



Cannabidiol and Other Phytocannabinoids as Cancer Therapeutics

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Abstract

Preclinical models provided ample evidence that cannabinoids are cytotoxic against cancer cells. Among the best studied phytocannabinoids, cannabidiol (CBD) is most promising for the treatment of cancer as it lacks the psychotomimetic properties of delta-9-tetrahydrocannabinol (THC). In vitro studies and animal experiments point to a concentration- (dose-) dependent anticancer effect. The effectiveness of pure compounds versus extracts is the subject of an ongoing debate. Actual results demonstrate that CBD-rich hemp extracts must be distinguished from THC-rich cannabis preparations. Whereas pure CBD was superior to CBD-rich extracts in most in vitro experiments, the opposite was observed for pure THC and THC-rich extracts, although exceptions were noted. The cytotoxic effects of CBD, THC and extracts seem to depend not only on the nature of cannabinoids and the presence of other phytochemicals but also largely on the nature of cell lines and test conditions. Neither CBD nor THC are universally efficacious in reducing cancer cell viability. The combination of pure cannabinoids may have advantages over single agents, although the optimal ratio seems to depend on the nature of cancer cells; the existence of a ‘one size fits all’ ratio is very unlikely. As cannabinoids interfere with the endocannabinoid system (ECS), a better understanding of the circadian rhythmicity of the ECS, particularly endocannabinoids and receptors, as well as of the rhythmicity of biological processes related to the growth of cancer cells, could enhance the efficacy of a therapy with cannabinoids by optimization of the timing of the administration, as has already been reported for some of the canonical chemotherapeutics. Theoretically, a CBD dose administered at noon could increase the peak of anandamide and therefore the effects triggered by this agent. Despite the abundance of preclinical articles published over the last 2 decades, well-designed controlled clinical trials on CBD in cancer are still missing. The number of observations in cancer patients, paired with the anticancer activity repeatedly reported in preclinical in vitro and in vivo studies warrants serious scientific exploration moving forward.

Key Points

Neither the non-psychotomimetic cannabidiol (CBD) nor the psychotomimetic delta-9-tetrahydrocannabinol (THC) are universally efficacious in reducing cancer cell viability.

In vitro, pure CBD is very often equally or more efficacious than CBD extracts, whereas pure THC is frequently less efficacious than THC-rich extracts.

Although cannabinoids have been shown to slow down tumour growth and/or extend survival in animals, similar observations in man are currently only supported by two pilot studies and a limited number of case reports.

1 Introduction

Cannabis has been used since ancient times as food, for its fibres and hallucinogenic properties, and as medicine. The high estimation of its use in the past is reflected by the discovery of plant remains, identified by both morphological and anatomical features as cannabis, in numerous, approximately 2800- to 2400-year-old tombs [1]. One of these tombs, radiometrically dated to the late 5th century BC and known as the tomb of the ‘Ukok princess’ or ‘Siberian Ice Maiden’, is of particular interest as this young woman died of metastasizing breast cancer [2]. It is presumed that cannabis found in her tomb was aimed at coping with the symptoms of her illness.

One of the first experimental studies in modern times that demonstrated anticancer activities of cannabinoids in vitro and in vivo, and which was supported by a grant from the National Institute on Drug Abuse, was published in 1975 [3]. Surprisingly, cannabinoids exhibited antitumour effects. Details will be discussed later in this review. However,

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despite encouraging observations, this study had no further consequences for almost 30 years, likely due to legal restrictions affecting the research on cannabis, in particular research with the psychotomimetic cannabinoid delta-9-tetrahydrocannabinol (THC). It was the discovery of the endocannabinoid system (ECS) at the end of last century that renewed and boosted scientific interest in cannabis and cannabinoids. The pioneering work of Massi et al. [4] on the effects of cannabidiol (CBD) on glioma cells, as well as the work of Ligresti et al. [5] and McAllister et al. [6], for example, on aggressive breast cancer cells stimulated the research on the antitumoral effects of cannabinoids. A few years later, public interest was fuelled by the case of Canadian Rick Simpson, who published his personal experiences with a self-made, THC-rich cannabis extract that he applied topically to a basal cell carcinoma that then completely disappeared. A similar testimony exists from a well-known physician and cannabis expert [7].

In contrast to synthetic cannabinoids, cannabinoids isolated from plants are 'products of nature' and are not patentable, which limits investment opportunities. Over the last 20 years, a large number of studies have demonstrated the antiproliferative properties of cannabinoids *in vitro* and *in vivo* against a wide range of tumour cells, such as breast, cervix, ovary, endometrial, colon, gastric, liver, pancreatic, glioma, leukaemia, prostate, bladder, skin and thyroid cancer cells.

In this narrative review, studies that primarily compared *in vitro* CBD head-to-head with THC are summarized, followed by *in vivo* studies of CBD against various types of cancer. The focus is on pure CBD as this is the second most documented phytocannabinoid after THC and the main non-psychotomimetic cannabinoid. Furthermore, CBD is freely available on prescription in a number of countries, such as the US, Europe, Australia and New Zealand. Phyto-CBD received marketing authorization for the treatment of rare forms of epilepsy in the US (June 2018) and Europe (September 2019); a European Union (EU) monograph on CBD is in preparation.

In case other substances were also included in the studies reviewed, comparisons are restricted to results with natural cannabinoids. In a pragmatic approach, the tumour inhibitory properties of CBD, as measured by the reduction in tumour cell viability *in vitro*, and tumour size *in vivo* or extension of survival time of animals, are reviewed as they are the most relevant endpoints for therapy. Finally, clinical publications describing the anticancer effects of CBD, THC and cannabis extracts in patients are summarized.

2 Literature Search Methodology

We conducted a search of the PubMed, Google Scholar, ResearchGate and medRxiv databases from 1975 through 1 July 2021 for relevant studies. The literature search was

performed using the following keywords and their combinations: 'cannabidiol', 'CBD', 'delta-9-tetrahydrocannabinol', 'THC', 'nabiximols', 'botanical drug substance', 'combinational therapy', 'cancer', 'xenograft', 'entourage effect', 'anticancer effect', 'cannabis extract' and similar terms ('botanical drug substance', 'cannabis drug preparation', 'CBD-oil'). To maximize the article search, 'citation chasing' was performed to identify *in vitro* and *in vivo* studies, clinical trials and case reports that evaluated CBD and that had been included in the reference list of relevant publications. There were no language restrictions. Additional searches included the Cochrane Library and clinical trial databases (ClinicalTrials.gov [32 trials] and ClinicalTrialsRegister.eu [9 trials]) but no additional results were found. For *in vitro* and *in vivo* experiments, only those that compared CBD or THC with other phytocannabinoids were included. Comparisons with synthetic cannabinoids or other substances have been excluded, as well as studies with formulations that are currently not available on the market (e.g., nanoparticles). A case regarding an ependymoma patient was supplemented with details provided on the internet by the patient's father, and a further case report on a lung cancer patient published during the review process was included.

Following the screening of titles and abstracts and the exclusion of duplicates, the final evaluation covered 27 articles describing the effects *in vitro*, 25 articles describing the effects *in vivo*, and 16 articles describing the anticancer effects of CBD and other phytocannabinoid preparations in patients with malignant diseases.

3 Antitumour Potency of Phytocannabinoids

Although being less 'translatable' to human therapy than animal studies, *in vitro* studies are commonly accepted as valuable first indicators for a possible effect also *in vivo*. A number of *in vitro* experiments including various cannabinoids have been conducted in the past, not only to investigate their toxic effects on cancer cell lines but also in order to gain insight into the mechanisms of action. Twenty-three *in vitro* studies have been identified that investigated the anticancer potency of CBD in comparison with other cannabinoids (mainly THC, but occasionally cannabiol [CBN], cannabigerol [CBG], cannabichromene [CBC], cannabidivarin [CBDV] and their extracts [CBD-E, THC-E]). Three other studies comparing THC and CBG with extracts are also included. In order to avoid confusion, the term extract (E) is used interchangeably with similar terms such as 'botanical drug substance' (BDS; CBD-BDS or THC-BDS), 'CBD-oil', 'CBD-tincture', 'CBD full spectrum extract' or 'cannabis drug preparation' (CDP). Overall, comparisons covered 86 cancer cell

lines, the large majority of which were of human origin. Some had been included in more than one study, allowing a comparison of results.

3.1 In Vitro Antitumour Potency of Pure, Isolated Phytocannabinoids

Even when differences were only minor in some studies, pure CBD was, in general, the most potent single compound, with lower half maximal inhibitory concentrations (IC_{50}) than THC in 54 of 62 tests (87%), being about equal in five (8%) and lower than THC in only three lung cancer cell lines [8]. Similar IC_{50} values of CBD were also slightly lower than those of CBG, CBN, CBC or CBDV; however, judgements are limited by the low number of experiments (Table 1).

In vitro anticancer effects of single cannabinoids such as CBD, THC, CBG, CBN or CBDV were commonly observed with concentrations in micromolar ranges, although they were influenced by the nature of cancer cells and test conditions [9–13]. Respective concentrations are in the order of micrograms/millilitre ($\mu\text{g}/\text{mL}$), and are therefore about 1000 times higher than blood levels achieved by oral doses. Similar concentrations were also inhibitory for targets such as cytochrome P450 (CYP) enzymes (e.g., CYP2C9, $IC_{50} = 2.16 \mu\text{M}$; CYP2C19, $IC_{50} = 2.51 \mu\text{M}$; CYP3A4, $IC_{50} = 11.7 \mu\text{M}$). Despite normal therapeutic blood levels being in the order of nanograms/millilitre (ng/mL), it is well known that CBD can increase the plasma levels of drugs such as warfarin S (metabolized by CYP2C9), warfarin R (metabolized by CYP3A4) or N-desmethyloclobazam (metabolized by CYP2C19) when administered concomitantly, by inhibition of their metabolism [14]. This demonstrates that effective blood concentrations in man are much lower than inhibitory concentrations observed in vitro.

The highest number of in vitro studies have been performed on tumour cells of the nervous system, followed by tests on breast cancer cell lines. IC_{50} values of pure CBD varied widely from about $0.6 \mu\text{M}$ to more than $22 \mu\text{M}$ for tumours of the nervous system, and were more effective than CBD-E, THC or CBG [15–20]. In ependymoma cell lines, CBD and THC were similarly effective, whereas CBD was more potent in medulloblastoma [20]. THC was less effective than THC-E [18] but more effective than CBG [16]. Inhibitory concentrations of pure CBD were also in a similar range for breast cancer cells, the next most frequently tested tumours ($1.2\text{--}25.8 \mu\text{M}$). Details are described in Table 1. Again, CBD was at least as potent or superior to other cannabinoids. The low number of comparative experiments must however be taken into account.

An ongoing problem in oncology is cancer stem cells perpetuating themselves via autorestitution, which is the main reason for treatment resistance and cancer recurrence [21, 22]. Intriguingly, CBD was the most potent cannabinoid in reducing the viability of glioma stem cells in vitro (order of potency: CBD > THC > CBG; mean $19.5\text{--}22.8\text{--}59.0 \mu\text{M}$) [16], and also demonstrated its potency in vivo [23, 24], although more studies on an eventually developing tumour resistance are necessary. CBD reduced both adherent lung cancer cells and lung cancer stem cells in vitro (CBD concentration $\geq 10 \mu\text{M}$) [10, 16]. Unsurprisingly, stem cells seem to be less sensitive to cannabinoids than primary cancer cells.

3.2 In Vitro Antitumour Potency of Combinations of Cannabinoids

The effects of a combination of CBD/THC have been investigated in 15 cell lines, with a ratio of CBD:THC varying between $\sim 1:6$ and $\sim 1:1$. The combination was synergistic in 12 but less than additive in 3 other cell lines (MDA-MB-231 and MCF-7 breast cancer cell lines, and U87 glioblastoma cell lines) [17, 25]. The potency of CBD against glioblastoma cell lines was also increased by a combination with CBG, with optimal ratios of 1:4 (CBD:CBG) for glioblastoma cells and 3:1 for glioblastoma stem cells; this effect was additive [16].

Furthermore, an increased anticancer potency was observed with a combination of $1 \mu\text{M}$ CBD + $1 \mu\text{M}$ THC in SK-MEL-28 melanoma cells where the effect on the cell viability was equivalent to about $4 \mu\text{M}$ pure THC [26].

A cocktail of pure cannabinoids, matching the composition in an extract fraction of a THC-rich cannabis chemotype, was also more potent than pure THC but less than the crude extract [27] (Table 1). Other combinations that have been studied were between CBD (THC) and CBG, CBC or CBV [28]. A CBD/CBG combination was also synergistic in acute lymphocytic leukaemia cells (CEM) [29].

It is worth mentioning that combinations of cannabinoids with canonical chemotherapeutics such as doxorubicin (hepatocellular carcinoma), cisplatin, 5-fluorouracil, paclitaxel (head and neck cancer) or temozolomide (glioma cell lines U251, U87MG, LN18, GL261) repeatedly potentiated anticancer effects [30–33].

Overall anticancer effects varied from synergistic to antagonistic, depending on the ratio of concentrations [31, 34]. Similarly, induction of apoptosis was increased after combined treatment of glioblastoma multiforme (GBM) cell lines (U87MG and U118MG) with $20 \mu\text{M}$ CBD and 5 Gy gamma irradiation (almost 90% in U87MG cells and almost 70% in U118MG cells after 72 h) [35].

Table 1 In vitro potency of cannabinoids with emphasis on CBD, THC and extracts

Cancer cell type; exposure duration	Cannabinoid	Comparison	Effect, potency (ranking: more effective, greater than)	Reference
2 human glioma cell lines: U87MG, U373MG; 2 canine glioma cell lines: J3TBG, SDT3G; 96 h exposure	CBD >99.9% (0–20 µg/mL)	CBD-E 96% (0–20 µg/mL)	CBD > CBD-E IC ₅₀ (µg/mL), CBD: 4.85–8.20 CBD-E: 5.15–11.20 In all 4 cell lines, glioma cells appeared to be marginally more sensitive to pure CBD isolate than CBD-E	[15]
3 human glioma cell lines: T98G, U87, U373; 2 GSCs: NCH644, NCH421k, and 5 other human glioblastoma cell lines; 48 h exposure	CBD, total of 10 cell lines; CBD 1.5 plus 5 µM for glioblastoma lines, and 5, 10, and 15 µM for GSCs	THC, CBG, CBD/CBG	CBD > THC (all 10 cell lines and 2 stem cell lines) IC ₅₀ (mean of 10 cell lines): CBD 22.0 µM, CBG 28.1 µM, THC 27.9 µM; IC ₅₀ (GSCs): CBD 20 µM, CBG 59 µM, THC 23 µM; CBD exhibited the lowest IC ₅₀ in all 10 cell lines as well as in 2 stem cell lines; combination is additive, significant with 5 µM CBD; 20 µM CBG (= 1:4); strongest with 15 µM CBD; 5 µM CBG (= 2:1); very low concentrations <10 µM (CBD) and 5 µM (CBG) increased GSC viability	[16]
3 human glioblastoma cell lines: SF126, U251 and U87; 72 h exposure	CBD	THC and THC/CBD (at predicted IC ₈₀ concentrations)	CBD > THC in all 3 cell lines; IC ₅₀ : SF126, CBD (THC) = 1.2 µM (2.5); U251 = 0.6 µM (3.3); U87 = 0.6 µM (3.3) CBD was significantly more potent than THC at inhibiting cancer cell growth; inhibition by combination was more than additive in SF126 and U251, but not in U87 Cell viability of SF126 cells: 90% in the presence of THC (1.6 µmol/L), 63% in CBD (1.1 µmol/L), and 25% in THC (1.6 µmol/L) + CBD (1.1 µmol/L), synergistic Viability of U251 cells: 71% in the presence of THC (1.7 µmol/L), 83% in CBD (0.4 µmol/L), and 7% in THC (1.7 µmol/L) + CBD (0.4 µmol/L), synergistic Both THC and CBD could significantly inhibit the invasiveness of U251 cells (no enhancement by combination); a combination enhanced apoptosis; no synergistic action of the combination in U87 cells	[17]
3 cell lines: human glioma line: T98G; astrocytoma line: U87MG; mouse glioma line: GL261; 24 h exposure	CBD (>96%)	CBD-E (CBD-BDS) [63.5% CBD, 3.6% THC, 5.2% CBG]; THC-BDS (65.4% THC, 0.4% CBD, 1.8% CBG); THC (>96%)	CBD > CBD-E and THC-E > THC in all cell lines; pure CBD was the most effective compound in all 3 cell lines (IC ₅₀ ~ 10–12 µMol/L); dose-dependent reductions of cell numbers; hyperadditive effect of combined CBD/THC (1:1)	[18]

Table 1 (continued)

Cancer cell type; exposure duration	Cannabinoid	Comparison	Effect, potency (ranking: more effective, greater than)	Reference
4 neuroblastoma cell lines: SK-N-SH, NUB-6, IMR-32, LAN-1; 24 h exposure	Synthetic CBD 0–50 µg/mL	Synthetic THC concentration: 0–50 µg/mL	CBD > THC Better response was observed in the SK-N-SH and NUB-6 cell lines (50% reduction in cell viability at 5 µg/mL CBD or THC concentrations) compared with 15 µg/mL for SK-N-SH and NUB-6, and compared with >20 µg/mL for IMR-32 and LAN-1; aneureoblastoma activity was better with CBD than with THC in all cell lines tested; induction of apoptosis was dose-dependent	[19]
3 human medulloblastoma cell lines: D283, D425, PER547 2 ependymoma cell lines: IC-1425EPN and DKFZEPINS; 72 h exposure	CBD, medulloblastoma, ependymoma	THC, medulloblastoma, ependymoma CBD/THC	CBD > THC, medulloblastoma, ED ₅₀ , CBD 3.2–5.5 µM, THC 4.4–8.4 µM CBD ≈ THC, ependymoma, ED ₅₀ 7.5–10.1 µM, THC ED ₅₀ 7.4–11.2 µM Both THC and CBD reduced cell viability in a dose-dependent manner; the combination of CBD/THC demonstrated significantly synergic effects in medulloblastoma and mild synergy in ependymoma cells	[20]
1 human glioma cell line: A172; 24–48 h exposure	THC	THC-rich fraction F5 of a THC-rich Cannabis sativa strain ‘DO’; standard mix (‘F5-SM’) at the appropriate percentages to be as close to F5 as possible	IC ₅₀ (µg/mL): F5-SM (4.61) > THC (4.83) > F5 (7.01); (F5, crude: 91.7% THC, 3.7% CBG, 4.6% CBN) Crude extract with 87.4% THC had the lowest potency (IC ₅₀ 10.17 µg/mL)	[27]
	CBG	CBG-rich fraction ‘F4’; F4-SM at the appropriate percentages of F4	IC ₅₀ (µg/mL): F4-SM (4.38) > CBG (5.0) > F4 (9.81) (F4, crude: 80.3% CBG + 11% THC + 5.8% CBD + 2.8% CBN) In both cases, the reconstituted ‘Standard Mix’ (SM) is slightly more cytotoxic than the respective natural fraction of the extract	[27]
6 human breast adenocarcinoma cell lines: MCF-7 and T47D (ER+, PR+, HER2-); BT474 and HCC1954 (HER2+); MDA-MB-231, SUM 159 (ER-, PR-, HER2-); 24–48 h exposure	THC (synthetic)	THC-E (THC ≈ 55%, THCA ≈ 0.3%, CBG ≈ 0.4%, CBD not tested)	THC < THC-E in all 6 cell lines; IC ₅₀ values, THC, THC-E: T47D, THC = 2.9 µM, E = 2.2 µM; MCF7, THC = 2.8 µM; E = 2.4 µM; HCC1954, THC = 2.7 µM, E = 2.0 µM; BT474, THC = 3.7 µM, E = 2.7 µM; MDA-MB-231 (triple-negative cancer), THC = 1.9 µM, E = 1.6 µM; SUM 159 (triple-negative cancer), THC = 2.8 µM, E = 2.1 µM; a terpene cocktail had no effect, also not with additional THC; MDA-MB-231 was not affected in combination with cisplatin	[40]

Table 1 (continued)

Cancer cell type; exposure duration	Cannabinoid	Comparison	Effect, potency (ranking: more effective, greater than)	Reference
2 human breast cancer cell lines: MDA-MB436, MDA-MB231; 72 h exposure	CBD	THC, CBN, CBG	MDA-MB436: CBD > CBG > THC > CBN, IC ₅₀ (μM): 1.6 – 2.1 – 2.5 – 2.6, respectively MDA-MB231: THC = CBN ≈ CBD > CBG, IC ₅₀ (μM): 1.2 – 1.2 – 1.3 – 2.3, respectively CBD was the most effective inhibitor of breast cancer cell proliferation and invasion CBD > THC; IC ₅₀ (μM): MDA-MB-231: CBD 1.9, THC 3.0; 4T1: CBD 1.8, THC 2.3	[6]
2 cell lines: 1 human breast cancer cell line: MDA-MB-231; 1 mouse breast cancer cell line: 4T1; 24–78 h exposure	CBD	THC (O-1663)		[61]
2 human breast cancer cell lines: MDA-MB-231, MCF-7; 48 h exposure	CBD	THC, CBN, CBG and combinations; cannabinoids were combined at equipotent ratios at double their respective IC ₅₀ ; IC ₅₀ concentrations	CBD > CBN > CBG > THC (both cell lines) IC ₅₀ (μM), MDA-MB-231: CBD (13.82), CBN (23.22), CBG (28.40), THC (30.13), IC ₅₀ (μM), MCF-7: CBD (20.62), CBN (28.19), CBG (31.45), THC (40.14), Lowest IC ₅₀ of combinations (μM): MDA-MB-231, CBD+CBN (31.62), MCF-7, CBD/THC (20.23 – synergistic); the majority of the combinations were antagonistic/less potent	[25]
2 human cell lines: 1 breast cancer cell line: MDA-MB-231; 1 colorectal cancer cell line: HCT-116; 48 h exposure	CBD	THC (and terpenoid fractions of a CBD-rich and THC-rich cultivar)	CBD ≈ THC IC ₅₀ (μM): MDA-MB-231, CBD: 17.65; THC: 15.18; CBD > THC; HCT-116, CBD: 20.64; THC: 29.75 Combination with the related terpenoid fraction increased the potency, depending on the ratio CBD > CBDV > CBC (order of reduction of cell viability at 10 μM concentration)	[44]
1 human colon adenocarcinoma cell line: Caco-2I; 24 h exposure	CBD	CBDV, CBC		[28]
1 human colorectal carcinoma cell line: HT-29; 24 h exposure	CBD	THC	CBD = THC Cytotoxicity (using MTT assay) on HT-29 cells was similar IC ₅₀ (μM) = 30.0 for both Oxidative damage was only induced by CBD	[121]

Table 1 (continued)

Cancer cell type; exposure duration	Cannabinoid	Comparison	Effect, potency (ranking: more effective, greater than)	Reference
4 prostatic carcinoma cell lines: 2 androgen-independent/castration resistant cell lines: DU-145, PC-3; 2 androgen-sensitive/responsive, low invasiveness cell lines: 22RV1, LNCaP; 2 different seeding techniques A and B; 72 h exposure	CBD ≥95% pure Technique A: IC ₅₀ DU-145, LNCaP = 25.3 and 25.0 μM respectively Technique B: IC ₅₀ DU-145, LNCaP = 5.4 and 5.7 μM, respectively	CBD-BDS (~65% CBD) Technique A: IC ₅₀ DU-145, LNCaP = 9.0 and 18.1 μM, respectively Technique B: IC ₅₀ DU-145, LNCaP = 7.8 and 6.6 μM, respectively THC ≥95% pure Technique A: IC ₅₀ DU-145, LNCaP = >25 and 16.9 μM, respectively Technique B: IC ₅₀ DU-145, LNCaP = 11.7 and 5.5 μM, respectively	CBD > or ≈ THC in DU-145, 22RV1 and PC-3 cells, both seeding techniques CBD < or ≈ THC in LNCaP, seeding technique A, PC-3 cells: IC ₅₀ CBD 17.4 μM, THC 25.0 μM; seeding technique B, PC-3 cells: IC ₅₀ CBD 5.2 μM, THC 9.9 μM; seeding technique A, 22RV1 cells: IC ₅₀ CBD 23.1 μM, THC >25 μM; seeding technique B, 22RV1 cells: IC ₅₀ CBD 8.4 μM, THC 15.3 μM; CBD < CBD-BDS (DU-145, LNCaP only); for seeding technique A CBD > CBD-BDS (DU-145, LNCaP only) for seeding technique B (test conditions influence the results); CBD, CBD-BDS were more effective than THC; CBDA and THCA were less active compared with their decarboxylated forms in all 4 cell lines, DU-145, LNCaP, PC-3, 22RV1 (technique A). With technique B only, THCA was more potent than THC in 22RV1	[9]
3 cell lines; 2 endometrial cancer cell lines: Ishikawa, estrogen-dependent; Hec50co, not estrogen-dependent; non-tumoral cell line: HFF-1; 24–78 h exposure	CBD, up to 25 μM	THC, up to 25 μM, endocannabinoids AEA and 2-AG, significant effect above 1 μM (up to 25 μM)	CBD > THC CBD (25 μM, 48 h) decreased cell viability of Ishikawa and Hec50co cell lines in a concentration-dependent manner; THC did not affect Ishikawa or Hec50co cells even after 72 h at a concentration of 25 μM HFF-1 was not affected by CBD or THC AEA and 2-AG have significant cytotoxic effects (LDH-release: AEA > 2-AG > CBD > THC); effects higher on Ishikawa and Hec50co cells, no effect on HFF-1 cells	[122]
3 cervical cancer cell lines: HeLa, metastatic ME-180, SiHa; 24 h exposure	CBD 0, 50, 100, and 150 μg/mL	Full spectrum cannabis butanolic- or hexane-extract Content of cannabinoids not specified; 0, 50, 100, and 150 μg E/mL (w/v)	CBD > CBD-E (all 3 cell lines) Extract IC ₅₀ , ME-180: viability reduced to 48.6% (at 100 μg/mL, butanolic extract), to 54% (at 50 μg/mL hexane extract) CBD IC ₅₀ , ME-180: viability reduced to 56% at 1.5 μg/mL, SiHa and HeLa reduced to 51% and 50%, respectively, at 3.2 μg/mL (effect concentration-dependent)	[39]

Table 1 (continued)

Cancer cell type; exposure duration	Cannabinoid	Comparison	Effect, potency (ranking: more effective, greater than)	Reference
3 NSCLC cell lines: A549, H469, H1792; 48 h exposure	CBD 10–100 μ M	THC, CBD+THC (1:1)	CBD < THC (all 3 cell lines) A549 cells, IC ₅₀ : THC: 27.25, CBD: 37.31 μ M; CBD+THC: 12.94 μ M; H469 cells, IC ₅₀ : THC: 30.64, CBD: 39.78, CBD+THC: 8.04 μ M; H1792 cells IC ₅₀ : THC: 33.39, CBD: 46.41, CBD+THC: 14.55 μ M Combination is significantly more potent	[8]
8 cell lines: 2 human breast cancer cell lines: MCF-7, MDAMB-231; 1 prostatic cancer cell line: DU-145; 1 colorectal cancer cell line: CaCo-2; 1 gastric adenocarcinoma cell line; AGS; 1 rat glioma cell line: C6; 1 rat thyroid cell line: KiMol; 1 rat basophilic leukaemia cell line: RBL-2H3; 96 h exposure	CBD	CBDA, THCA, THC, CBG, CBC, CBD-E (64.6% CBD, Nabidiotex [®]), THC-E (72.6% THC, Tetrabinex [®])	CBD > CBDA (more potent in all 8 cell lines) CBD > or \approx CBD-E (more potent or equal in 6 cell lines, less potent in C6, MCF-7) THC > THCA (only in CaCo-2, AGS); THC > THC-E (only in MCF-7, AGS, 14.2 vs. 21.0 and 19.3 vs. 22.0); both THC and THC-E were ineffective against prostatic cancer DU-145 cells CBD > THC in all 8 cell lines CBG and CBC were in no case more potent than CBD but more or equally potent as THC; pure THC was generally less potent or equally potent as THCA or THC-E. Overall, CBD was the most potent cannabinoid (IC ₅₀ \sim 6 to 20 μ M) except in MCF-7 and C6 cell lines	[5]
6 human cancer cell lines: 2 colorectal cancer cell lines: SW480, HCT116; 2 melanoma cell lines: 1205Lu, A375M; 2 glioblastoma cell lines: T98G, U87MG; 48 h exposure	Pure CBD, 10 μ M	3 CBD oils (A, B, C), normalized to 10 μ M CBD, with a content of CBD between \sim 32 and \sim 54 mg/mL (THC <0.3% in all oils)	CBD > CBD-E (in all 6 cell lines) IC ₅₀ values of pure CBD were consistently lower than values of the most potent CBD oil A in all 6 cell lines (between 5.8 and 17.8 μ M compared with 18 and 36.8 μ M for oil A). Oil C appeared to protect cancer cells from the toxic effects of CBD	[11]
4 human cancer cell lines: 1 alveolar basal epithelial adenocarcinoma cell line: A549; 1 epithelial colorectal adenocarcinoma cell line: Caco-2; 1 hepatoblastoma cell line: Hep G2; 1 epithelial breast adenocarcinoma cell line: MDA-MB-231; 72 h exposure	CBD	CBDV	CBD > CBDV in all carcinoma cell lines IC ₅₀ (μ M), CBD (CBDV): A54: 27.66 (32.91) Caco-2: 35.24 (46.02) Hep G2: 15.80 (19.74) MDA-MB-231: 25.84 (23.61)	[12]
7 human colorectal cancer cell lines: SW480, SW620, HT-29, DLD-1, HCT116, LS174, RKO; 48 h exposure	CBD	THC	CBD > THC IC ₅₀ (μ M), CBD: SW480 cells = 16.4; HT29 = 23.0; DLD-1 = 19.8 THC had no effect; synthetic cannabinoids had up to \sim threefold lower IC ₅₀ values than CBD; CBD was not effective against all cell lines	[38]

Table 1 (continued)

Cancer cell type; exposure duration	Cannabinoid	Comparison	Effect, potency (ranking: more effective, greater than)	Reference
3 cell lines: 2 human epithelial colon adenocarcinoma cells: HCT116 and DLD-1 compared with 1 healthy colonic epithelial cell line: HCEC; 24 h exposure	CBD (0.3–5 μM)	CBD-BDS (0.3–5 μM) [65.9% CBD, 2.4% THC, 1.0% CBG, 0.9% CBDV], normalized to CBD	CBD ≈ CBD-BDS CBD-BDS and CBD (0.3–5 μM) reduced cell proliferation in tumoral but not in healthy cells; CBD and CBD-BDS were almost equally active (reduction of proliferation to ~80% with 5 μM CBD, vs. ~85–77% with 5 μM CBD-BDS) with minimal differences between cell lines)	[59]
6 human pancreatic cancer cell lines: PANC1, CFPAC1, Capan2, SW1990, HPAF11, Mia-PaCa2; 48 h exposure	CBD	THC	CBD > THC IC ₅₀ CBD: ~2.5 μM, THC: ~5 μM; CBD and THC suppressed the proliferation of these 6 cell lines dose-dependently. CBD showed a more potent inhibitory effect than THC and achieved a reduction in proliferation to <40% of the control at 5 μM, and 10 μM for THC	[123]
2 human bladder transitional cell carcinoma lines: T24, TCCSUP; 48 h exposure	CBD	THC	CBD > THC IC ₅₀ T24 cells: CBD 5.5 μM, THC 8.5 μM IC ₅₀ TCCSUP cells: CBD 10.5 μM, THC 13.5 μM	[34]
2 human cancer cell lines: 1 acute lymphocytic leukaemia cell line: CEM; 1 promyelocytic leukaemia cell line: HL60; 48 h exposure	CBD	CBDA, CBG, CBGA, CBGV, CBGVA	CBD > CBDA The neutral form was always more active than its respective acid counterparts. The two cannabinoids with the greatest activities were CBD and CBG, with IC ₅₀ at 48 h of about 7 and 10 μM, respectively (IC ₅₀ of other cannabinoids were not given in the article)	[42]
2 human cancer cell lines: 1 acute lymphocytic leukaemia cell line: CEM; 1 promyelocytic leukaemia cell line: HL60; 72 h exposure	CBD	THC, CBG, CBD/THC, CBD/CBG, CBG/THC; cytarabine, vincristine	CEM: CBD/CBG > CBD/THC > CBD > CBG/THC > THC; IC ₅₀ CEM: CBD 7.8 μM, THC 13 μM, CBD/CBG 2.8 μM, CBD/THC 3.6 μM, Single CBD was more active than THC (single CBG was not tested); IC ₅₀ was higher (>10 μM) and the differences were less pronounced for HL60 cells (numerical values were not given in the article)	[29]

2-AG 2-arachidonoyl-glycerol, AEA anandamide, CBC cannabichromene, CBD cannabidiol, CBDA cannabidiolic acid, CBD-BDS cannabidiol botanical drug substance, CBDV cannabidivarin, CBG cannabigerol, CBGA cannabigerolic acid, CBGV cannabigerolic acid, CBGVA cannabigerolic acid, CBN cannabinol, E extract, ED₅₀ median effective dose, ER estrogen receptor, GSCs glioma stem cells, HER2 human epidermal growth factor receptor 2, IC₅₀ half-maximal inhibitory concentration, IC₈₀ 80% inhibitory concentration, LDH lactate dehydrogenase, NSCLC non-small cell lung cancer, PR progesterone receptor, THC tetrahydrocannabinol, THC-BDS tetrahydrocannabinol botanical drug substance, THCA tetrahydrocannabinolic acid, THCV tetrahydrocannabivarin

The sequence of administration of cannabinoids and chemotherapeutics may also play a role; a greater induction of apoptosis was observed in experiments with human leukaemia cell lines (CEM and HL60) when cells were first exposed to chemotherapeutics (cytarabine, vincristine), followed by exposure to cannabinoids then vice versa, irrespective of the cell line and agent [29]. Similar observations were made with cisplatin, 5-fluorouracil and paclitaxel [31]. Interestingly, prior exposure to gamma irradiation followed by pure CBD also enhanced apoptosis in HL60 cell lines [36].

3.3 In Vitro, Cannabidiol (CBD) was Often More Potent than CBD-Rich Extracts, Whereas the Opposite was Usually Observed for Delta-9-Tetrahydrocannabinol (THC)

An extract is usually defined as the result of a single-step process in which components are removed/enriched from a mixture of other (phyto-)components. Basically, cannabis chemotypes can be divided into three main categories: cannabis dominant in THC/low in CBD (slang name: ‘marijuana’; correct term: drug-type cannabis or Type I cannabis); cannabis with a ‘mixed’ ratio of CBD to THC (mixed or hybrid type/type II cannabis); and cannabis high in CBD/low in THC (‘hemp-type’ or ‘fibre-type’/type III) [37]. This therefore also defines the primary phytocannabinoid(s) in the extract. However, the final composition of phyto-compounds depends on many other factors, mainly on the extraction process and solvent(s) used. It should be noted that there are very few extracts that are well-characterized, standardized and of pharmaceutical quality; this limits comparisons between different extracts and any generalization of results, in particular when dealing with ‘CBD oils’ and other extracts from the internet.

Although extracts (‘CBD oils’) are considered, by consumers, to be more potent than pure cannabinoids, data from in vitro studies in cancer cell lines do not currently support this popular opinion. Results of in vitro studies suggest that in most cancer cell lines, CBD is often more potent or at least as potent as CBD-E. As shown in Table 1, CBD was superior to extracts rich in CBD (CBD-E) in 15 of 24 cell lines, about equal in 5, and inferior to CBD-E in only 4 cell lines, in DU-145, LNCaP prostate cancer [9] and MCF-7 and C6 breast cancer cell lines [5]. The higher potency of pure CBD is also reflected by a small study that compared three CBD oils (CBD-E) and pure CBD on six different cell lines [11]. Intriguingly, in a similar study that compared three commercial CBD oils with pure CBD in four carcinoma cell lines (colorectal cancer cell lines SW480 and HCT116; melanoma cell lines 1205Lu and A375M), the oils reduced cell viability to a much lower extent than pure CBD; one of the three CBD oils even protected cancer cells instead of killing

them [38]. In some cases, the potency of pure CBD was more than 10- to 15-fold higher than ‘full-spectrum cannabis extracts’ [39]. Even small differences in the composition can have a pronounced impact on cytotoxicity. A recent study compared pure CBD (>99.9%) with almost pure CBD-E (96%) and found that all four glioma cell lines tested (two human glioma cell lines U87MG and U373MG; two canine glioma cell lines J3TBG and SDT3G) were marginally more sensitive to pure herbal CBD than to CBD-E [15].

Whereas CBD seems to be more or at least equally potent to CBD-E in the majority of in vitro tests, the opposite is observed for THC; THC-rich extracts (THC-E) were more potent than THC in 15 (including six breast cancer cell lines [27, 40] and three brain tumour cell lines [18]) of 17 tests, and less potent in only two (breast cancer cell line MFC-7, gastric adenocarcinoma [AGS]); both THC and THC-E were ineffective against hormone-insensitive/androgen receptor-negative DU-145 prostate cancer cells [5]. Further factors of influence are the cell line and the nature of the extract (extraction process).

3.4 In Vitro Potency of Acid Forms of Cannabinoids

As unheated extracts are rich in acids, the in vitro potency of cannabidiolic acid (CBDA) and/or tetrahydrocannabinolic acid (THCA), which may increase the bioavailability of CBD or THC, respectively [41], is also of interest. The potency of CBD was higher than that of CBDA in all 14 cell lines investigated to date [5, 42]. Another study that compared the activity of CBD, CBG and cannabigerarin (CBGV), in both their neutral and acid forms, against the human cancer cell lines CEM (acute lymphocytic leukaemia) and HL60 (promyelocytic leukaemia), found that in all cases the neutral (decarboxylated) form (and also other cannabinoids [CBG, CBDV]) was more active than their respective acid counterpart. The two cannabinoids with the greatest activities were CBD and CBG, with IC_{50} values at 48 h of about 7 and 10 μ M, respectively [42]. This contrasts to the activity of THC, which was more potent than THCA in only 2 of 11 cell lines tested—colorectal carcinoma (CaCo-2) [5] and pancreatic carcinoma (PANC-1) [43].

3.5 Interaction of Phytocompounds in Extracts and the Entourage Effect

Two recent studies showed the complexity of the interaction of phytocompounds. In the first study, a crude extract of THC-rich cannabis sativa chemotype (87.4% THC) was fractionated into several cannabinoid-enriched fractions and the effects on the viability of A172 human glioblastoma cells were investigated. It was demonstrated that a standard mixture (SM) of pure cannabinoids, as present in the respective cannabinoid-rich fractions (THC- and CBG-rich fractions),

was more potent than pure THC or pure CBG, and the pure cannabinoids were more potent than the respective natural extract fraction. Although the potencies varied between the various fractions of the crude extract, the crude extract was the least potent [27].

In the second study, two different chemotypes of cannabis, one predominant in THC and the other predominant in CBD, have been separated into their cannabinoid and terpenoid fractions. These terpenoid fractions had no cytotoxic properties per se against breast cancer cells (MDA-MB-231) or colorectal carcinoma cells (HCT-116) in contrast to the cannabinoid fractions. However, when the terpenoid fraction of the THC-rich chemotype has been combined with pure THC, the cytotoxic effects of the latter were found to be significantly increased in both cancer cell lines. The effects of a respective combination of pure CBD with terpenoids of the CBD-rich chemotype were similar, although much weaker for HCT-116 than for MDA-MB-231 cells. Pure CBD was more potent than THC against HCT-116 colorectal carcinoma cells and about as equally effective against MDA-MB-231 breast carcinoma cells. Effects were also dependent on the ratio between the pure cannabinoid and the related 'terpenoid' fraction [44]; however, combinations with the 'unrelated' terpenoid fraction had no effects. Extraction with hexane resulted in about twofold higher amounts of terpenes than ethanolic extraction. In another similar study, the addition of a 'cocktail' of pure terpenes commonly found in cannabis did however not increase the potency of THC [40]. Minor cannabinoids such as CBC may also play a role but this would require further systematic investigations [45, 46].

Thus, isolated phytocannabinoids and their combinations may not fully mirror the complexity of natural extracts in *each* case. In addition, it should be mentioned that experimental conditions of *in vitro* studies, such as 2D versus 3D cultures [47], exposure time and serum starvation/concentration, also influence results [9, 11]. This may explain why results are sometimes conflicting.

Collectively, this demonstrates that cytotoxic effects of extracts and the 'entourage effect' depend on a multiplicity of factors, e.g. results vary between cannabinoids, their relative proportions in extracts, the presence of other phytochemicals, and the type of cancer cells. The interpretation of results with extracts is limited by the fact that there is no way of knowing exactly what these products contained. The large majority of commercial extracts are poorly characterized consumer products whose ingredients are incompletely, or even incorrectly, declared. If used for therapeutic purposes, results with different extracts can be highly variable, as has been demonstrated [48]. This limits any generalization of observations and the pharmacological use of extracts.

Taken together, these experiments demonstrate that no single cannabinoid or mixture is universally effective in reducing cancer cell viability, although the overall potency

of CBD seems to be often, but not always, higher than THC and also higher than extracts.

3.6 Exceptionally, Cannabinoids Have Enhanced Cancer Growth *In Vitro* and *In Vivo*

As has previously been mentioned, enhancement of the growth of primary cancer cells (melanoma cells, A375M; colorectal cancer cells, SW480) has been observed in an isolated case of a CBD-E (commercial CBD oil) *in vitro* [11]. Furthermore, increased viability of glioma stem cells occurred *in vitro* with moderate concentrations of CBD (below 10 μM) and CBG (below 5 μM), but not with higher concentrations (10–15 μM) [16]. Similar observations were made with very low concentrations of THC (1 nmol/L to 0.25–2 $\mu\text{mol/L}$), which enhanced, *in vitro*, the proliferation of primary DU-145 prostate cancer cells and human papilloma virus (HPV)-positive head and neck squamous cell carcinoma (HNSCC) cells (UD-SCC-2, UPCI:SCC090 and UM-SCC-47), whereas higher concentrations were tumour-inhibitory [5, 49, 50].

Enhancement of tumour growth was also seen *in vivo* in a xenograft model with human squamous cell carcinoma UD-SCC-2 cells, where nude mice received intraperitoneal THC 3 mg/kg/day [50], and in a murine lung cancer model where mice received THC 5 mg/kg intraperitoneally four times/week for 4 weeks [51]. THC exposure (25 and 50 mg/kg intraperitoneally every other day for 18–21 days) has also dose-dependently increased the growth of murine 4T1 breast cancer *in vivo*, as well as metastasization in BALB/c mice, supposedly related to a lack of expression of cannabinoid receptors and suppression of the antitumour immune response [51, 52].

No similar observations of growth enhancement in primary tumours have been reported to date for pure CBD. To note, tumour formation rates after subcutaneous injection of human tumour cells are higher in immunodeficient NOD/SCID mice than in BALB/c mice, whereby the tumorigenesis rate is influenced by the nature of carcinoma and by the mouse strain [53]. BALB7c mice lack a thymus (and therefore T cells), whereas NOD/SCID mice show multiple innate immune defects, including natural killer (NK) cell dysfunction, low cytokine production, and T- and B-cell dysregulation [54]. At present, such isolated observations in animal models are difficult to interpret as they do not reflect 1:1 the situation in man and conflict with many other studies. In most experiments with cannabinoids, a dose-dependent reduction of cell viability was observed.

In man, no enhancement of tumour growth has been observed with pure cannabinoids to date, despite the fact that they are administered orally, resulting in lower bioavailability than after intraperitoneal injection in animals. Only daily marijuana use correlated with the development of

HPV-positive HNSCC, as a result of past HPV oral infection [50]. It is worth mentioning that the viability of HPV-negative HNSCC was found to be significantly reduced by CBD in xenografts [31]. As systematic studies in man are still missing, it is not known whether conditions exist or whether subgroups of patients exist who might be less responsive or even at risk of enhanced tumour growth when receiving pure cannabinoids.

Overall, *in vitro* effects on cell viability depend on the experimental conditions, such as the cell line, the nature of cannabinoids, their ratio in the case of combinations, and on other phytosubstances in the case of extracts. Studies point to a dose-dependency of antiproliferative effects across a number of very different cell lines, with lower concentrations being cytostatic rather than inducing apoptosis [31, 42, 47, 52, 55, 56].

4 Effects of Cannabinoids on Tumour Growth and Survival in Animal Studies

After *in vitro* experiments, animal studies represent the next level of therapeutic relevance, whereby the effects on tumour growth and/or survival are the most important endpoints. Only five articles were identified that compared, head-to-head, CBD with THC or extracts in subcutaneous or orthotopic xenograft models. In addition, CBD has been investigated in a number of animal experiments that targeted aspects other than tumour volume or survival. A total of 25 articles describing comparative and non-comparative animal studies, excluding those that focused on mechanisms only, were identified. Significant antitumour effects of cannabinoids were observed in almost all articles and are summarized below and in Table 2.

The highest number of animal studies have been conducted on tumours of the nervous system, followed by breast cancer. Effective concentrations of pure CBD varied largely between 7.5 and 50 mg/kg/day five times a week in nervous system tumours, and between 1 mg/kg/day and 10 mg/kg on alternate days in breast cancer.

Three studies compared, head-to-head, the antitumour effects between CBD and THC. CBD was equally potent to THC in glioma [57], and superior in neuroblastoma cell xenografts [19]. In the third study on medulloblastoma xenografts, neither CBD nor THC could demonstrate a clear superiority compared with controls [20].

Another study that described a benefit for THC but not CBD relates to the very early experiment in mice with Lewis lung adenocarcinoma xenografts [3]. THC (25–50–100 mg/kg for 10 days) decreased tumour weight after 12 days, whereby differences to controls diminished over time and tumour growth approached control values after 3 weeks.

Lifespan was increased non-linearly by 17.4, 6.2 and 36% with daily doses of 25, 50 and 100 mg/kg, respectively. CBD (25 or 200 mg/kg daily until death) showed no tumour-inhibitory properties as measured by tumour size or survival time. However, CBD was administered in only two dosages, therefore the possible effects of dosages between 25 or 200 mg/kg/day would have been missed. Interestingly, experiments with D8-THC demonstrated a bell-shaped dose effect; intermediate doses of 100 and 200 mg/kg reduced tumour weight after 12 days more so than 50 mg/kg and a suprapharmacological dose of 400 mg/kg. Furthermore, the effects of D9-THC 100 mg/kg (range 25–50–100 mg/kg) were similar to a 50 mg/kg dose. Animal experiments with CBD were not conducted in parallel but as a separate study. Furthermore, according to the authors, the tumour growth rate of controls in this experiment with CBD was decreased compared with previous studies. Thus, experimental conditions for CBD and THC clearly differed, and the results for CBD are therefore inconclusive [3].

To date, only one study has compared pure CBD with CBD-E, in KiMol xenografts. CBD was superior to CBD-E, but equally effective in MBA-MD-231 breast cancer cell xenografts [5]. Conversely, pure THC was less effective than THC-E in xenografts of four different human breast cancer cell lines (HCC1954, MDA-MB-231, BT474 and T47D) [40].

Independent from the therapeutic animal studies that aimed to demonstrate an antitumour effect on xenografts, the prophylactic reduction of colon cancer formation induced by azoxymethane by CBD has been investigated in a mouse model [58]. CBD (1 or 5 mg/kg intraperitoneally 3 × weekly over 4 weeks), starting 1 week before the first administration of azoxymethane, significantly reduced tumour formation (–60% after 1 mg/kg vs. –30% after 5 mg/kg). Interestingly, the protective effect decreased with the higher dosage.

In a similar prophylactic experiment, CBD-E (CBD-BDS 5 mg/kg/day intraperitoneally 3 × weekly up to 3 months after the first injection of azoxymethane) also reduced azoxymethane-induced preneoplastic lesions (aberrant crypt foci/ACF) and polyps (ACF, 86% inhibition; polyps, 79% inhibition) [59]. However, in a therapeutic xenograft model (colorectal carcinoma HCT116 cells) reported in the same article, the average tumour volume in mice was significantly lower in mice treated with CBD-E (CBD-BDS 5 mg/kg intraperitoneally daily) compared with controls only on day 4, with no difference observed on day 7 [59].

Twelve other studies investigated pure CBD without a comparison with other phytocannabinoids. All reported significant effects against cancer cell xenografts [4, 23, 24, 31, 32, 58, 60–65].

Similar to *in vitro* experiments, animal studies seem to confirm a dose-dependent anticancer effect of cannabinoids on tumour growth, metastasis or survival. Overall,

Table 2 In vivo potency of cannabinoids with emphasis on CBD, THC and extracts in various models

Tumour model	Drug	Comparison	Efficacy, results	Reference
1 human glioblastoma cell line: U87; SC xenograft, athymic nude mice	0.5 mg CBD (i.e. ~2.5 mg/kg/day), 5 days/week for 23 days	Control	Tumours of animals treated with CBD were significantly smaller: Day 18, 572 mm ³ (control 1765 mm ³); Day 23, 1210 mm ³ (control 2212 mm ³)	[4]
2 intracranial glioma stem cell xenograft cell lines: 3832 and 387; female athymic nu/nu mice	CBD 15 mg/kg IP; 5 days/week for ~25 days after tumour induction	Control	CBD inhibited tumour growth and significantly improved the survival of mice bearing intracranial glioma initially but tumour resistance was observed later on Median survival with CBD in 3832 and 387 glioma stem cell lines was 33 days and 26 days, respectively, compared with 27 and 21 days, respectively, for controls	[24]
1 human glioblastoma cell line: U251; orthotopic intracranial xenograft, mice	CBD 15 mg/kg IP 5 days/week for 28 days	Control	Significant (~95%) decrease in tumour area; in 1 of 5 mice treated, no tumour cells were observed in any of the brain regions analysed	[64]
1 human glioblastoma cell line: U251 cells; SC xenograft, mice	CBD 20 mg/kg IP 5 days/week for 48 days	Control	A similar dose-dependent effect was observed in an SC model and with peritumoral injection of CBD; the tumour volume was ~30% lower with a 15 mg/kg dose and ~50% lower with a 20 mg/kg dose; CBD eradicated the tumour in 1 of 5 animals	[64]
1 human glioblastoma cell line: U87MG; orthotopic xenograft, nude mice	CBD 15 mg/kg/day IP for 21 days	TMZ 25 mg/kg/day, CBD/TMZ	50% survival: control ~42 days, CBD ~50 days, TMZ ~52 days, CBD/TMZ ~60 days; 0% survival: control 50 days, CBD 55 days, TMZ 60 days, CBD/TMZ 84 days	[32]
1 human glioma cell line: U87MG; heterotopic SC xenografts, nude mice	Synthetic CBD 15 mg/kg/d PO for 15 days	TMZ 5 mg/kg/day, CBD/TMZ	Tumour volume CBD > CBD/TMZ > TMZ Tumour volume was ~30% lower with CBD, ~70% lower with TMZ and ~50% lower with the combination compared with controls; no enhancement of anticancer activity by combination CBD/TMZ	[23]
1 human glioma cell line: U87MG; SC xenografts, nude mice		CBD/THC	Tumour growth was inhibited; no relevant difference between 1:4 ratio or 1:6 (extract) of THC:CBD compared with pure THC:CBD = 1:1; pure THC was not included	[23]
1 human glioma cell line: U87MG; SC xenografts, nude mice		CBD/THC/TMZ	Tumour volume THC/CBD (=1:4, extract) > TMZ > THC/CBD (1:4) + TMZ Combination with TMZ was more effective than THC/CBD extract combinations and more than TMZ alone	[23]

Table 2 (continued)

Tumour model	Drug	Comparison	Efficacy, results	Reference
1 human glioma cell line: U87MG; orthotopic intracranial xenografts, nude mice		THC/CBD (1:4) TMZ CBD/THC/TMZ	Oral administration of an extract (ratio THC + CBD = 1:4) did not significantly affect tumour size and survival (as determined by MRI), which was lower than with TMZ; combination with TMZ increased survival from ~30 days (control) to >50 days	[23]
1 human glioma cell line: 12O12 GICs; orthotopic intracranial xenografts, nude mice		THC/CBD (1:1 or 1:5) TMZ CBD+THC+TMZ	Oral administration of THC/CBD at a 1:5 ratio increased survival compared with a 1:1 ratio and controls; combination with TMZ increased survival further; pure CBD or THC were not included	[23]
2 human glioma cell lines: U87MG, T98G; SC xenograft, nude mice	CBD 7.5 mg/kg/day, peritumoral injection, for 14 days (15 mg/kg not tested)	THC 7.5 mg/kg/day or THC 15 mg/kg or CBD/ THC each 7.5 mg/kg/ day or a nabiximols-like preparation (15 mg/kg/ day)	CBD ≈ THC (7.5 mg/kg/day) Effect of a submaximal dose of THC 7.5 mg/kg increased when combined with CBD 7.5 mg/kg/day; THC + CBD (each 7.5 mg/kg) ≈ THC 15 mg/kg; a nabiximols-like combination of extracts reduced the growth of U87MG tumour xenografts to the same extent as an identical dose of pure THC	[57]
2 human medulloblastoma cell lines: D283, D425; intracranial xenografts, mice	Synthetic CBD 50 mg/kg IP or PO 5 days/ week for 5 weeks	D283: THC 15 mg/kg, combination: 50 mg CBD + 15 mg THC; D425: 22.5 mg/kg CBD; 45 mg/kg THC, or CBD/ THC 22.5/22.5 mg/kg	CBD ≈ THC ≈ vehicle No significant change in survival compared with vehicle was observed with CBD, THC or THC/CBD in both xenografts (D283 xenograft: THC 23 days, CBD 25 days, combination 21 days, vehicle 30 days); neither THC nor CBD/THC improved survival of mice with D425); results were similar for IP and PO administration	[20]
1 human neuroblastoma cell line: SK-N-SH; SC xenograft, Non-obese diabetic immunodeficient (NOD/SCID) mice	Synthetic CBD 20 mg/kg/day IP for 14 days	THC 20 mg/kg/day IP or ethanol vehicle or untreated	CBD > THC Response to treatment was better in the group with CBD; median xenograft volume at the end of treatment was 2.31 cm ³ in the CBD-treated group compared with 3.46 cm ³ (THC), 4.28 cm ³ (untreated) and 4.31 cm ³ in the vehicle-treated group	[19]
2 rat glioma cell lines: C6.9, C6.4; SC xenografts, mice	THC peritumoral 0.5 mg/day for 8 days	Vehicle	THC decreases tumour volume and downregulates MMP-2 expression of C6.9 by about 50% but not of C6.4 cells	[124]

Table 2 (continued)

Tumour model	Drug	Comparison	Efficacy, results	Reference
1 rat glioma cell line: C6; intracerebral implanted, rats (250–300 g BW)	THC intratumoral, total dose 2.5 mg/rat over 7 days (~THC 1.5–2 mg/kg/day)	0.25 mg/kg WIN-55,212-2 (synthetic cannabinoid similar to THC)	THC was ineffective in 3/15 rats, but tumour was completely eradicated in 3/15 rats, survival prolonged in 9 rats (up to 19–35 days vs. controls 12–18 days) WIN-55,212-2 was approximately similarly effective in the prolongation of survival (ineffective in 6 rats, complete eradication of tumours in 5 rats)	[68]
1 mouse glioma cell line: GL261; orthotopically implanted, mice		CBD/THC (each ~2 mg/kg IP) on Days 9, 13, and 16 after tumour implantation; X-ray (4 Gy) on Day 9; CBD-BDS (63.5% CBD, 3.6% THC, 5.2% CBC) or THC-BDS (65.4% THC, 0.4% CBD, 1.8% CBC) [P-purity >96%]	>85% decrease in tumour volume and vascularization on Day 21 (animals sacrificed); CBD/THC reduced progression, further enhanced by irradiation 4 h after drug administration (stagnant tumour sizes throughout the experiment); X-rays alone had no dramatic effects; pure CBD or THC were not included	[18]
4 human breast adenocarcinoma cell lines: 2 ER-; HCC1954, MDA-MB-231; and 2 ER+/PR+: BT474, T47D; SC xenografts, immune-deficient, nude mice	THC 45 mg/kg PO 3 × weekly for 30 days	THC-E, adjusted to THC 45 mg/kg PO 3 × weekly (extract [E] with 55% THC, 0.3% THCA, 0.4% CBG (CBD not done)	THC < THC-E; In all 4 animal tests, pure THC inhibited tumour growth less than THC-E; T47D, THC < THC-E, HCC1954, THC < THC-E, BT474, THC < THC-E, MDA-MB-231, THC < THC-E (triple-negative cancer)	[40]
2 breast cancer cell lines: 1 triple-negative murine cell line, 4T1.2; 1 triple negative human breast cancer cell line, MVT-1; orthotopically injected, BALB/c and FVB mice	CBD 10 mg/kg peritumoral injections on alternate days for 3 weeks	Control	CBD slowed down the growth of highly aggressive orthotopically injected, triple-negative murine breast cancer cells (4T1.2) by approximately 25–30% (tumour volume and weight). A similar dose-dependent inhibition of cancer growth was also seen after injection of a triple-negative human breast cancer (MVT-1)	[60]
1 human breast cancer cell line: MBA-MD-231; SC xenografts, athymic mice	CBD 5 mg/kg 2 × weekly intratumoral injection for 16 days	CBD-rich E 6.5 mg/kg 2 × weekly intratumoral injection	CBD ≈ CBD-E Extracts were injected intratumorally in the same inoculation region twice per week for 16 days; a significant reduction of the tumour volume was observed after both treatments, with no difference between CBD and CBD-E (~40% lower tumour volume)	[5]

Table 2 (continued)

Tumour model	Drug	Comparison	Efficacy, results	Reference
1 murine breast carcinoma cell line: 4T1; SC xenograft, BALB/c mice	THC 12.5, 25, or 50 mg/kg IP every other day for 18–21 days	Control	25 and 50 mg/kg THC led to a significant, dose-dependent increase in tumour mass and metastases, even more pronounced with 50 mg/kg	[52]
2 cell lines: 1 mouse breast cancer cell line: 4T1; 1 human breast cancer cell line: MDA-MB231; injected IV, mice	CBD 1 mg/kg/day, IP for ~1 month	Control	CBD increased survival significantly and reduced metastasis up to 75% (EC ₅₀ 0.3 mg/kg). Effects on metastasis were dose-dependent (CBD 0.5, 1 or 10 mg/kg/day IP)	[61]
1 mouse breast cancer cell line: 4T1; orthotopically injected into mammary glands, mice	CBD 1 mg/kg/day, IP for ~1 month	Control	CBD reduced metastasis even when administered only three times per week. However, CBD did not inhibit primary tumour growth	[61]
1 pancreatic tumour cell line: MiaPaCa2; SC xenograft, immunodeficient nude mice	THC peritumoral 15 mg/kg/day for 14 days	Vehicle or JWH-133 (1.5 mg/kg/day, CB2-selective agonist)	~50% reduced tumour growth, increased apoptosis	[125]
1 pancreatic ductal adenocarcinoma cell line, KPC mice	CBD 100 mg/kg/day	GEM 100 mg/kg/day IP, CBD/GEM	Mice receiving the combination CBD/GEM survived 2.8 times longer than mice not administered any treatment (1.3 times longer with CBD and 1.4 times longer with GEM alone); mean survival: no treatment 18.6 days, CBD 25.4 days, GEM 27.8 days, CBD/GEM 52.7 days	[65]
2 prostate cancer cell lines: LNCaP, DU-145; SC xenografts, athymic nude mice, 6 groups for each cell line	CBD-BDS (~65% CBD) 1, 10 or 100 mg/kg/day IP; initiated Day 15, terminated Day 38	Docetaxel 5 mg/kg IV 1 × weekly, Bicalutamide 25–50 mg/kg PO 3 × weekly, CBD-E 100 mg/kg/day IP plus either docetaxel 5 mg/kg IV 1 × weekly, or bicalutamide 25–50 mg/kg PO 3 × weekly	CBD-BDS (extract) dose-dependently inhibited the growth of xenografts from LNCaP, but not from DU-145 cells, CBD-E potentiated docetaxel/taxotere effects in DU-145, less so in LNCaP xenografts, CBD-E enhanced efficacy of bicalutamide on LNCaP only at the highest concentration tested (100 mg/kg IP), A group receiving pure CBD was not included	[9]
Colon cancer induced by AOM, mice	CBD 1 or 5 mg/kg IP 3 × weekly for 4 weeks	Control	CBD, starting 1 week before the first administration of AOM, reduced significantly aberrant crypt foci, polyps, as well as tumour formation (~60% after 1 mg/kg vs. ~30% after 5 mg/kg)	[58]
Colon cancer induced by AOM, mice	CBD-BDS 5 mg/kg IP, 3 × weekly up to 3 months after the first injection of AOM	Control	CBD-E reduced AOM-induced preneoplastic lesions and polyps (inhibition AOM-induced aberrant crypt foci by 86%, polyps by 79%), as well as tumour growth (by 40%)	[59]
1 human epithelial colon adenocarcinoma cell line: HCT116; SC xenograft, mice	CBD-BDS 5 mg/kg IP 1 × daily for 7 days	Control	CBD-E ≈ control Tumour growth between control and CBD-BDS was significantly different on Day 4 but there was no difference on Day 7	[59]

Table 2 (continued)

Tumour model	Drug	Comparison	Efficacy, results	Reference
Lewis lung adenocarcinoma, SC xenografts, mice	CBD 25 or 200 mg/kg/day until death	THC: 25, 50 or 100 mg/kg/day for 10 days, D8-THC: 50, 100, 200, or 400 mg/kg/day until death, CBN: 25, 50, or 100 mg/kg/day until death	CBD had no effect on tumour size or survival time. However, the tumour growth rate of controls in this experiment was decreased compared with previous studies. THC decreased tumour weight after 12 days, but differences approached control values after 3 weeks. Lifespan was increased non-linearly by 17.4, 6.2 and 36% with doses of 25, 50 and 100 mg/kg, respectively. D8-THC showed maximal effects after 100 and 200 mg/kg. The effects of CBN increased with the dose	[3]
1 human lung carcinoma cell line: A549, SC xenograft, mice	CBD 5 mg/kg IP every 72 h for 29 days	Control	CBD reduced tumour volume significantly by 80% compared with vehicle-treated animals (treatment started 7 days after tumour induction; after 29 days, animals were sacrificed and tumours were explanted)	[62]
1 human head and neck squamous carcinoma cell line: FaDu; SC xenograft and tongue xenograft, BALB/c nude mice	CBD 5 mg/kg PO 4 × weekly for 4 weeks (SC xenograft) or CBD 5 mg/kg IP, 3 × weekly for 4 weeks (tongue xenograft)	Cisplatin 2.5 mg/kg IP, CBD/cisplatin, control	CBD slowed down the tumour increase of tongue xenografts; tumour volume after 3 weeks of treatment with CBD was about 65% lower than controls (cisplatin ~50%); the combination with cisplatin (5 mg CBD PO + 2.5 mg cisplatin IP/kg) decreased the tumour volume further (tumour volume ~15% of controls); tumour size was measured after 3 weeks	[31]
1 murine melanoma cell line: B16F10; SC injection	CBD 5 mg/kg IP 2 × weekly	Cisplatin	CBD significantly reduced melanoma tumours and increased the survival of animals (less than cisplatin but demonstrating a better quality of life)	[63]
1 rat thyroid cell line: KiMol; SC xenografts, athymic mice	CBD 5 mg/kg every 72 h/2 × weekly injected intratumorally for 20 days	CBD-rich extract (Nabidiolex [®] , 64.6% CBD) 6.5 mg/kg 2 × weekly for 20 days, injected intratumorally in the same inoculation region	CBD > CBD-E Metastases were evaluated 21 days after injection; a significant reduction of the tumour volume was observed after both treatments (tumour volume was ~70% lower in the CBD group and ~40% lower in the CBD-E group compared with controls)	[5]

AOM azoxymethane, CBC cannabichromene, CBD cannabidiol, CBD-BDS cannabidiol botanical drug substance, CBG cannabigerol, CBN cannabinol, E extract, EC₅₀ half maximal effective concentration, ER estrogen receptor, GEM gemicitabine, GICs glioma-initiating cells, IP intraperitoneal, IV intravenous, MMP-2 matrix metalloproteinase 2, MRI magnetic resonance imaging, PO oral, PR progesterone receptor, SC subcutaneous, THC tetrahydrocannabinol, THCA tetrahydrocannabinolic acid, TMZ temozolomide

administrations and doses varied largely between two and seven times per week, and between 1 and 200 mg/kg (mainly 5–50 mg/kg), for CBD; doses for THC varied between 7.5 and 20 (to 45 mg/kg). CBD was at least equal or superior to THC or CBD-E; a combination of CBD with THC was additive or synergistic. THC-E was superior to THC, although the low number of head-to-head studies must be taken into account. Despite the fact that dose conversion from animals to human subjects is still a controversial area, it is assumed that the dose in mice (rats) is to be divided by a factor of ~12 (~6) [66, 67] to get the human equivalent dose. Accordingly, the lowest effective CBD doses in man (60 kg) would translate to about 25–250 mg/day of CBD. Most interestingly, one article describes the histopathologic eradication of glioblastoma tumours with CBD, in two different models, in 1 of 5 animals [64], and another study with THC in 3 of 15 rats [68].

Similar to *in vitro* observations, combinations of cannabinoids with chemotherapeutics influence tumour growth in animal models. Improved survival of animals or reduced tumour volume has been reported after combined treatment of CBD with gemcitabine (pancreatic ductal adenocarcinoma) [65] or cisplatin (FaDu head and neck squamous cell tumours) [31]. Conflicting results have been reported for a combination of CBD with temozolomide (U87MG glioma). Whereas an earlier article of the same group did not observe an enhancement of anticancer activity by a combination of CBD + temozolomide in subcutaneous xenografts [23], a very recent publication using a combination of CBD and temozolomide in an orthotopic cancer model reported a significantly longer survival [32]. Whereas a combination of neither THC nor THC-E with tamoxifen 2.5 mg/kg (T47D cells) had any impact in a breast cancer model, a combination of THC-E with cisplatin 3 mg/kg (MDA-MB-231 xenograft) reduced the tumour volume slightly more than cisplatin alone [40].

5 Anticancer Effects of Pure Cannabinoids and Extracts Described in Man

Cannabinoids and cannabis have become popular for cancer treatment in the community [69]. An uncountable number of anecdotal responses and testimonials after using cannabis oil for malignant diseases (extracts rich in THC or CBD oil) suggest anticancer effects [70–75]. The usual dosages are 1 g, up to 2 g per day. However, such reports must be interpreted with caution. Not only do they lack proper medical documentation, but, conversely, the story of the many others who were unsuccessful in their efforts to combat their tumour with cannabinoids remains unknown. In addition, the extracts that have been used often lack appropriate characterization, therefore the results may not be reproducible.

Although not an ideal form of evidence, they are nonetheless, to some extent, resources for identifying possible effects.

In contrast to the large number of reports in social media, only 16 articles were identified in the scientific medical literature describing nearly 180 cases. To date, CBD, CBD combined with THC, pure THC, as well as poorly characterized cannabis or hemp extracts, have been used for the treatment of malignant diseases in man. These articles reported measurable benefits, such as tumour regression and/or extension of survival. Although representing ‘real-world data’, the selection of cases responding, the paucity of similar cases, the heterogeneity of the data, the lack of head-to-head comparisons or of an independent ‘blinded’ assessment for possible confounders, and a sufficiently long follow-up in the majority of cases are factors limiting overall conclusions on efficacy (Table 3).

Treatment with pure CBD has been reported in only five articles, all of which are case reports. Brain tumours were the primary cancer described in these publications.

The largest collection of cases described the results from 119 patients with various cancers, most of which were metastatic [76]. Data were routinely collected, as part of a treatment programme with synthetic CBD, over a 4-year period. A minority of 28 patients received CBD as the sole treatment. Breast, prostate and colorectal cancer were the most often reported cancers, with 39, 16 and 13 patients, respectively; 8 other patients had non-Hodgkin's lymphoma and 7 had a diagnosis of GBM. One-third of the patients had a history of previous, self-initiated use of cannabis extracts, intriguingly without a response. CBD (10–30 mg twice daily for a minimum of 6 months) was administered according to a ‘3 days on, 3 days off’ schedule. In some cases, nabiximols was used in conjunction with CBD (two sprays twice daily, equivalent to 10.8 mg THC + 10 mg CBD/day).

Clinical responses were reported in 92% of the 119 cases with solid tumours, including a reduction in circulating tumour cells (test performed before and after treatment in the majority of patients). A number of patients had relevant scans. The authors noted that patients receiving continuous dosing did not do as well as those receiving this on/off pulsed regimen, unfortunately without supporting details. Some of the patients reverted to cannabis oil bought on the internet, and following this, 80% of these cases relapsed. According to the authors, <6 months of treatment had little effect; these patients were defined as ‘unassessable’ and were excluded. Such a selection can cause bias. Patients with <6 months of treatment may have had, for example, progressive disease or may have reverted to preparations from the internet because of the lack of a clear effect. However, many interesting aspects remain unanswered in that article, such as response criteria, number of patients who were stable/progressive when CBD was started or who had been treated for

Table 3 Anticancer effects of cannabinergic preparations in man

Disease, no. of patients	Drug	Results	Reference
Glioblastoma multiforme, grade IV, 15 patients	Pure CBD (mainly 400 mg/day PO) in addition to standard therapy (maximum resection of the tumour followed by radiochemotherapy)	Of 15 patients, 7 (46.7%) survived for at least 24 months, and 4 (26.7%) survived for at least 36 months. This is more than twice as long as has been previously reported in the literature. The mean overall survival is currently 24.2 months (median 21 months) and the 1-year survival rate was 87%	[77]
Glioblastoma multiforme, 9 patients (6 were grade IV)	Pure CBD (mainly 400 mg/day PO) in addition to standard therapy (maximum resection of the tumour followed by radiochemotherapy)	Of 9 patients, only 1 had died; mean survival time was 22.3 months (range 7–47 months)	[78]
Brain tumours, 2 patients	Pure CBD with <0.3% THC (case 1: glioblastoma multiforme; 300–450 mg/day; case 2: oligodendroglioma grade III: 100–200 mg/day following resection and chemoradiotherapy)	Treatment reduced oedema and inflammation and induced remission (MRI)	[79]
Piloicytic astrocytoma, WHO grade 1; 2 females: case 1 was aged 11 years and case 2 was aged 13 years at diagnosis	Consumption of cannabis of unknown composition via inhalation, on average 3 × weekly during the last 3 years of follow-up	The volume of tumour remnant was calculated using VOXAR volumetric software, and was found to be 1.28 cm ³ at 9 months and 0.27 cm ³ at 6 years post-surgery in the first case, and 3.3 cm ³ at 18 months and 0.28 cm ³ at 6 years postoperatively in the second case; the regular use of cannabis coincided with the time course of radiological tumour regression	[126]
Glioblastoma multiforme, 12 patients with nabiximols, 9 with placebo	Nabiximols (CBD:THC ≈ 1:1, maximum 32.4 mg THC + 30.0 mg CBD) following standard chemoradiotherapy treatment with dose-intense TMZ, as described by Stupp et al. [88]	Median survival in the placebo group was >550 days in the CBD:THC treatment group (not significant) and 369 days in the placebo group; 1-year survival was 83% and 56% in the CBD:THC and placebo groups, respectively (<i>p</i> = 0.042)	[89]
Glioblastoma multiforme, 9 patients with progressive tumours	THC solution, 20–40 mg at Day 1, increased for 2–5 days up to 80–180 mg/day, infused into the resection cavity, starting at Days 3–6 after surgery; median duration of an administration cycle was 10 days	5 patients received more than 1 cycle. In 3 of these 5 patients, a temporary reduction of tumour proliferation was observed. Median survival of the cohort from the beginning of THC administration was 24 weeks	[87]
Pancreatic cancer, 9 patients with advanced, metastatic disease	Pure CBD, 400 mg daily, concomitant to standard chemotherapy (2 patients with CBD as the only treatment)	The mean overall survival of these 9 patients was 11.5 months (median 11 months) and seems to be longer than the overall survival reported in the literature for metastatic disease (5.9 months)	[80]
Ovarian carcinoma, metastatic, low grade, 81-year-old female	CBD oil of unknown composition; 1 drop sublingually each evening, concomitant to laetrile tablets, 500 mg PO 4 × daily started 2 months after surgery	The CA125 level was 77 at the time of surgery and 46 before starting CBD; 1 month later, CA125 normalized to 22	[82]
Lung adenocarcinoma (T1c N3 M0, biopsy-confirmed), 81-year-old male	CBD 2 × 1.32 mg/day (2% CBD oil) as the sole therapy, increased to 2 × 6 mg/day after 1 week	11 months after the diagnosis of lung cancer, the patient started CBD; the tumour was progressive at that time. The CT 4 months later revealed near total resolution of the left lower lobe mass and a significant reduction in size and number of mediastinal lymph nodes (stable according to a CT control 2 months later)	[83]

Table 3 (continued)

Disease, no. of patients	Drug	Results	Reference
Lung cancer, terminal, 53-year-old male	Cannabis oil of unknown composition, daily inhalation with a vaporizer	After inhaling vaporized cannabis oil, the patient's lung cancer (biopsy confirmed) disappeared within about 3 months; other symptoms also improved, however the patient died from cardiac failure about 1 year after the diagnostic interview	[84]
Lung cancer, non-metastatic, female in her 80s	0.5 mL cannabis oil (20% CBD, 19.5% THC, 24% THCA) PO 2–3 × daily	Progressive shrinking of the tumour from 41 mm at diagnosis to 10 mm 32 months later; no chemotherapy or radiation, no surgical intervention	[85]
Various cancers, 119 patients; most frequent were breast, prostate and colorectal cancer, as well as glioblastoma	CBD (synthetic) 10–30 mg 2 × daily (administered 3 days on, 3 days off) for ≥6 months (28 patients with CBD as the only treatment); in some cases, nabiximols was administered as conjunctive treatment (2 sprays 2 × daily, in parallel with CBD, equivalent to 10.8 mg THC + 10 mg CBD)	Clinical responses were seen in 92% of the 119 cases; patients receiving continuous dosing did not do as well as those receiving this on/off repeating regimen. Some patients reverted to using cannabis oil bought on the internet, and 80% of these cases subsequently relapsed	[76]
Squamous cell cancer of the right buccal cavity, 44-year-old male	0.5–1.0 g dried cannabis/day (8.21% CBD, 7.25% THC), vaporized every 2–4 h and 15 min before the patient's daily wound dressing change; following resection and radiochemotherapy in the years before; when trismus and oral cutaneous fistula developed, the use of vaporized cannabis became technically difficult and was replaced by topical treatment (8.02% CBD, 5.24% THC)	The size of the malignant wound decreased by about 5% over the first 4-week interval; pain relief was so significant that the patient was able to discontinue pregabalin and dexamethasone while reducing hydromorphone to approximately 25% of his premedical cannabis dosage	[127]
Hodgkin lymphoma, stage IIB, 21-year-old pregnant female	History of Hodgkin lymphoma with incomplete remission after radiochemotherapy, 5 years later, the patient became pregnant; at 26 weeks of pregnancy, the patient began, on her own, a treatment with 'cannabis oil', supposed to be THC-predominant (1–5 mL 3 × daily, concomitant to opioids)	MRI scan revealed progression of disease; after starting cannabis, pain and general status improved and the tumour tissue decreased. The patient delivered a boy by C-section at 34 weeks who presented with withdrawal syndrome and intestinal invagination in the first 24 h postpartum, requiring care in the NICU and surgery with bowel resection	[128]
Acute lymphoblastic leukaemia, positive for Philadelphia chromosome mutation, 14-year-old female	Five different THC-rich extracts ('Rick Simpson oil') over a period of 78 days after unsuccessful bone marrow transplantation and chemotherapy	Extracts reduced blast cells but differed in their effects and adverse effects. With each new extract, the dose had to be adjusted again, starting with a lower dose; in parallel, blast cells increased. The appropriate dose was identified by the observation of adverse effects (euphoria, panic, appetite, nausea, fatigue) as guidance; the patient passed away due to the bowel perforation as a late effect of chemotherapy	[48]
Basal cell carcinoma, recurrent (nose), 74-year-old male	THC-rich extract (exact composition unknown), topical application 4 × daily for 2 weeks	After repeated surgical interventions, skin grafts and radiotherapy over the last 13 years, the malignant lesion completely disappeared within 2 weeks of daily treatment	[7]

CA125 cancer antigen 125, CBD cannabidiol, CT computed tomography, MRI magnetic resonance imaging, NICU neonatal intensive care unit, PO oral, THC tetrahydrocannabinol, THCA tetrahydrocannabinolic acid, TMZ temozolomide

<6 months (with reasons), or characteristics of patients who relapsed. It is therefore difficult to attribute the therapeutic success solely to CBD.

The publication describes six cases in more detail—four with breast cancer, one with prostate cancer and one female patient with oesophageal cancer, all receiving treatment with CBD alone and who demonstrated the most impressive response. In addition, the case of a 5-year-old boy with an anaplastic ependymoma, a rare form of brain tumour, who had exhausted standard treatment (twice surgery, chemotherapy, radiotherapy) and who experienced a reduction of his tumour by ~60% with CBD, is briefly described [76]. An updated description of this particular case, based on a publicly accessible interview with his father, is of interest as treatment was switched from pulse dosing with pure CBD to daily dosing with an extract. Details are provided below.

The boy, named William (as has been revealed from interviews with his father), has received much publicity over the years (e.g., <https://www.theextract.co.uk/health/pain/interview-father-of-tumour-sufferer/>). In 2014, at the age of slightly above 1½ years, William was diagnosed with an anaplastic ependymoma, Grade 3, of the 4th ventricle, the size of a golf ball. The tumour was resected, and chemotherapy, maintained for 9 months, was started. In late 2015, about 1 year after chemotherapy, the tumour was growing again and was surgically removed for a second time, followed by a 6-week course of radiotherapy. Around March 2016, the tumour had resumed growth and the family looked desperately for alternative treatments. Via a private clinic, the child received synthetic CBD oil at a dosage of only 5 mg CBD once daily for 3 days on and then 3 days off in parallel with a ketogenic diet. Not only did the child become more alert, but the magnetic resonance imaging (MRI) scan showed that the tumour had shrunk by more than two-thirds in comparison with 6 months earlier. However, in late 2018, the tumour had grown again slightly, and even significantly more 3 months later. In March 2019, the child underwent surgery for the third time, followed by further chemotherapy. At this time, the low-dose pulse treatment with synthetic CBD was stopped and replaced with a CBD extract (30 mg daily of a ‘full spectrum CBD oil’, likely containing 5.6% CBD, however the exact composition is unknown). Since then, the scans are stable and no growth of the tumour has been observed (latest update January 2021, ~7 years after diagnosis; <https://makewilliamwell.com/>).

Highly purified phyto-CBD (99.8%, mainly 200 mg twice daily) concomitant to standard radiochemotherapy with temozolomide was used in the treatment of 15 unselected, consecutive patients with GBM (see the study by Likar et al. [77]). Seven (46.7%) patients have now been living for at least 24 months, and four (26.7%) for at least 36 months; this is more than twice as long as has been previously reported in the literature. The mean overall survival is currently 24.2

months (median 21 months) and the 1-year survival rate 87%. The same authors had previously reported improved survival in a smaller cohort of nine consecutive patients with brain tumours (six with GBM grade IV) [78]. As with the previous case series, the heterogeneity of the patient population, the relatively small number of patients, and the lack of a control group are factors limiting definite conclusions.

Another publication describes the case of two male patients with brain tumours—one with GBM and the other with a grade III oligodendroglioma. Both patients underwent a partial surgical resection of tumours (both tumours were MGMT-methylated, with a mutation of IDH-1, indicative of chemotherapeutic resistance and enhanced cancer cell growth). The first patient developed temozolomide resistance and was administered chemoradiation with six cycles of PCV (procarbazine, lomustine, and vincristine) associated with a CBD regimen (300–450 mg/day; capsules made with hemp oil with <0.3% THC). Pseudoprogression (increased oedema, inflammation, extensive contrast enhancement and hypoperfusion), a marker of treatment response, was resolved within a short period. The second patient, also relapsing after temozolomide, was treated with a similar regimen of six cycles of PCV associated with CBD (100–200 mg/day). The patient also demonstrated a marked remission, which the authors considered as uncommon in both cases [79].

Pancreatic cancer is another example of a very aggressive and ‘orphan’ cancer, with an incidence of about 5/100,000. Pure phyto-CBD (99.8%, 200 mg twice daily) concomitant with standard chemotherapy improved the survival of nine consecutive, unselected patients with advanced, metastatic pancreatic cancer [80]. Whereas the overall survival reported in the literature for metastatic disease is 5.9 months [81], the mean overall survival of patients receiving CBD as additional treatment was almost twice as long (11.5 months). As noted previously, a collection of cases, widely differing in their characteristics, diagnosed at various stages of disease, and who also received other medications, does not have the same level of evidence as the results from controlled clinical trials that aim to include an homogeneous population. This limits definite conclusions regarding the therapeutic contribution of CBD.

Several other articles describe cases that have been treated with ‘CBD oil’, assumed to be extracts with CBD as the predominant cannabinoid. Compared with pure CBD, the lack of an appropriate characterization of the components of such extracts, as well as of the amounts administered, renders conclusions about the potential effects of CBD/cannabinoids even more difficult. Moreover, it cannot be excluded that patients who turn to cannabinoids may also take other alternative medicines, often without telling their physicians or only doing so long after initiation.

An 81-year-old female with a metastatic, low-grade, ovarian carcinoma, accidentally diagnosed during surgery (CA125 value 77 U/mL) received one drop of CBD oil (composition not reported) sublingually each evening concomitant with laetrile 500 mg four times daily. Chemotherapy was declined. The CA125 value dropped from borderline 46 U/mL after surgery to 22 U/mL after 1 month of CBD [82]; treatment was maintained. Assuming a volume of at least 35 μ L per drop and a concentration of 10% CBD, 1 drop contains about 3.5 mg of CBD. Repeated computed tomography (CT) imaging showed a dramatic reduction in the patient's disease burden, with near complete resolution of all previously identified lesions 7 months after surgery. CA125 values remained low at around 12 U/mL. The authors related this striking response to her intake of CBD. It was very likely tumour resection (CA125 value had considerably dropped after surgery) had a major effect on the further course of the disease, irrespective of CBD.

An 81-year-old male with biopsy-confirmed adenocarcinoma of the lung was administered a regimen with 2% CBD oil (1.32 mg CBD twice daily) 11 months after diagnosis. The tumour was progressive at that time. CBD, which was the sole therapy, was increased to 6 mg twice daily after 1 week. CT imaging 4 months later revealed near total resolution of the left lower lobe mass and a significant reduction in the size and number of mediastinal lymph nodes (stable according to a CT control 2 months later) [83].

Another article reported the case of a subject with terminal, biopsy-confirmed lung cancer. The patient, a 53-year old male, had a history of intense alcohol and drug abuse and repeated injuries to his spine after multiple car accidents. He suffered from very severe pain, insomnia, post-traumatic stress disorder, anxiety and depression, with a loss of bladder control in parallel. In a last attempt to save or improve his life, the patient joined Alcoholics Anonymous, where one of his fellow members advised him to inhale vaporized cannabis oil. To his surprise, he was not only able to stop substance abuse but also his lung cancer disappeared within about 3 months of inhaling vaporized cannabis oils (composition unknown) on a daily basis. He died from cardiac failure about 1 year later [84].

A similar observation has recently been published [85]. A female in her 80s was diagnosed with non-small cell lung cancer (tumour size 41 mm, with no evidence of local or further spread). As the patient refused treatment, she received only regular CT scans every 3–6 months. Surprisingly, a progressive shrinking of the tumour was observed over time, which reached a diameter of 10 mm 32 months after diagnosis, despite the fact that the patient continued to smoke (estimated 68 packs of cigarettes/year). Discussions with the physicians revealed that the patient had taken 0.5 mL of 'cannabis oil' two to three times daily since her diagnosis (20% CBD, 19.5% THC, 24% THCA, according to

the supplier), i.e. ~200–300 mg of CBD and THC per day. Other treatments the patient has been prescribed (for mild chronic obstructive pulmonary disease, osteoarthritis, and high blood pressure) cannot explain the shrinkage of the tumour by 76%.

Although no direct effects on the tumour have been reported, the case of an 11-year-old female with a pilocytic astrocytoma located in the medulla oblongata, diagnosed at the age of 4 years and treated with chemotherapy, has also been reviewed. Cerebellar pilocytic astrocytoma are a less malignant form of brain tumour, but, due to their location, they are usually accompanied by a range of neuropsychological sequelae; in this particular case, by mood instability, irritability, memory deficits, fatigue, nausea, decreased appetite and dysarthria. Multiple courses of chemotherapy in the past had left residues, which presented as a cyst. MRI showed stable conditions for 5 years from the time CBD was started, as well as during the entire period of treatment with CBD thereafter. Parents self-initiated treatment with over-the-counter CBD of unknown composition (likely 'CBD oil'), which considerably improved symptoms but was stopped for financial reasons; this was followed by worsening of irritability, fatigue and reduced appetite. Pharmacy-grade CBD (purity 99.8%, 200 mg/day for 3 months) was reintroduced, this time under medical surveillance, and improvement in, most notably, cognitive functions, working memory, behaviour and quality of life was observed [86].

The first clinical study in humans was a pilot phase I trial with THC in 9 patients who had recurrent GBM. All of these patients had previously not responded to standard therapy (surgery and radiotherapy) and had clear evidence of tumour progression at the time they received pure THC locally. An aliquot of a THC solution (100 mg/mL in ethanol) was dissolved in 30 mL of physiological saline solution supplemented with 0.5% (w/v) human serum albumin and infused into the resection cavity, at days 3–6 after surgery. After 20–40 mg on Day 1, the dose was progressively increased for 2–5 days up to 80–180 mg/day. The median duration of an administration cycle was 10 days; five patients received more than one cycle. In three of these five patients, a temporary reduction in tumour proliferation was observed. THC administration was well tolerated without overt psychoactive effects. Median survival of the cohort from the beginning of THC administration was 24 weeks [87].

In a two-part clinical study, six patients with recurrent GBM received standard chemoradiotherapy treatment, as described by Stupp et al. [88], followed by dose-intense temozolomide combined with nabixiol (a 1:1 CBD:THC oromucosal spray) to assess the maximum tolerated dose (12 sprays per day) and the safety of the combined treatment. In part two, 12 patients were randomized to CBD:THC (daily dose up to 32.4 mg THC + 30 mg CBD, i.e. 12 sprays)

and 9 patients were randomized to placebo (mean age 58 years, baseline median Karnofsky score 90 in both treatment groups). Median time from diagnosis of recurrence to the start of treatment (Day 1) was similar (3.6 and 3.0 weeks in the CBD:THC and placebo groups, respectively); however, there were relatively more males in the placebo group (5/12 vs. 8/9). The median number of days of dosing with CBD:THC or placebo was similar (155 days [range 50–356] and 134 days [range 13–359], respectively). Median survival in the CBD:THC treatment was >550 days, and 369 days in the placebo group (difference not significant); 1-year survival was 83% in the CBD:THC group and 56% in the placebo group ($p = 0.042$). Progression-free survival at 6 months was 42% in the CBD:THC group and 33% in the placebo group (not significant). Overall, the most common treatment-related adverse events were dizziness (11/18 patients) and nausea (7/18 patients) [89].

Although not planned as a therapeutic study, the effects observed with nabiximols in patients with a leukemic indolent B-cell lymphoma without treatment indication is of interest. Fifteen patients received the maximum tolerated dose containing 18.9 mg THC and 17.5 mg CBD as a single dose, and the effects on lymphocyte counts were measured after 2, 4, 6, 24 and 168 h in comparison with a non-treatment control day. A significant time-dependent decrease in lymphocyte counts (clonal B cells and lymphocytes to a similar extent) was observed with the nadir usually at 4 h after drug administration. A week after administration of nabiximols, all non-malignant lymphocytes had returned to baseline levels, but the clonal B cells had significantly increased. This transient decrease was not due to increased cell death, as measured by activated caspase-3, but was very likely to be a ‘homing’ of lymphoma B cells from blood into secondary lymphoid organs where they receive prosurvival signals. Therefore, the authors advise caution with nabiximols in patients with indolent leukemic lymphomas [90].

A recent publication presents the case of a pregnant patient with Hodgkin’s lymphoma (HL) who self-initiated topical and oral therapy with THC-rich cannabis oil (exact composition unknown) at 26 weeks of her second pregnancy. HL had been diagnosed 5 years previously. At that time, chemotherapy with adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) for 6 months, and radiotherapy (20 sessions of 20 Gy), achieved incomplete remission, with lymphoma tissue about 2 cm in diameter persisting in the thorax. Radiochemotherapy was poorly tolerated and the patient refused further treatment.

During the patient’s first pregnancy, an MRI scan revealed an HL of about 15 × 13 cm with left lung involvement, posterior and anterior mediastinal extension with anterior right pleural–pulmonary determination and extension to the anterior right ribs. Chemotherapy was refused. A healthy developed male was born by caesarean section at 37 weeks.

While still breastfeeding, the patient became pregnant again. As termination of the pregnancy was refused by the patient, disease progressed further, with terrible chest pain and numerous complications. At 26 weeks of pregnancy, the patient began topical cannabis oil application on the supraclavicular tumour and oral treatment with cannabis oil of unknown composition but obviously rich in THC, between 1 and 5 mL, three times daily. Remarkably, this not only reduced pain significantly but also the dimensions of her supraclavicular tumour, and improved the patient’s quality of life. Although the patient’s condition worsened, she delivered a male newborn, weighing 2380 g, by caesarean section at 34 weeks’ gestation. Postpartum, the mother’s disease progressed to HL stage IVB. Since her second pregnancy in 2015, the patient has received various treatments with chemo- and immune therapeutics, as well as a bone marrow autologous stem cell transplant. As at December 2019, the patient was still alive and possibly continued to use cannabis.

Also of particular interest is the case of a 14-year-old female with terminal acute lymphoblastic leukaemia (ALL) with a Philadelphia chromosome mutation, a very aggressive form of ALL, diagnosed in March 2006. When standard treatment options were unsuccessful, the patient received a bone marrow transplant 6 months after diagnosis. Six months later, aggressive chemotherapy was again initiated along with imatinib mesylate, a tyrosine kinase inhibitor, 500 mg orally twice daily. Nine months after the transplant, the presence of premature blast cells was observed. In February 2008, 23 months after diagnosis, another tyrosine kinase inhibitor, dasatinib, was administered at 78 mg twice daily with no additional rounds of chemotherapy. Five months later, cerebellitis was noted after conducting a CT scan, and 10 treatments of radiation therapy were administered to the brain. Almost 3 years after diagnosis, blood was noted in the patient’s stools and a blood cell count revealed the presence of blast cells. All treatment was then suspended. Within about 2 weeks, the patient’s blast cell counts rose from 51,490 to 194,000. At this time, the patient’s family decided to administer a THC-rich extract (‘Rick Simpson oil’), in increasing daily doses. Blast cells decreased from a peak of 374,000 to 61,000 after 15 days; common adverse effects of THC, such as an increase in euphoria symptoms, a disoriented memory, an increase in alertness, and decreased use of morphine for pain were observed in parallel. The original extract had been consumed after 2 weeks and a new extract from a new cannabis cultivar was started. However, with the same dose as before, a decreased response in terms of the adverse effects of euphoria and appetite was noted; blast cells began to increase again to a peak of 66,000. After increasing the daily dose, blast cell counts decreased rapidly. Four weeks later, the second extract had been consumed and a new, third and fourth batch of another cannabis

cultivar was started, with more pronounced adverse effects that necessitated a reduction in dose. As blast cells began to increase, dosages were also increased. The level of blast cells could be maintained at around 0.5–0.6 per thousand. This extract was used up after 25 days, 68 days after the initial administration of cannabis oil. At this time, blast cells had increased again to 79,000. A new batch, batch number 5, was administered and blast cells decreased very rapidly as before. However, on Day 78, gastrointestinal bleeding occurred and the patient passed away. Neutropenic colitis with perforation was diagnosed as the cause of death, very likely resulting from the aggressive chemotherapy the patient had received previously [48].

Another case report describes a 44-year-old male with a painful, non-healing malignant, exophytic wound (recurrence of squamous cell cancer of the right buccal cavity treated surgically and with radiochemotherapy 3 years earlier). Despite using high-dose hydromorphone, pregabalin, and dexamethasone, the patient continued to experience continuous (background) generalized right hemifacial pain along with volitional incident pain (wound-related procedural pain) occurring with wound dressing changes, rated by the patient as 9 out of 10 on a daily average. After starting to inhale medical cannabis (MC; THC 7.25% + CBD 8.21%, 0.5–1.0 g/day, vaporized every 2–4 h and 15 min before the patient's daily wound dressing change), the patient's pain improved significantly. He was able to discontinue pregabalin and dexamethasone while reducing hydromorphone to approximately 25% of his pre-MC dosage. Despite continued vaporizing, the patient's malignant wound increased in size. Therefore, topical treatment with 1–2 mL of MC (THC 5.24% + CBD 8.02%) dissolved in sunflower oil, applied both externally and intrabuccally four times daily on the entire malignant wound, was started. With this treatment, the size of the patient's malignant wound decreased by about 5% over the following 4 weeks. Unfortunately, the initial success was not maintained. After acute hospitalization for hypovolaemia and interruption of MC, the patient passed away.

Grotenhermen [7] reported the successful topical treatment of a basal cell carcinoma with a THC-rich cannabis extract (exact composition not reported). The patient, a 74-year-old male, had a recurrent basal cell carcinoma on his nose, diagnosed and surgically treated 13 years earlier, followed by resection, skin transplant and radiation on two further occasions. After topical application of a THC-rich extract four times daily, the tumour completely disappeared within 2 weeks. Ten months later, the patient was still free of recurrences. Impressive pictures were presented by Grotenhermen at the Cultiva Congress in Vienna, October 2017.

Such observations with extracts have inherent limitations, on the one hand due to the insufficient characterization of components and their dosages, and on the other hand,

because of the limited information provided with most of these case reports. Nonetheless, the number of observations cannot be completely ignored as these articles were written by medical professionals. In line with *in vitro* and *in vivo* results of experiments with various cannabinoids, anticancer effects in man seem to be at least plausible. In short, 16 articles report the anticancer effects of CBD and other cannabinoid preparations against various malignancies in approximately 180 patients; 5 publications report the use of pure CBD, and 11 others report the use of (medical) cannabis, THC or extracts. Remarkably, oral daily doses of CBD for adults varied widely over a range of 20–600 mg/day, which is within the range of human-equivalent doses used in animal experiments.

Five of 16 publications independently reported positive effects on brain tumours. As no comparative trials between cannabinergic products exist in man in contrast to preclinical studies, a therapeutic advantage for a specific substance, combination or product cannot be delineated. A majority of patients with GBM has been treated with CBD concomitant with standard radiochemotherapy. A closer look at the survival data shows that those patients who received CBD 200 mg/day have survived for a mean of 10.5 months (four patients) compared with a mean of 24.2 months for the entire cohort of 15 patients [77].

All articles described some benefit, such as an extension of survival or tumour regression; however, bias in reporting patients who improved with cannabinoids cannot be excluded, particularly for case reports. Currently, there is no unambiguous evidence for a definite long-term 'cure', as maintained in social media. Conversely, no prominent harmful effects have been reported in any of these articles. As for extracts, it has to be kept in mind that each has its own characteristics in terms of composition, anticancer potency and adverse reactions [91]. Caution is therefore advised as results may not be reproducible with another extract.

6 Mechanism

The exact mechanism by which CBD exerts its anti-tumour action is still a mystery and cannot be pinned down to just a few targets. In contrast to THC, which is an agonist on CB1 receptors and, although weaker, on CB2 receptors, CBD does not bind significantly to either receptor. A recent review identified 76 different molecular targets of CBD, most being ion channels/ionotropic receptors or enzymes [92]. Furthermore, CBD affects the expression of a number of genes involved in zinc (Zn) homeostasis; the regulation of Zn levels plays a crucial role in antioxidant

and anti-inflammatory processes. One study found 1204 gene transcripts that were significantly up- or downregulated by CBD, many of them being directly involved in Zn homeostasis, whereas only 94 gene transcripts were regulated by THC [93].

CBD also interacts with a wide range of transient receptor potential ion channels known to play a role in carcinogenesis, such as TRPA1, TRPM8, TRPV1, TRPV2, TRPV3, TRPV4, as well as the voltage-dependent anion channel VDAC1, the peroxisome proliferator-activated receptor- γ (PPAR γ) and the G-protein coupled ‘orphan’ receptor, with GPR55 being the most relevant [37, 94, 95]. CBD acts as an agonist of PPAR γ , but is an antagonist of GPR55. Activation of PPAR γ suppresses nuclear factor- κ B (NF- κ B), which is constitutively active in cancer cells and is responsible for cancer cell proliferation and the formation of inflammatory cytokines such as tumour necrosis factor (TNF)- α . In addition, overall effects are further influenced by endocannabinoids (anandamide, 2-arachidonoylglycerol), which demonstrate anticancer activities of their own [96–100]. Anandamide and 2-arachidonoylglycerol bind to both receptors (CB1 and CB2; anandamide more to CB1 than CB2, and 2-arachidonoylglycerol more to CB2 than CB1). Most intriguingly, CBD is able to interfere with fatty acid amide hydrolase (FAAH) and to increase blood levels of anandamide. CBD also inhibits, concentration dependently, ID-1, which controls cancer invasiveness and metastasis [64, 101]. On a molecular basis, CBD is able to induce programmed cell death by autophagy as well as by apoptosis.

Compared with healthy tissues, the expression of cannabinoid receptors is altered in tumours [102], and the effects of cannabinoids therefore vary among cancer cell lines. A detailed review of the mechanisms is beyond the scope of this article; the complexity of the ECS in cancer has recently been reviewed elsewhere [103].

7 Circadian Rhythmicity and Dosing Schedule

Most biological processes, including the endocannabinoid and immune systems, are subject to circadian rhythmicity. In healthy adults, endocannabinoid blood levels demonstrate marked circadian rhythmicity, with 2-arachidonoylglycerol levels more than three times higher around 12 noon to 1.00 pm than at 3.30–4.30 am [104]. Rhythmicity was also observed for anandamide, with a first peak occurring during early sleep (2.00 am) and a second peak occurring in the mid-afternoon (3.00 pm), while a 1.5-fold lower nadir was detected in the mid-morning (10.00 am) [105]. Theoretically, administering CBD once daily at noon would increase anandamide peak levels in

the mid-afternoon. Day/night-dependent changes have also been observed for the expression of CB1 and CB2 receptors in liver tissue of rats, with the highest amount of RNA during the resting (light) phase [106]. Circadian rhythmicity can be disrupted by a number of conditions, such as obesity, after intensive exercise, after sleep restriction, and in tumour cells where the circadian clock may even be suppressed.

In addition to the combination of cannabinoids, their dose timing and dosage schedule possibly influence the results. Two *in vitro* studies suggest that an intermittent dosage of CBD could decrease the viability of cancer cell lines better than continuous dosing [42, 107]. Until now, only one publication has also described the application of such a ‘pulse dosing’ regimen in man [76]. Remarkably, low daily doses of CBD have been administered according to a ‘3 days on, 3 days off’ schedule and were considered to be successful.

8 Conclusions

A major goal in cancer therapy is to kill malignant cells via the natural process of apoptosis, avoiding eventual ‘recyclation’ of cancer cells by autophagy. In addition to tumour-selective action of agents, this requires intact immune competence of the host.

In diseased subjects, the ECS is dysregulated. As an example, although the results were somewhat conflicting, anandamide levels seemed to be lower and 2-arachidonoylglycerol levels were upregulated in glioblastomas [108]. Glioma invasiveness has been linked to the tumour suppressor p38 MAPK; the anti-invasive effect of CBD interferes with this pathway [109]. Increased expression and activity of p38 MAPK correlates with poor prognosis in GBM. Intriguingly, the levels of phosphorylated p38 MAPK are significantly reduced in clock-deficient glioma cells, indicating that the circadian clock plays an important role in the activation of this pathway [110]. Other big players on inflammation and cell survival, such as PPAR γ , also exhibit circadian expression.

In vitro, a dose-dependent increase in the apoptotic effects of CBD in glioblastoma and other human carcinoma cell cultures has been repeatedly reported whereby the ‘apoptotic threshold’ likely varies, not only between different cancer cell types but also between cannabinoids. Intriguingly, in a few experiments and in a few animals, complete eradication of tumour cells has been observed with CBD [64] as well as with THC [68]. However, it is worth noting that there are distinct differences between animal experiments and patients. Tumours in animals are artificial, whereas they are

spontaneous in man. Cannabinoids have been administered during the day, which corresponds to the rest/sleep phase in mice and rats; products are most often injected once daily in animals, resulting in 100% bioavailability, and the frequency of applications varied between two and seven times per week. In contrast, oral bioavailability in man is low and administration is usually on a continuous, twice-daily basis. Patients referred to in the studies by Likar et al., as an example, have been advised to take CBD daily after breakfast and after the evening meal. In any case, the problem of translatability from preclinical results to therapy in man remains; there are considerable physiological differences between humans and animals that impact drug effects.

The circadian rhythmicity, briefly mentioned above, particularly of the endocannabinoid and immune system, suggests that an optimal daytime period may exist during which cannabinoids have the highest impact on cancer cells; however, our knowledge of exactly when during the diurnal cycle rhythmically expressed targets are most sensitive is still insufficient. In cancer patients, the delivery of cytotoxic agents such as 5-fluorouracil or cisplatin at early night-time [111, 112] or bortezomib at the beginning of the rest (sleep) phase [113] was shown to improve treatment efficacy and tolerability. Similarly, docetaxel, another proapoptotic drug, displayed least toxicity and highest antitumour efficacy following dosing during the rest/sleep phase in mice [114]. On the other hand, temozolomide administration in the morning may improve the survival of patients with MGMT-methylated glioblastoma, according to a recent retrospective analysis [115]. It has to be stressed that any optimized intervention would need to be timed to the patient's individual biological diurnal rhythm (personal sleep cycle). The right timing of drug administration may be as important as the right dose.

There is mounting evidence from preclinical studies that cannabinoids are effective against cancer cells. An increasing number of in vitro studies have described not only the cytotoxic effects of cannabinoids against numerous cancer cell lines but also putative mechanisms that finally lead to an inhibition of metastasization, angiogenesis, tumour growth, enhancement of autophagy, and, ultimately, to apoptosis of cancer cells. Observations in man support the idea of a potential life extension of cancer patients. Nonetheless, there is currently no medical proof of a long-term cancer cure in man. Although sensitivities vary between cell lines and vary in the dependence of the nature of the cannabinoid, most articles report dose- (or concentration-) dependent effects. In general, CBD has demonstrated a favourable overall efficacy and safety profile, with a potency that has exceeded, in many in vitro tests, that of its psychotomimetic counterpart THC and of extracts. Combinations of cannabinoids have been reported to increase potency further, although the optimal

ratio between cannabinoids is variable and depends on the cancer cell line. With fixed combinations of CBD and THC, the pronounced psychotomimetic effect of THC would limit the use of higher dosages.

Caution is advised against the indiscriminate use of products ('CBD oils', 'cannabis oils') from the internet; the large majority of them are insufficiently characterized. According to a retrospective analysis of US FDA warning letters to CBD product-promoting companies, 87.2% of the letters cited unapproved therapeutic claims in cancer [116]. Products are often extracted and processed without adhering to good manufacturing practice principles and without adequate oversight; product consistency and quality is not assured, as it is for products of pharmaceutical grade. Often, the concentration of cannabinoids and the composition of such extracts is unknown, and differences between chemotypes, batch variations, even of the same chemotype, may have unpredictable consequences. In the worst-case scenario, the uncontrolled use of such extracts can induce unexpected, severe intoxication, particularly if administered in high dosages, as is often the case for cancer patients [117–119]. Caution is also advised against the use of cannabis by cancer patients during immune therapy with monoclonal antibodies, as this may result in a decrease in time to tumour progression and decreased overall survival [120].

Systematic, well-designed clinical trials are necessary to confirm preclinical results and improve cancer treatment with cannabinoids.

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