by activating CB1 receptors in the brain, the spinal cord and nerve terminals. Endocannabinoids naturally function to suppress pain by inhibiting nociceptive neurotransmission.⁹⁶ Clinical trials on cannabinoid analgesia are very heterogeneous; nonetheless there are some human data to support the effectiveness of cannabinoids in alleviating pain associated with cancer (Table 3). In particular, findings from propensity-score analysis of data obtained from advanced cancer patients suggest that nabilone offers benefits beyond its original indication to treat chemotherapy-induced nausea and vomiting. Nabilone administration improved management of

Table 3

Clinical studies and case reports of palliative properties of cannabinoids in cancer patients.

Diagnosis/study	Medications	Results	Reference
A prospective observational study assessed the effectiveness of adjuvant nabilone therapy in managing pain and symptoms experienced by advanced cancer patients	Nabilone	Patients receiving nabilone experienced reduced pain, nausea, and anxiety and relief of overall distress. Beneficial but non-significant effect on appetite	82
Phase-III clinical trials will test the drug's ability to treat pain in advanced cancer patients who have not found relief through conventional opioid medications (e.g. codeine, morphine, and/or their synthetic analogues)	Sativex	In progress	
Cancer-related anorexia	Dronabinol	Dronabinol alone improved appetite in almost 50% of patients	83
CINV 5-day double-blind, placebo-controlled study	Dronabinol	Dronabinol was as effective as ondansetron in reducing nausea and vomiting; combination of therapy was not more effective	84
CINV Appetite loss, weight loss	Nabilone	Nabilone treatment improved pain, nausea, appetite and several other symptoms	85
CINV Appetite loss, weight loss	Dronabinol	A significant increase in appetite and decrease in nausea in most patients	86
Appetite loss, weight loss in patient with CACS	Cannabis extract, THC	No difference between cannabis, THC and placebo	87
Multicentre, phase-III, randomized, double-blind, placebo-controlled clinical trial			
CINV	Nabilone	Significant improvement in one case of intractable neuropathic pain and on case of refractory CINV	88
Appetite loss/weight loss in cancer patients with CACS	Dronabinol	Megestrol acetate was superior to dronabinol	89
CINV eight children, aged 3–13 years with various haematological cancers, treated with different anti-neoplastic drugs for up to 8 months	Δ^8 -THC	Vomiting was completely prevented. The side-effects observed were negligible	90
A phase-II study of Δ^9 -tetrahydrocannabinol for appetite stimulation in cancer-associated anorexia	Δ^9 -THC	THC is an effective appetite stimulant in patients with advanced cancer. It is well tolerated at low doses	91
Dronabinol and prochlorperazine were tested alone and in combination in a randomized, double-blind, parallel-group, multicentre study	Dronabinol	The combination was significantly more effective than was either agent alone in controlling chemotherapy-induced nausea and vomiting	92

CINV, chemotherapy-induced nausea and vomiting; CACS, cancer anorexia-cachexia syndrome; THC, tetrahydrocannabinol.

pain and was associated with a lower overall use of drugs such as opioids and non-steroidal anti-inflammatory drugs.⁸²

Health Canada approved Sativex in 2007, with conditions, as adjunctive analgesic treatment in adult patients with advanced cancer who experience moderate to severe pain during the highest tolerated dose of strong opioid therapy for persistent background pain. This authorization reflects the promising nature of the clinical evidence which will be confirmed with further clinical trials on

cannabinoids in the treatment of cancer pain – including terminal care – which are now in progress. Indeed, the US FDA accepted GW's Investigational New Drug (IND) Application for Sativex, allowing GW to launch phase-III clinical trials in the United States. These trials will test the drug's ability to treat pain in advanced cancer patients who have not found relief through conventional opioid medications.

Appetite stimulation (orexigenic effects)

Cancer-related anorexia and associated cachexia are prevalent manifestations of disease in people with malignancies, and the cancer anorexia–cachexia syndrome (CACS) is an important risk factor for morbidity and mortality in people with cancer. Many studies have reported that THC and other cannabinoids have a stimulatory effect on appetite and increase food intake in animals.⁹⁷ This circuit is well known, and the orexigenic effect occurs through the inhibition of leptin at hypothalamic level.⁹⁸ Anecdotal information from cannabis smokers and numerous clinical trials support the appetite-stimulating properties of THC. In fact, the synthetic cannabinoid dronabinol is approved by the FDA for the treatment of anorexia associated with weight loss in AIDS patients.

Clinical evidence for the use of cannabinoids in patients with CACS is limited (Table 3). A phase-II study of THC for appetite stimulation in cancer-associated anorexia showed that THC is an effective stimulant in patient with advanced cancer.⁹¹ In contrast, Jatoi et al demonstrated that megestrol acetate (an orexigenic agent) provided palliation of anorexia in advanced cancer patients superior to that of dronabinol alone, and that the combination therapy did not confer additional benefit.⁸⁹ Moreover, it was showed that dronabinol produced a small, although not significant, increase in body weight in residents of geriatric facilities.⁹⁹ Finally, the first phase III in patients with CACS comparing the effects of cannabinoids with placebo and standardized cannabis extract showed no differences between the three groups for stimulation of appetite, quality of life, or secondary endpoint such as mood or nausea.⁸⁷ Further research should elucidate the clinical relevance and the real benefit of cannabinoids for cancer anorexia.

Conclusions

The discovery of the endocannabinoid system and the recognition of its potential impact in a plethora of pathological conditions led to the development of therapeutic agents related to either agonism or antagonism of CB1 and CB2 receptors, the majority of which are in preclinical studies. A few medications that belong to the endocannabinoid system have been subjected to clinical studies and are now available and useful as palliative drugs in disease states associated with cancer, such as chemotherapy-induced nausea and vomiting, pain relief, and anorexia/weight loss. Cannabinoid agonists show interesting potential as anticancer drugs, and the preclinical studies carried out so far have yielded encouraging results in different *in vitro* and *in vivo* models of cancers. The use of cannabinoid agonists as palliative drugs, and results obtained in the unique clinical trial in glioma patients, has demonstrated that cannabinoid agonists show a good-safety profile. Although their use in medicine could be limited by their known psychotropic effects, this could be bypassed by the development of selective agonists devoid of psychotropic effects (such as cannabidiol) or unable to pass the blood–brain barrier. It is noteworthy that THC delivery in glioma patients was safe and achieved without psychoactive effects. Moreover, the potential adverse effects of cannabinoid agonists are within the range believed acceptable for other drugs, especially anticancer drugs. It is well known that the therapeutic activity of most anticancer drugs in clinical use is limited by their general toxicity to proliferating cells, including normal cells. Novel cytotoxic agents with known mechanisms of action have been developed, but they still lack tumour selectivity and have not been therapeutically useful. Cannabinoid agonists do seem to selectively target tumour cells, while normal cells are less sensitive or even protected. Further clinical studies will clarify their efficacy in treating cancer in humans, not only as palliative drugs but also as therapeutic agents which could be used alone or in combination with other chemotherapeutic drugs in order to avoid resistance and exert

a more potent clinical impact. As our knowledge of the endocannabinoid system becomes more defined, it can be expected that more drugs acting directly on this system will be available for therapeutic exploitation.

Practice points

- cannabinoid agonists show a good-safety profile
- the psychotropic effects are limited to CB1 potent agonists, whereas CB1/CB2 mixed agonists or CB2 selective agonists are devoid of psychoactivity
- intracranial administration of THC in glioma patients was safe and did not induce tumour growth or psychotropic effects
- cannabinoid agonist-based medications are used as palliative drugs in chemotherapy-induced nausea and vomiting, pain relief, and anorexia/weight loss in cancer patients, in multiple sclerosis and in AIDS patients

Research agenda

- to clarify molecular mechanisms involved in the action of cannabinoid agonists
- to synthesize and develop less hydrophobic and non-psychotropic cannabinoid agonists in order to improve their efficacy and reduce side-effects
- to perform exhaustive clinical trials aimed at evaluating the potential anti-tumour efficacy of cannabinoid agonists in several tumours when used alone or in combination with other chemotherapeutic drugs
- to evaluate the cannabinoid receptor expression in tumour versus normal tissues and their association with well-known prognostic markers in order to assess their prognostic value
- to study the CB receptor genes at the promoter regions and to elucidate their regulation in carcinogenesis and tumour progression
- to perform further clinical trials on cannabinoids in the treatment of cancer pain and cancer anorexia

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References

1. Peters H & Nahas GG. A brief history of four millennia (B.C. 2000–A.D. 1974). In Nahas GG, Sutin KM, Harvey D et al (eds.). *Marihuana and medicine*. Totowa, NJ: Humana Press, 1999, pp. 3–7.
2. Gaoni Y & Mechoulam R. Isolation, structure and partial synthesis of an active constituent of hashish. *Journal of the American Chemical Society* 1964; **86**: 1646–1647.
3. Howlett AC, Barth F, Bonner TI et al. Classification of cannabinoid receptors. *Pharmacological Reviews* 2002; **54**: 161–202.
- *4. De Petrocellis L, Cascio MG & Di Marzo V. The endocannabinoid system: a general view and latest additions. *British Journal of Pharmacology* 2004; **141**: 765–774.
5. Di Marzo V. Endocannabinoids and other fatty acid derivatives with cannabimimetic properties: biochemistry and possible physiopathological relevance. *Biochimica et Biophysica Acta* 1998; **1392**: 153–175.
- *6. Pacher P, Batkai S & Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacological Reviews* 2006; **58**: 389–462.
7. Munro S, Thomas KL & Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993; **365**: 61–65.
8. Howlett AC, Qualy JM & Khachatryan LL. Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. *Molecular Pharmacology* 1986; **29**: 307–313.

9. Bouaboula M, Poinot-Chazel C, Bourrie B et al. Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *The Biochemical Journal* 1995; **312**: 637–641.
10. Gomez Del Pulgar T, De Ceballos ML, Guzman M et al. Cannabinoids protect astrocytes from ceramide-induced apoptosis through the phosphatidylinositol 3-kinase/protein kinase B pathway. *The Journal of Biological Chemistry* 2002; **277**: 36527–36533.
11. Mackie K & Hille B. Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proceedings of the National Academy of Sciences of the United States of America* 1992; **89**: 3825–3829.
- *12. Bifulco M & Di Marzo V. The endocannabinoid system as a target for the development of new drugs for cancer therapy. *Nature Medicine* 2002; **8**: 547–550.
- *13. Bifulco M, Laezza C, Pisanti S et al. Cannabinoids and cancer: pros and cons of an antitumour strategy. *British Journal of Pharmacology* 2006; **148**: 123–135.
- *14. Bifulco M, Malfitano AM, Pisanti S et al. Endocannabinoids in endocrine and related tumours. *Endocrine-related Cancer* 2008; **15**: 391–408.
15. De Petrocellis L, Melck D, Palmisano A et al. The endogenous cannabinoid anandamide inhibits human breast cancer cell proliferation. *Proceedings of the National Academy of Sciences of the United States of America* 1998; **95**: 8375–8380.
16. Melck D, Rueda D, Galve-Ropher I 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 Letters* 1999; **463**: 235–240.
17. Melck D, De Petrocellis L, Orlando P et al. Suppression of nerve growth factor Trk receptor and prolactin receptors by endocannabinoids leads to inhibition of human breast and prostate cancer cell proliferation. *Endocrinology* 2000; **141**: 118–126.
18. Laezza C, Pisanti S, Crescenzi E et al. Anandamide inhibits Cdk2 and activates Chk1 leading to cell cycle arrest in human breast cancer cells. *FEBS Letters* 2006; **13**(580): 6076–6082.
19. Grimaldi C, Pisanti S, Laezza C et al. Anandamide inhibits adhesion and migration of breast cancer cells. *Experimental Cell Research* 2006; **15**(312): 363–373.
20. McKallip RJ, Nagarkatti M & Nagarkatti PS. Delta-9-tetrahydrocannabinol enhances breast cancer growth and metastasis by suppression of the antitumor immune response. *Journal of Immunology* 2005; **15**(174): 3281–3289.
21. Caffarel MM, Sarrio D, Palacios J et al. Delta9-tetrahydrocannabinol inhibits cell cycle progression in human breast cancer cells through Cdc2 regulation. *Cancer Research* 2006; **66**: 6615–6621.
22. Ligresti A, Moriello AS, Starowicz K et al. Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *The Journal of Pharmacology and Experimental Therapeutics* 2006; **318**: 1375–1387.
23. Sarnataro D, Pisanti S, Santoro A et al. The cannabinoid CB1 receptor antagonist rimonabant (SR141716) inhibits human breast cancer cell proliferation through a lipid rafts mediated mechanism. *Molecular Pharmacology* 2006; **70**: 1298–1306.
24. Mimeault M, Pommery N, Wattez N et al. Antiproliferative and apoptotic effects of anandamide in human prostatic cancer cell lines: implication of epidermal growth factor receptor down-regulation and ceramide production. *Prostate* 2003; **56**: 1–12.
25. Ruiz L, Miguel A & Diaz-Laviada I. Delta9-tetrahydrocannabinol induces apoptosis in human prostate PC-3 cells via a receptor-independent mechanism. *FEBS Letters* 1999; **458**: 400–404.
26. Sarfaraz S, Afaq F, Adhami VM et al. Cannabinoid receptor as a novel target for the treatment of prostate cancer. *Cancer Research* 2005; **65**: 1635–1641.
27. Sanchez MG, Sanchez AM, Ruiz-Llorente L et al. Enhancement of androgen receptor expression induced by (R)-methanandamide in prostate LNCaP cells. *FEBS Letters* 2003; **555**: 561–566.
28. Galve-Ropher I, Sanchez C, Cortes ML et al. Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. *Nature Medicine* 2000; **6**: 313–319.
29. Sanchez C, de Ceballos ML, del Pulgar TG et al. Inhibition of glioma growth in vivo by selective activation of the CB2 cannabinoid receptor. *Cancer Research* 2001; **61**: 5784–5789.
30. Ellert-Miklaszewska A, Kaminska B & Konarska L. Cannabinoids downregulate PI3K/Akt and Erk signalling pathways and activate proapoptotic function of Bad protein. *Cellular Signalling* 2005; **17**: 25–37.
31. McAllister SD, Chan C, Taft RJ et al. Cannabinoids selectively inhibit proliferation and induce death of cultured human glioblastoma multiforme cells. *Journal of Neuro-oncology* 2005; **74**: 31–40.
32. Hinz B, Ramer R, Eichele K et al. R(+)-methanandamide-induced cyclooxygenase-2 expression in H4 human neuroglioma cells: possible involvement of membrane lipid rafts. *Biochemical and Biophysical Research Communications* 2004; **324**: 621–626.
33. Bifulco M, Laezza C, Portella G et al. Control by the endogenous cannabinoid system of ras oncogene dependent tumor growth. *The FASEB Journal* 2001; **15**: 2745–2747.
34. Portella G, Laezza C, Laccetti P et al. Inhibitory effects of cannabinoid CB1 receptor stimulation on tumor growth and metastatic spreading: actions on signals involved in angiogenesis and metastasis. *The FASEB Journal* 2003; **17**: 1771–1773.
35. Bifulco M, Laezza C, Valenti M et al. A new strategy to block tumor growth by inhibiting endocannabinoid inactivation. *The FASEB Journal* 2004; **18**: 1606–1608.
36. Maccarrone M, Lorenzon T, Bari M et al. Anandamide induces apoptosis in human cells via vanilloid receptors: evidence for a protective role of cannabinoid receptors. *The Journal of Biological Chemistry* 2000; **275**: 31938–31945.
37. McKallip RJ, Lombard C, Fisher M et al. Targeting CB2 cannabinoid receptors as a novel therapy to treat malignant lymphoblastic disease. *Blood* 2002; **100**: 627–634.
38. Flygare J, Gustafsson K, Kimby E et al. Cannabinoid receptor ligands mediate growth inhibition and cell death in mantle cell lymphoma. *FEBS Letters* 2005; **579**: 6885–6889.
39. Gustafsson K, Christensson B, Sander B et al. Cannabinoid receptor-mediated apoptosis induced by R(+)-methanandamide and Win55, 212-2 is associated with ceramide accumulation and p38 activation in mantle cell lymphoma. *Molecular Pharmacology* 2006; **70**: 1612–1620.
40. Herrera B, Carracedo A, Diez-Zaera M et al. p38 MAPK is involved in CB2 receptor-induced apoptosis of human leukaemia cells. *FEBS Letters* 2005; **579**: 5084–5088.
41. Ligresti A, Bisogno T, Matias I et al. Possible endocannabinoid control of colorectal cancer growth. *Gastroenterology* 2003; **125**: 677–687.
42. Patsos HA, Hicks DJ, Dobson RR et al. The endogenous cannabinoid, anandamide, induces cell death in colorectal carcinoma cells: a possible role for cyclooxygenase 2. *Gut* 2005; **54**: 1741–1750.

43. Izzo AA, Aviello G, Petrosino S et al. Increased endocannabinoid levels reduce the development of precancerous lesions in the mouse colon. *Journal of Molecular Medicine* 2008; **86**: 89–98.
44. Wang D, Wang H, Ning W et al. Loss of cannabinoid receptor 1 accelerates intestinal tumor growth. *Cancer Research* 2008; **68**: 6468–6476.
45. Casanova ML, Blazquez C, Martinez-Palacio J et al. Inhibition of skin tumor growth and angiogenesis in vivo by activation of cannabinoid receptors. *The Journal of Clinical Investigation* 2003; **111**: 43–50.
46. Munson AE, Harris LS, Friedman MA et al. Antineoplastic activity of cannabinoids. *Journal of the National Cancer Institute* 1975; **55**: 597–602.
47. Hart S, Fischer OM & Ullrich A. Cannabinoids induce cancer cell proliferation via tumor necrosis factor alpha-converting enzyme (TACE/ADAM17)-mediated transactivation of the epidermal growth factor receptor. *Cancer Research* 2004; **64**: 1943–1950.
48. Carracedo A, Gironella M, Lorente M et al. Cannabinoids induce apoptosis of pancreatic tumor cells via endoplasmic reticulum stress-related genes. *Cancer Research* 2006; **66**: 6748–6755.
49. Blazquez C, Casanova ML, Planas A et al. Inhibition of tumor angiogenesis by cannabinoids. *The FASEB Journal* 2003; **17**: 529–531.
50. Pisanti S, Borselli C, Oliviero O et al. Antiangiogenic activity of the endocannabinoid anandamide: correlation to its tumor-suppressor efficacy. *Journal of Cellular Physiology* 2007; **211**: 495–503.
51. Ramer R & Hinz B. Inhibition of cancer cell invasion by cannabinoids via increased expression of tissue inhibitor of matrix metalloproteinases-1. *Journal of the National Cancer Institute* 2008; **100**: 59–69.
52. Klein TW, Newton C, Larsen K et al. The cannabinoid system and immune modulation. *Journal of Leukocyte Biology* 2003; **74**: 486–496.
53. Malfitano AM, Matarese G, Pisanti S et al. Arvanil inhibits T lymphocyte activation and ameliorates autoimmune encephalomyelitis. *Journal of Neuroimmunology* 2006; **171**: 110–119.
54. Pockaj BA, Basu GD, Pathangey LB et al. Reduced T-cell and dendritic cell function is related to cyclooxygenase-2 over-expression and prostaglandin E2 secretion in patients with breast cancer. *Annals of Surgical Oncology* 2004; **11**: 328.
55. Newton CA, Klein TW & Friedman H. Secondary immunity to *Legionella pneumophila* and Th1 activity are suppressed by Δ -9-tetrahydrocannabinol injection. *Infection and Immunity* 1994; **62**: 4015.
56. Vieweg J, Su Z, Dahm P et al. Reversal of tumor-mediated immunosuppression. *Clinical Cancer Research* 2007; **13**: 727s–732s.
57. Zhu Li X, Sharma S, Stolidi M et al. Δ -9-Tetrahydrocannabinol inhibits antitumor immunity by a CB2 receptor-mediated, cytokine-dependent pathway. *The Journal of Immunology* 2000; **165**: 373–380.
58. Yuan M, Kiertscher SM, Cheng Q et al. Δ 9-Tetrahydrocannabinol regulates Th1/Th2 cytokine balance in activated human T cells. *Journal of Neuroimmunology* 2002; **133**: 124–131.
59. Xu X, Liu Y, Huang S et al. Overexpression of cannabinoid receptors CB1 and CB2 correlates with improved prognosis of patients with hepatocellular carcinoma. *Cancer Genetics and Cytogenetics* 2006; **171**: 31–38.
60. Islam TC, Asplund AC, Lindvall JM et al. High level of cannabinoid receptor 1, absence of regulator of G protein signalling 13 and differential expression of Cyclin D1 in mantle cell lymphoma. *Leukemia* 2003; **17**: 1880–1890.
61. Calatozzolo C, Salmaggi A, Pollo B et al. Expression of cannabinoid receptors and neurotrophins in human gliomas. *Neurological Sciences* 2007; **28**: 304–310.
- *62. Balkwill F & Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539–545.
63. Philip M, Rowley DA & Schreiber H. Inflammation as a tumor promoter in cancer induction. *Seminars in Cancer Biology* 2004; **14**: 433–439.
64. Coussens LM & Werb Z. Inflammation and cancer. *Nature* 2002; **420**: 860–867.
65. Yang CR, Hsieh SL, Ho FM et al. Decoy receptor 3 increases monocyte adhesion to endothelial cells via NF- κ B-dependent up-regulation of intercellular adhesion molecule-1, VCAM-1, and IL-8 expression. *Journal of Immunology* 2005; **174**: 1647–1656.
66. Molina-Holgado F, Molina-Holgado E & Guaza C. The endogenous cannabinoid anandamide potentiates interleukin-6 production by astrocytes infected with Theiler's murine encephalomyelitis virus by a receptor-mediated pathway. *FEBS Letters* 1998; **433**(1–2): 139–242.
67. Mazzari S, Canella R, Petrelli L et al. N-(2-hydroxyethyl)hexadecanamide is orally active in reducing edema formation and inflammatory hyperalgesia by down-modulating mast cell activation. *European Journal of Pharmacology* 1996; **300**(3): 227–236.
68. Richardson JD, Aanonsen L & Hargreaves KM. Antihyperalgesic effects of spinal cannabinoids. *European Journal of Pharmacology* 1998; **345**(2): 145–153.
69. Michalski CW, Laukert T, Sauliunaite D et al. Cannabinoids ameliorate pain and reduce disease pathology in cerulein-induced acute pancreatitis. *Gastroenterology* 2007; **132**(5): 1968–1978.
70. Massa F, Marsicano G, Hermann H et al. The endogenous cannabinoid system protects against colonic inflammation. *The Journal of Clinical Investigation* 2004; **113**(8): 1202–1209.
71. Joseph J, Niggemann B, Zaenker KS et al. Anandamide is an endogenous inhibitor for the migration of tumor cells and T lymphocytes. *Cancer Immunology, Immunotherapy* 2004; **53**(8): 723–728.
72. Zheng D, Bode AM, Zhao Q et al. The cannabinoid receptors are required for ultraviolet-induced inflammation and skin cancer development. *Cancer Research* 2008; **68**(10): 3992–3998.
- *73. Guzman M, Duarte MJ, Blazquez C et al. A pilot clinical study of D9-tetrahydrocannabinol in patients with recurrent glioblastoma multiforme. *British Journal of Cancer* 2006; **95**: 197–203.
- *74. Bifulco M, Grimaldi C, Gazerro P et al. Rimonabant: just an antiobesity drug? Current evidence on its pleiotropic effects. *Molecular Pharmacology* 2007; **71**: 1445–1456.
75. Bouaboula M, Perrachon S, Milligan L 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. *The Journal of Biological Chemistry* 1997; **272**: 22330–22339.
76. Gary-Bobo M, Elachouri G, Scatton B et al. The cannabinoid CB1 receptor antagonist rimonabant (SR141716) inhibits cell proliferation and increases markers of adipocyte maturation in cultured mouse 3T3 F442A preadipocytes. *Molecular Pharmacology* 2006; **69**: 471–478.

77. Teixeira-Clerc F, Julien B, Grenard P et al. CB1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis. *Nature Medicine* 2006; **12**: 671–676.
- *78. Walsh D, Nelson KA & Mahmoud FA. Established and potential therapeutic applications of cannabinoids in oncology. *Support Care in Cancer* 2003; **11**: 137–143.
79. Andrews PL, Naylor RJ & Joss RA. Neuropharmacology of emesis and its relevance to anti-emetic therapy. Consensus and controversies. *Support Care in Cancer* 1998; **6**: 197–203.
80. Cichewicz DL. Synergistic interactions between cannabinoid and opioid analgesics. *Life Sciences* 2004; **74**: 1317–1324.
81. Carlo Di & Izzo AA. Cannabinoids for gastrointestinal diseases: potential therapeutic applications. *Expert Opinion on Investigational Drugs* 2003; **12**: 39–49.
82. Maida V, Ennis M, Irani S et al. Adjunctive nabilone in cancer pain and symptom management: a prospective observational study using propensity scoring. *The Journal of Supportive Oncology* 2008; **6**: 119–124.
83. Walsh D, Kirkova J & Davis MP. The efficacy and tolerability of long-term use of dronabinol in cancer-related anorexia: a case series. *Journal of Pain and Symptom Management* 2005; **30**: 493–495.
84. Meiri E, Jhangiani H, Vredenburg JJ et al. Efficacy of dronabinol alone and in combination with ondansetron versus ondansetron alone for delayed chemotherapy-induced nausea and vomiting. *Current Medical Research and Opinion* 2007; **23**: 533–543.
85. Maida V. The synthetic cannabinoid Nabilone improves pain and symptom management in cancer patients. SABCS, 2006, abstract 3145.
86. Zutt M, Hänssle H, Emmert S et al. Dronabinol for supportive therapy in patients with malignant melanoma and liver metastases. *Der Hautarzt* 2006; **57**: 423–427.
87. Strasser F, Luftner D, Possinger K et al. Comparison of orally administered cannabis extract and delta-9-tetrahydrocannabinol in treating patients with cancer-related anorexia-cachexia syndrome: a multicenter, phase III, randomized, double-blind, placebo-controlled clinical trial from the Cannabis-In-Cachexia-Study-Group. *Journal of Clinical Oncology* 2006; **24**: 3394–3400.
- *88. Sutton IR & Daeninck P. Cannabinoids in the management of intractable chemotherapy-induced nausea and vomiting and cancer-related pain. *The Journal of Supportive Oncology* 2006; **4**: 531–535.
89. Jatoi A, Windschitl HE, Loprinzi CL et al. Dronabinol versus megestrol acetate versus combination therapy for cancer-associated anorexia: a North Central Cancer Treatment Group study. *Journal of Clinical Oncology* 2002; **20**: 567–573.
90. Abrahamov A, Abrahamov A & Mechoulam R. An efficient new cannabinoid antiemetic in pediatric oncology. *Life Sciences* 1995; **56**: 2097–2102.
91. Nelson K, Walsh D, Deeter P et al. A phase II study of delta-9-tetrahydrocannabinol for appetite stimulation in cancer-associated anorexia. *Journal of Palliative Care* 1994; **10**: 14–18.
92. Lane M, Vogel CL, Ferguson J et al. Dronabinol and prochlorperazine in combination for treatment of cancer chemotherapy-induced nausea and vomiting. *Journal of Pain and Symptom Management* 1991; **6**: 352–359.
93. Cherny NI. The pharmacologic management of cancer pain. *Oncology* 2004; **18**: 1499–1515.
94. Walker JM & Huang SM. Cannabinoid analgesia. *Pharmacology & Therapeutics* 2002; **95**: 127–135.
95. Potenzieri C, Harding-Rose C & Simone DA. The cannabinoid receptor agonist, WIN 55, 212-2, attenuates tumor-evoked hyperalgesia through peripheral mechanisms. *Brain Research* 2008; **1215**: 69–75.
96. Pertwee RG. Cannabinoid receptors and pain. *Progress in Neurobiology* 2001; **63**: 569–611.
97. Cota D. Role of the endocannabinoid system in energy balance regulation and obesity. *Frontiers of Hormone Research* 2008; **36**: 135–145.
98. Di Marzo V, Goparaju SK, Wang L et al. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 2002; **410**: 822–825.
99. Wilson MM, Philpot C & Morley JE. Anorexia of aging in long term care: is dronabinol an effective appetite stimulant?—a pilot study. *The Journal of Nutrition, Health & Aging* 2007; **11**: 195–198.