Lipogenesis inhibitors: therapeutic opportunities and challenges

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Abstract | Fatty acids are essential for survival, acting as bioenergetic substrates, structural components and signalling molecules. Given their vital role, cells have evolved mechanisms to generate fatty acids from alternative carbon sources, through a process known as de novo lipogenesis (DNL). Despite the importance of DNL, aberrant upregulation is associated with a wide variety of pathologies. Inhibiting core enzymes of DNL, including citrate/isocitrate carrier (CIC), ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), represents an attractive therapeutic strategy. Despite challenges related to efficacy, selectivity and safety, several new classes of synthetic DNL inhibitors have entered clinical-stage development and may become the foundation for a new class of therapeutics.

NAFLD
Nonalcoholic fattly liver disease (NAFLD) includes a spectrum of metabolic diseases ranging from simple steatosis to cirrhosis in the liver.

Fatty acids are essential for cell survival as they serve as key structural components of cell membranes and important signalling molecules. Fatty acids are also the most calorically dense form of energy storage with the conversion of excess glucose into fatty acids protecting against glucotoxicity and providing a much larger energy reserve than glycogen for times of nutrient scarcity. Given the vital roles of fatty acids, cells have evolved mechanisms to maintain them at adequate levels. This includes mechanisms to take up exogenous fatty acids but also to generate fatty acids from alternative carbon sources through a series of enzymatic reactions, a process highly conserved across phyla known as de novo lipogenesis (DNL)[1].

DNL is initiated when excess substrate availability, relative to cellular energy demands, leads to increases in mitochondrial citrate, which is exported from mitochondria into the cytosol by the mitochondrial citrate/isocitrate carrier (CIC; also known as CTP and SLC25A1) (FIG. 1). This cytosolic citrate is then converted into fatty acids by a series of biosynthetic reactions catalysed by ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACC; also known as ACACA) and fatty acid synthase (FAS; also known as FASN). The expression of these enzymes differs across tissues and stages of development (for example, proliferation or quiescence). Expression and activity are also acutely and chronically regulated through transcriptional control and post-translational modifications that are linked to nutritional status (for example, fasting and feeding) and substrate availability (for example, fatty acids suppress DNL) (BOX 1).

Although DNL is vital to maintain whole-body and cellular homeostasis, chronic elevations are associated with the development of a broad spectrum of diseases and disorders including cardiovascular disease (CVD)[2,3], nonalcoholic fatty liver disease (NAFLD)[4,5], type 2 diabetes (T2D)[5,6], numerous cancers[7,8], viral infections[9,10], autoimmune diseases[11,12], acne vulgaris[13], neurodegeneration[14] and ageing[15]. This suggests that pharmacological inhibition may be beneficial across multiple disease areas (BOX 2; Supplementary Fig. 1). Several natural products have been identified as inhibitors of DNL and these have been adopted as a cornerstone for the development of synthetic inhibitors that display improved bioavailability, efficacy and specificity. Recently, some of these compounds have reached clinical stage development and have been approved for the treatment of hyperlipidaemia[16] or are in late-stage development for NAFLD and oncology.

However, there are still many important questions that remain to be answered as it is currently unclear whether systemic or organ-specific inhibition of DNL should be targeted and what degree of inhibition is necessary to avoid potential side effects such as defects in fetal development[17], platelet production[18] or muscle dysfunction[19]. It is also unclear under what conditions inhibition of the classical DNL pathway may be bypassed by the scavenging of alternative carbon sources, through acetyl-CoA synthetase[20,21], ketoacid dehydrogenase[22] or isocitrate dehydrogenases[23–25], and therefore whether combination therapies or changes in diet may be required. Although there are several important targets that indirectly influence lipogenesis (for example, fructokinase, glucokinase or the glucagon receptor, sterol regulatory element-binding protein 1 (SREBP1) and liver X receptor (LXR))[26,27] or are involved in downstream lipid processing (for example, SCD1, DGAT1 and DGAT2)[28,29], here, we specifically review recent advances in the development of inhibitors that directly target core components within the lipogenic pathway,

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namely, citrate/isocitrate carrier (CIC), ATP-citrate lyase (ACLY), ACC and fatty acid synthase (FAS).

Physiological and pathological roles of DNL enzymes

The physiological regulation of DNL is complex, differing widely between cell types and with nutritional status. As discussed in this Review, studies from mice genetically lacking *Slc25a1*, *Acly*, *Acaca* and *Fas* have confirmed the importance of DNL in lipogenic tissues such as liver and adipose tissue, but also revealed unexpected biological consequences in cell types thought to have limited capacity for DNL. These findings indicate a much broader role for DNL in regulating the production of lipids under a wider array of physiological functions than initially appreciated (FIG. 2). Although a detailed description of the complex physiology that regulates lipogenesis (reviewed in REFS[30,31]) is not within the scope of this Review, an overview of the key physiological and pathological roles of DNL enzymes is provided below. Importantly, the information obtained from genetic studies provides crucial insight into both the opportunities and challenges of developing DNL inhibitors.

Energy intake and expenditure

Activity of the DNL pathway appears to regulate energy intake and expenditure. For example, mice that lack *Fas* have reduced food intake[32], whereas food intake is

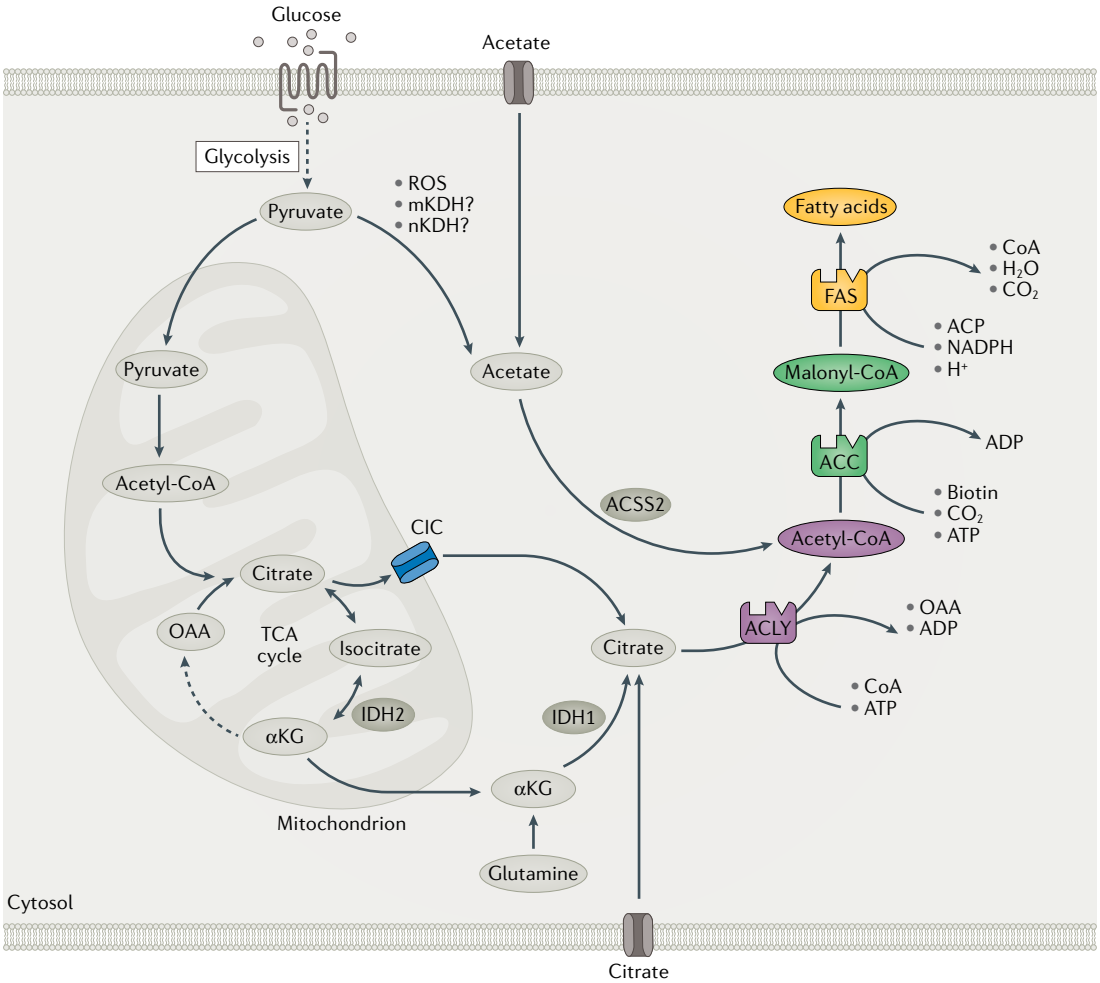


Fig. 1 | **Overview of DNL.** A series of coordinated enzymatic reactions takes place during fatty acid biosynthesis. Typically, pyruvate produced by glycolysis is converted in the mitochondrion into acetyl-CoA, which enters the tricarboxylic acid (TCA) cycle to produce citrate. In conditions of carbohydrate excess, citrate is exported to the cytosol by the citrate/isocitrate carrier (CIC) and is broken down to acetyl-CoA and oxaloacetate (OAA) by ATP-citrate lyase (ACLY). Acetyl-CoA is subsequently carboxylated by acetyl-CoA carboxylase (ACC) to generate malonyl-CoA, which is considered the first committed metabolic intermediate in fatty acid synthesis. Utilizing seven malonyl-CoA molecules and one acetyl-CoA primer, the synthesis of palmitate (16:0 fatty acid) is completed by repeating a cycle of condensation, reduction, condensation and dehydration catalysed by fatty acid synthase (FAS). An alternative carbon source of de novo lipogenesis (DNL) is acetate, which can be produced de novo from glucose through non-enzymatic and enzymatic reactions. Acetyl-CoA synthetase 2 (ACSS2) catalyses the reaction of acetate and CoA to form acetyl-CoA, which is subsequently used for fatty acid biosynthesis. With hypoxia or CIC deficiency another alternative pathway for DNL is reductive carboxylation of glutamine via cytosolic isocitrate dehydrogenase 1 (IDH1) and mitochondrial IDH2. αKG, α-ketoglutarate; ACP, acyl carrier protein; mKDH, mitochondrial ketoacid dehydrogenase; nKDH, nuclear ketoacid dehydrogenase; ROS, reactive oxygen species.

Box 1 | **Transcriptional control of DNL enzymes**

De novo lipogenesis (DNL) is fundamental for the survival of multicellular organisms. However, it is also an energy-intensive process that requires 7 ATP and 14 NADPH molecules to convert acetyl-CoA into palmitate. As such, numerous overlapping biological pathways have been developed to tightly match pathway flux with nutrient availability.

Transcriptional regulation of the citrate/isocitrate carrier (CIC), ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) is governed by a set of three transcription factors called sterol regulatory element-binding proteins (SREBPs), carbohydrate-responsive element-binding proteins (ChREBPs) and liver X receptors (LXRs)[29,281] (FIG. 3). Although cholesterol and fatty acids are both synthesized from acetyl-CoA, their biosynthetic pathways are largely regulated by distinct SREBPs with DNL being dependent on SREBP1a and SREBP1c whereas cholesterol synthesis is primarily regulated by SREBP2 (REF.[282]). In the liver, the expression of CIC, ACLY, ACC and FAS are increased by SREBP1c in response to glucose and insulin, while being repressed by fatty acids[283]. The expression of DNL genes is also altered by ChREBP, which exists as two isoforms, ChREBPα and ChREBPβ, that have differential tissue expression profiles.

Upon ingestion of carbohydrates, ChREBPα is activated causing nuclear import and binding to carbohydrate-responsive elements in promoters of lipogenic genes, promoting transcription; a response that is further amplified by ChREBPβ, which is constitutively active[281]. LXRs have a central role in the transcriptional control of SREBP1c by insulin[283], and although ChREBP was originally suggested to be a target gene of LXRs[284], this has not been observed in all studies[285]. The activity of all three transcription factors is altered by a multitude of adaptor proteins, ubiquitin ligases, transcriptional activators and repressors as well as epigenetic and post-translational modifications[29,281].

increased in mice that lack *Acc*[33]. Mechanistically, the divergent effects of FAS and ACC on food intake can potentially be explained through differential effects on malonyl-CoA, which has been shown to suppress food intake: FAS inhibition increases malonyl-CoA, whereas ACC inhibition reduces it. Consistent with changes in malonyl-CoA, stereotactic delivery of malonyl-CoA decarboxylase (MCD) into the hypothalamus increases food intake[34], whereas food intake is reduced in mice with constitutively active ACC1 and ACC2 isoforms[35]. These data suggest that inhibition of FAS suppresses food intake whereas inhibition of ACC stimulates food intake.

In both mice and humans, brown adipose tissue (BAT) is switched on in response to increases in nutrients (diet-induced thermogenesis) or cold (adaptive thermogenesis)[36,37]. The activation of BAT in rodents and humans increases glucose uptake; however, recent studies have found that surprisingly, the primary function of this glucose is not to support glycolysis but instead to fuel DNL[38]. Consistent with this concept, both cold exposure and high carbohydrate availability increase DNL within BAT[39], suggesting that BAT primarily utilizes endogenous triglycerides to fuel thermogenesis. Paradoxically, reductions in adipose tissue *Fas* leads to reductions in body mass[40] due to enhanced local sympathetic nervous system activity, which causes the browning of the white fat[41]. These data suggest that counterintuitively inhibiting DNL in adipose tissue may increase whole-body energy expenditure. Future studies are required to selectively inhibit ACLY, ACC and FAS within BAT and white adipose tissue (WAT) to directly quantify the importance of the DNL pathway for regulating futile cycling and energy expenditure.

Futile cycling
A process of using energy in two opposing metabolic pathways without a net effect.

NASH
Nonalcoholic steatohepatitis (NASH) is an advanced form of nonalcoholic fatty liver disease that is characterized by liver steatosis, inflammation and fibrosis.

Obesity
A condition characterized by the excessive accumulation of fat in the body.

Insulin resistance
An impaired glucose-lowering effect of insulin.

Lipid deposition: NAFLD–NASH

Individuals with nonalcoholic fatty liver disease (NAFLD) have increased rates of DNL and this is a major factor contributing to increased lipid deposition[4,5]. Consistent with these observations, the expression of CIC, ACLY, ACC and FAS are increased in the liver of patients with NAFLD or nonalcoholic steatohepatitis (NASH)[42]. In mice fed a high-fat diet (HFD), liver-specific deletion of the *Slc25a1* gene reduces liver steatosis[43]. In *ob/ob* mice fed a high-carbohydrate diet, transient genetic inhibition of ACLY using small interfering RNA (siRNA) reduces liver lipid content[44]. Similarly, when *Acly* is selectively removed from hepatocytes of adult mice with NASH induced by a high-fat and high-fructose diet, there are reductions in liver steatosis (G.R.S., unpublished observations). By contrast, lifelong inhibition of liver *Acly* in mice fed high levels of fructose does not lower liver fat, potentially because of upregulation of acetate production by the gut microbiome and compensatory upregulation of acetate-CoA synthetase 2 (ACSS2) (REF.[45]). However, it should be noted that the high levels of fructose used in this study did not promote obesity, NAFLD or NASH[45]; thus, the therapeutic relevance of the findings is currently unclear.

Genetic inhibition of liver *Acc1* (REF.[46]) or both *Acc1* and *Acc2* (REFS[47,48]) lowers liver fat in mice fed a high-carbohydrate diet independently of changes in adiposity. Conversely, mice with constitutively active ACC1 and ACC2 isoforms, owing to lack of AMP-activated protein kinase (AMPK) inhibitory phosphorylation, develop greater steatosis and fibrosis than controls when fed a high-carbohydrate diet[49]. These data indicate that inhibition of ACC can exert positive effects on lowering steatosis and fibrosis. However, an important consequence of inhibiting liver ACC is the development of hypertriglyceridaemia owing to reductions in liver polyunsaturated fatty acids, which increase SREBP1c and the expression of glycerol-3-phosphate acyltransferase (GPAT), the rate-limiting enzyme for triglyceride synthesis[48]. Although most studies have indicated positive effects of ACC inhibition on liver steatosis, in one study genetic inhibition led to increases in liver lipids attributed to hyperacetylation of mitochondrial proteins, potentially due to accumulation of acetyl-CoA and lower fatty acid oxidation[50]. The phenotype of these liver-specific ACC null mice was similar to that of mice lacking FAS, which also have increased steatosis owing to reductions in fatty acid oxidation[51]. These studies in ACC-null and FAS-null mice highlight complex interactions that may limit therapeutic application in NAFLD and NASH.

Insulin sensitivity: type 2 diabetes

Insulin resistance is a hallmark of type 2 diabetes (T2D). Rates of DNL in the liver are inversely correlated with hepatic and whole-body insulin sensitivity in humans[5,52]. Liver-specific deletion of *Slc25a1* improves glucose tolerance in mice fed a control carbohydrate diet or HFD[43]. siRNA-mediated suppression of ACLY expression reduces fasting glucose and improves glucose tolerance in *ob/ob* mice fed a high-carbohydrate diet[44]. Similar observations are made when ACLY is selectively deleted from hepatocytes of mice fed a high-fat and high-fructose

diet for 16 weeks (G.R.S., unpublished observations). Genetic inhibition of ACC2 in the muscle[53] or ACC1 and ACC2 in the liver[47] leads to improvements in muscle and liver insulin sensitivity, respectively, whereas mice with constitutively active ACC1 and ACC2 isoforms have worse insulin resistance[49,54]. These data indicate that inhibition of DNL and/or upregulation of fatty acid oxidation within muscle and liver of mice can improve insulin sensitivity.

In addition to muscle and liver, adipose tissue is also crucial for regulation of insulin sensitivity[55,56]. In rodents, WAT has rates of DNL comparable to those of liver during the postprandial period[57]. However, with obesity, rates of adipose tissue DNL decline in both rodents[58] and humans[59], despite only modest reductions in insulin-simulated glucose uptake[60]. Recent studies indicate that this reduction in adipose tissue DNL occurs rapidly and continues to decline with worsening obesity and insulin resistance and is associated with reductions in the expression of ACLY, ACC, FAS[61] and carbohydrate-responsive element-binding protein-β (ChREBPβ)[62], suggesting that inhibition of adipose tissue DNL may be detrimental to whole-body insulin sensitivity. Consistent with this concept, when ACLY fat-specific null mice are challenged with a high-sucrose diet, female mice develop a lipodystrophy-like phenotype that is associated with hepatic lipid accumulation and insulin resistance[63]. Similar observations are also observed in ACC1 fat-specific null mice[64]. These data indicate an important role for adipose tissue DNL in maintaining whole-body insulin sensitivity, potentially by protecting the liver from developing steatosis. Additional studies characterizing transient knock-down of ACLY and ACC in fully differentiated adipocytes of obese adult mice, rather than lifelong deletion[20,63,64], could better inform the potential protective role of adipose tissue DNL in insulin resistance.

The inability of pancreatic β-cells to secrete sufficient amounts of insulin to maintain euglycaemia is a hallmark of T2D. Available evidence suggests that ACC may be important for pancreatic β-cell function, as deletion of ACC impairs glucose-stimulated insulin secretion ex vivo and insulin tolerance in vivo[65]. Mechanistically, ACC is abundant in β-cells, whereas FAS is expressed at low levels, leading to a rise in malonyl-CoA levels following glucose stimulation[66]. As a result, fatty acid oxidation is inhibited by elevated malonyl-CoA, increasing the availability of cytosolic long-chain fatty acyl-CoA (LCFA-CoA) for lipid signalling to cellular processes involved in insulin secretion[67]. In addition, recent studies have found that ACC is important for promoting β-cell mass in mice[65]. These data suggest that inhibition of ACC in pancreatic islets may lead to impaired insulin secretion and reduced β-cell mass that could contribute to T2D.

Cardiovascular disease

Elevations in liver DNL lead to increases in plasma VLDL and LDL, major risk factors of cardiovascular disease (CVD) mortality[68,69]. Recent studies have found that incident heart failure and CVD mortality[2,70] are positively associated with increases in fatty acids derived from DNL. Consistent with this concept, genetic variants in *ACLY* are associated with reduced plasma LDL and decreased cardiovascular events[3]. Similarly, polymorphisms of *ACLY* and *ACC* are associated with lower triglycerides following dietary fish oil supplementation[71]. Surprisingly, to the best of our knowledge, no studies have examined the effects of genetic inhibition of liver *Acly*, *Acc* or *Fas* on established models of atherosclerotic development. However, given the effects of ACC inhibition in promoting hypertriglyceridaemia[48], it might be anticipated to enhance atherosclerosis. By contrast, a deficiency in liver ACLY lowers triglycerides in mice (G.R.S., unpublished observations). The reasons for the opposing effects of ACLY and ACC inhibition on circulating triglycerides is unclear; however, it could be linked to the concomitant suppression of cholesterol synthesis unique to ACLY inhibition and/or the reciprocal effect that ACLY and ACC inhibition has on levels of acetyl-CoA, which may be important to support protein acetylation. These data indicate that while ACC inhibition may be effective at reducing liver steatosis, it has detrimental effects on CVD risk profile that would need to be managed with other lipid-lowering therapies such as fish oil[71], DGAT inhibition[72] or PPARα agonists such as fenofibrate[73].

Macrophages are also crucial for the development of atherosclerotic CVD as they take up and store lipid, triggering inflammation within the atherosclerotic lesion. The activity of ACLY[74] and FAS[75] is increased in

Box 2 | **Emerging applications of lipogenesis inhibitors**

In addition to the widely studied therapeutic application of inhibitors of de novo liopogenesis (DNL) enzymes in metabolic diseases and cancer, emerging evidence indicates that lipogenesis inhibitors may also be beneficial in several other disorders.

During viral infections, lipogenesis is upregulated to meet the high demand for membrane synthesis required for viral replication. As such, blocking lipogenesis has emerged as a potential antiviral therapeutic option. For instance, lipogenesis inhibitors have been demonstrated to block the infection of hepatitis C virus[286], HIV[287], West Nile virus[288], rotavirus[289], Epstein–Barr virus[290], dengue virus[291], Japanese encephalitis virus[291] and chikungunya virus[291]. Lipogenesis inhibitors could also potentially be beneficial against the COVID-19 pandemic, as like all viruses, SARS-CoV-2 relies on newly synthesized phospholipids for its replication[292]. Consistent with this concept, orlistat and TVB-2640 inhibit the replication of SARS-CoV-2 variants[293].

Another emerging area for lipogenesis inhibitors involves the treatment of acne vulgaris. Excess sebum production is an important cause of acne vulgaris, and studies using isotope labelling have revealed that ~80% of the sebum lipid in humans is derived from DNL[13]. In early-stage clinical trials, topical applications of acetyl-CoA carboxylase (ACC) inhibitors olumacostat glasaretil[294] and PF-05175157 (REF.[13]) showed beneficial effects for treating acne vulgaris; however, it appears that neither agent has proceeded further with clinical development.

Multiple studies of early inhibitors soraphen A and TOFA suggest a potential benefit of ACC inhibition on several other pathological conditions. Soraphen A attenuates T helper 17 (T_H17) cell-mediated autoimmune disease[98], inhibiting effector T cell expansion in graft-versus-host disease[295], and improves neurological outcomes after ischaemic stroke by preserving the regulatory T cell and T_H17 cell balance[296]. More recently, it was demonstrated that soraphen A blocks autophagy in ageing yeast[297] and attenuates undirected endothelial cell migration, which is a process implicated in many severe diseases[298]. Recent studies suggest that TOFA is involved in survival of memory T cells during chronic infections[299] and in reduction of pro-inflammatory signalling in cystic fibrosis[300]. Although these findings are intriguing, owing to the potential off-target effects of these compounds, conclusions about the therapeutic benefit of ACC inhibition derived from these studies should be interpreted with caution until they are replicated using more-specific inhibitors.

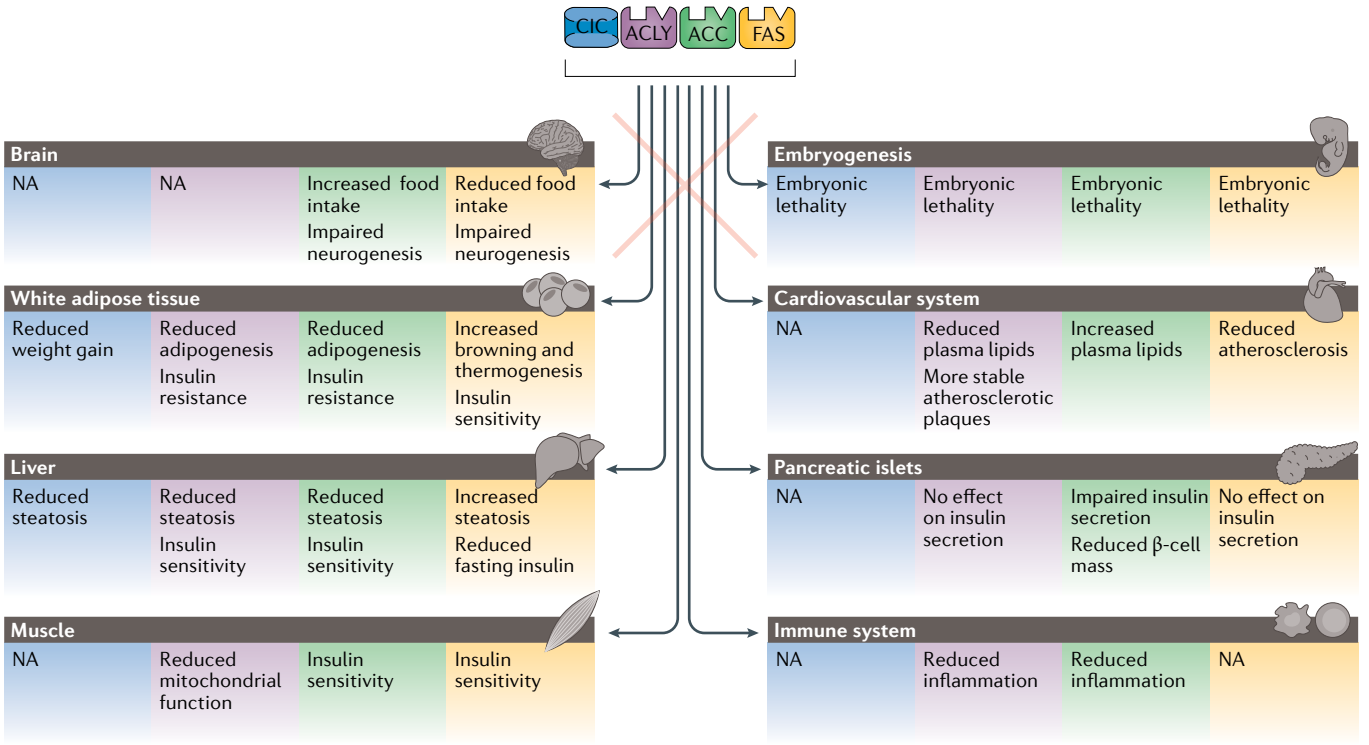


Fig. 2 | **Tissue-specific actions of DNL.** Important insights into distinct actions of citrate/isocitrate carrier (CIC) and de novo lipogenesis (DNL) enzymes in various tissues have been postulated from studies that employed animal models that lack one of these core components of the DNL pathway. The actions of each enzyme is colour coded: CIC-mediated effects are in blue boxes, ATP-citrate lyase (ACLY) in purple boxes, acetyl-CoA carboxylase (ACC) in green boxes and fatty acid synthase (FAS) in yellow boxes. NA, not available.

atherosclerotic plaques of mice and humans, suggesting that inhibition may be beneficial. Consistent with this concept, genetic inhibition of *Acly*[74] or *Fas*[76] within macrophages of ApoE mice reduces atherosclerosis. Surprisingly, there have been no studies examining the effects of genetic deletion of macrophage ACC on atherosclerosis.

Cancer

DNL is constitutively active in many cancer cells and contributes most of the intracellular lipid mass[77]. It is now well recognized that common genetic mutations (for example, in the gene encoding p53 (REF.[78]) or the gene encoding PTEN[79]) and growth factor signalling (epidermal growth factor[80], HER2 (REF.[81]) and keratinocyte growth factor[82], ERK1/ERK2 MAPKs[82,83]) enhance the activity of SREBP1 leading to upregulation of lipogenic genes. In addition to transcriptional control, enhanced glucose uptake — common in glycolytic tumours — increases citrate availability, which allosterically activates ACLY and ACC. Mutations in *PTEN*, which lead to constitutive activation of AKT, also increase the activity of ACLY through phosphorylation at Ser454, while at the same time enhancing ACC activity by suppressing AMPK[84]. Similarly, mutations in LKB1 reduce activating AMPK phosphorylation, thereby increasing ACC activity and cell proliferation[85]. Thus, tumours use multiple overlapping mechanisms to enhance DNL.

Genetic evidence that supports a crucial role for DNL enzymes in regulating tumorigenesis has been obtained from multiple studies involving knock-down of ACLY[7,86,87], ACC[88] and FAS[89,90] in a wide variety of distinct tumour types (reviewed in REFS[91,92]). Inhibition of DNL also increases sensitivity to standards of care such as radiation[93], androgen deprivation[94] chemotherapies or tyrosine kinase inhibitors such as sorafenib[95]. Survival analysis examining the relationship between DNL-expressing genes also supports an important relationship across different tumour types[96]. These data suggest that inhibiting DNL may exert favourable effects in multiple types of cancer.

Infection and immunity

Inflammation and metabolism are intimately linked, as the maintenance of cellular defence systems and removal of pathogens is an energetically demanding process. Upon activation, immune cells undergo metabolic reprogramming to meet the energy demand for cell proliferation, differentiation and cytokine production[97]. One of the metabolic switches that occurs during these processes is the induction of DNL[98,99]. It is known that immune cells that subsume a pro-inflammatory role (M1-like) use aerobic glycolysis and have high rates of DNL, whereas anti-inflammatory immune cells (M2-like) use predominantly oxidative metabolism and have low rates of DNL. Although this is an oversimplification of an extremely complicated non-binary relationship, it does provide a general context for understanding the link between inflammation and metabolism[100]. The triggers for immune activation vary, but findings in

Metabolic diseases
A group of diseases characterized by disrupted metabolism that adversely affect normal cellular function and structure.

chronic metabolic diseases such as obesity, NASH and CVD have found an important role for microbial products such as lipopolysaccharide (LPS) and ectopic lipid accumulation (cholesterol and fatty acids)[101].

Both ACC and ACLY regulate immune function. The inhibition of ACLY inhibits LPS-induced inflammation[102] in some but not all studies[74] and is important for mediating the anti-inflammatory effects of IL-4 (REF.[103]) and IL-2 (REF.[104]). In human and mouse naive T cells, deletion of ACC1 restrains the development of pro-inflammatory T helper 17 (T_H17) cells and promotes the formation of anti-inflammatory regulatory T cells; a finding that translates in vivo into the attenuation of T_H17-mediated autoimmune disease[98] and infection-associated intestinal inflammation[105]. ACC1 is also crucial in obesity-induced T_H17 cell differentiation in humans[12]. Deletion of ACC1 also reduces antigen-specific clonal expansion of cytotoxic $CD8^+$ T cell populations during *Listeria* infection[106]. Interestingly, this increase in DNL is exploited by many viruses for the formation of their replication complex, and as a result, inhibition of SREBP, ACLY, ACC1 and FAS reduces viral replication[9,10,107]. These studies suggest that inhibiting DNL may be effective at reducing inflammation in chronic disease settings such as obesity and NASH and may exert positive effects to reduce viral replication. However, whether this may be exploited therapeutically without suppressing host immunity, which requires activation of DNL to promote an M1 phenotype, remains to be determined.

Neurogenesis

Neural differentiation occurs throughout life[108] and is strongly associated with upregulation of DNL in neural stem and progenitor cells[14]. The inactivation of FAS and ACC reduces neurodifferentiation[14], and this has been linked to neurodegenerative diseases, including multiple sclerosis[109], Parkinson disease[110] and Alzheimer disease[111]. Interestingly, individuals with a variant of FAS (FAS-R1819W) have cognitive disorders[112], a finding that has been shown to be consistent in rodents overexpressing this variant[113]. In addition to neural stem and progenitor cells, DNL is central for the proper morphological formation and function of neurons. FAS is highly expressed in neurons and is crucial for dendrite branching and function[114]. DNL is also essential for myelination, as lipids comprise a large portion of the myelin membrane[115]. Recent studies have demonstrated that FAS is necessary for the correct onset of myelination and proper myelin growth by Schwann cells in the peripheral nervous system[116] and oligodendrocytes in the central nervous system[109]. These data suggest that inhibition of DNL in the nervous system, not only in fetal development but also in adulthood, could potentially promote the progression of neurodegenerative disease.

CIC

Regulation and structure

CIC is encoded by the *SLC25A1* gene and is ubiquitously expressed, with highest expression in liver, reproductive organs, gastrointestinal tract and adipose tissue (see Related links). It is located within the inner mitochondrial membrane, and primarily catalyses the efflux of tricarboxylates such as citrate and isocitrate in exchange for tricarboxylates, dicarboxylates and phosphoenolpyruvate[117]. LCFA-CoAs inhibit CIC in a reversible manner, competitive with citrate[118], while acetylation can increase allosteric activation by citrate[119] (FIG. 3).

Little is known about how the structural components of CIC are implicated in physiological regulation of activity. Structurally, eukaryote CIC is composed of three homologous domains, each of which form two hydrophobic membrane-spanning α-helices that are connected by hydrophilic loops that span from the intermembrane space to the mitochondrial matrix[117] (FIG. 4). There are at least two citrate binding sites, residing at different depths within the membrane bilayer and serving as the binding site of CIC inhibitors. Residues within sites 1 and 2 form six and eight hydrogen bonds with citrate, respectively[120]. CIC site 1 is kinetically accessible to anions from the inner surface and determines specificity to internal substrate as it moves through the CIC[121]. After binding to site 1, citrate is transferred to site 2, before being released into the intermembrane space where it then diffuses through a voltage-dependent anion transport channel within the mitochondrial outer membrane, into the cytoplasm.

Pharmacological inhibitors

The first-generation CIC inhibitor to have a higher affinity for the transporter than any substrate was benzenetricarboxylate (BTC)[117] (TABLE 1; Supplementary Table 1), which inhibits the CIC in a mixed competitive and uncompetitive manner[117,122]. BTC is structurally similar to citrate and primarily interacts with citrate binding site 2 (REF.[122]) (FIG. 4). Although this inhibitor has been widely used for structural and functional characterization of the CIC, potential binding of the inhibitor to other citrate binding proteins has limited its therapeutic development.

CTPI-1, also known as compound 792949, is a next-generation competitive CIC inhibitor identified via in silico screening of commercially available small molecules. It has a slightly higher affinity for CIC than BTC does and binds to residues from both citrate binding sites simultaneously[122]. This inhibitor was identified in yeast CIC and later it was found to exhibit suboptimal binding for human CIC, potentially owing to a key amino acid difference in the citrate binding sites between the species[123].

Attempts to optimize compounds specific for human CIC led to the identification of CTPI-2, which exhibited a 20-fold improvement in binding affinity relative to CTPI-1 and inhibited citrate transport at lower concentrations[123]. Furthermore, it has shown several favourable effects in preclinical models as discussed below, indicating the potential of the inhibitor for development.

Therapeutic indications

Metabolic diseases. Lowering cytosolic citrate would be expected to inhibit DNL by reducing both substrate availability and allosteric activation of ACLY and ACC[124,125]. Consistent with this concept, BTC

lowers triglyceride content in primary hepatocytes[126] (Supplementary Fig. 1). CIC expression is increased in the liver of people with NASH, and CTPI-2 treatment of HFD-fed mice reversed steatohepatitis and liver injury, with a concomitant reduction in serum cholesterol and triglycerides[43]. In addition, CTPI-2 reduced fasting glucose and normalized glucose tolerance and insulin sensitivity, potentially through targeting gluconeogenesis. However, as CTPI-2 also lowered body mass through mechanisms that are not understood, this may have

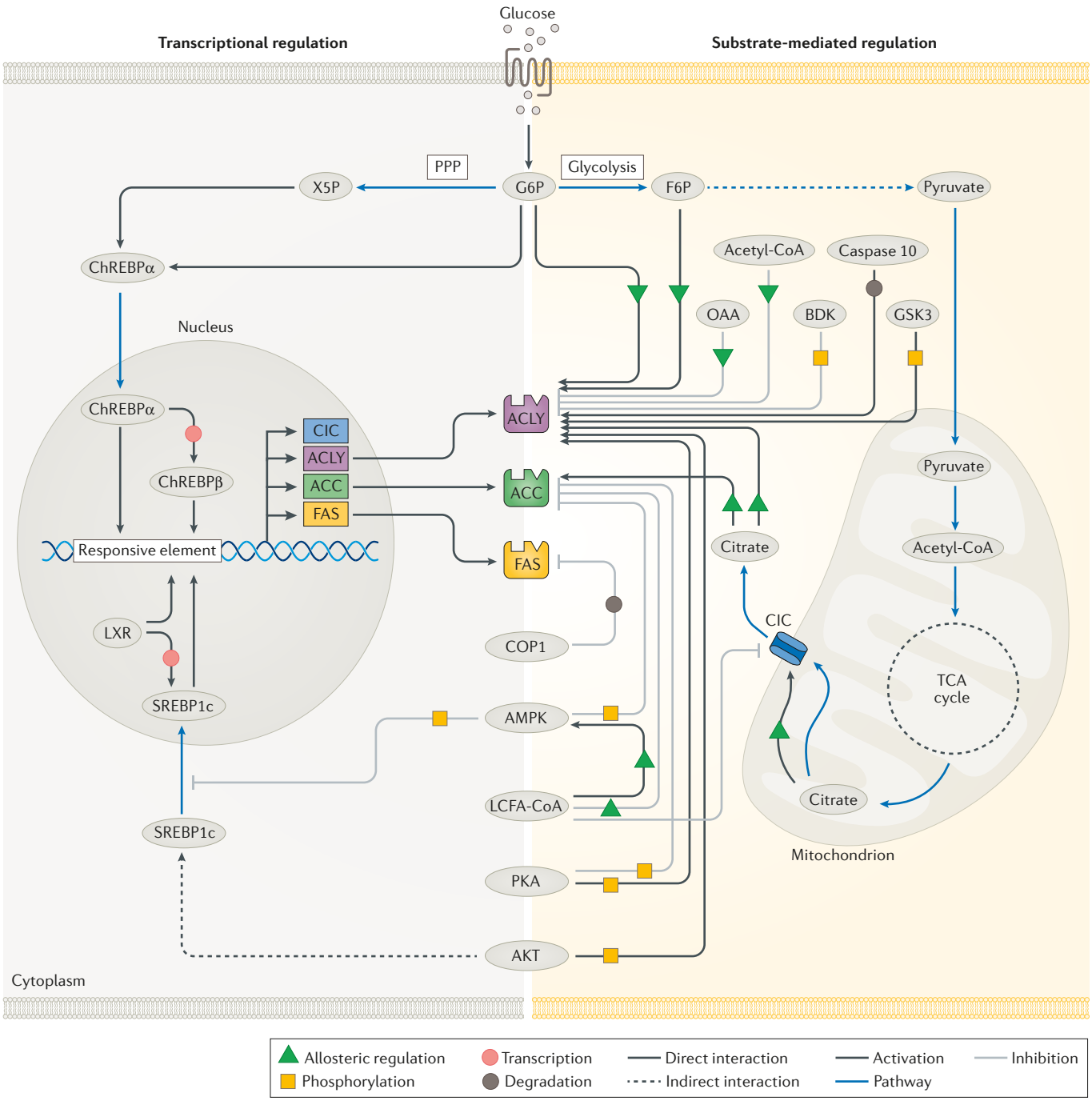
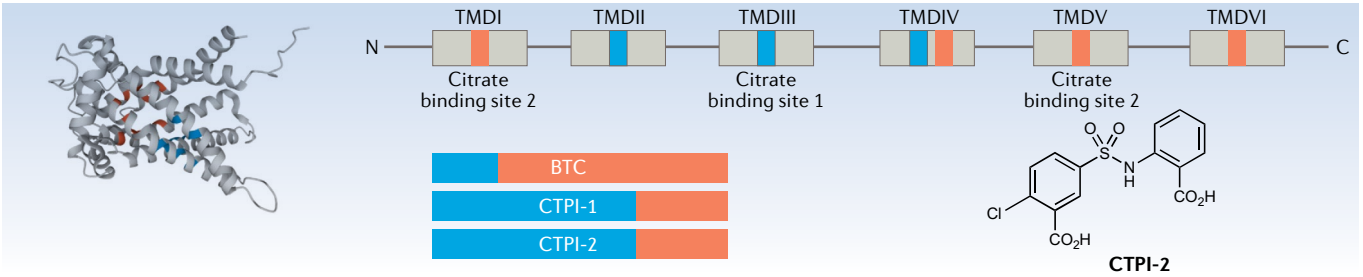
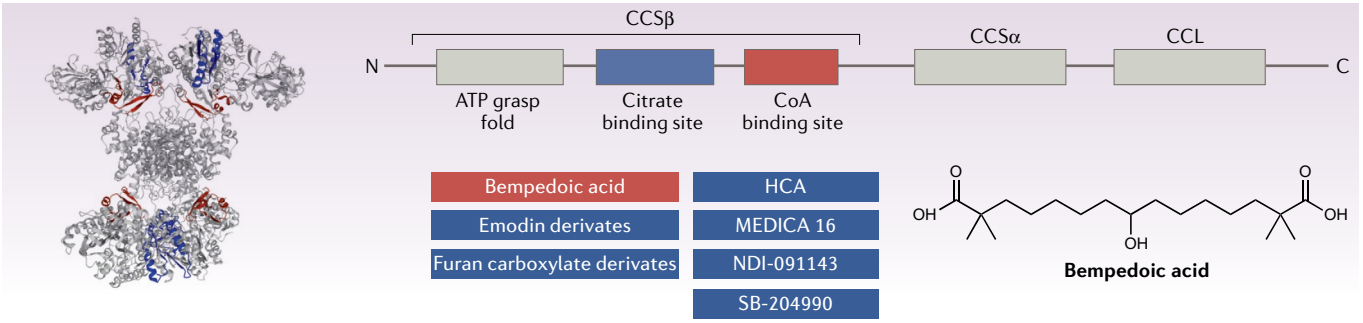


Fig. 3 | **Physiological regulation of DNL.** Regulatory mechanisms of de novo lipogenesis (DNL) involve allosteric regulation, covalent modifications and transcriptional changes. Allosteric activators include citrate, glucose 6-phosphate (G6P) and fructose 6-phosphate (F6P) while oxaloacetate (OAA) and long-chain fatty acyl (LCFA)-CoAs are allosteric inhibitors. Regulatory phosphorylation is facilitated by several enzymes including AMP-activated protein kinase (AMPK), AKT, branched-chain α-keto dehydrogenase kinase (BDK), glycogen synthase kinase 3 (GSK3) and protein kinase A (PKA), whereas caspase 10 and constitutive photomorphogenic 1 (COP1) facilitate the degradation of ATP-citrate lyase (ACLY) and fatty acid synthase (FAS), respectively. Transcriptional modifications are regulated by two major transcription factors, sterol regulatory element-binding protein 1c (SREBP1c) and carbohydrate-responsive element-binding protein (ChREBP). Additional transcription factors, such as liver X receptor (LXR) are also implicated in the transcriptional regulation to varying degrees of importance depending on the cell type. ACC, acetyl-CoA carboxylase; CIC, citrate/isocitrate carrier; FAS, fatty acid synthase; PPP, pentose phosphate pathway; TCA, tricarboxylic acid; X5P, xylulose 5-phosphate.

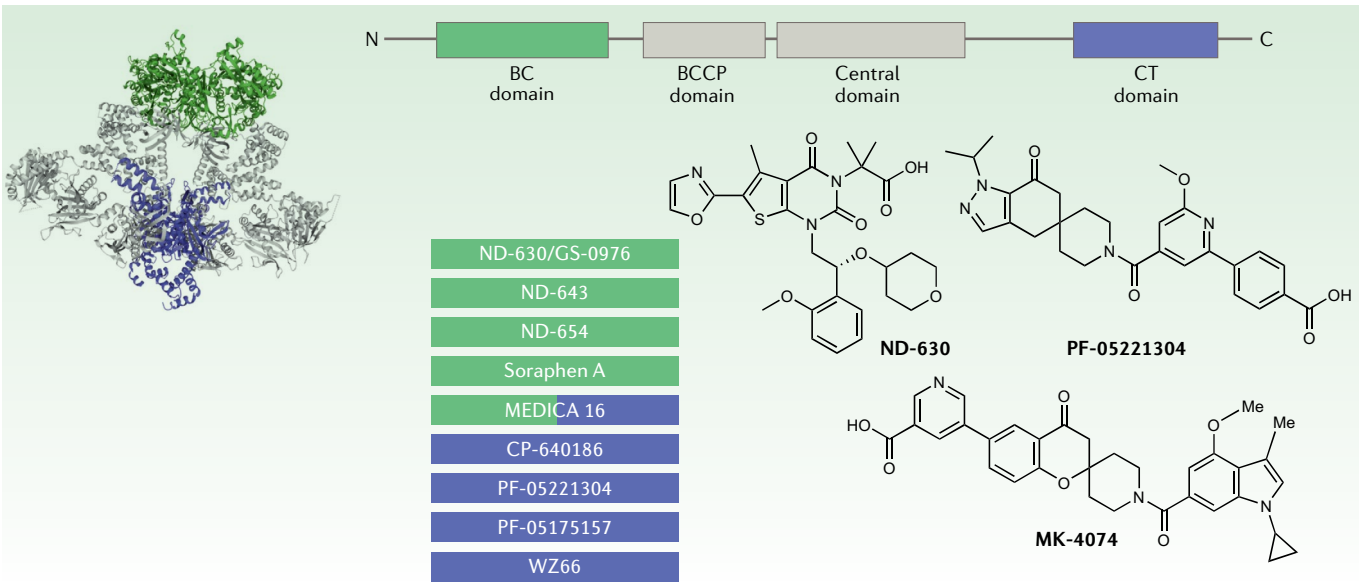
a CIC inhibitors



b ACLY inhibitors



c ACC inhibitors



d FAS inhibitors

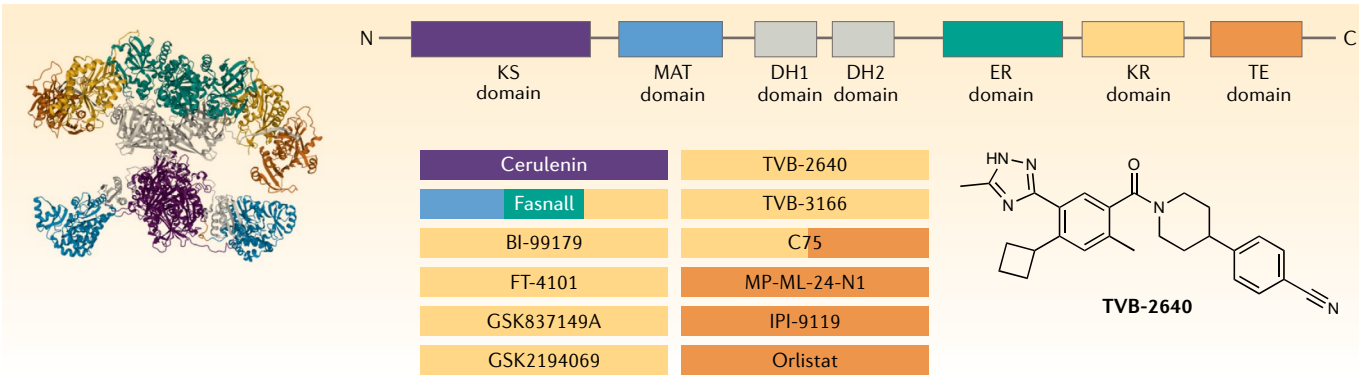


Fig. 4 | **Structural domains and binding sites of DNL inhibitors with chemical structures of the most advanced inhibitors.** Lipogenesis inhibitors interact with one or more druggable sites of the enzyme to exhibit an inhibitory effect. Linear organization and model representation of each enzyme are shown with known inhibitor binding sites in colours. Inhibitors with known enzyme binding sites are colour coded with their respective interaction sites. **a** | Citrate/isocitrate carrier (CIC) inhibitors: compounds that bind to citrate binding site 1 are shown in light blue and those that bind to citrate binding site 2 in orange. The chemical structure of CTPI-2 is shown. **b** | ATP-citrate lyase (ACLY) inhibitors: compounds that interact with the CoA binding site are highlighted in red and those that interact with citrate binding site in dark blue. The chemical structure of bempedoic acid is shown. **c** | Acetyl-CoA carboxylase (ACC) inhibitors: compounds that target the biotin carboxylase (BC) domain are highlighted in green and those that target the carboxyl transferase (CT) domain are highlighted in violet. Chemical structures of clinical stage inhibitors are shown. **d** | Fatty acid synthase (FAS) inhibitors: inhibitors that bind to the β-ketoacyl synthase (KS) domain are highlighted in purple, inhibitors that bind to malonyl-acetyl transferase (MAT) are in blue, inhibitors that bind to enoyl reductase (ER) are in green, inhibitors that bind to β-ketoreductase (KR) in yellow and those that bind to thioesterase (TE) in orange. The chemical structure of TVB-2640 is shown. BCCP, biotin carboxyl carrier protein; BTC, benzenetricarboxylate; CCL, citryl-CoA lyase; CCS, citryl-CoA synthetase; DH, dehydratase; HCA, (–)-hydroxycitric acid; TMD, transmembrane domain. Part **a** CIC structure adapted from P53007, CC BY 4.0; part **b** ACLY structure adapted from PDB ID 6POF, CC BY 1.0; part **c** ACC structure adapted from PDB ID 5CSK, CC BY 1.0; part **d** FAS structure adapted from PDB ID 2VZ8, CC BY 1.0.

contributed to improvements in glucose homeostasis. BTC acutely inhibits glucose-stimulated insulin secretion in INS1 cells and islets[127]; however, in vivo effects on pancreatic islets have not been evaluated. These data suggest that inhibition of CIC may exert favourable effects on obesity, NAFLD and T2D. Future studies are needed to determine the mechanisms that contribute to weight loss and whether this is required for the beneficial effects on liver steatosis and glucose homeostasis.

One potential mechanism by which CIC inhibitors may exert positive effects in metabolic disease independently of reductions in body mass, may involve inhibition of inflammation. Inflammatory stimuli such as LPS, TNFα and IFNγ, elicit metabolic reprogramming in macrophages and natural killer cells that leads to increases in CIC expression, suggesting that inhibition may exert anti-inflammatory effects[128,129]. In cultured macrophages, CTPI-1 inhibits inflammatory responses induced by TNFα and IFNγ[128]. Similarly, in obese mice CTPI-2 blocks inflammatory macrophage infiltration into the liver and adipose tissue and this is accompanied by a decrease in circulating pro-inflammatory cytokines[43]. These data suggest that inhibiting CIC may reduce inflammation and this may exert a positive effect on metabolic diseases.

Table 1 | **CIC inhibitors**

Compound (developer)	Potency in biochemical assays	Indication and/or preclinical effects	Refs
Preclinical stage inhibitors			
Benzenetricarboxylate	K_i: 0.07–0.16 mM	Decreased triglyceride synthesis, reduced glucose-stimulated insulin secretion and inhibited cancer cell growth	126, 127,132
CPTI-1 (Rosalind Franklin University of Medicine and Science, USA)	K_i: 0.048–0.07 mM	Reduced inflammation and inhibited cancer cell growth	43,128
CPTI-2 (Lombardi Comprehensive Cancer Center, USA)	K_d: 3.5 μM	Decreased hepatic steatosis, improved lipid and glucose homeostasis, and inhibited cancer cell growth	43,129

CIC, citrate/isocitrate carrier; K_d, dissociation constant; K_i, inhibition constant.

Cancer. As CIC expression is increased in several different cancer cells[130] and inhibition of CIC blunts cell growth[131], CIC has emerged as an attractive target for anticancer drug development. All three CIC inhibitors have been found to exert anticancer activity in cultured cancer cell lines[123,132,133] and reduce tumour growth in vivo[132,133]. Future studies are needed to evaluate the effects of these inhibitors in clinical settings.

ACLY

Regulation and structure

ACLY expression is highest in adipose and liver and lowest in skeletal muscle (see Related links). Although ACLY is predominantly a cytosolic enzyme, it is also found within the nucleus (see Related links). The activation of ACLY involves four steps: first, Mg–ATP binding, and phosphorylation of His760, which catalyses the formation of an enzyme-bound citryl-phosphate, followed by a CoA attack and formation of a citryl-CoA intermediate and finally, citryl-CoA cleavage into final products acetyl-CoA and oxaloacetate (OAA). This reaction is allosterically activated by the glycolytic intermediates glucose 6-phosphate (G6P) and fructose 6-phosphate (F6P), with the latter being more potent[134] (FIG. 3). Citrate also allosterically activates ACLY while the products of the reaction, acetyl-CoA and OAA, inhibit enzyme activity[124].

In addition to allosteric activation, early studies identified that insulin[135] and glucagon[136] increase Ser/Thr phosphorylation on ACLY (FIG. 3), with subsequent studies establishing that Thr446 and Ser450 are phosphorylated by glycogen synthase kinase 3 (GSK3)[137], and Ser454 is phosphorylated by protein kinase A (PKA)[136] and the 'insulin-stimulated kinase'[138] that was subsequently identified as AKT (also known as PKB)[139]. mTORC2 was also suggested to phosphorylate ACLY Ser454 (REF.[8]), an effect shown to require AKT[140]. More recently, branched-chain ketoacid dehydrogenase kinase was also found to phosphorylate Ser454, thus linking ACLY activity with amino acid availability and insulin resistance[141]. Although initial studies found that Ser454 phosphorylation had minimal effects on ACLY activity[142], further work established that phosphorylation of this residue decreased sensitivity to allosteric activation by F6P and G6P[134]. Subsequent studies in various cell lines have confirmed the important effects of this phosphorylation event in enhancing ACLY activity[8,104,143]. In addition, ACLY is regulated by cleavage of the enzyme at a post-transcriptional level[144].

The structural underpinnings of the chemical reactions mediated by ACLY are still not fully understood[124,145]. ACLY consists of two modules, ACLY-A and ACLY-B, which are present as separate domains (heteromeric) in prokaryotes, but are linked (homomeric) in animals[146] to form a functional tetrameric structure[124] (FIG. 4). Each monomer consists of an N-terminal citryl-CoA synthetase (CCS) module, containing CCSβ and CCSα regions, and a C-terminal citryl-CoA lyase (CCL) domain[145]. The ACLY-A module forms the CCSβ

Table 2 | **ACLY inhibitors**

Compound (developer)	Potency in biochemical assays	Indication and/or preclinical effects	Clinical trial ID or refs
Clinical stage inhibitors			
Bempedoic acid (Esperion Therapeutics, USA)	K_i: 2 μM	Primary hypercholesterolaemia and established atherosclerotic cardiovascular disease	Approved
Hydroxycitrate	K_i: 0.15 μM	Obesity and type 2 diabetes	NCT01238887 and NCT00699413 (terminated, phases I and IV)
		Urine chemistries	NCT03348228 (in progress)
Preclinical stage inhibitors			
BMS-303141 (Bristol-Myers Squibb Pharmaceutical Research Institute, USA)	IC_{50}: 0.13 μM	Reduced weight gain, plasma lipids and glycaemia, and inhibited cancer cell growth	94,160
Emodin derivates (Harvard Medical School, USA)	IC_{50}: 3–30 μM	Inhibited cancer cell growth	155
Furan carboxylate derivates (Harvard Medical School, USA)	IC_{50}: 4.1–11.9 μM	Inhibited cancer cell growth	159
MEDICA 16 (Hadassah Medical School, Israel)	K_i: 16 μM	Reduced weight gain, hepatic steatosis, plasma lipids and atherosclerosis	162,177,178
SB-204990 (SmithKline Beecham Pharmaceuticals, UK)	K_i: 1 μM	Lowered plasma lipids and inhibited tumour growth	86,158
Discovery stage inhibitor			
NDI-091143 (Nimbus Therapeutics, USA)	K_i: 0.07 μM	No functional studies reported	161

ACLY, ATP-citrate lyase; IC_{50}, half-maximal inhibitory concentration; K_i, inhibition constant.

region, while ACLY-B forms the remaining domains of the monomer, with Ser/Thr phosphorylation occurring within a linker region between the modules[145]. Recent studies have provided a detailed structural basis for the multistep catalytic mechanism of ACLY, showing that citrate binds to the CCSβ region, while the CCSα region serves as a CoA binding site[124]. An additional CoA binding site is in the CCL domain[124,145]; however, it is controversial whether binding at this site is required for acetyl-CoA formation.

Pharmacological inhibitors

ACLY is an attractive drug target owing to its strategic position at the nexus of fatty acid, cholesterol and carbohydrate metabolism. As such, several ACLY inhibitors have been identified on the basis of their ability to mediate concomitant inhibition of fatty acid and cholesterol synthesis, while activating fatty acid β-oxidation.

The first identified and extensively studied inhibitor of ACLY was (−)-hydroxycitric acid (HCA), a derivative of citric acid found in tropical plants *Garcinia cambogia* and *Hibiscus subdariffa*[147]. HCA inhibits ACLY by competing with citrate[147], which results in concomitant suppression of DNL and cholesterol biosynthesis[148] (TABLE 2; Supplementary Table 2). However, HCA possesses poor physicochemical properties and was later found to allosterically activate ACC[149]. Although numerous attempts were made to improve on the HCA scaffold, these efforts were unsuccessful as off-target effects continued to be observed[150].

Additional screening of natural products identified several other ACLY inhibitors, including purpurone[151], a series of anthrones and anthraquinones derived from *Penicillium* sp.[152], radicicol, a 14-membered macrolide originally isolated from *Monosporium bonorden*[153], and cucurbitacin B, a natural bioactive compound abundant in cucumber[154]. Another series of inhibitors was developed on the chemical scaffold of the natural product emodin[155]. Although many of these compounds suppressed ACLY activity in biochemical assays, their specificity for ACLY and mechanisms of action remained unresolved[156].

Several inhibitors were designed on the basis of an alternative targeting strategy aimed to disrupt the formation of a stable citryl-CoA intermediate bound to the active site. SB-201076 was a promising compound that demonstrated activity against purified rat ACLY, but was inactive in cell-based DNL assays owing to poor cell permeability[157]. Improved cell permeability was achieved by generating the lactone prodrug analogue, SB-204990, which undergoes hydrolysis and activation once inside the cell to yield the active metabolite, SB-201076 (REF.[158]). However, development of this series was halted before clinical development. More recently, in silico screening identified four subtypes of furans and benzofurans that inhibit ACLY by also binding to the citrate binding domain[159].

A novel chemical scaffold discovered by high-throughput screening (HTS) led to the identification of BMS-303141, a 2-hydroxy-*N*-arylbenzenesulfonamide[160].

This compound inhibits ACLY, but also ACC1 and ACC2, although nearly 100-fold less potently[160]. Building on the BMS-303141 chemical scaffold, Nimbus Therapeutics used rational computer-aided design to develop a new series of ACLY inhibitors. The most potent compound, NDI-091143, inhibited human ACLY competitively with respect to citrate. The use of cryo-electron microscopy revealed an unexpected allosteric mechanism of inhibition whereby NDI-091143 bound next to the citrate binding site in a hydrophobic cavity, resulting in an extensive conformational change that prevented citrate binding to the enzyme. Although no cell-based or in vivo data were reported, the identification of this novel allosteric mechanism provides a new approach to discover novel ACLY inhibitors with improved drug-like properties[161].

One of the first synthetic fatty acid-like ACLY inhibitors was MEDICA 16. This compound was designed by modifying long-chain dicarboxylic fatty acids to generate ββ′-methyl-substituted α,ω-dicarboxylic acids, with the aim of maintaining the lipid-regulating properties of natural long-chain fatty acids while improving drug-like properties by preventing β-oxidation and enzymatic esterification[162]. MEDICA 16 inhibited ACLY competitively with citrate[163] and ACC competitively with acetyl-CoA and ATP[164]. A similar dicarboxylic acid, 3-thiadicarboxylic acid, was synthesized by replacing the dimethyl substitution in the β-position with a sulfur atom. This molecule inhibited ACLY and FAS, resulting in reduced levels of plasma triglycerides and cholesterol[165]. However, neither molecule advanced beyond preclinical studies.

Using a phenotypic screen based on inhibiting fatty acid and sterol synthesis in primary rat liver cells, Esperion Therapeutics discovered a liver-specific inhibitor, ETC-1002, 8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid, also known as bempedoic acid and ESP-55016 (REF.[166]). Studies have established that bempedoyl-CoA potently inhibits recombinant human ACLY competitively with CoA and that the prodrug (bempedoic acid) is inactive[167]. Importantly, conversion into the CoA conjugate is dependent on very long-chain acyl-CoA synthetase (ACSVL1/FATP2/SCL27A2), an enzyme highly expressed in the liver, but not in most other tissues including skeletal muscle, thus enabling the liver-specific actions of bempedoic acid[167]. And while bempedoyl-CoA also inhibits ACC[166] and activates AMPKβ1-containing heterotrimers[167], the relevance of these activities has not been demonstrated, since potency towards ACC is relatively weak and bempedoic acid continues to suppress liver DNL even in the absence of the AMPKβ1 isoform[167].

Therapeutic indications

Obesity. Preclinical studies in rodents found that HCA reduces body mass through a mechanism related to caloric restriction[168,169], but this effect is not observed in humans[170,171] (Supplementary Fig. 1). Bempedoic acid and BMS-303141, two of the better-characterized ACLY inhibitors, have strengthened a potential connection between weight loss and ACLY, with both reducing body weight gain and adiposity independently of changes in food intake in preclinical models[160,166,167,172]. Importantly, recent evidence has emerged from pooled analyses of clinical trials that bempedoic acid elicits modest weight loss in humans[173]. Studies examining the potential mechanisms by which ACLY inhibitors exert weight loss are warranted.

NAFLD–NASH and type 2 diabetes. Liver lipids and insulin sensitivity are directly linked; as such there is a very close connection between NAFLD and T2D. MEDICA 16 reduced liver lipid content[162], hepatic glucose production, and improved peripheral insulin sensitivity[174,175] in several distinct rodent models of obesity-induced insulin resistance. Bempedoic acid also reduced hepatic triglycerides and markers of inflammation in *Ldlr*$^{-/-}$ mice fed a diet high in fat and cholesterol. Importantly, the liver lipid-lowering effects of bempedoic acid are independent of liver AMPK activation[167]. In multiple mouse models, bempedoic acid also reduced fasting glucose, fasting insulin and glucose intolerance, suggesting improvements in insulin sensitivity[172]. Importantly, these effects appear to be translated to humans, as a meta-analysis of randomized trials suggests that bempedoic acid reduces new incidence or worsening of diabetes[176]. Whether bempedoic acid is effective at reversing NASH and fibrosis remains to be determined.

Cardiovascular disease. Given its dual impact on cholesterol and fatty acid biosynthesis, pharmacological ACLY inhibition has been studied for CVD. Early studies in animal models demonstrated the broad lipid-lowering effects of MEDICA 16 (REFS[177,178]) on circulating levels of cholesterol and triglycerides and associated beneficial effects on vascular and myocardial lesions[178]. Studies of BMS-303141 and SB-204990 further support ACLY as a target for hyperlipidaemia, as both compounds effectively lowered circulating triglycerides and cholesterol in animal models of hyperlipidaemia[158,160]. Bempedoic acid also suppresses hepatic cholesterol and fatty acid biosynthesis[172] and its hypolipidaemic actions have been demonstrated in hyperlipidaemic hamsters[172], obese Zucker rats[166] and in mice deficient for ApoE[167] or the LDL receptor[179] in which atherosclerosis is also reduced. In humans, bempedoic acid promotes dose-dependent LDL-cholesterol lowering effects as monotherapy, and when combined with a statin or ezetimibe[180]. Unlike rodents, in which bempedoic acid has a profound effect in reducing both plasma triglycerides and cholesterol, the primary effect of bempedoic acid in humans appears to be a reduction in plasma LDL-cholesterol[180]. The lack of effect on plasma triglycerides may potentially be due to lower liver DNL in humans compared with rodents. In addition to lowering LDL-cholesterol, bempedoic acid also reduces several plasma markers associated with atherosclerotic CVD such as total cholesterol, non-HDL cholesterol, plasma apoB, LDL particle numbers and high-sensitivity C-reactive protein[180]. More recently, bempedoic acid has been demonstrated to be a safe and effective therapeutic option for patients on maximally tolerated statin therapy[181] or patients intolerant of statins[182]. Bempedoic acid was recently approved for patients with heterozygous familial

hypercholesterolaemia (HeFH) (USA and Switzerland), established atherosclerotic CVD (ASCVD) (USA and Switzerland) who need additional LDL-cholesterol lowering, as an adjunct to diet and maximally tolerated statins (USA, EU, Switzerland and UK), and alone or with other lipid-lowering therapies in patients who are statin-intolerant or for whom a statin is contraindicated (EU and UK). The effect on cardiovascular morbidity and mortality has not been established.

Cancer. Substantial evidence supports the positive effects of pharmacological inhibition of ACLY using SB-204990 and BMS-303141 in cultured cancer cells[94]. However, despite the robust in vitro evidence, there is surprisingly few data supporting the concept that pharmacological inhibition of ACLY may be effective in vivo. For example, although early studies of HCA showed antitumour effects[183], target specificity and suppression of food consumption and body mass confounded interpretation of the primary mechanism of action. Similarly, SB-204990 reduced tumour growth in several distinct xenograft models; however, weight loss was again a confounding variable and no direct effect on metabolic reprogramming within the tumour was reported[86]. More recently, bempedoic acid has been demonstrated to inhibit hepatocellular carcinoma in mice, but given the requirement for bempedoic acid to be converted into a CoA by ASCVL1, it is currently unclear whether this is due to direct tumour effects or is secondary to inhibition of ACLY in the liver[87]. Future studies in mouse models examining the effects of pharmacological ACLY inhibitors in conjunction with measures of target engagement within the tumour (for example, suppression of DNL) are needed to evaluate whether pharmacological inhibition of ACLY may be effective in cancer.

ACC

Regulation and structure

In mammals, there are two isoforms of ACC, ACC1 (also known as ACCα) and ACC2 (also known as ACCβ). ACC1 is ubiquitously expressed (see Related links), whereas ACC2 is predominantly found in skeletal muscle, breast, adipose and liver (see Related links). ACC isoforms exhibit amino acid sequence similarity of 82% and 76% in their biotin carboxylase (BC) and carboxyl transferase (CT) domains, respectively[184], with the major difference being an N-terminal extension in ACC2 resulting in a higher molecular weight compared with ACC1 (REF.[184]). Although both ACC isoforms are expressed in the cytosol, it was hypothesized that the N-terminal extension in ACC2 might facilitate a preferential role (compared with ACC1) in controlling fatty acid oxidation, because of proximity to carnitine palmitoyltransferase 1, which is allosterically inhibited by malonyl-CoA[184]. Genetic evidence supports significant overlap between ACC isoforms in regulating fatty acid oxidation and DNL[47,49], suggesting that the relative importance of each isoform in regulating DNL and fatty acid oxidation is related to tissue-specific expression profiles rather than cellular localization.

The mechanisms linking tricarboxylic acid (TCA) cycle intermediates with increases in DNL were first uncovered in 1962 when it was found that citrate stimulated the activity of ACC[125] (FIG. 3). Subsequent studies identified that activation was mediated by promoting polymerization of the enzyme, an effect inhibited by LCFA-CoAs[185]. In addition to allosteric control, ACC is inactivated by phosphorylation[186], which is sufficient to overcome allosteric activation under physiological concentrations of citrate[187] (FIG. 3). This suggested that phosphorylation, not allosteric control, may be the predominant mechanism regulating ACC activity. ACC is phosphorylated and inhibited by the AMPK at Ser79 (REF.[188]). ACC2 activity is similarly inhibited by phosphorylation at a homologous site (Ser221). In hepatocytes, mutations in both ACC1 and ACC2 were necessary to exert maximal effects on DNL and fatty acid oxidation[189]. Interestingly, a recent study found that LCFA-CoAs directly activate AMPK[190], thus enacting a bimodal mechanism involving both allosteric and covalent inhibition of ACC activity, to inhibit DNL and increase fatty acid oxidation.

The ACC enzyme consists of three domains — BC, biotin-containing carboxyl carrier protein (BCCP) and CT — which are assembled in a single chain in most eukaryotes including mammals (FIG. 4). In eukaryotes, ACC functions as dimers and higher oligomers with the BC and CT domain dimers located at the top and bottom of the structure, respectively, while the BCCP is located within the CT active site[191]. The ACC reaction involves two steps: the first is ATP-dependent carboxylation of a biotin moiety by the BCCP, followed by transfer of a carboxyl group from biotin to acetyl-CoA to produce malonyl-CoA, within the CT[192]. Recent structural studies have detailed the dynamic interactions that occur between polymerization state and filament structures upon exposure to citrate and palmitoyl-CoA, effectively locking the enzyme into catalytically competent or incompetent conformational states, respectively[193]. Phosphorylation at Ser79 in ACC1, and presumably also Ser221 in ACC2, also induces a large conformational change involving the BC dimer interface, which promotes dissociation of the dimer and inactivation[194]. Thus, both allosteric regulation of ACC by citrate and palmitoyl-CoA and phosphorylation by AMPK regulate enzyme activity through alterations in conformational state[188,193].

Pharmacological inhibitors

Inhibition of ACC lowers malonyl-CoA, which is also an allosteric inhibitor of carnitine palmitoyl transferase 1, the rate-limiting enzyme that controls the flux of fatty acids into the mitochondria for β-oxidation. Thus, ACC inhibition represents an attractive approach to simultaneously suppress DNL and increase fatty acid oxidation. Given the embryonic lethality associated with ACC1 inhibition, differential tissue-specific expression profiles and potential compensation by the alternative isozyme, isozyme-specific (ACC1 or ACC2) and nonspecific (ACC1 and ACC2) inhibitors, as well as tissue-selective inhibitors, have been pursued.

The first generation of ACC inhibitors to be studied were soraphen A and TOFA (5-(tetradecyloxy)-2-furancarboxylic acid). Soraphen A is a macrocyclic

polyketide, originally isolated from the soil myxobacterium *Sorangium cellulosum* for its potent antifungal activity. It was later identified as an inhibitor of yeast and rat ACC1 (REF.[195]). In eukaryotes soraphen A binds to the BC domain, allosterically inhibiting enzyme activity by disrupting oligomerization; however, this does not occur in prokaryotes owing to large structural differences within the BC domain[196] (TABLE 3; Supplementary Table 3). TOFA is intracellularly converted into an ester, TOFyl-CoA, which acts as an ACC inhibitor but also reduces cholesterol synthesis[197], suggesting that it may inhibit ACLY. Given limited bioavailability and lack of specificity, neither agent was commercially developed.

Pfizer identified a series of isozyme-nonspecific ACC inhibitors through HTS which, following a series of optimizations, led to CP-640186 (REF.[198]). The crystal structure of the yeast CT domain of ACC in complex with CP-640186 indicates that the compound has tight associations with the active site of the enzyme, blocking biotin binding to the CT domain[199]. Taisho Pharmaceutical[200] and Takeda Pharmaceuticals[201] subsequently developed derivatives of CP-640186; however, these compounds had relatively poor metabolic stability and, while subsequent reductions in lipophilicity led to more favourable pharmacokinetics[202], and the introduction of a 7-methoxy group led to greater potency and metabolic stability[203], none of these compounds entered clinical testing. Also binding to the CT domain is WZ66, a compound identified via structure-based drug design studies by China Pharmaceutical University, which

Table 3 | **ACC inhibitors**

Compound (developer)	Potency in biochemical assays	Indication and/or preclinical effects	Clinical trial ID or refs
Clinical stage inhibitors			
Firsocostat (Nimbus Therapeutics, USA)	IC_{50}: 2.1 nM (hACC1), 6.1 nM (hACC2)	NASH	NCT02856555 (completed, phase II)
PF-05221304 (Pfizer Inc., USA)	IC_{50}: 13 nM (hACC1), 9 nM (hACC2)	NAFLD–NASH	NCT03248882 (completed, phase II)
PF-05175157 (Pfizer Inc., USA)	IC_{50}: 27 nM (hACC1), 33 nM (hACC2)	Type 2 diabetes	NCT01792635 (terminated, phase II)
		Acne vulgaris	NCT02100527 (withdrawn)
MK-4074 (Merck &Co., USA)	IC_{50}: ~3 nM (hACC1), ~3 nM (hACC2)	NAFLD	NCT01431521 (completed, phase I)
Preclinical stage inhibitors			
A-908292 (Abbott Laboratories, USA)	IC_{50}: >30 μM (hACC1), 0.023 μM (hACC2)	Reduced plasma lipids and glycaemia	226
Carboxamide derivative-1k (Takeda, Japan)	IC_{50}: 170 nM (hACC1), 2 μM (hACC2)	Decreased malonyl-CoA in xenograft tumour	209
CP-640186 (Pfizer Inc., USA)	IC_{50}: 53 nM (rACC1), 61 nM (rACC2)	Reduced weight gain, hepatic steatosis, plasma lipids and glycaemia, and inhibited cancer growth	216,230
Monocyclic derivate-1q (Takeda, Japan)	IC_{50}: 0.58 nM(hACC1), >10 μM (hACC2)	Decreased malonyl-CoA in xenograft tumour	208
ND-654 (Nimbus Therapeutics, USA)	IC_{50}: 3 nM (hACC1), 8 nM (hACC2)	Inhibited hepatocellular carcinoma growth, reduced hepatic steatosis and plasma lipids	189
ND-646 (Nimbus Therapeutics, USA)	IC_{50}: 3.5 nM (hACC1), 4.1 nM (hACC2)	Inhibited tumour growth	207
Olefin derivate-2e (Shionogi & Co., Japan)	IC_{50}: 1,950 nM (hACC1), 1.9 nM (hACC2)	Improved glucose homeostasis	213
(*S*)-9c (Boehringer Ingelheim Pharma GmbH & Co., USA)	IC_{50}: >30 μM (hACC1), 0.07 μM (hACC2)	Improved glucose and lipid homeostasis	212
Soraphen A	K_i: 2.1 nM (yACC)	Reduced weight gain, improved insulin sensitivity and inhibited cancer cell growth	214,228
TOFA	IC_{50}: 2.5 μM (rACC)	Reduced lipid synthesis, inflammation and cancer cell growth	229
WZ66 (China Pharmaceutical University, China)	IC_{50}: 435.9 nM (hACC1), 141.3 nM (hACC2)	Reduced hepatic steatosis and hepatic stellate cell activation	204

ACC, acetyl-CoA carboxylase; hACC, human ACC; IC_{50}, half-maximal inhibitory concentration; K_d, dissociation constant; K_i, inhibition constant; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; rACC, rat ACC; yACC, yeast ACC.

inhibits recombinant human ACC1 and ACC2 in cells and in rodents[204].

Several pan-ACC inhibitors have recently entered clinical testing in humans. Studies by Merck revealed MK-4074 as a liver-selective ACC inhibitor that dose-dependently inhibited DNL and lowered liver lipids in both rodent and humans[48] (TABLE 3; Supplementary Table 3). Although the exact binding site of the compound has not been published, it does not appear that clinical development is continuing, potentially owing to the observed induction of hypertriglyceridaemia[48]. Following on from studies with CP-640186, Pfizer developed PF-05221304, as a selective, orally bioavailable and reversible ACC inhibitor that is preferentially distributed to the liver[205], thereby avoiding potential toxicity related to the inhibition of platelet formation[18] and developmental defects[17]. Phase II clinical studies with PF-05221304 alone or in combination with a DGAT inhibitor, PF-06865571, in NAFLD–NASH have been completed[72].

In contrast to PF-05221304, which binds to the CT domain, Nimbus Therapeutics developed several potent ACC inhibitors by focusing on compounds that bind to the BC domain, blocking dimerization. Briefly, using crystal structure of the human ACC2 BC domain in complex with soraphen A and subsequent optimization of noncovalent interactions with the dimerization site, they developed a reversible ACC1 and ACC2 inhibitor, ND-630 (REF.[206]). In contrast to previous ACC inhibitors, this compound disrupted subunit dimerization by mimicking the effects of AMPK phosphorylation[206]. A second compound of this series, ND-646, exhibited similar potency and mode of inhibition to ND-630, but was developed to be more broadly distributed among peripheral tissues[207]. The third compound, ND-654, was modified to allow for enhanced hepatic uptake[189]. ND-630, also known as GS-0976 (Firsocostat), entered clinical development and is currently in phase II clinical trials for NASH.

In addition to the pan-ACC inhibitors described above, isozyme-specific ACC inhibitors have been developed. Takeda identified novel monocyclic derivatives[208] and carboxamide derivatives[209] that demonstrated specificity for ACC1 over ACC2. Abbott Laboratories developed a series of ACC2-specific inhibitors, one of the most potent and selective being A-908292, which exhibited more than 1,000-fold selectivity against ACC2 compared with ACC1. This compound dose-dependently lowered malonyl-CoA in muscle but not liver of rodents[210]. However, a preliminary safety assessment showed neurological and cardiovascular side effects that were resolved by replacement of the alkyne moiety with a heteroaryl linker[211]. Building on this series, Boehringer Ingelheim Pharma and Shionogi research laboratory also identified a series of new molecules that selectively inhibited ACC2 compared with ACC1 without toxicity[212,213]. To the best of our knowledge, none of these agents has entered clinical testing.

Therapeutic indications

NAFLD–NASH and type 2 diabetes. Significant preclinical evidence supports the benefits of ACC inhibition on liver steatosis, but they have also revealed potential on-target liabilities. In rodents, soraphen A lowered steatosis and this was associated with improved insulin sensitivity[214] (Supplementary Fig. 1). However, these improvements in insulin sensitivity did not translate to reductions in blood glucose, as consistent with studies in ACC null mice, ACC inhibition reduced insulin secretion from pancreatic β-cells[215]. To avoid this problem, TOFA derivatives such as WZ66 were developed that have a preferential liver distribution, reducing hepatic steatosis and hepatic stellate cell activation in mice with diet-induced obesity[204]. CP-640186 also lowers hepatic steatosis and insulin resistance in mice fed a HFD; however, this may be secondary to reductions in adiposity[216]. Lastly, short-term treatment of *db/db* mice with a selective ACC2 inhibitor, (*S*)-9c, reduced muscle malonyl-CoA levels and intramyocellular lipids while long-term treatment increased muscle glucose uptake and glucose tolerance while reducing HbA_{1c}, postprandial glucose and plasma triacylglycerol levels[212]. These studies suggest that inhibition of ACC is associated with reductions in liver steatosis and modest improvements in glycaemic control.

Given the promising effect of studies evaluating the effects of genetic and pharmacological inhibition of ACC on liver steatosis, several new-generation ACC inhibitors have advanced to clinical trials in NAFLD–NASH. In a phase I clinical study, PF-05175157 reduced DNL and increased whole-body fatty acid utilization[217]. However, subsequent clinical trials were terminated owing to extra-hepatic activity leading to reduced platelet count[18,205]. The liver-optimized inhibitor, PF-05221304, was subsequently shown to inhibit DNL, stimulate fatty acid oxidation and reduce triglyceride accumulation in primary human hepatocytes, along with reducing DNL, hepatic steatosis, fibrosis and immune cell activation in preclinical models[218]. Preclinical studies designed to assess the level of liver targeting achieved with PF-05221304 showed a maximal reduction of 82% in hepatic DNL compared with up to 33% reductions in lung and bone marrow[219]. In phase I clinical trials, PF-05221304 inhibited hepatic DNL in a dose-dependent manner without affecting platelet count, consistent with its more liver-targeted actions[205]. And in phase II clinical trials for NAFLD, PF-05221304 dose-dependently reduced liver fat by up to 65% at the highest dose while also lowering HbA_{1c} (REF.[72]).

Studies using the liver-specific inhibitor MK-4074 in rodents and humans have supported the therapeutic potential of potent ACC inhibition, but they have also raised additional questions about on-target liabilities. In a phase I clinical trial, MK-4074 reduced hepatic steatosis by 36% after 4 weeks of treatment[48]. In a preclinical model of NASH, the compound decreased liver fibrosis by 19% in addition to reducing liver fat[220]. However, clinical development of MK-4074 has been discontinued.

ND-630 is preferentially distributed to liver[206] and shows favourable effects in both preclinical and clinical studies. ND-630 dose-dependently reduces hepatic steatosis and hyperinsulinaemia in rodents[206]. An independent group has confirmed these effects on hepatic steatosis in animals fed a Western diet and also observed reductions in fibrosis[221]. In line with these studies,

HbA_{1c}
Haemoglobin A_{1c} (HbA_{1c}) is a glycated haemoglobin — a measurement used for the assessment of glycaemic control.

ND-630 inhibits hepatic stellate cell activation to reduce fibrogenic activity[222]. In a phase I clinical study, ND-630/GS-0976 was well tolerated and inhibited hepatic DNL in subjects with obesity and/or overweight[223]. In an open labelled, non-placebo-controlled study, patients with NASH treated with ND-630 had a median decrease of 22% in hepatic DNL and a significant reduction in hepatic fat and liver stiffness after 12 weeks of treatment[4]. In a subsequent larger randomized, placebo-controlled study, ND-630 treatment reduced hepatic steatosis by 21%[224]. ND-630 has recently been tested in combination with the apoptosis signal-regulating kinase 1 (ASK1) inhibitor selonsertib and farnesoid X receptor (FXR) agonist cilofexor in patients with bridging fibrosis or compensated cirrhosis, with combinations showing greater improvement in NASH activity than ND-630 alone[225]. Phase II studies with ND-630 and the GLP1R-agonist semaglutide are currently in progress.

Cardiovascular disease. Circulating triglycerides are an important risk factor for CVD, and in preclinical studies in mice, several ACC inhibitors, such as CP-640186 (REF.[216]), A-908292 (REF.[226]), (*S*)-9c[212], ND-630 (REF.[206]) and ND-654 (REF.[189]) lowered plasma triglyceride levels. However, in humans, ACC inhibitors dose-dependently increase plasma triglycerides and/or VLDL. As originally described for MK-4074 (REF.[48]), but also observed with PF-05221304 (REF.[72]), this increase in circulating triglycerides is likely mediated by the effects of decreased liver polyunsaturated fatty acids to stimulate SREBP1c activation, leading to increased GPAT and VLDL secretion[48]. ND-630 also increases plasma triglycerides[224], an effect associated with reduced triglyceride clearance[227]. To avoid this adverse effect, preclinical studies have been undertaken that combine ACC inhibitors with agents that reduce plasma triglycerides such as omega-3 fatty acids[48] and PPARα agonists such as fenofibrate[227]. Similarly, DGAT2 inhibition also blocked the effects of PF-05221304, to increase liver SREBP1c and circulating triglycerides while exerting additive effects on liver fibrosis in rodent models[72]. Importantly, in phase II clinical trials, DGAT2 inhibition also mitigated PF-05221304-induced increases in serum triglycerides and ApoaC3 (REF.[72]). These data suggest that combination strategies may be an effective means to improve the cardiovascular risk profile of ACC inhibitors in patients with NASH.

Cancer. Studies of early ACC inhibitors, soraphen A[228] and TOFA[229] demonstrated inhibitory effects on multiple cancer types. Although the contribution of ACC inhibition to the effects of these early inhibitors is unknown, the anti-tumorigenesic effect of ACC inhibition has been supported by more recent studies using inhibitors with improved potency and specificity. For example, CP-640186 reduced the growth of human lung cancer cells[230], while ND-646 and ND-654 reduced tumour burden in mouse models of non-small-cell lung cancer[207] and hepatocellular carcinoma[189], respectively, when used alone and in combination with existing standards of care.

FAS

Regulation and structure

Human FAS is highly expressed in adipose tissues and reproductive organs (see Related links). In contrast to ACLY and ACC, few allosteric and covalent mechanisms regulating the activity of FAS have been described (FIG. 3). In yeast, phosphorylation of Ser1140, Ser1640 and Ser1827 are associated with increased 18:0-CoA production[231], while in a breast cancer cell line, FAS is phosphorylated at Tyr66 when in complex with human epidermal growth factor receptor 2 (REF.[232]). In addition, FAS is degraded by E3 ubiquitin ligase COP1 in the presence of adaptor protein SHP2 and deubiquitinated by ubiquitin-specific protease 2a[233] (FIG. 3). These data suggest that under some conditions and cell types, FAS may be regulated by covalent and ubiquitylation mechanisms; however, it appears that the regulation of FAS primarily occurs through transcriptional control.

FAS has evolved structurally and functionally different variants that are generally divided into two types based on the organization of their catalytic units — type 1 and type 2 FAS[234,235]. In eukaryotes, the multifunctional complex of type 1 FAS is expressed in the cytosol and regulated by only one gene[234]. Type 2 FAS is present in mitochondria and functions completely independently of the cytosolic type 1 FAS[235]. Type 1 FAS typically generates only palmitate, whereas type 2 FAS is able to produce diverse fatty acids, including unsaturated fatty acids[236]. Structurally, mammalian FAS is a homodimer protein that consists of two multifunctional polypeptides, containing seven functional domains and two non-enzymatic or pseudo domains in each chain[237], all of which are required for fatty acid biosynthesis (FIG. 4).

Pharmacological inhibitors

Several natural products and their derivatives have been found to inhibit FAS. Cerulenin, an antifungal antibiotic originally isolated from *Cephalosporium caerulens* inhibits FAS in both mammals and bacteria[238]. And although cerulenin inhibits FAS by binding covalently to the active cysteine thiol in the β-ketoacyl synthase (KS) domain (FIG. 4), it also inhibits HMG-CoA synthetase activity[238], blocking sterol synthesis. The reactivity and lack of specificity of cerulenin led to the development of the synthetic analogue C75, which binds to the KS[239] and thioesterase (TE) domains of human FAS[240].

Several novel small-molecule FAS inhibitors have been developed that inhibit the TE domain. Orlistat, also known as tetrahydrolipstatin, is a derivative of lipstatin, a naturally occurring inhibitor of pancreatic lipase, and was found to also inhibit FAS through irreversible binding to the TE domain of the enzyme[241]. Owing to poor oral bioavailability and metabolic instability, several reformulation efforts were initiated, including a hydrophilic nanoparticle delivery system[242], poly(ethylene glycol)-conjugated poly(lactic-co-glycolic acid) nanoparticles[243] and folate receptor-targeted micellar nanoparticles[244]; however, none of these has moved into clinical development. Fasnall, a thiophenopyrimidine derivative also targets cofactor binding sites in multiple domains and in pharmacokinetic and toxicity studies shows no toxic effects[245]. More recently, triazole

Table 4 | **FAS inhibitors**

Compound (developer)	Potency in biochemical assays	Indication and/or preclinical effects	Clinical trial ID or refs
Clinical stage inhibitors			
Orlistat	IC_{50}: 100 nM	Obesity	Approved
TVB-2640 (Sagimet Biosciences, USA)	IC_{50} < 0.05 μM	NASH	NCT04906421 (in progress, phase II)
		Lung carcinoma, breast cancer, astrocytoma, colon cancer	NCT03808558, NCT03179904, NCT03032484, NCT02980029 (in progress, phase I/II)
FT-4101 (Forma Therapeutics, USA)	IC_{50}: 40 nM	NASH	NCT04004325 (terminated, phase I/II)
Preclinical stage inhibitors			
BI-99179 (Boehringer Ingelheim Pharma GmbH & Co, Germany)	IC_{50}: 79 nM	Inhibited cancer cell growth	249
Cerulenin	IC_{50}: 4.5 μM	Reduced weight gain and inhibited cancer cell growth	253,261
C75 (Johns Hopkins Medical Institutions, USA)	IC_{50}: 15.5 μM	Reduced body weight, hepatic steatosis and blood glucose, and inhibited cancer growth	255,257
Fasnall (Duke University School of Medicine, USA)	IC_{50}: 3.71 μM	Inhibited cancer cell growth	245
GSK2194069 (GlaxoSmithKline, USA)	IC_{50}: 7.7 nM	Inhibited cancer cell growth	246
IPI-9119 (Dana-Farber Cancer Institute, USA)	IC_{50}: 0.3 nM	Inhibited cancer cell growth	247
MP-ML-24-N1 (University Hospital Tübingen, Germany)	IC_{50}: 1.6 μM	Inhibited cancer cell growth	246
TVB-3166 (Sagimet Biosciences, USA)	IC_{50}: 42 nM	Inhibited cancer cell growth	250

FAS, fatty acid synthase; IC_{50}, half-maximal inhibitory concentration; NASH, nonalcoholic steatohepatitis.

urea-based substitutions to Fasnall, led to the identification of MP-ML-24-N1, which inhibits FAS through the TE domain and has good cellular permeability[246]. Lastly, IPI-9119 irreversibly inhibits the TE domain by promoting acetylation of the catalytic serine residue and has shown high potency and selectivity against FAS as well as good pharmacological properties[247].

FAS inhibitors that target the β-ketoacyl reductase (KR) domain have also been developed with some recently entering clinical testing. GSK identified GSK2194069 using an HTS approach based on compound competition with NADPH for binding in the KR domain[248]. Inhibitor-bound X-ray crystal structures showed that the compound forms hydrogen bonds with residues Ser2021 and Thyr2034, while studies in various cell lines indicated good cellular activity towards inhibiting DNL[248]. Using a similar approach, Boehringer Ingelheim Pharma GmbH & Co identified BI-99179 as a potent and selective inhibitor for human FAS that is also assumed to bind to the KR domain[249]. BI-99179 showed high metabolic stability in rat and human microsomes and high oral bioavailability in rats[249]. Lastly, Sagimet Biosciences developed TVB-3166, an orally bioavailable, reversible, potent and selective inhibitor of FAS that also likely inhibits the KR domain[250]. A related but more advanced compound, TVB-2640, is under clinical development. Recently, Forma Therapeutics reported that FT-4101 inhibited human FAS by also targeting the KR domain[251] (TABLE 4; Supplementary Table 4).

Therapeutic indications

Obesity. The link between FAS inhibition and reductions in body weight is not well understood. Initial observations that cerulenin induced weight loss in both lean[252] and obese[253] mice suggested that effects may be primarily related to toxicity rather than inhibition of FAS. However, subsequent studies showed that C75 also had a profound effect on body weight[254,255] (Supplementary Fig. 1), suggested that effects may involve an increase in energy expenditure and suppression of appetite. Although the FAS inhibitor orlistat has been approved for weight management in obesity it is important to note that owing to poor bioavailability the inhibition of pancreatic lipase, not FAS, is the primary mechanism by which this agent induces weight loss. Importantly, data from selective FAS inhibitors such as TVB-2640 do not support a link with weight loss.

NAFLD–NASH and type 2 diabetes. Early studies demonstrated that treatment with C75 decreases hepatic steatosis[256] and blood glucose in mouse models of obesity[252,257]. These findings supported the development

of TVB-2640, which was found to lower DNL by up to 90% in individuals with obesity and insulin resistance[258]. Phase II clinical studies are currently underway in patients with NASH. FT-4101 has been tested in humans with obesity, where it suppressed DNL and lowered steatosis[251]. However, clinical development has ceased for reasons that have not been disclosed. Although these studies support a potential therapeutic benefit of inhibiting FAS in NASH, results from the ongoing phase II studies will be imperative to better understand safety and efficacy.

Cancer. Although cerulenin has beneficial effects in various cancer cell lines and xenograft tumour models[259–261], reactivity and the profound weight loss caused by this compound limited its potential. Antitumour effects of C75 were also demonstrated in various cancer cell lines and xenograft tumours alone[261] and in combination with radiotherapy[93]. In addition, orlistat exhibits cytostatic and cytotoxic effects in tumour cells, and nanoparticle technology has been used to improve bioavailability and subsequent cytotoxicity in xenografts[241,243,244]. Studies have been conducted with more-specific inhibitors that strengthen FAS as an anticancer target. Fasnall was shown to inhibit breast cancer[245] and prostate cancer cell growth[262], and GSK2194069 inhibits the proliferation of non-small-cell lung cancer cell lines[248]. IPI-9119 (REF.[247]) and TVB-3166 (REF.[250]) also inhibit cancer cell proliferation in several distinct preclinical models. Interestingly, recent studies have found that owing to limited exogenous fatty acids within the brain, metastasis of breast cancer is highly sensitive to FAS inhibition by TVB-3166 and BI-99179 (REF.[263]). Phase I clinical trials with TVB-2640 have been completed in healthy subjects and in patients with solid malignant tumours[264], and phase II studies in multiple cancers are underway. Results from these studies will be important to better understand the potential therapeutic effects of FAS inhibition in cancer.

Challenges

In the past 50 years tremendous progress has been made in understanding the biochemical mechanisms and physiological significance of DNL in regulating cellular metabolism and whole-body energy homeostasis. Important steps along the way have included biochemical identification of the key metabolic intermediates in the conversion of glucose to fatty acids, the molecular cloning of the key enzymes regulating the process and the discovery of crucial allosteric and covalent mechanisms that regulate flux through the pathway. Subsequent studies in genetically modified mice revealed the physiological role of DNL, broadening our understanding of the complex connections that exist between multiple cellular processes and tissues well beyond the simple storage of excess calories in adipose tissue. Meanwhile, advances in structural biology laid the foundation for the molecular underpinnings by which natural products and new-generation small molecules inhibited enzyme activity. Collectively, this information led to an explosion in the number of novel small molecules that have been developed and tested in preclinical models for cardiometabolic disease, cancer and other indications. Although these compounds vary in their molecular target, chemical structure and physicochemical properties, the common action in which DNL is inhibited supports the notion that it is beneficial for the prevention and treatment of a broad spectrum of diseases. However, the development and testing of this new generation of highly selective and efficacious small molecules has also revealed several surprising new biological insights that have important implications for safety, efficacy and the need for combination therapies as they progress into clinical trials.

Safety-related side effects

There are several safety concerns associated with inhibiting DNL. The first and most significant to be identified was related to embryonic development. Mice homozygous deficient for ACLY[265], ACC1 (REF.[266]) or FAS[267] die during embryonic development, and CIC deficiency is characterized by severe neurodevelopmental disorders[268]. The ACC inhibitor PF-05175157 also induces developmental toxicity (growth retardation and dysmorphogenesis associated with disrupted midline fusion) in rats and rabbits[17]. Thus, avoiding systemic inhibition of DNL is essential to safeguard embryonic development.

Another major concern with inhibiting DNL in lipogenic organs such as liver and adipose tissue is that this simply redirects carbon into other parts of the body where toxicity may be even greater. Experimental evidence of this effect was first established with the ACC inhibitor MK-4074, which dose-dependently increased SREBP1c and serum triglycerides; an effect that was shown to be 'on-target' as mice genetically lacking ACC in the liver had a similar phenotype[48]. Subsequent studies with ND-630 (REF.[224]) and PF-05221304 (REF.[205]) in humans with NASH revealed similar effects. Importantly, increases in serum triglycerides by PF-05221304 can be inhibited by co-administration of a DGAT2 inhibitor[72]. Interestingly, inhibiting ACC by activating AMPK[269,270] lowers serum triglycerides and cholesterol effects. Similar results are also observed with the inhibition of ACLY[173,181,182]. A greater understanding of the mechanism behind these differential responses to inhibiting liver DNL will be important for alleviating potential adverse events.

The upstream and downstream consequences associated with blockade at different steps in the DNL pathway, leading to the accumulation of metabolic intermediates, must also be considered. For example, inhibition of FAS leads to a build-up of malonyl-CoA. This increase in malonyl-CoA as a result of FAS inhibition impairs physiological and pathological angiogenesis via the malonylation of mTOR[271]. Malonylation has also been implicated in various metabolic pathways[272], histone modifications[273] and immune cell reprogramming[274], suggesting that FAS inhibition may exhibit broader biological effects than the anticipated DNL inhibition. CIC inhibition may also pose safety concerns, as a deficiency of CIC increases mitochondrial citrate and isocitrate and this is associated with severe neurometabolic effects[268]. Lastly, ACLY inhibition, which lowers acetyl-CoA, may affect the acetylation profile of many different histones, potentially having a wide array of differential effects on gene expression profiles and epigenetic programming[275].

Thus, it is pivotal to consider the effects of metabolic intermediates when developing DNL inhibitors as a therapeutic approach.

Bypassing DNL inhibition/compensatory pathways

The concept of cellular metabolic flexibility is another key concern for DNL inhibitors. Metabolism is fluid and interconnected: a block in one pathway enhances flux through alternative pathways. This has been studied most intensely in cancer in which plasticity is a hallmark of chemoresistance. For example, in response to reduced flux from glucose to acetyl-CoA under hypoxic conditions, mutations in *KRAS* have been shown to provide cancer cells with increased ability to scavenge exogenous lipids, thereby diminishing dependence on DNL[276]. The reciprocal relationship is also observed whereby inhibiting exogenous fatty acid uptake by blocking CD36 enhances the efficacy of the FAS inhibitor C75 (REF.[277]). Similarly, in breast cancer brain metastasis, where exogenous fatty acids are limited, inhibiting FAS exerts greater lethality compared with the liver where exogenous fatty acids are abundant[263]. Inhibition of ACLY can also be bypassed in some cancer cells[278] and in the liver of mice fed a high-fructose diet[45] through upregulation of ACSS2. These data suggest that depending on the nutritional context and cell types involved, for DNL inhibitors to be highly effective it may be necessary to also target compensatory mechanisms. For example, might it be important for people being treated with an ACLY inhibitor to limit their alcohol and fructose intake to maximize effects? Alternatively, might an individual having their cancer treated with a FAS inhibitor need to limit fatty foods? Despite these compelling data in preclinical models, data generated in clinical settings with respect to ACLY inhibition for LDL lowering[173,181,182], ACC inhibitors for NASH[48,72,205,224] and FAS inhibition for cancer[264] suggest that the degree of DNL inhibition achieved may be sufficient to overcome any compensatory pathways. However, further studies investigating this will be important.

Systemic/organ-specific inhibition

A significant challenge with the clinical development of DNL inhibitors has been the discovery of differential responses of certain cells between rodents and humans. An example of this occurred with the clinical development of the systemic ACC inhibitor PF-05175157, which was found to have no toxicity in rodents but dose escalation studies in people revealed reduced platelet count[18,205]. Subsequent studies established that the reduction in platelet count was due to ACC inhibition within bone marrow, which impaired megakaryocyte maturation[18]. This finding demonstrated a requirement for DNL in humans but not rodents, which was especially surprising given that DNL in tissues such as liver and adipose tissue is much higher in rodents than in humans[279]. Importantly, these effects have been avoided with new-generation liver-targeted ACC inhibitors ND-630 (REF.[224]) and PF-05221304 (REF.[205]). Similarly, genetic inhibition of ACLY in muscle and adipose tissue results in muscle weakness and lipodystrophy, respectively, but has been avoided by the development of the liver-targeted prodrug bempedoic acid[167]. Liver-specific inhibition of DNL may also be helpful to avoid the potential detrimental effect of adipose tissue DNL inhibition on whole-body insulin sensitivity, given that adipose tissue DNL directly correlates with insulin sensitivity[61,280]. Lastly, FAS inhibition is associated with alopecia, an effect that has been reduced with the new generation of liver-directed FAS inhibitors TVB-2640 (REF.[264]) and FT-4101 (REF.[251]). Thus, data presented to date suggest that inhibition of FAS, ACC and ACLY can be safely achieved through liver targeting; however, whether chronically inhibiting DNL in other organs or cell types can be safely achieved requires further study.

Outlook

Over the past decade, many challenges have been addressed leading to DNL inhibitors entering clinical development for cancer, CVD, NASH and acne vulgaris. In the era of single-cell analysis of cellular metabolism, an especially exciting avenue of research will be to determine whether there may be specific cell types in which DNL is upregulated under different pathological conditions. The identification of these potential cell types may lead to cell-specific DNL inhibitors that could be applied during certain windows of development for alleviating or potentially preventing disease. Another important consideration will be related to balancing efficacy and safety as enhanced potency may lead to greater compensatory upregulation and/or toxicity. An example of this is seen with the ACC inhibitors whereby inhibition of DNL closely parallels concomitant increases in circulating triglycerides and inhibition of platelets. As DNL is transiently upregulated after a meal, one way to potentially avoid upregulation of compensatory pathways and/or toxicity may involve the use of inhibitors with a shorter half-life so that DNL is maintained at basal levels. Ultimately, whether DNL inhibitors are safe and effective enough to be used as a monotherapy or will be used in combination with other therapies to enhance efficacy or offset liabilities, as has been proposed for ACC inhibitors in NASH, will depend on clinical trial results. In this regard, findings with the ACLY inhibitor bempedoic acid are encouraging as they suggest that chronic inhibition of DNL in the liver is safe when used alone or with other standards of care. Whether DNL inhibitors that target other cell types and organ systems become widely adopted as a cornerstone for treating other disease indications beyond CVD and NASH remains to be determined.

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Human Protein Atlas, ACC1 in tissues: https://www.proteinatlas.org/ENSG00000278540-ACACA/tissue
Human Protein Atlas, ACC2 in tissues: https://www.proteinatlas.org/ENSG00000076555-ACACB/tissue
Human Protein Atlas, ACLY in cells: https://www.proteinatlas.org/ENSG00000131473-ACLY/cell
Human Protein Atlas, ACLY in tissues: https://www.proteinatlas.org/ENSG00000131473-ACLY/tissue
Human Protein Atlas, CIC in tissues: https://www.proteinatlas.org/ENSG00000100075-SLC25A1/tissue
Human Protein Atlas, FAS in tissues: https://www.proteinatlas.org/ENSG00000169710-FASN/tissue