Inhibitors of lipogenic enzymes as a potential therapy against cancer

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Abstract
Cancer cells rely on several metabolic pathways such as lipid metabolism to meet the increase in energy demand, cell division, and growth and successfully adapt 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 that target 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.
1 | INTRODUCTION

Many types of cancers are detected every year worldwide. According to the World Health Organization, cancer is the second cause of death globally. It was responsible for the 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 made up of fatty acids (FAs) of different chain lengths, 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 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 accelerated lipid synthesis to generate biological membranes.\(^2,5,6\)

**KEYWORDS**
cancer drugs, lipid metabolism, lipogenic enzyme inhibitors

**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 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. In addition, lipid stores can be used to obtain FFA due to high activity of monoacylglycerol lipase (MAGL). Cancer cells with a high proliferation rate need free fatty acids for phospholipid formation of new membranes, for signaling molecules such as ceramides or sphingolipids or to obtain energy via β-oxidation. All the indicated enzymes have been studied as potential therapeutic targets against cancer.
The metabolic cycles of cancer cells are altered, through a series of oncogenic events and the tumor microenvironment, to meet energy and lipid requirements.\textsuperscript{6,7} The best known perturbation in the metabolic cycle of cancer cells is the Warburg effect, which involves an increase in glucose uptake and subsequent lactate production by glycolysis (Figure 1).\textsuperscript{8} Other important perturbations that are enhanced as part of cancer-associated metabolic reprogramming are the biosynthesis of proteins, nucleic acids, and lipids.\textsuperscript{3,6} Specifically, lipid biosynthesis is induced as part of the anabolic metabolism of cancer cells. It is the process of converting nutrient-derived carbons (that are normally an energy source) into FAs (Figure 1) and cholesterol.\textsuperscript{3,4,6}

### 2 | LIPIIDS’ ROLE IN CANCER

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

Cancer cells can obtain FAs either from exogenous sources or from de novo lipogenesis (DNL).\textsuperscript{2,3} In the presence of oxygen and abundant extracellular nutrients, most cancer cells synthesize FAs from citrate by DNL (Figure 1).\textsuperscript{9} During this process, glucose is converted into pyruvate and subsequently transported to the mitochondria, where it is converted to acetyl-CoA. Acetyl-CoA enters the tricarboxylic acid (TCA) cycle and finally the citrate is obtained. However, under hypoxic conditions, cancer cells can use glutamine to produce citrate by its reductive metabolism \textsuperscript{6,10} and synthesize acetyl-CoA directly from acetate by the action of the cytoplasmic acetyl-CoA synthetase (ACSS2).\textsuperscript{11} Moreover, under conditions of metabolic stress, cancer cells collect extracellular lipids as an adaptation to survive.\textsuperscript{12} This adaptation involves a reduction of the carbon supply and power for the FA synthetic pathway.\textsuperscript{7} Finally, FAs can be used as an energy source when mitochondrial oxidation (β-oxidation) occurs (Figure 1). They produce more than twice as much ATP per gram as glucose or amino acid oxidation.\textsuperscript{2,4,6,7} Consequently, some cancer cells prefer to use FAs as an energy source even under nutrient-replete conditions.\textsuperscript{7}

Another important biosynthetic process within lipid metabolism is the mevalonate pathway, which facilitates the synthesis of cholesterol.\textsuperscript{2,4,6} Cholesterol is one of the main components of biological membranes, as it modulates the fluidity of the lipid bilayer and forms detergent-resistant microdomains called lipid rafts that coordinate the activation of some signal transduction pathways.\textsuperscript{2,4,6} In cancer cells, many signaling proteins, such as protein kinase B (Akt), and receptors regulating prooncogenic and apoptotic pathways reside in lipid rafts.\textsuperscript{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 tumor cell proliferation is reduced.\textsuperscript{13}

Some of the aforementioned 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.\textsuperscript{4} LDs are typically found in some aggressive cancers, and high levels of saturated FAs are found in some aggressive breast cancers.\textsuperscript{14} Cancer cells have higher amounts of LDs than normal tissue, which enhances their resistance to chemotherapy.\textsuperscript{15} Moreover, the high levels of saturated FAs increase the levels of saturated PLs in cancer cells, which reduces membrane fluidity and protects cancer cells from oxidative damage.\textsuperscript{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.\textsuperscript{6,16} In addition, phosphoinositides act as specific binding sites for the coupling of effector proteins into specific membrane sites.\textsuperscript{6} Other lipids that act as second messengers are lysophosphatidic acid (LPA), phosphatidic acid (PA), and DAG.\textsuperscript{6} Moreover, sphingolipids are important signaling molecules (Figure 1). The simplest of the sphingolipids is ceramide.\textsuperscript{6} In cancer cells, ceramides mediate growth inhibitory signals and is involved in initiation of the apoptotic process and growth arrest.\textsuperscript{6} Furthermore, the enzymes involved in the sphingolipid metabolism pathway are normally deregulated in cancer cells, which produces low ceramide levels and consequently increased resistance to chemotherapy.\textsuperscript{17}

Besides the aforementioned lipid use in cancer cells, lipids play an important role in post-translational modification of proteins.\textsuperscript{8} Palmitate and myristate are saturated acyl chains that are normally (covalently) coupled to proteins and improve the protein interaction with membrane rafts.\textsuperscript{18} Lipid metabolism is also involved in the autophagic process, which is a self-degradation mechanism that is required to remove 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. Apart from the importance of lipids in cancer cell proliferation and survival, they are also implicated in more complex processes such as cell migration, invasion, tumor angiogenesis, and metastasis formation. Many proteins are involved in the supply of fatty acids. We could divide them into the (a) proteins involved in de novo synthesis of palmitate, (b) palmitate modification proteins such as elongase or proteins that introduce unsaturation in the aliphatic chain, (c) lipases that liberate FAs from energy storage as adipose tissue, and (d) proteins that transport FAs or lipids from outside the cell. As previously mentioned, the preferred source of FAs in many cancer cells is de novo synthesis. Nevertheless, new studies have shown that cancer cells can switch to another FA source in specific metabolic conditions such as hypoxia or nutrient shortage that are present in solid tumors. Consequently, those proteins are important and could be targeted pharmacologically against cancer disease. The overexpression of lipogenic enzymes (Figure 1), such as ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and the enzyme responsible for liberating fatty acid from energy storage as monoacylglycerol lipase (MAGL) represent a nearly universal phenotypic alteration in most tumors and cancer cells (Figure 1). A lot of effort was made in the discovery of new inhibitors of these enzymes. In this review, we focus on the aforementioned enzymes and on some inhibitors of each of them as potential therapeutic targets against cancer (Table 1). We focus on de novo fatty acid synthesis enzymes (ACLY, ACC and FAS) and one of the most studied lipases (MAGL), as their inhibition directly interrupts the supply of fatty acid, have a high pharmacological potential and a proven cytotoxic response. The positive perspective of this strategy is reinforced by a promising FAS inhibitor reaching clinical trials recently (see Section 3.3). Other proteins involved in fatty acid metabolism will be briefly presented in an anticancer context at the end of this review.

3  LIPOGENIC ENZYMES’ INHIBITORS

3.1  ACLY

3.1.1  Function and description

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

In more detail, 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. Initially, the catalysis starts by autophosphorylation of a histidine residue, resulting in a citrly-phosphate within the active site. Subsequently, a covalent citrly-enzyme complex is produced and is attacked by the CoA to form the citrly-CoA. Finally, the enzyme catalyzes the cleavage of citrly-CoA to acetyl-CoA and OAA.

ACLY is overexpressed in many cancer cell lines, stimulated by insulin, growth factors, and high levels of glucose. Moreover, the stimulation mechanism occurs through the phosphoinositide 3-kinase (PI3K)/Akt pathways. The Akt pathway upregulates ACLY by activating the sterol regulatory element-binding protein 1 (SREBP-1), which is a transcription factor for genes involved in FA and cholesterol synthesis. PI3K/Akt pathways stimulate ACLY through its phosphorylation (which contributes to protein stabilization) rather than transcriptional upregulation. In addition, ACLY is regulated by other pathways, depending on the cell line. When ACLY is deregulated, the expression of the acyl-CoA synthetase short-chain family member 2 (ACSS2) increases, which catalyzes the conversion of exogenous acetate to acetyl-CoA using ATP.

3.1.2  Inhibitors and cytotoxic effects

The inhibition of ACLY produces cytotoxic effects by disrupting FA synthesis, similar to FAS and ACC inhibition (mentioned in the next sections). Moreover, ACLY inhibition affects the mevalonate pathway, which disrupts cholesterol and isoprenoid synthesis and thus enhances its cytotoxic effect. Furthermore, ACLY inhibition produces stronger cytotoxic effects in cells with elevated glucose metabolism than in others with low aerobic glycolysis. This shows that some cancer cells do not need ACLY to produce acetyl-CoA, like those that use ACSS2. Another important effect of ACLY inhibition is the increase in intracellular amounts of reactive oxygen species. This enhances the phosphorylation of an important regulator of lipid metabolism: AMP-activated protein kinase (AMPK).
Several studies have shown that many ACLY inhibitors have cytotoxic effects against some cancer cell lines, and can be enhanced in combination with other agents that block oncogenic receptor signaling. The human ACLY has been widely studied and many potent inhibitors have been developed (Figure 2). First, (–)-Hydroxycitric acid (1, Figure 2) is a naturally occurring compound that was extracted for the first time from *Garcinia* fruits. 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 agent that binds DNA). In vivo studies showed that the combination of these three drugs attacks the altered metabolism and DNA of cancer cells and thus brings some improvement compared to cisplatin treatment (monotherapy).

Another natural product, 2-chloro-1,3,8-trihydroxy-6-methylanthrone (2, Figure 2), was found to be a strong inhibitor of hACLY with an IC50 of 283 nM. 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.

Another compound of natural origin is Cucurbitacin B (3, Figure 2), found in cucumber, among other members of the *Cucurbitaceae* family, with a tetracyclic triterpenoid structure. In vitro studies showed that Cucurbitacin B is cytotoxic against various cancer cell lines, including breast SK-BR-3 (IC50 = 4.6 µg/mL), MCF7 (IC50 = 88.7 µg/mL), and MDA-MB-231 (IC50 = 38.9 µg/mL), lung NCI-H460 (about 87% inhibition after 48 hours with 0.1 µM of drug), central system SF-268 (about 92% inhibition after 48 hours with 0.05 µM of drug), colon HCT-116 (about 80% inhibition after 48 hours with 0.4 µM of drug), prostate PC-3 and LNCaP (IC50 ~0.3 µM), and hepatocellular BEL-7402 (IC50 = 0.32 µM). The mechanism of action of Cucurbitacin B was linked to several targets and it is not fully elucidated.

However, Xiao et al demonstrated an in vitro and in vivo

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**FIGURE 2** Structure and half-maximal inhibitory concentration (IC50) of selected ACLY inhibitors. The organism from which ACLY was extracted and the cell line of the cytotoxicity studies are indicated in parentheses.
dose depending on ACLY inhibition by this compound.\textsuperscript{35} Additionally, several in vivo studies in xenograft rat models showed tumor reduction after treatment with Cucurbitacin B.\textsuperscript{21,35} Recently, the pharmacokinetics of Cucurbitacin B was studied. Limited 10% low oral bioavailability was found, along with a large volume of distribution in internal organs (Table 1).\textsuperscript{37}

Synthetic inhibitors of ACLY have been extensively reviewed.\textsuperscript{21} However, it is worth mentioning the most potent, promising compounds (Figure 2). **ETC-1002** or Bempedoic acid (4, Figure 2) was developed by Esperion Therapeutics Inc and is currently in phase III of clinical trials to reduce low-density lipoprotein cholesterol (LDL-C), and prevent cardiovascular-related diseases.\textsuperscript{38} In the liver, it is converted to its active form (ETC-1002-CoA) by acyl-CoA synthase. ETC-1002-CoA inhibits ACLY completely, reducing the OAA and acetyl-CoA levels to finally disrupt DNL. Moreover, it increases AMPK activity, which inhibits the phosphorylation of ACC and HMG-CoA reductase, and thus reduces glucose and lipid biosynthesis.\textsuperscript{21}

In 2007, a series of 2-hydroxy-N-arylbenzenesulfonylamide compounds were reported as strong inhibitors of ACLY.\textsuperscript{39} The most potent was compound 5 (Figure 2) with an IC\textsubscript{50} of 130 nM. Although it showed high enzymatic inhibition activity, compound 5 has weak cytotoxicity (>50 mM) against human liver carcinoma HepG2 cell line. The biological activity study of this compound was focused once again on decreasing cholesterol and TGs, and not on FAs.\textsuperscript{39}

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), which maintains the benzenesulfonylamide connected to the biphenyl moiety but substitutes one of the chlorides with a methoxycarbonyl group on the phenolic part, and incorporates two fluorine atoms on the biphenyl moiety.\textsuperscript{40} These changes greatly improve the inhibitory effect of NDI-091143. This compound has become the strongest inhibitor of ACLY known to date, with a Ki of 7.0 nM and an IC\textsubscript{50} between 2.1 and 4.8 nM. Interestingly, the authors were able to obtain the ACLY-NDI-091143 co-crystal structure using a cryo-electron technique. They 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 in amino acid residues allowed the binding of the drug, which led the authors to conclude that NDI-091143 is the first allosteric inhibitor of ACLY.\textsuperscript{40,41} These recent discoveries will probably 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.\textsuperscript{42} 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.\textsuperscript{42} Recently, it was found that when prostate cancer is treated in vitro and in vivo with Leelamine, FA synthesis is disrupted by the downregulation of protein and/or mRNA expression of ACLY.\textsuperscript{43}

Compound 8 (Figure 2), also known as **DCV** (10,11-dehydrocurvularin), is a macrolide and fungus-derived natural-product recently connected with the ACLY target. It shows cytotoxic activity against leukemia cancer cells.\textsuperscript{44} This recent study performed a proteome-wide analysis using classical chemo-proteomic profiling in living cell models treated with DCV.\textsuperscript{44} Deng et al have found that DCV is a strong, irreversible ACLY inhibitor with an IC\textsubscript{50} of 0.93 µM. Moreover, some SAR studies have been performed with a few derivatives of DCV. The reduction in the conjugated double bond in the lactone moiety causes the loss of cytotoxic activity. This suggests that the macrolide binds with its target by the Michael addition mechanism, probably with the protein cysteine-thiol nucleophile.

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

### 3.2 | ACC

#### 3.2.1 | Function and description

ACC is the rate-limiting enzyme in FA synthesis as it catalyzes the formation of malonyl-CoA (Figure 1), which is one of the necessary substrates for the next step (catalyzed by FAS).\textsuperscript{45} In more detail, ACC is a biotin-dependent multienzyme located in the endoplasmic reticulum. It contains biotin carboxylase (BC) and carboxyltransferase (CT) active sites. The full crystal structure of human ACC has not been elucidated yet, but it is believed to be similar to ACC from yeast, in which the BC and CT domains are the active sites. Biotin is covalently linked to the biotin carboxyl carrier protein (BCCP) domain, which is translocated during catalysis.\textsuperscript{46} In the first step of the reaction, the BC catalyzes the ATP-dependent carboxylation of biotin with bicarbonate serving as the CO\textsubscript{2} source. In the second step, the CT promotes carboxyl transfer from biotin to acetyl-CoA, and malonyl-CoA is formed.\textsuperscript{45,47}

There are two known isoforms of ACC in mammals: ACC1 and ACC2.\textsuperscript{45} ACC1 is mainly found in the cytosol
| #  | Compound                          | Target enzyme | Outcome                                                                 | Development stage                  | Cancer type                      | References                |
|----|-----------------------------------|---------------|-------------------------------------------------------------------------|-----------------------------------|---------------------------------|---------------------------|
| 1  | (–)-Hydroxycitric acid            | ACLY          | In combination with cisplatin, it improves tumor reduction in vivo when compared to cisplatin monotherapy against cancer | Pre-clinical                      | In vivo: lung (LL/2) and bladder (MBT-2) | (31)                      |
| 2  | 2-Chloro-1,3,8-trihydroxy-6-methylanthrone | ACLY         | Strong inhibitor. Not studied in the context of cancer                  | Pre-clinical                      | –                               | (32)                      |
| 3  | Cucurbitacin B                    | ACLY          | Cytotoxic for many cancer cell lines, it produces tumor reduction in xenograft models, and has low oral bioavailability in pharmacokinetics studies | Pre-clinical                      | In vitro: breast (BRCA-1, SK-BR-3, MCF-7, MDA-MB-231), lung (NCI-H460), brain (SF-268), colon (HCT-116), prostate (PC-3, LNCaP), and liver (BEL-7402) In vivo: prostate (PC-3 xenograft) and liver (BEL-7402 xenograft) | (33,35-38,171) |
| 4  | ETC-1002                          | ACLY          | A prodrug of active metabolite ETC-1002-CoA. AMPK activator. In Phase III of clinical trials, it decreases levels of low-density lipoprotein-cholesterol. Not studied in the context of cancer | Clinical—phase III (for treatment of hypercholesterolemia) | –                               | (21)                      |
| 5  | Unnamed                           | ACLY          | Nanomolar range inhibitor designed and studied for the treatment of cardiovascular disease. Studies in HepG2 cell line show low cytotoxic activity in the micromolar range | Pre-clinical                      | In vitro: liver (HepG2)          | (40)                      |
| 6  | NDI-091143                        | ACLY          | Similar structure to compound 5 but with structural changes to improve the inhibitory activity to a low nanomolar range. Not studied in the context of cancer | Pre-clinical                      | –                               | (41)                      |
| 7  | Leelamine                         | ACLY          | Natural product, micromolar cytotoxic activity in several cancer cell lines, it reduces tumor growth in xenograft models. Recently reviewed in the context of antitumoral activity | Pre-clinical                      | In vitro: prostate (LNCaP, 22Rv1, PC-3) In vivo: prostate (22Rv1 xenograft) | (43,44)                   |
| 8  | DCV                               | ACLY          | Fungus-derived macrolide, low micromolar range inhibitor. Cytotoxic activity against leukemia cancer cells showing low micromolar activity. Targets other than ACLY may be involved in its cytotoxic activity | Pre-clinical                      | In vitro: leukemia (Jurkat)       | (45)                      |
| 9  | Soraphen A                        | ACC           | Natural myxobacterial metabolite product, active against several cancer cell lines. Antifungal, inhibits human immunodeficiency virus and hepatitis C virus at nanomolar concentration | Pre-clinical                      | In vitro: liver (HepG2) and prostate (LNCaP, PC-3M) | (61,64-67,71) |
| 10 | CP-640186                         | ACC           | Inhibition of isoenzyme ACC1 and ACC2 at nanomolar concentration, triglyceride synthesis inhibitor, reduction of body fat content in vivo. It reduces lung cancer cell proliferation at micromolar concentration | Pre-clinical                      | In vitro: liver (HepG2) and lung (H460) | (72-74)                   |

(Continues)
| #  | Compound | Target enzyme | Outcome | Development stage | Cancer type | References |
|----|----------|---------------|---------|-------------------|-------------|------------|
| 11 | unnamed  | ACC           | CP-640186-based inhibitor with improved activity. Not studied in the context of cancer | Pre-clinical | –            | (75)       |
| 12 | MK-4074  | ACC           | Low nanomolar inhibitory activity. it reduces DNL but increases plasma TGs, producing hypertriglyceridemia. Not studied in the context of cancer | Clinical - phase I (for treatment of nonalcoholic fat liver disease) | –            | (76)       |
| 13 | Unnamed  | ACC2          | Strong selective ACC2 inhibitor, 3 orders of magnitude higher than ACC1. Good bioavailability and safety profile in vivo after oral administration. Not studied in the context of cancer | Pre-clinical | –            | (77)       |
| 14 | Unnamed  | ACC1          | Strong nanomolar range selective inhibitor. Inhibition of Malonyl-CoA in xenograft models of cancer. Good pro-drug properties. Not studied in xenograft tumor inhibition | Pre-clinical | In vivo: colorectal (HCT-116 xenograft) | (78)       |
| 15 | Unnamed  | ACC1          | Strong nanomolar range selective ACC1 inhibitor, 2 orders of magnitude higher than ACC2. Strong tumor growth inhibition in xenograft studies after oral administration with moderate body weight reduction side effect | Pre-clinical | In vivo: colorectal (HCT-116 xenograft) and renal cell (786-O xenograft) | (79)       |
| 16 | ND-630   | ACC           | Strong ACC1/2 dimerization inhibition; liver specific. Reduces fatty acid synthesis, stimulates fatty acid oxidation, reduces weight gain without affecting food intake and improves insulin response in diabetic fatty rat model. Weak cytotoxic effect against lung tumors | Clinical - phase II (for treatment of nonalcoholic fat liver disease) | In vitro: liver (HepG2) and non-small-cell lung (A549) | (80,81) |
| 17 | ND-646   | ACC           | Potent inhibition of ACC and FA synthesis, lung tumor growth suppression, only moderate body weight loss in high-dose treatment and in vivo | Pre-clinical | In vitro: non-small-cell lung (A549, H157, H1355, H460) In vivo: non-small-cell lung (A549 xenograft) | (81,82) |
| 18 | Unnamed  | ACC1          | Similar structure to ND-646, it inhibits ACC1 and presents cytotoxic activity against lung cancer cell line at low nanomolar concentration. Leads to apoptosis of non-small-cell lung carcinoma cells | Pre-clinical | In vitro: non-small-cell lung (A549) | (81)       |
| 19 | (–)-C75  | FAS           | It is often used as a racemic mixture. (+)-C75 is a carnitine palmitoyltransferase inhibitor and has an anorexic effect. (–)-C75 is a weak FAS inhibitor at micromolar range, nevertheless it has shown cytotoxic activity against several cell lines in vitro. The racemic mixture inhibits tumor growth in many xenograft models | Pre-clinical | In vitro: breast (MCF-7, SKBr-3, MDA-MB-231), ovarian (OVCAr-3), pancreas (MIAPaCa-2), prostate (PC-3), and colorectal (HCT-116) | (101,104-107,172) |
| 20 | (–)-UB006| FAS           | Based on (–)-C75 with improved inhibition and cytotoxic properties, especially in an ovarian cancer cell line. No body change or food intake change after intraperitoneal administration in mice. Not studied in xenograft tumor inhibition | Pre-clinical | In vitro: breast (MCF-7, SKBr-3, MDA-MB-231), ovarian (OVCAr-3), pancreas (MIAPaCa-2), prostate (PC-3), and colorectal (HCT-116) | (101)       |

(Continues)
| #  | Compound      | Target enzyme | Outcome                                                                 | Development stage       | Cancer type                                                                 | References |
|----|---------------|---------------|-------------------------------------------------------------------------|-------------------------|----------------------------------------------------------------------------|------------|
| 21 | Orlistat      | FAS           | It inhibits tumor growth in *xenograft* models of prostate, colon and melanoma cancer, reduces proliferation and enhances apoptosis of breast cancer. Lipase inhibitor that is commercialized to treat obesity by preventing fat absorption and calorie intake | FDA-approved (as an anti-obesity drug) | In vitro: breast, prostate, ovarian, endometrium, colon, retina, oral cavity, head & neck, gastric and brain using many different cell lines In vivo: prostate (PC-3 and LNCaP *xenografts*), colon (HT29 *xenograft*), and other models of breast, oral, lung and murine melanoma | (98,173) |
| 22 | GSK2194069    | FAS           | Nanomolar range inhibitor, inhibits tumor growth of *xenograft* model without significant loss of weight | Pre-clinical | In vitro: gastric (KATO-III, MKN45), and non-small-cell lung (A549) In vivo: prostate (C42b *xenograft*) | (113,114) |
| 23 | TVB-3166      | FAS           | Nanomolar range inhibitor that stops FA synthesis, disrupts the lipid raft structure, and in vivo inhibits tumor growth in lung, ovarian and pancreatic tumors | Pre-clinical | In vitro: lung (CALU-6, A-549), breast (MDA-MB-231, MDA-MB-453, MDA-MB-468), colorectal (COLO-205, HT-29), prostate (22RV1), and ovarian (OVCAR-5, OVCAR-8) In vivo: pancreas (PANC-1 *xenograft*), ovarian (OVCAR-8 *xenograft*), and non-small-cell lung (CTG-0165, CTG-0160, CTG-0743 *xenografts*) | (116) |
| 24 | TVB-2640      | FAS           | In phase I clinical trials, antitumor activity in monotherapy and co-treatment with paclitaxel, and in phase II trials, it was well-tolerated in humans in co-treatment with bevacizumab | Clinical - phase II (ongoing) | In vivo: lung, colon, breast, and astrocytoma | (118,119) |
| 25 | Fasnall       | FAS           | Micromolar concentration inhibitor, produces a significant change in the global cellular lipid profiles, induces apoptosis, and critically affects the lipid raft structure and functioning, in vivo study shows improved effect in co-treatment with carboplatin regarding tumor reduction and survival | Pre-clinical | In vitro: liver (HepG2), and breast (MCF7, MDA-MB-468, BT474, SKBR-3) In vivo: breast (MMTV-Neu model) | (120) |
| 26 | JNJ-54302833  | FAS           | Nanomolar range inhibitor with cytotoxic activity among many cell lines from different tissues, fatty acid synthesis inhibition in vivo, no reported tumor reduction in vivo | Pre-clinical | In vitro: ovarian (A2780), prostate (PC-3M, LNCaP), lymphoma (OCI-LY1), leukemia/lymphoma/myeloma (MV4-11), lung (H460, A549), and breast (MDA-MB-468) In vivo: lung (H460 *xenograft*) | (121) |
| 27 | IPI-9119      | FAS           | Nanomolar concentration FAS irreversible inhibitor binding to TE domain that reduces tumor growth in *xenograft* models | Pre-clinical | In vivo: prostate (22Rv1, LNCaP-95 *xenografts*) | (122) |
| #  | Compound   | Target enzyme | Outcome                                                                 | Development stage | Cancer type                                      | References            |
|----|------------|---------------|-------------------------------------------------------------------------|-------------------|------------------------------------------------|-----------------------|
| 28 | FT113      | FAS           | Low micromolar FAS inhibitor and nanomolar cytotoxic activity in cancer cells that inhibits tumor growth in vivo | Pre-clinical      | In vitro: prostate (PC-3), breast (BT474), and leukemia (MV-411) | (123)                 |
|         |            |               | In vivo: leukemia (MV-411 xenograft)                                     |                   |                                                |                       |
| 29 | CAY10499   | MAGL          | Nanomolar range reversible inhibitor with micromolar cytotoxic properties in several cancer cell lines | Pre-clinical      | In vitro: ovarian (OVSAHO, OVCAR3, COV318, CAOV3, SKOV3), breast (MDA-MB-231), and colorectal (HCT116) | (141-143)             |
|         |            |               |                                                                         |                   |                                                |                       |
| 30 | JZL184     | MAGL          | Irreversible inhibitor. Shows anticancer effects in vivo against several cancer cell lines, also has analgesic, antinoceptive, anti-inflammatory, gastroprotective, antidepressant and anxiolytic effects | Pre-clinical      | In vitro: colorectal (HCT116, SW480, LoVo), melanoma (C8161, MUM2B, MUM2C), ovarian (SKOV3, OVCAR3), breast (231MFP, MCF7), and liver (HepG2, SMMC-7721, L02) | (125,134,145-147)     |
|         |            |               |                                                                         |                   |                                                |                       |
| 31 | KML29      | MAGL          | Developed based on JZL184. In vitro, selective inhibition at nanomolar range concentration | Pre-clinical      | –                                              | (148)                 |
| 32 | PF-0679071 | MAGL          | Potent inhibitor at low nanomolar concentration that was studied in vitro and in vivo against neuroinflammatory disease. Not studied in the context of cancer | Pre-clinical      | –                                              | (150)                 |
| 33 | JKK-048    | MAGL          | Ultra-potent irreversible selective inhibitor. Not studied in the context of cancer. | Pre-clinical      | In vitro: melanoma (C8161)                     | (151)                 |
| 34 | Unnamed    | MAGL          | Low nanomolar reversible inhibitor. In in vivo studies it showed a dramatic reduction of arachidonic acid and an increase of 2-AG. Studied against neurodegenerative diseases and not cancer. | Pre-clinical      | –                                              | (153)                 |
| 35 | Unnamed    | MAGL          | Micromolar reversible inhibitor with antiproliferative activity against ovarian cancer cell lines | Pre-clinical      | In vitro: ovarian (OVCAR3, CAOV3)               | (143)                 |
| 36 | Unnamed    | MAGL          | Nanomolar reversible inhibitor with antiproliferative activity at micromolar concentration against human breast, colorectal and ovarian cancer cells. In vivo tumor growth inhibition study has not been carried out | Pre-clinical      | In vitro: breast (MDA-MB-231), colorectal (HCT116), and ovarian (CAOV3, OVCAR3, and SKOV3) | (142)                 |

**TABLE 1** (Continued)
of lipogenic tissues such as liver, adipose tissue, and lactating mammary gland and catalyzes malonyl-CoA formation in FA synthesis.\textsuperscript{45,48} It has been reported as upregulated in some types of human cancer, including breast, prostate, lung, ovary, and colon.\textsuperscript{49} ACC2 is commonly found in the mitochondrial outer membrane of more oxidative tissues such as skeletal muscle and heart and in the metabolically active liver. In these tissues, ACC2 functions as a regulator of FA β-oxidation (Figure 1) by inhibition of carnitine palmitoyltransferase 1 (CPT1) via malonyl-CoA.\textsuperscript{50} Human ACC1 contains 2,346 amino acid residues with a molecular weight of 265 kDa and ACC2 contains 2,483 amino acid residues with a molecular weight of 280 kDa.\textsuperscript{50} The difference of about 140 amino acids in the N-terminus of ACC2 explains the difference in location and function when compared to ACC1.\textsuperscript{50,51}

Both ACCs isofoms are mainly regulated by AMPK, which inactivates the enzyme by phosphorylation, and protein phosphatase 2A, which dephosphorylates the enzyme and activates it.\textsuperscript{45,51} In addition, AMPK is activated by AMP and deactivated by ATP. When AMP levels are low, AMPK is inactive and ACC is active.\textsuperscript{45} Other molecules that regulate ACC are citrate (activation), palmitoyl-CoA (inactivation) and CoA.\textsuperscript{45,48,50,51} At transcriptional level, ACC is regulated by some transcription factors such as the carbohydrate response element-binding protein (ChREBP) and SREBP-1c by some transcription factors such as the carbohydrate receptor complex 56 and the PPARγ co-activator (PGC).\textsuperscript{57} During the catalytic reaction of ACC (Figure 1), bicarbonate plays an important role as it is the CO\textsubscript{2} source for biotin carboxylation. This bicarbonate molecule is synthesized from CO\textsubscript{2} and water by α-carboxylases (CAs), which shows that CAs are also important regulators of ACC.\textsuperscript{45} One CA, CA9/CA12, has been identified as overexpressed in many tumors and is associated with cancer progression.\textsuperscript{51} There are also other novel regulators of ACC activity, such as BRCA1 and AKR1B10 that are overexpressed in human carcinomas and are associated with an increase in FA synthesis.\textsuperscript{45}

The ACC1 isoform, in particular, is regulated by a series of transcription factors controlled by glucose, insulin, thyroid hormones, and catabolic hormones.\textsuperscript{51,54} These transcription factors are SREBP-1,\textsuperscript{55} the liver X receptor/retinoid X receptor complex\textsuperscript{56} and the PPARγ co-activator (PGC).\textsuperscript{57} Specifically, SREBP-1 is a key regulator of ACC1 (and of other lipogenic enzymes) at transcriptional level as it is an effector of MAPK and PI3K.\textsuperscript{50,51} At translational level, ACC1 is activated by the human epidermal growth factor receptor-2 (HER2) mediated by the PI3K/Akt/mTOR signaling pathway, as observed in breast cancer.\textsuperscript{58}

### 3.2.2 Inhibitors and cytotoxic effects

The overexpression of ACC, mainly the ACC1 isoform, plays an important role in cancer treatment because it contributes to the survival of cancer cells when therapies targeting the Warburg effect are applied.\textsuperscript{59} The mechanism of action is not fully understood yet, but it is associated with the response of cancer cells to AMPK activation-induced inhibition of ACC.\textsuperscript{60} In addition, it has been observed that the exogenous uptake of palmitic acid completely saves cancer cells from death. The addition of palmitic acid to culture media where LNCaP and PC-3M cells were incubated with Soraphen A (ACC inhibitor, see below) rescues cells from drug-induced cell death. An exogenous source of fatty acid could affect the treatments that target ACC or in general treatment targeting de novo synthesis enzymes. For effective treatment, a combination of membrane proteins that transport FAs and lipids together with pharmacological agents that interrupt de novo synthesis may be needed.

Although ACC1 overexpression has been observed in many tumors, both isoforms of ACC seem to contribute almost equally to lipid synthesis, as studies have shown.\textsuperscript{61} Moreover, the pharmacological inhibition of ACC leads to cancer cell cycle arrest and/or apoptosis in several cell lines.\textsuperscript{35}

ACC inhibitors have been reviewed in a previous study,\textsuperscript{52} but it is worth mentioning the most important ones (Figure 3). First, Soraphen A (9, Figure 3) is a natural product isolated from the soil bacterium \textit{Sorangium cellulosum}, with a structure that contains a lactone formed by 17 carbon atoms, where the C3 and C7 of the ring are connected as a hemiketal.\textsuperscript{63} It was initially recognized as a potent antifungal drug. Further studies showed that it is also a potent inhibitor of eukaryotic ACC that binds specifically to the BC domain.\textsuperscript{64} However, it has no effect on the BC domain of prokaryotic ACC (bacterial).\textsuperscript{65} 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, which inhibits ACC.\textsuperscript{64} Further studies evidenced that Soraphen A inhibits DNL in human hepatoma (HepG2 cell line) and prostatic cancer (LnCaP cell line),\textsuperscript{66} and antitumor activity has been observed in other cancer cells.\textsuperscript{67-69} In particular, Soraphen A inhibits FA synthesis, which promotes FA β-oxidation and reduces the PLs level in prostate cancer cells (LnCaP and PC-3M cell lines), which inhibits their proliferation.\textsuperscript{60} Additionally, it shows antiviral activity.\textsuperscript{70} However, Soraphen A has not been clinically used due to its poor drug-like properties.

Pfizer researchers developed a metabolically stable piperidinyl derived analog, CP-640186 (10, Figure 3).\textsuperscript{71} It is a nonselective, reversible, ATP noncompetitive inhibitor of ACC1/2. It has been found to inhibit the synthesis of FAs and TGs in HepG2 cells\textsuperscript{71} and shows cytotoxic properties in...
The authors reported an IC$_{50}$ of 53 nM for rACC1 (rat) and 61 nM for rACC2. A study of the co-crystal structure of CP-640186 and the CT domain of ACC (yeast) revealed 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.

Another piperidinyl-derived compound that interacts similarly with the CT domain is compound 11 (Figure 3), which has 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

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**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 parentheses.

| Compound | ACC IC$_{50}$ | Cytotoxicity IC$_{50}$ |
|----------|---------------|------------------------|
| 9, Sorafen A | 5 nM (hACC1/2) | - |
| 10, CP-640186 | 53 nM (rACC1) | 61 nM (rAA2) |
| 11 | 76 nM (hACC1/2) | - |
| 12, MK-4074 | 3 nM (hACC1/2) | - |
| 13 | 1.9 nM (hACC2) | 1.95 µM (hACC1) |
| 14 | 0.58 nM (hACC1) | >10 µM (hACC2) |
| 15 | 1.5 nM (hACC1) | 140 nM (hACC2) |
| 16, ND-630 | 2.1 nM (hACC1) | 6.1 nM (hACC2) | >20 µM (A549) |
| 17, ND-646 | 3.5 nM (hACC1) | 4.1 nM (hACC2) | 16.2 nM (A549) |
| 18 | 6.87 nM (hACC1) | 9.4 nM (A549) |
In 2016, Nimbus therapeutics developed an efficient, reversible, specific ACC1/2 non-selective inhibitor: \textit{ND-630} (16, Figure 3). It inhibits ACC by interacting with its phosphopeptide-acceptor and dimerization site, which interferes with dimerization.\textsuperscript{79} In the same way as \textit{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. However, ND-630 is more potent because it fills a narrow, deep pocket in the BC domain.\textsuperscript{79} Despite its potent ACC inhibitory effect, further studies showed that ND-630 has weak cytotoxicity against lung tumors.\textsuperscript{80} In contrast, its primary amide, \textit{ND-646} (17, Figure 3), showed both properties: potent ACC inhibition and FA synthesis,\textsuperscript{81} and a strong cytotoxic effect against lung tumors.\textsuperscript{80} 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 cannot dimerize.\textsuperscript{81}

In 2019, Li et al performed small structural changes in ND-646, which led to the discovery of several compounds that have a better cytotoxic effect.\textsuperscript{80} One is compound 18 (Figure 3), which acts by the same mechanism of \textit{ND-646} and has an \(I_{50}\) of 6.87 nM for hACC1. In non-small-cell lung cancer (NSCLC), the ACC1 mRNA is overexpressed (as in many other types of cancer mentioned above). By using compound 18, the growth of A549 cells was inhibited (\(I_{50} = 16.2\) nM), leading to apoptosis.\textsuperscript{80}

To sum up, 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 cancer therapy could have some non-desired side effects.

### 3.3 | FAS

#### 3.3.1 | Function and description

Human fatty acid synthase (hFAS) is a complex homodimeric cytosolic enzyme of 552 kDa that catalyzes the formation of palmitate (C\textsubscript{16}) from acetyl-CoA and malonyl-CoA in the presence of NADPH (Figure 1).\textsuperscript{82} FAS has seven catalytic domains, which are (in linear order from the carboxyl terminus): thioesterase (TE), acyl-carrier protein (ACP), \(\beta\)-ketoacyl reductase (KR), enoyl reductase (ER), \(\beta\)-hydroxyacyl dehydratase (DH), malonyl/acyetyl transferase (MAT), and \(\beta\)-ketoacyl synthase (KS). Moreover, there are two additional nonenzymatic domains, pseudoketoreductase (\(\PsiKR\)), and the peripheral pseudomethyltransferase (\(\PsiME\)).\textsuperscript{83}

As mentioned above, FAS catalyzes the final step of 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.\textsuperscript{84} The ACP passes the acetyl moiety over the active site cysteine of the KS. Subsequently, MAT transfers the malonyl group of malonyl-CoA to the ACP, and the KS catalyzes 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.84

FAS overexpression is one of the most frequent phenotypic alterations in cancer cells. Moreover, it is related to a higher risk of cancer recurrence and death.85 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.85,86 Normally, FAS is mainly regulated by nutritional signals and is expressed in hormone-sensitive cells and cells with high lipid metabolism.87 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.83 Two well-studied pathways involved in FAS regulation are the MAPK and the PI3K/Akt pathways.88 HER2 and epidermal growth factor (EGF) receptors are involved in the downstream PI3K/Akt and MAPK signaling pathways, which subsequently activate FAS expression transcriptionally.89 Moreover, FAS expression can be amplified by crosstalk between sex hormones, growth factors and their receptors.28 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.83 In addition, SREBP-1c is directly regulated by the proto-oncogene FBI-1 (Pokemon) through its DNA-binding domain, and thus synergistically activates FAS transcription.90 Another transcription factor that is regulated as SREBP-1c and is highly implicated in FAS expression is ChReBP.91 There are other factors such as NAC1, acetyltransferase P300 and some microRNAs that regulate FAS expression in tumor cells.92

In breast cancer, FAS mediates the overexpression of S14, a lipogenesis-related nuclear protein that is regulated by SREBP-1c and supports cell growth and survival.93 Moreover, in SK-BR-3 and BT-474 breast cancer cell lines, FAS might be regulated by another mechanism via mTOR-mediated translational induction.58 By this mechanism, HER2 is overexpressed and with it higher levels of FAS are observed.83 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.94 USP2a is regulated by androgen and its inactivation results in FAS protein decrease and enhanced apoptosis.83

Microenvironmental effects such as hypoxia and acidity have important roles in the regulation of FAS.83 It has been evidenced that under hypoxic conditions in human breast cancer cell lines, FAS is upregulated.95 Moreover, Furuta et al found that SREBP-1c is also upregulated as an effect of phosphorylation of Akt with subsequent activation of the hypoxia-inducible factor HIF1. Finally, excessive extracellular acid conditions could result in changes in transcriptional activation of the FAS gene in breast cancer cells.96

### 3.3.2 Inhibitors and cytotoxic effect

Since the discovery of FAS as an oncogenic target, many inhibitors have been developed and tested against several cancer cell lines. It has been evidenced that FAS inhibition stops proliferation and induces the apoptosis of cancer cells, with minimal effects on normal cells.92 Most of the FAS inhibitors have been previously reviewed,83,97,98 but it is worth mentioning the most important, promising, potent inhibitors that are found in the literature (Figure 4). The first is the synthetic compound C75, a weak irreversible FAS inhibitor with an IC\(_{50}\) between 200-500 µM (racemic mixture).96-100 C75 interacts with FAS in different domains, specifically with KS, TE, and ER domains,101 and shows anticancer activity in many cancer cell lines and xenograft models.102,103 However, in vivo studies showed that C75 has a negative side effect: it reduces food intake and induces body weight loss.104,105 Further studies showed that the (−)-C75 enantiomer (19, (Figure 4) can inhibit FAS in vitro, which has a cytotoxic effect in several cancer cell lines without affecting food consumption.106 It was also evidenced that the (+)-C75 enantiomer inhibits CPT1 and produces anorexic effects. With these results, Makowski et al developed a series of C75-based inhibitors, taking into account the enantiomeric selectivity of FAS.100,107 They found that elongation of the aliphatic chain or the introduction of larger groups in the β-position of the lactone cause a decrease in inhibitory activity of FAS.107 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 FAS from rat.108 In vitro studies showed that (−)-UB006 is more cytotoxic than C75 against several cancer cells. Specifically, against the OVCAR3 cell line, it was found to be 40 times more cytotoxic than C75. Furthermore, in vivo administration of (−)-UB006 evidenced that it does not affect food intake and body weight. There are other C75-based inhibitors with no apparent negative side effects, like C93 and C247.108

**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.97 Orlistat forms a covalent adduct with the serine of the TE domain and has an IC\(_{50}\) of 0.9 µM.98,109 It has shown tumor growth inhibition in xenograft models of prostate cancer and melanoma, and 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. 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.

In 2014, the pharmaceutical company GlaxoSmithKline developed a highly potent, reversible, specific inhibitor of the KR domain of hFAS. 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 decreases the DNL, which produces potent inhibition in cancer cell growth and proliferation in gastric and non-small-cell lung cancer cell lines. Moreover, they determined 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 tumor growth with no apparent side effects.

**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 parentheses.
Sagimet Biosciences (previously 3-V Biosciences) developed a new generation of highly potent, reversible, FAS inhibitors. One of them is TVB-3166 (Figure 4), that has an IC\textsubscript{50} of 0.042 \mu M for FAS (from rabbit). It can stop FA synthesis and disrupt the lipid raft structure, which affects all the membrane-associated molecules and signaling pathways, such as Ras, Akt-mTOR, and Wnt-\beta-catenin. In vivo studies showed that a single daily dose can inhibit FAS for 10-12 hours every day, which induces xenograft tumor growth inhibition in lung, ovarian, and pancreatic tumor models. These results showed that an irreversible inhibitor is not necessary to stop tumor growth in vivo. Moreover, TVB-3166 does not have any apparent negative side effects.

Another compound developed by Sagimet Biosciences is TVB-2640 (24, Figure 4), which is the first and only FAS inhibitor that has reached clinical trials to date. TVB-2640 is described as a highly potent, selective, reversible FAS inhibitor that acts in the KR domain and has an IC\textsubscript{50} of 0.05 \mu M. In 2017, the phase 1 clinical trial of TVB-2640 in patients with solid tumors was completed. Its antitumor activity was demonstrated in monotherapy and co-treatment with paclitaxel. Some common negative side effects were observed including alopecia, palmar-plantar erythrodysesthesia, and decreased appetite, among others. Now, phase 2 of clinical trials of TVB-2640 (monotherapy and/or co-treatment) is underway, and includes the treatment of lung, colon, breast, and astrocytoma cancer (NCT03808558, NCT02980029, NCT03179904, and NCT03032484). Moreover, partial results of the phase 2 trial of TVB-2640 in combination with Bevacizumab in patients with the first relapse of high-grade astrocytoma showed that co-treatment is well-tolerated in humans.

In 2016, Alwarawrah et al discovered a potent thiophenopyrimidine-based FAS inhibitor with broad antitumor activity against various non-tumorigenic and aggressive tumor-forming breast cancer cell lines. Fasnall (25, Figure 4) with an IC\textsubscript{50} of 3.71 \mu M for hFAS, can produce a significant change in the global cellular lipid profile. Its mechanism of action includes an increase of intracellular levels of ceramide (also in DAGs and unsaturated FA), which increases the apoptosis of cancer cells. Moreover, Fasnall inhibits the formation of PLs with saturated acyl chains and promotes the uptake of unsaturated FAs, which critically affects the lipid raft structure and functioning. Fasnall treatment has no apparent negative side effects and its combination with other chemotherapeutic agents such as carboplatin augments the tumor volume reduction and survival in vivo studies. All these characteristics and the ease of adaptability of the Fasnall synthetic route suggest that it can be further optimized to develop new derivatives with better pharmacological properties.

In 2018, Lu et al developed a series of spirocyclic imidazolinone FAS inhibitors. One of them showed high FAS inhibitory activity with good cellular activity and oral bioavailability. The compound JNJ-54302833 (26, Figure 4) has an IC\textsubscript{50} of 28 nM for hFAS and effectively inhibits the proliferation of several cancer cell lines including ovarian, prostate, lymphoma, leukemia, lung, and breast. The authors found that one compound of the series of spirocyclic imidazolinone FAS inhibitors (not exactly JNJ-54302833) binds to the KR domain by H-bonds. Furthermore, hydrophobic interactions occurred with the KR and non-catalytic domains of FAS. In 2019, Infinity Pharmaceuticals published the discovery of a potent, irreversible inhibitor of hFAS: IPI-9119 (27, Figure 4). It has an IC\textsubscript{50} of 0.3 nM for hFAS and inhibits the TE domain by promoting acylation of the catalytic serine. The authors evidenced that IPI-9119 significantly reduced prostate cancer cell growth and induced cell cycle arrest and apoptosis in PCA cells. Moreover, FAS inhibition generated an entire lipid homeostasis change, including the accumulation of polyunsaturated FAs produced by the uptake and use of exogenous FAs. In addition, cholesterol synthesis was increased as a type of redirection of the unused acetyl-CoA. Therefore, PCA cells tried to compensate for the DNL deficiency by upregulating genes encoding enzymes and transcription factors involved in lipid synthesis. Further investigations are necessary to understand these anomalies caused by IPI-9119 treatment.

FORMA Therapeutics developed a series of novel piperazine derivative FAS inhibitors. One of them is the compound FT113 (28, Figure 4) with an IC\textsubscript{50} of 0.213 \mu M for hFAS. The authors reported that several H-bond interactions occurred between the hydroxyl and carbonyl of the hydroxy-cyclopropyl amide and the active site residues of the KR domain. These observations were determined by the X-ray co-crystal structure of FT113 bound to a \Psi ME-\Psi KR-KR tridomain FAS construct. FT113 was the compound with the best balance between physicochemical and pharmacokinetic properties and potency. Moreover, FT113 showed anti-proliferative activity against prostate (PC3 cell line), breast (BT-474 cell line), and leukemia (MV-411 cell line) cancer cells. After 16 days of treatment with FT113, an increase in malonyl-CoA levels was evidenced in tumors, as well as tumor growth inhibition of 32% and 50% by treatment with 25 and 50 mg/kg, respectively, compared to the vehicle.

It has been evidenced that FAS is a viable target for the inhibition of FA biosynthesis, as many compounds showed high FAS inhibitory activity and cytotoxic effects against several cell lines, with no apparent side effects. Moreover, one compound (TVB-2640) is currently being tested in humans, which suggests that FAS targeting has great potential for anticancer therapy. We now have new tools for discovering FAS inhibitors, such as computational screening. With tools of this kind, it is easier to discover and optimize new compounds, with lower costs and less time in the process.
3.4 | MAGL

3.4.1 | Function and description

In DNL, MAGL liberates the stored fatty acids for metabolic and signaling purposes, which supports and promotes the migration, invasion, survival, and growth of tumors in aggressive human cancers.124 This enzyme has been found at elevated levels, together with free fatty acids (FFAs), in several aggressive human cancer cell lines.124 MAGL controls the FFAs level in cancer cells through the hydrolysis of monoacylglycerols (MAGs) (Figure 1).124 In normal cells, MAGL controls the levels of MAGs and not the FFAs level, which shows that in cancer cells this pathway is altered (as others have already mentioned) to satisfy pathogenic requirements.124,125 The mechanism of MAGL-stimulation for cancer aggressiveness is through the action of FFA-derived products.124 Some secondary lipid metabolites such as LPL (including lysosphatidylcholine [LPC], LPA, lysosphatidylethanolamine [LPE] and PA), prostaglandin E2 (PGE2), and ether lipids (monoalkylglycerol [MAGE] and alkyl LPE) are regulated by MAGL. All of them support cancer malignancy but LPA and PGE2 to a 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 Besides, it has been observed that CB2 receptor activation promotes colon cancer. Recently, it was evidenced that the attenuation of CB2 signaling suppress tumor growth.130 In addition, 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. 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 the inaccessible or solvent-exposed active site.134 This special characteristic evidenced that MAGL regulates the entering of 2-AG to the catalytic site through CPE flexible control of the lid domain opening.134 It was hypothesized that the hydrophobic character of the lid domain serves to locate MAGL close to the PL membrane, which facilitates 2-AG recruitment.134 When the substrate enters the active site, MAGL takes its closed form and dissociates from the membrane in parallel to cleaving 2-AG. Then, MAGL again takes the open conformation and reassociates with the membrane.134 The mechanism of action of MAGL has not been fully elucidated yet. However, as mentioned before, an important part of this is now understood and might lead to the development of better inhibitors for this enzyme.

Regarding the regulation of MAGL, very little is known about post-transcriptional and post-translational modifications. There is no evidence of phosphorylation or other modifications to date.135 However, slight variations have been found between the MAGL enzymes from adipose tissues, liver, heart, lung, stomach, kidney, spleen, kidney, and adrenal gland, and the MAGL enzymes from brain, testis, and skeletal muscle.135,136 Therefore, post-transcriptional and/or post-translational modifications may 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 human MAGL) and thus design better and more selective inhibitors.

3.4.2 | Inhibition and cytotoxic effect

It has been evidenced that by inhibiting MAGL, tumorigenesis and cancer progression are suppressed in several cancer cell lines.41,137 However, 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.139,140 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 new ones (Figure 5). In 2008, Muccillo et al developed a good inhibitor of MAGL: CAY10499 (29, Figure 5).140 The carbamate derivative CAY10499 is a covalent irreversible inhibitor of MAGL, with an IC₅₀ of 134 nM for hMAGL.141 However, 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(3H)-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, COV3, 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 of its administration, including analgesic, antinociceptive, anti-inflammatory, gastroprotective, antidepressant, and anxiolytic effects. JZL184 has shown anticancer effects against colorectal cancer and hepatocellular carcinoma (HCC). In colorectal cell lines, it reduced tumor cell progression and increased apoptosis (correlated with MAGL inhibition). In HCC cell lines, it significantly increased apoptosis and reduced tumor growth, and even decreased the invasion ability of the HCC cell line SMMC-772. Moreover, in C8161 and SKOV3 aggressive

| Compound | MAGL IC$_{50}$ | Cytotoxicity IC$_{50}$ |
|----------|----------------|------------------------|
| 29, CAY10499 | 0.134 µM | 34 µM (SKOV3) |
| 30, JZTL184 | 6 nM | - |
| 31, KML29 | 5.9 nM | - |
| 32, PF-06795071 | 3 nM | - |
| 33, JJKK-048 | 0.36 nM | - |
| 34 | 3.6 nM | - |
| 35 | 0.51 µM | 11 µM (CAOV3) |
| 36 | 80 nM | 15 µM (SKOV3) |

**FIGURE 5** Structure and half-maximal inhibitory concentration (IC$_{50}$) of selected hMAGL inhibitors. The cell line of the cytotoxicity studies is indicated in parentheses in each case.
cancer cells, the tumor growth and migration rate were reduced by the administration of JZL184 once per day (40 mg/kg). 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 generated a more selective JZL184 derivative with O-hexafluoroisopropyl as a leaving group: KML29 (31, Figure 5). This compound has an IC50 value of 5.9 nM for hMAGL and has shown complete selectivity for MAGL over FAAH. KML29, like other carbamate derivatives, binds covalently and irreversibly to MAGL by the formation of a carbamylated enzyme-inhibitor adduct. However, there have been no studies of the anticancer activity of this compound to date.

After a series of optimizations of carbamate derivatives, Pfizer’s research group developed the irreversible inhibitor PF-06795071 (32, Figure 5) with an IC50 of 3 nM for hMAGL. This compound has great drug-like properties due to its novel stereo-defined trifluoromethyl glycol leaving group. PF-06795071 was studied against neuroinflammatory disease and showed high in vivo efficacy, 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 IC50 value of 0.36 nM against hMAGL. This compound irreversible inhibitor was proved to be selective for MAGL against other serine hydrolases such as FAAH and ABHD6. The proposed mechanism of action is very similar to that of the carbamate-derived inhibitors. JJKK-048 also forms a carbamate adduct with a serine in the active site of MAGL, and the triazole (1,2,4-triazolateanion) acts as the leaving group. In vitro studies of JJKK-048 in C8161 melanoma cells show that it is highly selective for MAGL, but further information about its impact on cell proliferation, migration, and invasiveness has not been reported.

The irreversible inhibition of MAGL has many negative effects in vivo, such as loss of analgesic effects and cross-tolerance to CB1 agonists. In addition, it generates physical effects in vivo, such as loss of analgesic effects and cross-tolerance to any of the CB1 and CB2 cannabinoid receptors. Recently, Granchi et al developed a benzoylpiperidine-based potent reversible inhibitor of MAGL: compound 36 (Figure 5). Compound 36 has a value of 80 nM for hMAGL and showed high selectivity over FAAH, ABHD6, ABHD12, CB1, and CB2 (IC50 > 10 μM in all cases). In addition, 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 cancer treatment could result in the disruption of cancer cell proliferation but could also 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.

4.1 | SREBP-1: Blocking overexpression of lipogenic enzymes

Sterol regulatory element binding proteins (SREBPs) are transcription factors that are responsible for cholesterol and fatty acid biosynthesis gene activation. Pharmacological inhibition of this protein could block the expression of lipogenic enzymes in cancer cells. Indeed, some small molecules such as fatostatin were found to block fatty acid synthesis by inhibiting the activation of SREBP-1 and they reduced the malignant phenotype of esophageal cancer.

4.2 | IDH and SLC25A1: Blocking citrate supply for FA synthesis

Citrate is a key building block for de novo fatty acid synthesis. In normal oxygen supply conditions, it is obtained from
glucose. Under hypoxic conditions, such as in cells from the solid tumor, another nutrient like glutamine might become a citrate precursor. In this case, de novo synthesis can be interrupted by inhibition of the key enzyme in that pathway: isocitrate dehydrogenase (IDH), which in reductive metabolism conditions catalyzes the conversion of α-ketoglutarate into citrate (Figure 6), the precursor of acetyl-CoA. Nevertheless, IDH has become a target for myeloid leukemia rather than solid tumor treatment because of other mechanisms. The neomorphic mutants of IDH present in blood cancer produce abnormal accumulation of R-2-hydroxyglutarate, which promotes leukemogenesis. The FDA approved Enasidenib and Ivosidenib for treatment of relapsed or refractory acute myeloid leukemia. These are mutant IDH2 and IDH1 isoforms inhibitors. Another protein that can interrupt the citrate supply for fatty acid synthesis is SLC25A1. The pyruvate obtained from glucose can be converted into lactate. In mitochondria, lactate can be transformed into citrate. SLC25A1 transports lactate from mitochondria into the cytosol, where it becomes a starting material for acetyl-CoA, a fatty acid precursor. Overexpression of SLC25A1 was found in some ovarian and most lung cancer cell lines and is associated with chemotherapy resistance. Pharmacological inhibition of SLC25A1 inhibits tumor growth alone and increases sensitivity for chemotherapeutic agents such as cis-platin.

4.3 ELOVL6, SCD1, FASD: FA modification proteins

The aliphatic chain of de novo synthesized palmitate can be elongated by ELOVL enzyme and/or unsaturations can be introduced by SCD or FASD enzymes (Figure 6). Recent findings show that one of the members of the ELOVL protein family (ELOVL1-7) ELOVL6 could be a promising target for lung squamous carcinoma. Pharmacological inhibition in vitro with an indole core derivative called compound A did not produce changes in cell proliferation, but did decrease colony formation. Oral and local intra-tumor administration of ELOVL6 inhibitor produces tumor growth inhibition.

**FIGURE 6** Proteins involved in lipid metabolism and proposed as pharmacological targets for cancer therapy. Citrate is a starting material for palmitate de novo synthesis and could be biosynthesized in normal oxygen conditions from glucose. In the glycolytic pathway, pyruvate is obtained and could be converted into lactate, which in mitochondria is transformed into citrate. Inhibition of SLC25A1 protein that transports citrate from mitochondria into cytosol can block de novo fatty acid synthesis. In hypoxic conditions, cancer cells can switch from glucose to glutamine as a source for citrate synthesis. An intermediate protein in that catabolic pathway is isocitrate dehydrogenase, whose inhibition leads to a reduction of the citrate pool. Synthesis of complex lipids requires modification of de novo synthesized palmitate. Proteins involved in FA modification are chain elongation ELOVL and proteins that introduce unsaturations: SCD and FASD. Inhibition of SCD and FASD may also induce a lipotoxicity effect. Cancer cells might obtain FAs from lipid stores such as triglycerides from lipid droplets. The most important lipase that catalyzes FA liberation from glycerides is MAGL. Recently, new evidence has shown the importance of ATGL and DAGL lipases in cancer cells. The key function of lipid droplets is energy storage and they are very important in tumor growth and cell survival, especially in hypoxic conditions. Rather than targeting catabolism of LD, the biogenesis of LD is a more attractive approach in cancer studies. This includes the study of LPCAT2 protein, which is involved in LD formation. Extracellular lipids could be transported through the cellular membrane by the actions of several proteins such as CD36, which is the most studied from the perspective of a pharmacological target in cancer.
Accumulation of saturated fatty acids and a disequilibrium with unsaturated fatty acids can produce an increased concentration of reactive oxygen species and endoplasmic reticulum stress. The appropriate ratio of saturated and unsaturated FAs is maintained with the action of stearoyl-CoA desaturases (SCDs) and fatty acid desaturases (FADS). The inhibition of SCD1 in vivo responds very well. It stops tumor growth in many cancer cell lines including metastatic cell lines, and leads to a better prognosis in animals. Recently, the SCD1 inhibitors were reviewed. Nevertheless, some cancer cells resist SCD1 inhibition. A new study showed that some cancer cells show plasticity in adapting mechanisms in the synthesis of unsaturated fatty acids. Resistance to SCD1 inhibition in lung and liver carcinomas is developed by an alternative pathway using fatty acid desaturase 2 (FASD2) enzyme, which catalyzes sapienate formation from palmitate. This finding reveals new details of cancer lipid metabolism and the possibility of treatments for SCD1-inhibition-resistant carcinoma with drug combinations.

4.4 CD36, LDLR, FATPs, FABPs: Blocking exogenous fatty acid sources

In addition to de novo synthesis, cancer cells can meet FA requirements from the exogenous microenvironment. However, this requires transportation through cell membranes using proteins such as CD36, LDLR, FATPs, and FABPs (Figure 6). The importance of interrupting this pathway in cancer was recently proved, as an exogenous source of oleic acid reversed the effect of SCD1 inhibitor and this effect was restored in CD36 knockdown breast cancer cells. This suggests that combinatorial treatment targeting lipogenesis together with FA transport would be more effective in cancer treatment. Probably the best characterized protein is CD36. Pharmacological CD36 targeting is a very promising approach since its two ligands reached clinical trials. Although ABT-510 failed in phase 2 and CVX-045 in phase 1, both molecules gave very important input and expectations for new molecules with improved activity targeting CD36 and other membrane FA transport proteins.

4.5 LPCAT2: Lipid droplet formation

One of the established hallmarks of cancer is lipid droplet (LD) accumulation, which is associated with chemotherapy resistance in colon cancer cells. The excess of cholesterol esters is stored as LDs that cancer cells can use in a situation of nutrient depletion or hypoxia, when synthesis of de novo FA is compromised. Proteins responsible for the formation of LDs are attractive targets, since increased concentration of non-stored cholesterol can cause lipotoxic effects and lipid supply would be compromised. Some inhibitors of lysophosphatidylcholine acyltransferase 2 (LPCAT2) have been developed such as TSI-01, which has been found to prevent LD accumulation in the treatment of HT29 colon cancer cells.165

4.6 ATGL, DAGL, and HSL: Tri and diglycerol catabolism

In this review, we mainly focused on one lipase, MAGL, which catalyzes the breakdown of FA from triglyceride, since it is the most extensively studied enzyme in cancer. Adipose triglyceride lipase (ATGL), diacylglycerol lipase (DAGL), and hormone-sensitive lipase (HSL) are other important lipases in adipose tissue that are responsible for FA mobilization (Figure 6). HSL and DAGL have not been widely studied in the context of cancer. However, DAGL was recognized recently as a participant in tumorigenesis in oral squamous cell carcinomas and may be a therapeutic target in this kind of diseases. Targeting ATGL for cancer treatment must be tissue specific. A recent study showed that this enzyme can have a tumor-suppressive function. Low levels of mRNA of this enzyme were associated with low survival in patients suffering from different cancers. In a study by Gerald Hoefler et al, the authors found pulmonary neoplasia in mice lacking ATGL. However, others studies demonstrated that, similar to MAGL, inhibition of ATGL may have a cytotoxic effect. Altogether, these contradictory effects may be a big obstacle in drug development targeting ATGL in specific tissues.

4.7 CPT1: Blocking fatty acid oxidation

Carnitine palmitoyltransferase 1 (CPT1) is a mitochondrial membrane enzyme (Figure 6) that catalyzes the transport of long-chain fatty acids (previously activated with Coenzyme A) from the cytosol into the mitochondria, where FA could be β-oxidized to produce ATP, acetyl-CoA, NADH, and FADH2. Inhibition of CPT1 may produce lipotoxic effects and depletion of the required energy source that cancer cells need for growth and survival. The in vivo study of CPT1 inhibition by ST1326 in Eµ-myc transgenic mice prevented tumor formation. However, pharmacological targeting of CPT1 in cancer therapy may have some side effects, since the inhibition of this enzyme is associated with an anorexic effect.

5 CONCLUSIONS

Lipid metabolism is a key player in cancer cell survival. 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 being evaluated in clinical trials. Inhibitors that target ACC and MAGL should focus on selectivity to avoid possible side effects, because inhibition of these enzymes affects other metabolic diseases. However, inhibitors focused on ACLY need to be studied more exhaustively to assess their effectiveness for cancer. In addition to the de novo pathway, the simultaneous inhibition of proteins responsible for exogenous FAs transportation should be considered, as new evidence suggests that cancer cells can recover using that pathway, reversing drug effects.

Tumor microenvironment plays a critical role in drug delivery and efficiency. Tumor microenvironment might be different in solid vs hematological cancers such as CML, AML, or CLL (chronic myeloid leukemia, acute myeloid leukemia, and chronic lymphocytic leukemia respectively). In fact, different solid tumors such as prostate or colon cancer might also have distinct microenvironments and thus, distinct metabolic profile. Tridimensional heterogeneity and cellular complexity and interaction could be higher in solid tumors. This might directly affect drug delivery, diffusion, sensitivity, uptake, and metabolism. Hematological cancers could potentially be more sensitive than solid tumors to different pharmacological drugs since in hematological tumor cells are directly exposed in the circulation. A tumor that highly relies on lipogenesis for biomass production would potentially present higher efficiency to lipogenic inhibitors. The reliance would be lower in a tumor with high availability to circulating lipids in its microenvironment as in the liver. In addition, it must be taken into account the different mutations and enzyme expression levels present in distinct tumors that might directly affect the metabolic fluxes and drug efficiency. Therefore, it would be interesting to study the metabolic reprogramming of each tumor according to its own microenvironment.

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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
K. Makowski conceived the idea; N. Montesdeoca, M. López, X. Ariza, L. Herrero, K. Makowski wrote the manuscript; K. Makowski designed the illustrations.

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