Inhibitors of lipogenic enzymes as a potential therapy against cancer

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Abbreviations

FA, Fatty acid; PL, Phospholipid; LPL, lysophospholipids; CoA, Coenzyme A; DAG, Diacylglyceride; TG, Triacylglyceride; LD, Lipid droplets; ATP, Adenosine triphosphate; DNL, *De novo* lipogenesis; AKT, Protein kinase B; LPA, lysophosphatidic acid; PA, phosphatidic acid; ACLY, ATP-citrate lyase; ACC, Acetyl-CoA carboxylase; FAS, Fatty acid synthase; MAGL, Monoacylglycerol lipase; OAA, oxaloacetate; PI3K, Phosphoinositide 3-kinase; SREBP-1, sterol regulatory element-binding protein 1; ACSS2, acyl-CoA synthetase short-chain family member 2; AMP, Adenosine monophosphate; AMPK, AMP-activated protein kinase; hACLY, Human ACLY; LDL-C, Low-density lipoprotein cholesterol; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; RTK, receptor tyrosine kinase; STAT3, signal transducer and activator of transcription 3; MAPK, Mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; SAR, Structure–activity relationship; BC, biotin carboxylase; CT, carboxyltransferase; BCCP, biotin carboxyl carrier protein; CPT1, carnitine palmitoyltransferase 1; ChREBP, Carbohydrate-responsive element-binding protein; CAs, α-carbonic anhydrases; PPAR, peroxisome proliferator-activated receptor; HER2, human epidermal growth factor receptor-2; H-bond, hydrogen bond; rACC, rat ACC; GPAT1, glycerol-3-phosphate acyltransferase; VLDL, very low-density lipoprotein; NSCLC, non-small-cell lung cancer; hFAS, Human FAS; TE, Thioesterase domain; ACP, Acyl-carrier protein domain; KR, β-ketoacyl reductase domain; ER, Enoyl reductase domain; DH, β-hydroxyacyl dehydratase; MAT, Malonyl/acetyl transferase; KS, β-ketoacyl synthase; YKR, pseudoketoreductase; YME, pseudomethyltransferase; NADPH, Nicotinamide adenine dinucleotide phosphate; EGF, Epidermal growth factor; SREBP-1c, Sterol regulatory element-binding protein 1; USP2a, Ubiquitin-specific cysteine protease 2a; FFAs, free fatty acids; MAGs, monoacylglycerols; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PA, phosphatidic acid; PGE2, Prostaglandin E2; MAGE, Monoalkylglycerol; 2-AG, 2-arachidonoylglycerol; CB1, Cannabinoid receptor type 1; CB2, Cannabinoid receptor type 2; FAAH, fatty acid amide hydrolase; ABHD6, α/β hydrolase domain 6; ABHD12, α/β hydrolase domain 12; hMAGL, human MAGL; HCC, hepatocellular carcinoma.

Abstract

Cancer cells rely on several metabolic pathways such as lipid metabolism to fulfill the increase in energy demand, cell division and growth and successful adaptation to challenging environments. Fatty acid synthesis is therefore commonly enhanced in many cancer cell lines. Thus, relevant efforts are being made by the scientific community to inhibit the enzymes involved in lipid metabolism to disrupt cancer cell proliferation. We review the rapidly expanding body of inhibitors targeting lipid metabolism, their side effects, and current status in clinical trials as potential therapeutic approaches against cancer. We focus on their molecular, biochemical, and structural properties, selectivity and effectiveness and discuss their potential role as antitumor drugs.

Keywords

Cancer drugs, lipid metabolism, lipogenic enzyme inhibitors

Introduction

Many types of cancers are detected each year around the globe. According to the World Health Organization cancer is the second cause of death, bringing a disturbing number of 9.6 million deaths in 2018 (1). Cancer is a disorder of cell growth and proliferation that requires high amounts of energy and cellular building blocks including nucleic acids, proteins, and lipids (2). Lipids comprise a wide group of biomolecules make-up of fatty acids (FAs) of different chain length, number and location of double bonds, and backbone structure (3). Lipid metabolism is of special interest in cancer therapy because lipids are involved in multiple biochemical processes during cancer initiation and development (2). Lipids participate in the growth, energy and redox homeostasis of cancer cells. Moreover, they have structural roles as passive components of cell membranes, like cholesterol and sphingolipids that are important components of membrane rafts (2, 4). Furthermore, they initiate some signal transduction cascade processes and also can be broken down into bioactive lipid mediators that regulate cancer cell growth, migration, and metastasis formation (4–6).

The high rate of cancer cell proliferation requires an accelerated synthesis of lipids for the generation of biological membranes (2, 5, 6). The metabolic cycles of cancer cells are altered, due to a series of oncogenic events and the tumor microenvironment, to satisfy the

energy and lipids requirements (6, 7). The most well-known perturbation in the metabolic cycle of cancer cells is the Warburg effect, which implies an increase in glucose uptake and the use of aerobic glycolysis (*Figure legends*

Figure 1) (8). Other important perturbations enhanced as part of cancer-associated metabolic reprogramming includes the biosynthesis of proteins, nucleic acids, and lipids (3, 6). Specifically, lipid biosynthesis is induced as part of the anabolic metabolism of cancer cells and it is the process to convert nutrient-derived carbons (that normally are an energy source) into FAs (*Figure legends*

Figure 1) and cholesterol (3, 4, 6).

Lipids' role in cancer

The main building blocks of cell membranes are phospholipids (PLs), sterols, sphingolipids and also lysophospholipids (LPL) (*Figure legends*

Figure 1). All of them are derived from acetyl-CoA and many contain FAs (3). The FAs structure consists of a terminal carboxyl group and a hydrocarbon chain (usually with an even number of carbons) that can be saturated or unsaturated (3). FAs can be used to generate many different types of lipids including diacylglycerides (DAGs) and triacylglycerides (TGs); this last one is mainly used for energy storage in the form of lipid droplets (LDs) (2, 4, 6). Moreover, DAGs and TAGs are synthesized via glycerol phosphate pathway, which uses the glycolytic intermediate glycerol-3-phosphate to form the glycerol backbone of these lipids; the intermediates in this process can be converted into different phosphoglycerides that are the major structural components of biological membranes (3, 6).

Cancer cells can obtain FAs either from exogenous sources or from *de novo* lipogenesis (DNL) (2, 3). In the presence of oxygen and abundant extracellular nutrients, most cancer cells synthesize FAs *de novo* (*Figure legends*

Figure 1); but under conditions of metabolic stress, they collect extracellular lipids as an adaptation to survive (9). This adaptation implies a reduction of the carbon supply and power for the FAs synthetic pathway (7). Moreover, FAs can be used as an energy source when mitochondrial oxidation (β-oxidation) occurs (*Figure legends*

Figure 1); they produce more than the double of ATP per mol when compared to the glucose or aminoacids oxidation (2–4, 6, 7). Consequently, some cancer cells prefer to use FAs as an energy source even under nutrient-replete conditions (7).

Another important biosynthetic process within lipid metabolism is the mevalonate pathway, which facilitates the synthesis of cholesterol (2, 4, 6). Cholesterol is one of the main components of biological membranes, as it modulates the fluidity of the lipid bilayer, and also forms detergent-resistant microdomains called lipid rafts, that coordinate the activation of some signal transduction pathways (2, 4, 6). In cancer cells, many signaling proteins as protein kinase B (Akt), and receptors regulating prooncogenic and apoptotic pathways reside in lipids rafts (2). Moreover, the activation of oncogenic signaling pathways only depends on the lipid rafts integrity, therefore, by disrupting them, the activation of the anchored-lipid raft Akt protein is inhibited and the tumor cell proliferation is reduced (10).

Some of the already mentioned characteristics of cancer cells, improve their proliferation and resistance to chemotherapy. TGs and cholesteryl esters are stored in LDs, which are highly ordered intracellular structures formed in the endoplasmic reticulum (4). LDs are typically found in some aggressive cancers as well as high levels of saturated FAs in some aggressive breast cancers (11). Cancer cells have higher amounts of LDs compared with normal tissue, which enhances their resistance to chemotherapy (12). Moreover, the high levels of saturated FAs increase the levels of saturated PLs in cancer cells, reducing the membrane fluidity and protecting cancer cells from oxidative damage (6).

Lipids are also important signaling molecules. For example, phosphoinositides are a family of second messengers that transmit signals from activated growth factor receptors to the cellular machinery (6, 13). Besides, the phosphoinositides act as specific binding sites for the coupling of effector proteins into specific membrane sites (6). Other lipids that act as second messengers are lysophosphatidic acid (LPA), phosphatidic acid (PA) and DAG (6). Moreover, sphingolipids are other important signaling molecules (*Figure legends* **Figure 1**); the simplest of them is ceramide (6). In cancer cells, ceramide mediates growth inhibitory signals and is involved in the initiation of the apoptotic process and growth arrest (6). Furthermore, the enzymes involved in the sphingolipid metabolism pathway are normally deregulated in cancer cells, producing low ceramide levels and the consequently increased resistance to chemotherapy (14).

Besides the already mentioned lipids employment in cancer cells, lipids have an important role in post-translational modification of proteins (6). Palmitate and myristate are saturated acyl chains that are normally (covalently) coupled to proteins and improve the protein interaction with membrane rafts (15). Lipid metabolism is also involved in the autophagic process, which is a mechanism of self-degradation required for the removal of defective

proteins and organelles. Moreover, the autophagic process is favored under conditions of nutrient scarcity and enhances the survival of cancer cells by contributing to the maintenance of energy supply during tumorigenesis (16).

Apart from the importance of lipids in cancer cells proliferation and survival, they are also implicated in other more complex processes as cell migration, invasion, tumor angiogenesis and metastasis formation (6). Finally, the overexpression of lipogenic enzymes (*Figure legends* **Figure 1**), such as ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and monoacylglycerol lipase (MAGL), represent a nearly-universal phenotypic alteration in most tumors and cancer cells (*Figure legends* **Figure 1**) (3, 7). In this review, we will focus on the description of the lipogenic enzymes mentioned above, and some inhibitors of each of them as potential therapeutic targets against cancer.

Lipogenic enzymes' inhibitors

ACLY

Function and description. The ACLY is the key enzyme of the conversion of citrate derived from glycolytic metabolism into acetyl-CoA (*Figure legends* **Figure 1**); which is the starting material of two highly important processes in DNL, the FA synthesis and mevalonate pathway (17). The human ACLY is a homotetramer of about 0.5 MDa, where each polypeptide chain is formed by 1,101 amino-acid residues. It consists of an *N*-terminal citryl-CoA synthetase module and a C-terminal citryl-CoA lyase domain (18, 19). It has five functional domains divided into two subunits. The α-subunit has two domains, the domain 1 binds CoA, and the domain 2 contains the phosphorylated histidine residue. In the β-subunit, the domain 3 and 4 adopt an ATP-grasp fold and bind ATP, and the domain 5 stimulates domain 2 to form a helix dipoles called "power helices" that stabilize the phosphorylated histidine residue at the *N*-terminal domain and arranges the binding site of citrate (20, 21).

In more detail, the ACLY is a cytosolic enzyme that catalyzes the conversion of citrate (transported from the mitochondria) and CoA into acetyl-CoA and oxaloacetate (OAA) in the presence of magnesium complex Mg-ATP (17). Initially, the catalysis starts by autophosphorylation of a histidine residue, resulting in a citryl-phosphate within the active site. Subsequently, a covalent citryl-enzyme complex is produced and is attacked by the CoA

to form the citryl-CoA. Finally, the enzyme catalyzes the cleavage of citryl-CoA to acetyl-CoA and OAA (22).

The ACLY is overexpressed in many different cancer cell lines, stimulated by insulin, growth factors, and high levels of glucose (23). Moreover, the stimulation mechanism occurs through the phosphoinositide 3-kinase (PI3K)/Akt pathways. The Akt pathway upregulates the ACLY by the activation of the sterol regulatory element-binding protein 1 (SREBP-1), which is a transcription factor for genes involved in FA and cholesterol synthesis (22, 24). On the other hand, the PI3K/Akt pathways stimulates the ACLY through its phosphorylation (contributing to the protein stabilization) rather than transcriptional upregulation (17, 25). Also, ACLY is regulated by other pathways depending on the cell line (22, 24). Furthermore, when ACLY is deregulated, the expression of the acyl-CoA synthetase short-chain family member 2 (ACSS2) increases, catalyzing the conversion of exogenous acetate to acetyl-CoA using ATP (18, 24).

Inhibitors and cytotoxic effects. The inhibition of ACLY produces cytotoxic effects by disrupting FA synthesis, similar to the FAS and ACC inhibition (mentioned in next sections) (17, 26). Moreover, ACLY inhibition affects the mevalonate pathway, disrupting the cholesterol and isoprenoids synthesis, and thus, enhancing its cytotoxic effect (17). Furthermore, the ACLY inhibition produces stronger cytotoxic effects in cells with elevated glucose metabolism than in others with a low aerobic glycolysis (27). It evidences that some cancer cells are independent of ACLY to produce acetyl-CoA, like the ones that use ACSS2 (23). Other important effect of the ACLY inhibition, is the increase of intracellular amounts of reactive oxygen species; enhancing the phosphorylation of an important regulator of lipid metabolism, the AMP-activated protein kinase (AMPK) (21).

Several studies have shown that many ACLY inhibitors produce cytotoxic effects against some cancer cell lines, and can be enhanced in combination with other agents that block oncogenic receptor signaling (23). The human ACLY has been widely studied and many potent inhibitors have been developed (Figure 2). First, the **(–)-Hydroxycitric acid** (**1**, Figure 2), it is a naturally occurring compound extracted for the first time from fruits of *Garcinia* (28). It is a competitive inhibitor of ACLY with a Ki value of 300 μM. Some preclinical studies for cancer therapy were performed with this compound, in co-treatment with lipoic acid (a pyruvate dehydrogenase kinase inhibitor) and cisplatin (a classical chemotherapeutic that binds DNA). *In vivo* studies, showed that the combination of those three drugs, by

attacking the altered metabolism and DNA of cancer cells, brings some improvement when compared to cisplatin treatment (monotherapy) (28).

Other natural product, the 2-chloro-1,3,8-trihydroxy-6-methylanthrone (**2**, Figure 2), was found to be a strong inhibitor of hACLY with an IC_{50} of 283 nM (29). Compound **2** was extracted from active microbial metabolite derived from a soil fungus (*Penicillium sp*). Despite promising results, no cytotoxic study was carried out using this compound against cancer cells.

Other compound of natural origin is **Cucurbitacin B** (**3**, Figure 2), found in cucumber among other members of the *Cucurbitaceae* family with tetracyclic triterpenoid structure (30). *In vitro* studies showed that Cucurbitacin B is cytotoxic against different cancer cell lines, including breast SK-BR-3 (IC_{50} = 4.6 μg/mL), MCF7 (IC_{50} = 88.7 μg/mL (31), 48.6 μg/mL), MDA-MB-231 (IC_{50} = 38.9 μg/mL), lung NCI-H460 (about 87% inhibition after 48h with 0.1 μM of drug), central system SF-268 (about 92% inhibition after 48h with 0.05 μM of drug) (30), colon HCT-116 (about 80% inhibition after 48h with 0.4 μM of drug) (32), pancreas PC-3 and LNCaP (IC_{50} ~ 0.3 μM) (33), and hepatocellular BEL-7402 (IC_{50} = 0.32 μM) (34). The mechanism of action of Cucurbitacin B, was linked to several targets and it is not fully elucidated. However, Xiao *et al.* demonstrated *in vitro* and *in vivo* dose depending ACLY inhibition by this compound (33). Additionally, several *in vivo* studies in xenograft rat models showed tumor reduction after treatment with Cucurbitacin B (18, 33). Recently, the pharmacokinetic of Cucurbitacin B was studied demonstrating limited 10% low oral bioavailability, however, large volume of distribution into internal organs (35).

Synthetic inhibitors of ACLY has been extensively reviewed (18), however, it is worth mentioning the most potent and promising compounds (Figure 2). **ETC-1002** or Bempedoic acid (**4**, Figure 2) was developed by Esperion Therapeutics Inc, and is currently in the phase III of clinical trials to reduce low-density lipoprotein cholesterol (LDL-C), and prevent cardiovascular related diseases (36). In the liver, it is converted to its active form (ETC-1002-CoA) by the acyl-CoA synthase. The ETC-1002-CoA inhibits the ACLY completely, reducing the OAA and acetyl-CoA levels to finally disrupt DNL. Moreover, it increases the AMPK activity, inhibiting the phosphorylation of ACC and HMG-CoA reductase, and thus, reducing the glucose and lipid biosynthesis (18).

In 2007 a series of 2-hydroxy-*N*-arylbenzenesulfonamides compounds were reported as strong inhibitors of ACLY (37). The most potent one, was the compound **5** (Figure 2) with an IC_{50} of 130 nM. Although it showed high enzymatic inhibition activity, compound **5** has

weak cytotoxicity (> 50 μM) against human liver carcinoma HepG2 cell line. The biological activity study of this compound was focus once again in decreasing cholesterol and TGs, and not for FAs (37).

Very recently, a novel series of compounds were synthesized with similar structure to compound **5**. Specifically, Wey *et al*. developed the compound **NDI-091143** (**6**, Figure 2)**,** maintaining the benzenesulfonamide connected to biphenyl moiety but substituting one of the chlorides with a methoxycarbonyl group on the phenolic part, and incorporating two fluorine atoms on the biphenyl moiety (38). Those changes improve greatly the inhibitory effect of NDI-091143, and it became the strongest inhibitor of ACLY known so far with a Ki of 7.0 nM and an IC_{50} between 2.1- 4.8 nM. Interestingly, the authors were able to obtain ACLY-NDI-091143 co-crystal structure using a cryo-electron technique and observed that NDI-091143 occupies the polar citrate domain of ACLY; which is surprising because of the lack of carboxylic moieties in the drug. The conformational changes of the amino acid residues, allowed the binding of the drug, leading the authors to conclude that NDI-091143 is the first allosteric inhibitor of ACLY (38, 39). These recent discoveries most likely will permit the finding of novel ACLY inhibitors with similar or even better inhibition activity which is very exciting in terms of anticancer drug discovery targeting lipogenic enzymes.

A natural compound found in the bark of pine trees, is a diterpene amine called **Leelamine** (**7**, Figure 2), it demonstrated cytotoxic activity against many cell lines including melanoma, prostate and breast cancer (40). Leelamine targets key oncogenic pathways including the receptor tyrosine kinase (RTK)-Akt/signal transducer and activator of transcription 3 (STAT3)/ mitogen-activated protein kinase (MAPK), and the Akt/mammalian target of rapamycin (mTOR) pathways (40). Recently, it has been found that treating prostate cancer *in vitro* and *in vivo* with Leelamine, the FA synthesis is disrupted by the downregulation of protein and/or mRNA expression of ACLY (41).

The compound **8** (Figure 2), also known as **DCV** (10,11-dehydrocurvularin), is a macrolide and fungus-derived natural-product recently connected with ACLY target; showing cytotoxic activity against some cancer cell lines (42). This recent work, performed a proteome-wide analysis using classical chemo-proteomic profiling in living cell models treated with DCV (42). Deng *et al.* have found that DCV is a strong irreversible ACLY inhibitor with an IC_{50} of 0.93 μM. Moreover, some SAR studies were performed with some few derivatives of DCV. The reduction of the conjugated double bond in the lactone moiety causes the loss of

cytotoxic activity; which suggests that the macrolide binds with its target by Michael addition mechanism probably with protein cysteine-thiol nucleophile.

The presented inhibitors of ACLY enzyme are promising in terms of cancer treatment. However, many studies with ACLY targeting drugs are focusing exclusively on treatment of cardiovascular diseases and for that reason less information regarding the cytotoxicity and effectiveness for cancer treatment is available.

ACC

Function and description. The ACC is the rate-limiting enzyme in FA synthesis as it catalyzes the formation of malonyl-CoA (*Figure legends* **Figure 1**), which is one of the necessary substrates for the next step (catalyzed by FAS) (43). In more detail, the ACC is a biotin-dependent multidomain enzyme located in the endoplasmic reticulum, it contains a biotin carboxylase (BC) and a carboxyltransferase (CT) active sites. The full crystal structure of the human ACC has not been elucidated yet but it is believed that it is similar to the ACC from yeast, in which the BC and CT domains are the active sites; and the biotin is covalently linked to the biotin carboxyl carrier protein (BCCP) domain, which is translocated during catalysis (44). In the first step of the reaction, the BC catalyzes the ATP-dependent carboxylation of biotin with bicarbonate serving as the CO_2 source and in the second step, the CT promotes the carboxyl transfer from biotin to acetyl-CoA and malonyl-CoA is formed (43, 45).

There are two known isoforms of ACC in mammals, the ACC1 and the ACC2 (43). The ACC1 is mainly found in the cytosol of lipogenic tissues such as liver, adipose tissue, and lactating mammary gland and catalyzes the malonyl-CoA formation in the FA synthesis (43, 46). It has been reported as up-regulated in some types of human cancer including breast, prostate, lung, ovary, and colon (47). On the other hand, the ACC2 is commonly found in the outer membrane of more oxidative tissues such as skeletal muscle and heart and also in the metabolically active liver. In these tissues, the ACC2 functions as a regulator of the FA β-oxidation (*Figure legends* **Figure 1**) by the inhibition of carnitine palmitoyltransferase 1 (CPT1) via malonyl-CoA (48). The human ACC1 contains 2,346 amino acid residues with a molecular weight of 265 kDa and the ACC2 contains 2,483 amino acid residues with a molecular weight of 280 kDa (48). The difference of about 140 amino acids in the *N*-terminus of ACC2, explains the difference in location and function when compared to ACC1 (48, 49).

The both ACCs isoforms are mainly regulated by AMPK, that inactivates the enzyme by phosphorylation, and the protein phosphatase 2A, that dephosphorylate the enzyme and activates it (43, 49). Besides, the AMPK is activated by AMP and deactivated by ATP; when AMP levels are low, the AMPK is inactive and the ACC is active (43). Other molecules that regulates ACC are citrate (activation), palmitoyl-CoA (inactivation) and CoA (43, 46, 48, 49). In the transcriptional level, ACC is regulated by some transcription factors as the carbohydrate response element-binding protein (ChREBP) and SREBP-1c (normally activated by glucose and insulin, respectively) (50). During the catalytic reaction of ACC (*Figure legends*

Figure 1), bicarbonate plays an important role as it is the CO_2 source for the biotin carboxylation; this bicarbonate molecule is synthesized from CO_2 and water by α-carbonic anhydrases (CAs), thus, evidencing that CAs are also important regulators of ACC (43). One of these CAs, the CA9/CA12 has been determined as overexpressed in many tumors and it is associated with cancer progression (51). There are also other novel regulators of the ACC activity as BRCA1 and AKR1B10 that are overexpressed in human carcinomas and are associated with an increase in the FA synthesis (43).

The ACC1 isoform, in particular, is regulated by a series of transcription factors controlled by glucose, insulin, thyroid hormones and catabolic hormones (49, 52); these transcription factors are the SREBP-1 (53), the liver X receptor/retinoid X receptor complex (54), and the PPARγ co-activator (PGC) (55). Specifically, the SREBP-1 is a key regulator of ACC1 (also of other lipogenic enzymes) in the transcriptional level as it is an effector of MAPK and PI3K (48, 49). In the translational level, ACC1 is activated by the human epidermal growth factor receptor-2 (HER2) mediated by PI3K/Akt/mTOR signaling pathway, as observed in breast cancer (56).

Inhibitors and cytotoxic effects. The overexpression of ACC, mainly the ACC1 isoform, has an important role in cancer treatment because it contributes to the survival of cancer cells when therapies targeting the Warburg effect are applied (57). The mechanism of action is not fully understood yet, but it is the response of cancer cells to the AMPK activation-induced inhibition of ACC (58). Also, it has been observed that the exogenous uptake of palmitic acid completely saves cancer cells from death, thus affecting the treatments that target ACC and FAS (mentioned in next section). Despite the ACC1 overexpression has been observed in many tumors, both isoforms of ACC seems to contribute almost equally to the lipid synthesis

as a studies have shown (59). Moreover, the pharmacological inhibition of ACC leads to cancer cell cycle arrest and/or apoptosis in several cell lines (43).

The ACC inhibitors have been reviewed in a previous study (60), but it is worth to mention the most important ones (*Figure 3*). First, **Soraphen A** (**9**, *Figure 3*) is a natural product isolated from the soil bacterium *Sorangium cellulosum*, with a structure that contains a lactone formed by seventeen carbon atoms, where the C3 and C7 of the ring are connected as a hemiketal (61). It was initially recognized as a potent antifungal drug, but, further studies showed that it is also a potent inhibitor of eukaryotic ACC, binding specifically to the BC domain (62). However, it has no effect on the BC domain of prokaryotic ACC (bacterial) (63). By analyzing the co-crystal structure of **Soraphen A** and the BC domain of ACC (yeast), it was evidenced that the entire macrocyclic portion binds to the BC domain, the methoxy groups at C11 and C12 positions act as H-bond acceptors, and the hydroxyl groups at C3 and C5 positions act as H-bond donors. Moreover, it was established that the binding of Soraphen A to the BC domain, interferes with the oligomerization of the domain, inhibiting ACC (62). Further studies, evidenced that Soraphen A inhibits DNL in human hepatoma (HepG2 cell line) and prostatic cancer (LnCaP cell line) (64), and also anti-cancer activity has been observed in other cancer cells (65–67). In particular, Soraphen A inhibits the FA synthesis, promoting the FA β-oxidation and reducing the PLs level in prostate cancer cells (LnCaP and PC-3M cell lines), inhibiting their proliferation (58). Additionally shows antiviral activity (68). However, Soraphen A has not been clinically used due to its poor drug-like properties.

Pfizer researchers developed a metabolically stable piperidinyl derived analog, the **CP-640186** (**10**, *Figure 3*) (69). It is a nonselective, reversible, and ATP noncompetitive inhibitor of ACC1/2, and has been shown to inhibit the synthesis of FAs and TGs in HepG2 cells (69) and shows cytotoxic properties in lung cancer line (70) . The authors reported an IC_{50} of 53 nM for rACC1 (rat) and 61 nM for rACC2 (69). By studying the co-crystal structure of CP-640186 and the CT domain of ACC (yeast), it was elucidated that the anthracene flat ring, the carbonyl group next to it, and the piperidine rings interact with the CT domain to produce the inhibitory effect (71).

Other piperidinyl derived compound that interacts similarly with the CT domain, is compound **11** (*Figure 3*), with a higher activity against ACC1/2 than compound **10**. The metabolically stable compound **11** developed by Taisho has an IC_{50} of 101 nM for rACC1, 23 nM for rACC2 and 76 nM for hACC1/2 (human). The authors reported that compound **11**

inhibited the FA synthesis with an IC_{50} of 0.34 μM and increased the fatty acid oxidation with an EC_{50} of 0.58 μM in HepG2 cells (72). Moreover, they found that the two aryl rings at the 2,6-position of the pyridine ring have distinct activities, one provides close hydrophobic space and the other interacts with the acetyl-CoA binding site.

The potential of piperidine derived compounds, inspired researchers to develop spiropiperidine derived ACC inhibitors by fusing piperidine rings with side chains or aromatic rings, giving rise to more rigid compounds with better binding activity to the CT domain (69). A potent inhibitor of hACC1/2 was developed by researchers from Merck Sharp & Dohme Corp and the University of Texas Southwestern Medical Center, the compound **MK-4074** (**12**, *Figure 3*) with an IC_{50} of approximately 3 nM (73). Clinical studies in healthy young male subjects have shown that MK-4074 reduces DNL in 96%. However, the plasma TGs were significantly elevated compared to the placebo group (73). In this way, inhibition of ACC can decrease the malonyl-CoA levels, interfering with the synthesis of polyunsaturated FA, but it also leads to the activation of SREBP-1c and glycerol-3-phosphate acyltransferase (GPAT1), which increase the secretion of very low-density lipoprotein (VLDL), and thus produces hypertriglyceridemia (73).

After a series of optimizations and previous studies, the researchers of Shionogi & Co. developed a benzothiazole derivative with high activity and selectivity for ACC2, the compound **13** (*Figure 3*). Their results showed that it has good drug-like properties and suggested that the linker between the side chain and the aromatic ring was critical for the safety and efficacy of these types of compounds (74).

By optimizing a 2-azetidinyl-1,3-benzoxazole derivative, Takeda researchers developed a novel and selective ACC1 inhibitor, compound **14** (*Figure 3*). They reported an IC_{50} of 0.58 nM for hACC1 and an IC_{50} of more than 10 μM for hACC2, and also it has good drug-like properties (75). After a series of analyses, they found that the cyclopropyl ether aryl side chain enhances the selectivity of ACC1, and several bicyclic cores improve the inhibitory activity (75). Recently, Takeda researchers developed a series of 1,3 benzoxazole derivatives (with a similar structure to compound **14**); one results to be a potent ACC1 inhibitor with good drug-like properties (76). The compound **15** (*Figure 3*) inhibits both ACC isoforms, but is more potent against hACC1 (IC_{50} = 1.5 nM) than against hACC2 (IC_{50} = 140 nM). *In vivo* studies showed that compound **15** reduced the malonyl-CoA levels in HCT-116 xenograft tumors and also that inhibits tumor growth in a 786-O xenograft mouse model at doses of more than 30 mg/kg (76).

In 2016, Nimbus therapeutics developed an efficient, reversible and specific ACC1/2 non-selective inhibitor, the **ND-630** (**16**, *Figure 3*) (77). It inhibits ACC by interacting with its phosphopeptide-acceptor and dimerization site, interfering with dimerization (77). In the same way as **Soraphen A**, ND-630 interacts within the dimerization site of the enzyme with the same residues as the AMPK-phosphorylated ACC peptide tail to disrupt subunit dimerization and inhibit ACC activity, but ND-630 is more potent because it fills a narrow, deep pocket in the BC domain (77). Despite its potent ACC inhibitory effect, further studies showed that ND-630 has weak cytotoxicity against lung tumors (78). In contrast, its primary amide, **ND-646** (**17**, *Figure 3*), showed both properties, a potent ACC inhibition and FA synthesis (79), and a strong cytotoxic effect against lung tumors (78). By the same inhibition mechanism of ND-630, ND-646 binds the BC domain of ACC and generates its consecutive dephosphorylation, this prevents the action of AMPK and then ACC is not able to dimerize (79).

In 2019, Li *et al*. performed small structural changes in ND-646, leading to the discovery of several compounds with a better cytotoxic effect (78). One is compound **18** (*Figure 3*), which acts by the same mechanism of **ND-646** and has an IC_{50} of 6.87 nM for hACC1. In non-small-cell lung cancer (NSCLC), the ACC1 mRNA is overexpressed (like in many other types of cancer mentioned above). By using compound **18**, the growth of A549 cells was inhibited (IC_{50} = 16.2 nM), leading apoptosis (78).

To sum up, the ACC inhibition is not only a target for cancer but also for other metabolic diseases like diabetes, obesity and fatty liver with positive results. Therefore, targeting ACC in the cancer therapy could implicate some non-desired side effects.

FAS

Function and description. The human fatty acid synthase (hFAS) is a complex homodimeric cytosolic enzyme of 552 kDa, that catalyzes the formation of palmitate (C_{16}) from acetyl-CoA and malonyl-CoA in the presence of NADPH (*Figure legends* **Figure 1**) (80). FAS has seven catalytic domains, which are (in linear order from the carboxyl terminus): thioesterase (TE), acyl-carrier protein (ACP), β-ketoacyl reductase (KR), enoyl reductase (ER), β-hydroxyacyl dehydratase (DH), malonyl/acetyl transferase (MAT) and β-ketoacyl synthase (KS). Moreover, there are two additional nonenzymatic domains, the pseudoketoreductase (ΨKR) and the peripheral pseudomethyltransferase (ΨME) (81).

As mentioned above, FAS catalyzes the final step of the FA biosynthesis. Starting with a load of acetyl (from acetyl-CoA) onto the terminal thiol of the phosphopantetheine cofactor of the ACP, this process is performed by the MAT (82). The ACP passes the acetyl moiety over the active site cysteine of the KS. Subsequently, the MAT transfers the malonyl group of malonyl-CoA to the ACP, and the KS catalyzes the decarboxylative condensation of the acetyl and malonyl moieties to an ACP-bound β-ketoacyl intermediate. Then, the β-carbon position is modified by the NADPH-dependent KR, DH, and NADPH-dependent ER domains to finally generate a saturated acyl group product with two extra carbon units. This molecule is the starting substrate for the next reactions of elongation until a fatty acid of 16 to 18 carbon atoms of length is obtained. Finally, the products are released from ACP as free FAs by the TE domain (82).

In cancer cells, the FAS overexpression is one of the most frequent phenotypic alterations, moreover, it is related to a higher risk of cancer recurrence and death (83). FAS overexpression has been evidenced in many human cancer cell lines including breast, colorectum, prostate, bladder, ovary, esophagus, stomach, lung, oral tongue, oral cavity, head and neck, thyroid and endometrium, among others (83, 84). Normally, FAS is mainly regulated by nutritional signals and is expressed in hormone-sensitive cells and cells with high lipid metabolism (85). In contrast, FAS regulation in cancer cells implicates the activity of several transcriptional and post-translational factors (growth factors, hormones, and their receptors), in parallel with microenvironmental effects (81). Two well-studied pathways involved in the FAS regulation, are the MAPK and the PI3K/Akt pathways (86). The HER2 and epidermal growth factor (EGF) receptors are involved in the downstream of PI3K/Akt and MAPK signaling pathways, which subsequently activates FAS expression transcriptionally (87). Moreover, FAS expression can be amplified by the crosstalk between sex hormones, growth factors, and their receptors (26). Both Akt and MAPK transduction pathways regulate FAS by the same mechanism. They regulate the expression of SREBP-1c which interacts with regulatory elements in the FAS promoter (81). Also, SREBP-1c is directly regulated by the proto-oncogene FBI-1 (Pokemon) through its DNA-binding domain, and thus synergistically activates the FAS transcription (88). Another transcription factor that is also regulated as SREBP-1c and is highly implicated in the FAS expression, is the ChREBP (89). There are also other factors as NAC1, the acetyltransferase P300, and some microRNAs that regulate FAS expression in tumor cells (90).

In breast cancer, FAS mediates the overexpression of S14, a lipogenesis-related nuclear protein that is regulated by SREBP-1c, supporting cell growth and survival (91). Moreover, in SK-BR-3 and BT-474 breast cancer cell lines, FAS might be regulated by another mechanism, via mTOR-mediated translational induction (56). By this mechanism, HER2 is overexpressed and with-it higher levels of FAS are observed (81). In prostate cancer, the ubiquitin-specific protease 2a (USP2a) is overexpressed and plays a critical role in cell survival. It may interact with FAS to stabilize it through the removal of ubiquitin (92). USP2a is regulated by androgen and its inactivation results in the FAS protein decrease and enhanced apoptosis (81).

Microenvironmental effects as hypoxia and acidity have important roles in the regulation of FAS (81). It has been evidenced that under hypoxic conditions in human breast cancer cell lines, FAS is upregulated (93). Moreover, Furuta *et al.* found that SREBP-1c is also upregulated as an effect of the phosphorylation of Akt with the subsequent activation of the hypoxia-inducible factor HIF1. Finally, excessive extracellular acid conditions could result in changes in the transcriptional activation of the FAS gene in breast cancer cells (94).

Inhibitors and cytotoxic effect. Since the discovery of FAS as an oncogenic target, many inhibitors have been developed and proved against several cancer cell lines. It has been evidenced that the FAS inhibition, stops the proliferation and induce the apoptosis of cancer cells, with minimal effects on normal cells (90). Most of the FAS inhibitors have been previously reviewed (81, 95, 96), but it is worth to mention the most important, promising and potent inhibitors that are found in the literature (Figure 4). First, the synthetic compound **C75**, a weak irreversible FAS inhibitor with an IC_{50} between 200 - 500 μM (racemic mixture) (96–98). C75 interacts with FAS in different domains, specifically with the KS, TE, and ER domains (99); showing anticancer activity in many cancer cell lines and xenografts models (100–102). However, *in vivo* studies showed that C75 has a negative side effect; it reduces food intake and induces body weight loss (103, 104). Further studies showed that the (–)-C75 enantiomer (**19**, Figure 4) is capable of inhibiting FAS *in vitro*, producing a cytotoxic effect in several cancer cell lines without affecting food consumption (105). On the other hand, it was evidenced that the (+)-C75 enantiomer inhibit CPT1 and produce anorexic effects. With these results, Makowski *et al.* developed a series of C75-based inhibitors taking into account the enantiomeric selectivity of FAS (98, 106). They found that the elongation of the aliphatic chain or the introduction of larger groups in the β-position of the lactone causes a decrease in the inhibitory activity of FAS (106). This reduced structure-activity relationship

study led to the development of a better FAS inhibitor, the **(–)-UB006** (**20**, Figure 4) with an IC_{50} of 220 μM for hFAS (98). *In vitro* studies showed that (–)-UB006 is more cytotoxic than C75 (racemic mixture) against several cancer cells; but specifically, against the OVCAR3 cell line, it showed to be 40 times more cytotoxic than C75. Furthermore, *in vivo* administration of (–)-UB006, evidenced that it does not affect the food intake and body weight. There are other C75-based inhibitors with no apparent negative side effects as C93 and C247 (107).

Orlistat (**21,** Figure 4) is a potent FAS inhibitor that was initially designed for obesity treatment, moreover, it is an FDA-approved pancreatic lipase inhibitor (95). Orlistat forms a covalent adduct with the serine of the TE domain and has an IC_{50} of 0.9 μM (96, 108). It has shown tumor growth inhibition in xenograft models of prostate cancer and melanoma, and also reduced proliferation and enhanced apoptosis in breast cancer that overexpresses HER2. However, Orlistat has poor oral bioavailability and metabolic stability, and thus it is difficult to use for cancer treatment (95). Moreover, the use of a drug-delivery system based on nanoparticles for Orlistat can improve its bioavailability, water-solubility and even its cytotoxic effect on aggressive breast cancer models (109, 110).

In 2014, GlaxoSmithKline pharmaceutical company developed a highly potent, reversible and specific inhibitor of the KR domain of hFAS (111). The compound **GSK2194069** (**22**, Figure 4), has an IC_{50} of 7.7 nM for hFAS and showed acceptable solubility and permeability. The authors demonstrated that GSK2194069 decrease the DNL, producing a potent inhibition in the cancer cell growth and proliferation in gastric and non-small-cell lung cancer cell lines. Moreover, they identified that GSK2194069 interacts specifically with the KR domain and works as a competitive inhibitor. Further studies evidenced that treatment with GSK2194069 in prostate cancer C42b cell xenografts inhibited the tumor growth with no apparent side effects (112).

Sagimet Biosciences (previously 3-V Biosciences) developed a new generation of highly potent, reversible, FAS inhibitors (113). One of them is **TVB-3166** (**23**, Figure 4), that has an IC_{50} of 0.042 μM for FAS (from rabbit). It is capable of stopping the FA synthesis and disrupt the lipid raft structure, affecting all the membrane-associated molecules and signaling pathways as Ras, Akt-mTOR, and Wnt-β-catenin (114). *In vivo* studies showed that a single daily dose can inhibit FAS for 10-12 h each day, inducing xenograft tumor growth inhibition in lung, ovarian and pancreatic tumor models (114). These results showed that an

548 irreversible inhibitor is not necessary to stop the tumor growth *in vivo*. Moreover, TVB-3166
549 does not have any apparent negative side effects.
550 Another compound developed by Sagimet Biosciences, is the **TVB-2640** (**24**, Figure 4),
551 which is the first and only FAS inhibitor that has reached the clinical trials until date (95).
552 TVB-2640 is described as a highly potent, selective and reversible FAS inhibitor that acts in
553 the KR domain and has an IC_{50} of 0.05 μM (115). In 2017, the phase 1 clinical trial of TVB-
554 2640 in patients with solid tumors was finished, demonstrating its antitumor activity in
555 monotherapy and co-treatment with paclitaxel (116). Some common negative side effects
556 were observed including alopecia, palmar-plantar erythrodysesthesia, decreased appetite,
557 among others. Nowadays, the phase 2 of clinical trials of TVB-2640 (monotherapy and/or co-
558 treatment) is underway, including the treatment of lung, colon, breast, and astrocytoma
559 cancer (NCT03808558, NCT02980029, NCT03179904, and NCT03032484). Moreover,
560 partial results of the phase 2 trial of TVB-2640 in combination with Bevacizumab in patients
561 with the first relapse of high-grade astrocytoma, showed that the co-treatment is well
562 tolerated in humans (117).
563 In 2016, Alwarawrah *et al.* discover a potent thiophenopyrimidine-based FAS inhibitor
564 with broad antitumor activity against various non-tumorigenic and aggressive tumor-forming
565 breast cancer cell lines (118). **Fasnall** (**25**, Figure 4) with an IC_{50} of 3.71 μM for hFAS, can
566 produce a significant change in the global cellular lipid profile. Its mechanism of action
567 includes the increase of intracellular levels of ceramide (also in DAGs and unsaturated FA)
568 which increases the apoptosis of cancer cells. Moreover, Fasnall inhibits the formation of PLs
569 with saturated acyl chains and promotes the uptake of unsaturated FAs, affecting critically
570 the lipid raft structure and functioning (119). Fasnall treatment has no apparent negative side
571 effects and its combination with other chemotherapeutic agents as carboplatin augments the
572 tumor volumes reduction and survival *in vivo* studies (118). All these characteristics and the
573 ease of adaptability of the Fasnall synthetic route, suggest that it can be further optimized to
574 developed new derivatives with better pharmacological properties.
575 In 2018, Lu *et al.* developed a series of spirocyclic imidazolinone FAS inhibitors; one of
576 them showed high FAS inhibitory activity with good cellular activity and oral bioavailability
577 (120). The compound **JNJ-54302833** (**26**, Figure 4) has an IC_{50} of 28 nM for hFAS and
578 effectively inhibits the proliferation of several cancer cell lines including ovarian, prostate,
579 lymphoma, leukemia, lung and breast. The authors found that one compound of the series of
580 spirocyclic imidazolinone FAS inhibitors (not exactly JNJ-54302833) binds to the KR

581 domain by H-bonds and also hydrophobic interactions occurred with the KR and non-
582 catalytic domains of FAS (120). In 2019, Infinity Pharmaceuticals published the discovery of
583 a potent and irreversible inhibitor of hFAS, the **IPI-9119** (**27**, Figure 4) (121). It has an IC_{50} of
584 0.3 nM for hFAS and inhibits the TE domain by promoting acylation of the catalytic serine.
585 Authors evidenced that IPI-9119 significantly reduced prostate cancer cell growth, and
586 induced cell cycle arrest and apoptosis in PCa cells. Moreover, FAS inhibition generated an
587 entire lipid homeostasis change, including the accumulation of polyunsaturated FAs produced
588 by the uptake and use of exogenous FAs. Also, the cholesterol synthesis was increased as a
589 type of redirection of the unused acetyl-CoA. Therefore, it is evidenced that PCa cells tried to
590 compensate for the DNL deficiency by up-regulating genes encoding enzymes and
591 transcription factors involved in lipid synthesis (121). Further investigations are necessary to
592 understand these anomalies caused by IPI-9119 treatment.
593 FORMA Therapeutics developed a series of novel piperazine derivative FAS inhibitors;
594 one of them is the compound **FT113** (**28**, Figure 4) with an IC_{50} of 0.213 μM for hFAS (122).
595 The authors reported that several H-bond interactions occurred between the hydroxyl and
596 carbonyl of the hydroxy-cyclopropyl amide and the active site residues of the KR domain.
597 These observations were determined by the X-ray co-crystal structure of FT113 bound to a
598 ΨME-ΨKR-KR tridomain FAS construct (122). FT113 was the compound with the best
599 balance between physicochemical and pharmacokinetic properties and potency. Moreover,
600 FT113 showed anti-proliferative activity against prostate (PC3 cell line), breast (BT-474 cell
601 line) and leukemia (MV-411 cell line) cancer cells. After 16 days of treatment with FT113, it
602 was evidenced an increase in malonyl-CoA levels in the tumors as well as a tumor growth
603 inhibition of 32% and 50%, by treatment with 25 and 50 mg/kg respectively, compared to the
604 vehicle.
605 It has been evidenced that FAS is a viable target for the inhibition of FA biosynthesis, as
606 many compounds showed high FAS inhibitory activity and cytotoxic effect against several
607 cell lines, with no apparent side effects. Moreover, there is one compound (**TVB-2640**) that is
608 currently been tested in humans, suggesting that FAS targeting has a great potential for
609 anticancer therapy. It is important to mention that nowadays, we have new tools for the
610 discovery of FAS inhibitors, like the computational screening (123). With new tools of this
611 kind, there is more ease of discovery, and optimization of new compounds with lower
612 expenses and time in the process.
613

MAGL

Function and description. In DNL, the MAGL liberates the stored fatty acids for metabolic and signaling purposes, supporting and promoting the migration, invasion, survival, and growth of tumors in aggressive human cancers (124). This enzyme has been found in elevated levels, together with free fatty acids (FFAs), in several aggressive human cancer cell lines (124). The MAGL controls the FFAs level in cancer cells through the hydrolysis of monoacylglycerols (MAGs) (*Figure legends* **Figure 1**) (124). In normal cells, the MAGL controls the levels of MAGs and not the FFAs level, evidencing that in cancer cells this pathway is altered (as others already mentioned) to satisfy the pathogenic requirements (124, 125). The mechanism of MAGL-stimulation for the cancer aggressiveness is through the action of FFA-derived products (124). Some secondary lipid metabolites such as LPL (including lysophosphatidylcholine (LPC), LPA, lysophosphatidylethanolamine (LPE), PA), Prostaglandin E2 (PGE2), and ether lipids (Monoalkylglycerol (MAGE), alkyl LPE) are regulated by MAGL; all of them support cancer malignancy but LPA and PGE2 in greater extent (124, 126). Moreover, MAGL is the primary enzyme that degrades endogenous cannabinoid 2-arachidonoylglycerol (2-AG) *in vivo* (127), moreover, 2-AG is the principal signaling molecule of MAGs and activates the CB1 and CB2 receptors (124, 128). In particular, the CB1 receptor has been highly implicated in the aggressiveness of prostate cancer (127, 129). Therefore, it can be said that MAGL controls the FA and endocannabinoid pathways that support the aggressiveness in prostate cancer (127). Besides, it has been observed that the CB2 receptor activation promotes colon cancer and recently, it was evidenced that the attenuation of CB2 signaling suppress the tumor growth (130). Also, these endocannabinoid (CB1 and CB2) receptors have other functions related to pain, inflammation, neurodegeneration, and anxiety (131).

MAGL is part of the α/β hydrolase superfamily of enzymes (132). It is found as a dimer of 33 kDa with 313 residues, it has two protein molecules per asymmetric unit with the catalytic site facing the PL membrane (133). MAGL is located in the cytosol and cell membranes (amphipathic behavior), so it is soluble in the cytosol and at the same time, it can interact with the PL membrane to recruit its substrate (134). Moreover, it has been observed that MAGL exist in two distinct conformations, closed and open, these correspond to inaccessible or solvent-exposed active site (134). This special characteristic evidenced that MAGL regulates the entering of 2-AG to the catalytic site through the CPe flexible control of the lid domain opening (134). It was hypothesized that the hydrophobic character of the lid

domain serves to located the MAGL close to the PL membrane, facilitating the 2-AG recruitment (134). When the substrate enters the active site, the MAGL takes its closed-form and dissociates from the membrane in parallel to the cleaving the 2-AG, then the MAGL takes again the open conformation and re-associates to the membrane (134). The mechanism of action of the MAGL has not been fully elucidated yet, but as mentioned before, an important part of it is now understood and might lead to the development of better inhibitors for this enzyme.

About the regulation of MAGL, very little is known of the post-transcriptional and post-translational modifications; there is no evidence of phosphorylation or other modifications to date (135). There is evidence pointing out that there are slight variations between the MAGL enzymes from adipose tissues, liver, heart, lung, stomach, kidney, spleen, kidney and adrenal gland, with the ones from brain, testis and skeletal muscle (135, 136). Therefore, there is the possibility that the post-transcriptional and/or post-translational modifications could occur depending on the particular need of the cell in a distinct tissue or physiological state (135). In general terms, the regulation of MAGL is unknown, therefore, more studies are required to understand it (especially the human MAGL) and thus design better and more selective inhibitors.

Inhibition and cytotoxic effect. It has been evidenced that by inhibiting the MAGL, the tumorigenesis and cancer progression are suppressed in several cancer cell lines (39, 137), but also its inhibition can have other implications in neurodegeneration, inflammation and metabolic disorders (138). Moreover, there are other serine hydrolases such as fatty acid amide hydrolase (FAAH), α/β hydrolase domain 6 (ABHD6), and α/β hydrolase domain 12 (ABHD12) that have similar binding site properties (to MAGL); although they exert different functions and have different endogenous substrates in human (138). Therefore, the analysis of the selectivity profile of MAGL inhibitors is very important for the development of new and better compounds.

Some MAGL inhibitors have been reviewed previously (138, 139), but it is important to mention the most promising and new ones (Figure 5). In 2008, Muccioli *et al*. developed a good inhibitor of MAGL, the **CAY10499** (**29**, Figure 5) (140). The carbamate derivative CAY10499, is a covalent irreversible inhibitor of MAGL, with an IC_{50} of 134 nM for hMAGL (141). Although, CAY10499 is not a selective inhibitor of MAGL, as it is also active against FAAH (140). The authors suggested that the active moiety of CAY10499 is the 5-methoxy-1,3,4-oxadiazol-2(3*H*)-one moiety and not the carbamate. Further studies,

showed that CAY10499 is active against five tumor cell lines, human breast (MDA-MB-231), colorectal (HCT116), and ovarian (OVSAHO, CAOV3, COV318, OVCAR3, and SKOV3) cancer cell lines (141, 142).

The first selective and *in vivo* active, irreversible inhibitor of MAGL was synthesized in 2009 by Long *et al*. (143). The **JZL184** (**30**, Figure 5) is a piperidine carbamate compound, that binds covalently and irreversibly by carbamylating a serine residue in the active site of MAGL; it has an IC_{50} of 6 nM for hMAGL. *In vivo* studies showed several beneficial effects by its administration, including analgesic, antinociceptive, anti-inflammatory, gastroprotective, antidepressant and anxiolytic effects (144, 145). Moreover, JZL184 has shown anticancer effects against colorectal cancer (146) and hepatocellular carcinoma (HCC) (137). In colorectal cell lines, it reduced the tumor cell progression and increased apoptosis (correlated with MAGL inhibition) (146). In HCC cell lines, it significantly increased apoptosis and reduced tumor growth, and it even decreases the invasion ability HCC cell line SMMC-772 (137). Moreover, in C8161 and SKOV3 aggressive cancer cells, the tumor growth and migration rate were reduced by the administration of JZL184 once per day (40 mg/kg) (124). Nomura *et al*. evidenced that JZL184 blocks the conversion of LPA and PGE2 from MAGs in aggressive cancer cells, which correlates with the tumor growth and migration reductions. Further investigations (147), generate a more selective JZL184 derivative with *O*-hexafluoroisopropyl as a leaving group, the **KML29** (**31**, Figure 5). This compound has an IC_{50} value of 5.9 nM for hMAGL and has shown a complete selectivity for MAGL over FAAH (148). KML29, as other carbamate derivatives, binds covalently and irreversibly to MAGL by the formation of a carbamylated enzyme-inhibitor adduct (147). Although, there is not any study of the anticancer activity of this compound until the date.

After a series of optimizations of carbamate derivatives, the Pfizer's research group developed the irreversible inhibitor **PF-06795071** (**32**, Figure 5) with an IC_{50} of 3nM for hMAGL (149). This compound has great drug-like properties thanks to its novel stereo-defined trifluoromethyl glycol leaving group. PF-06795071 was studied against neuroinflammatory disease and showed high *in vivo* efficacy (149), but there are no studies about its use against cancer. In 2013, a series of urea-based MAGL inhibitors were developed by Aaltonen *et al*.; the most potent of them was **JJKK-048** (**33**, Figure 5) with an IC_{50} value of 0.36 nM against hMAGL (150). This compound irreversible inhibitor was proved to be selective for MAGL against other serine hydrolases as FAAH and ABHD6. The proposed mechanism of action is very similar to the carbamates derived inhibitors, JJKK-048 also

forms a carbamate adduct with a serine in the active site of MAGL and the triazole (1,2,4-triazolate anion) acts as the leaving group. *In vivo* studies of JJKK-048 in C8161 melanoma cells, shows that it is highly selective for MAGL but further information about its impact on cell proliferation, migration, and invasiveness has not been reported (150).

The irreversible inhibition of MAGL has many negative effects *in vivo*, as the loss of the analgesic effects and cross-tolerance to CB1 agonists (151). Also, it generates physical dependence, damages the endocannabinoid-dependent synaptic plasticity and desensitizes brain CB1 receptors. The chronic inhibition of MAGL has many obstacles to be an anticancer treatment, therefore, the development of reversible inhibitors is of great importance. In 2018, a selective and reversible inhibitor of MAGL has been developed by Takeda Pharmaceutical, the compound **34** (**Figure 5**) (152). This compound with piperazinyl pyrrolidin-2-one structure, showed great balance between metabolic stability (29 μL/min/mg) and inhibition activity (IC_{50} = 3.6 nM). The authors reported that it was selective against MAGL over FAAH, but selectivity over other serine hydrolases has not been reported yet. The **34** produced a dramatic reduction of arachidonic acid (25%) and an increase of 2-AG (340%) in mice brain *in vivo*. This compound was designed for the treatment of neurodegenerative diseases and no anti-cancer studies have been developed yet.

In 2018, Aghazadeh *et al*. developed a pyrazole-3-carboxamide derivative after a series of optimization (142). The compound **35** (**Figure 5**), a pyrazole-3-carboxamide derivative that acts as an efficient reversible inhibitor of MAGL, with an IC_{50} of 0.51 μM against hMAGL. *In vitro* studies showed that compound **35** has promising antiproliferative activity against two ovarian cancer cell lines (OVCAR-3 and CAOV-3). Moreover, compound **35** has selectivity for MAGL over FAAH, ABHD6, and ABHD12; also, it did not show significant binding to any of the CB1 and CB2 cannabinoid receptors. Recently, Granchi *et al*. developed a benzoylpiperidine-based potent and reversible inhibitor of MAGL, the compound **36** (**Figure 5**) (141). Compound **36** has a value of 80 nM for hMAGL and showed high selectivity over FAAH, ABDH6, ABDH12, CB1 and CB2 (IC_{50} > 10 μM in all cases). Also, *in vitro* studies showed antiproliferative activities in human breast MDA-MB-231, colorectal HCT116, and ovarian CAOV3, OVCAR3, and SKOV3 cancer cells at micromolar concentrations. *In vivo* studies proved that compound **36** inhibits MAGL, as it significantly increased the 2-AG levels in brain and plasma after intraperitoneal injection into C57BL6 mice.

Targeting MAGL for the cancer treatment could result in the disruption of cancer cells proliferation but also could lead to neurodegeneration, inflammation and metabolic disorders.

The development of future MAGL inhibitors must focus on the selectivity and reversibility of the inhibition to avoid possible side effects.

Conclusions

Lipid metabolism is a key player in cancer cell survival. Those enzymes that regulate the synthesis of FAs are often overexpressed in many cancer cell lines and their inhibition can result in the disruption of cancer cell proliferation. In this review, inhibitors of the three enzymes involved in the anabolism of FFAs from citrate (ACLY, ACC and FAS) and one involved in the catabolism of FFAs from MAG (MAGL) have been analyzed as potential antitumor drugs. Among them, inhibitors of FAS are very promising, since they showed cytotoxicity against several cancer cell lines and one of them (TVB-2640) is currently evaluated in clinical trials. Inhibitors that target ACC and MAGL should focus on selectivity to avoid possible side effects, because the inhibition of these enzymes affects other metabolic diseases. On the other hand, inhibitors addressed to ACLY need to be studied more exhaustively to assess their effectiveness for cancer.

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Author Contributions

KM conceived the idea; NM, ML, XA, LH and KM wrote the manuscript; KM designed the illustrations

Conflicts of Interest: The authors declare no conflicts of interest.

Figure legends

Figure 1. Overview of lipogenesis in cancer and four target enzymes responsible for fatty acid synthesis. Cancer cells obtain free fatty acids (FFA) primarily from de novo biosynthesis. Glucose is converted to pyruvate, via aerobic glycolysis. Pyruvate is metabolized to citrate within the mitochondria in the Krebs cycle to produce ATP. The citrate excess is expelled to the cytosol where it enters to the lipogenic pathway. ATP-citrate lyase (ACLY) catalyzes the production of acetyl-CoA, which is then carboxylated to malonyl-CoA by Acetyl-CoA carboxylase (ACC). The FAS then catalyzes palmitate biosynthesis from acetyl-CoA and malonyl-CoA. On the other hand, lipid stores can be used to obtain FFA due to a high activity of Monoacylglycerol lipase (MAGL). Cancer cells with a high proliferation rate need free fatty acids for phosolipids formation of the new membranes, for signaling molecules as ceramides or sphingolipids or to obtain energy via β-oxidation. All the indicated enzymes have been studied as potential therapeutic targets against cancer.

Figure 2. Structure and half-maximal inhibitory concentration (IC_{50}) of selected ACLY inhibitors. The organism from which ACLY was extracted and the cell line of the cytotoxicity studies are indicated in parenthesis.

Figure 3. Structure and half-maximal inhibitory concentration (IC_{50}) of selected ACC inhibitors. The organism from which ACC was extracted, the isoform, and the cell line of the cytotoxicity studies are indicated in parenthesis.

Figure 4. Structure and half-maximal inhibitory concentration (IC_{50}) of selected FAS inhibitors. The organism from which FAS was extracted and the cell line of the cytotoxicity studies are indicated in parenthesis.

Figure 5. Structure and half-maximal inhibitory concentration (IC_{50}) of selected hMAGL inhibitors. The cell line of the cytotoxicity studies is indicated in parenthesis in each case

Figure 6. The inhibitors reviewed in this work. Inhibition of lipogenic enzyme produce cancer cells death due fatty acid starvation which are the building blocks for membranes, pro-survival signaling molecules and source of energy in hypoxic environment as is a solid tumor.

Table 1. Summary of selected lipogenic enzyme inhibitors, their targets and relevance in cancer therapy

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