Management of Cancer Cachexia: Attempting to Develop New Pharmacological Agents for New Effective Therapeutic Options

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Cancer cachexia (CC) is a multifactorial syndrome characterized by systemic inflammation, uncontrolled weight loss and dramatic metabolic alterations. This includes myofibrillar protein breakdown, increased lipolysis, insulin resistance, elevated energy expediture, and reduced food intake, hence impairing the patient's response to anti-cancer therapies and quality of life. While a decade ago the syndrome was considered incurable, over the most recent years much efforts have been put into the study of such disease, leading to the development of potential therapeutic strategies. Several important improvements have been reached in the management of CC from both the diagnostic-prognostic and the pharmacological viewpoint. However, given the heterogeneity of the disease, it is impossible to rely only on single variables to properly treat patients presenting this metabolic syndrome. Moreover, the cachexia symptoms are strictly dependent on the type of tumor, stage and the specific patient's response to cancer therapy. Thus, the attempt to translate experimentally effective therapies into the clinical practice results in a great challenge. For this reason, it is of crucial importance to further improve our understanding on the interplay of molecular mechanisms implicated in the onset and progression of CC, giving the opportunity to develop new effective, safe pharmacological treatments. In this review we outline the recent knowledge regarding cachexia mediators and pathways involved in skeletal muscle (SM) and adipose tissue (AT) loss, mainly from the experimental cachexia standpoint, then retracing the unimodal treatment options that have been developed to the present day.

Keywords: cancer cachexia, muscle tissue and adipose tissue loss, cachexia mediators, animal models, clinical trials, therapeutic strategies

INTRODUCTION

Cancer-associated cachexia (CC) is a paraneoplastic syndrome whose outbreak is governed and driven by inflammation. It involves various organs and is characterized by changes in body composition (1–3). Although loss of appetite is frequently associated with cancer cachexia, tissue wasting under cachectic conditions occurs through different modalities compared to those triggered by starvation, which primarily affects the adipose tissue (AT). The major feature of cachexia is, instead, represented by loss of skeletal muscle (SM) mass, not necessarily accompanied by AT wasting (4).

Loss of muscle mass in cachectic individuals is due to altered proteostasis, which results from a combination of enhanced proteolysis and decreased protein synthesis (5, 6). Here, the high rate of proteolysis largely depends on upregulation of ubiquitin–proteasome and autophagy–lysosomes pathways, while calpains and caspases have been found to contribute to a lesser extent, though valuably. For instance, the calpains-mediated protein degradation is thought to act upstream of the ubiquitin–proteasome pathway, thus allowing the release of myofilaments from the myofibrils and their subsequent ubiquitylation and degradation (7).

Patients with CC often manifest generalized hypermetabolism with lower energy intake and higher energy expenditure. In particular, anomalous increases in resting energy expenditure (REE) are nowadays considered the major contributor to energy consumption. In this context, systemic inflammation and changes in the immune system both represent important determinants of this condition, albeit the precise mechanisms remained elusive (8, 9). Increases in energy expenditure are also explained by enhanced thermogenesis and "browning" of white adipose tissue (WAT), which consists of a rapid increase of beige adipocytes (10, 11). Moreover, WAT of cachectic individuals can undergo to consistent dissolution of fatty acids and glycerol. Such an event seems to occur in consequence to increased lipolytic activity of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), two key enzymes involved in catabolism of triglycerides. On the contrary, some studies suggest that no significant downregulation in lipogenesis or expression/activity of lipoprotein lipase (LPL) occur in patients with CC (12, 13).

Incidence and prevalence of CC are not homogenous across cancer patients, but they rather vary depending on the tumor type and stage. CC has been mainly associated with incurable cancers and is highly prevalent at the end of life (14, 15); however, it can also occur in curable cancers and may be reversed by properly treating the underlying tumor (16). Meanwhile, this syndrome is notoriously influenced by additional endogenous and environmental factors, such as comorbidities, genetic risk factors, sex, age, and anti-cancer treatment (17–19). A report based on information provided by two independent studies showed a prevalence of ~70% for pancreatic cancer, ~60% for gastro-esophageal and head-neck cancers, and 50–40% for non-small-cell lung cancer, colorectal cancer, and certain hematological malignancies (20). Similarly, a recent systematic review (15) reported very high risk (80–90%) of developing cachexia for patients with liver, pancreatic and lung cancers, followed by head-neck, gastric and colorectal cancers (60–70%). On the contrary, patients with thyroid, breast and prostate cancers and melanoma of the skin represented the groups at lowest risk (~20%). In the same study, cachexia was defined for the first time an orphan disease (15).

Approximately 20–30% of mortalities in cachectic tumor patients are thought to derive from cachexia rather than from the tumor burden itself (6, 21). In addition, cachexia can lead to lower responsiveness to anticancer therapies, worsening of life quality and poor prognosis in advanced tumor patients (14, 22, 23). In this regard, it has been reported that treating cachectic patients with conventional chemotherapeutic regimens further enhances hypercatabolism of SMs and could cause changes in fat and bone mass. This would exacerbate the pathological condition, thus requiring dosage limitation or therapy interruption (3).

Clinically, combinations of metastatic tumor, muscle wasting, debilitation, and refractoriness to chemotherapy seriously limit benefits from treatments of cachexia, even when multimodal options are adopted. Thus, the identification of reliable diagnostic markers, predictive of CC, is of fundamental importance to prevent the patient's physiological decline. According to the most recent consensus report, published by Fearon et al., the current standard diagnostic criterion for cachexia is represented by a weight loss >5% over the past 6 months, or any degree of weight loss >2% in individuals showing a Body Mass Index (BMI) < 20 or sarcopenia (14). In the same report, a classification criterion was proposed to clinically subdivide CC into three stages, aiming to properly treating cachectic tumor patients. In line with the diagnostic criterion, such classification would be based on three established parameters of cachexia, i.e., percentage of weight loss, BMI values and metabolic changes (14). Specifically, patients manifesting anorexia, impaired glucose tolerance and weight loss ≤5% over the past 6 months would be classified as pre-cachectic. Patients with systemic inflammation, BMI < 20, weight loss > 5% over the past 6 months and ongoing weight loss of more than 2% would be classified as cachectic. Absence of responsiveness to anticancer treatments or preterminal cancer stage would instead determine refractory cachexia (14). However, this classification cannot currently be given as officially accepted by clinicians, but is rather to be considered as a proposal under evaluation. Indeed, additional diagnostic parameters such as hemoglobin (Hb) and albumin levels were previously proposed (4). The same parameters were then re-evaluated by two more recent studies, with the addition of C reactive protein levels (24, 25). However, none of these were included in the diagnostic definition of CC neither in its classification by the last consensus, although it was stated that several other components of cachexia (thus including the aforementioned ones) should be further evaluated (14).

Interestingly, some research groups investigated on possible causative genetic variants underlying CC or cachexia-related appetite loss in cancer patients [e.g., (26, 27)]. Although these studies put into evidence dozens of single nucleotide polymorphisms as potentially involved in such processes, no significant findings were brought out. Moreover, some discrepancies emerged when results from some of these studies were compared to one another (26). Nonetheless, a role for genetics in the pathophysiology of CC cannot be excluded, since it should be considered that this type of analysis is in its early stage. Thus, further genome-wide approaches are needed, considering the complexity and the variability of the syndrome.

From the molecular standpoint, numerous *in vitro* and *in vivo* studies have demonstrated that several pro-inflammatory cytokines, toll-like receptors (TLRs) and growth/differentiation factors (GDFs) act as mediators of CC. In general, most of these molecules are purposely used as signaling molecules in cell-to-cell communication and mechanisms involved in innate immunity, and exert pleiotropic effects. For instance, cytokines are primarily produced by immune cells, although several other cells of the organism as well as tumor cells are capable to express

them (28). In the pathogenesis of cancer, the tumor-induced inflammatory response leads to expression and secretion of a number of immune-suppressive and pro-inflammatory cytokines by immune cells, aiming to eradicate tumor cells from the host (29). However, inappropriate accumulation/regulation of leukocytes in the tumor site can cause an imbalance between pro- and anti-inflammatory mechanisms, eventually leading to chronic inflammation and subsequent immunosuppression (30), as occurs in advanced cancer patients. As a result, the chronic presence of such mediators of inflammation in both the tumor microenvironment and circulation causes systemic deregulations and metabolic dysfunctions in the host, including CC (2, 29).

MEDIATORS OF CC: WHAT HAVE WE LEARNED FROM *IN VITRO* AND *IN VIVO* STUDIES

Experimental research on CC has experienced an exponential increase in terms of gained knowledge during the last three decades. In particular, the identification of several endogenous factors functioning as mediators of CC and the uncovering of their relative mechanisms of action has led to the achievement of important frontiers in this field of oncology. This has allowed the development of potential effective pharmacological agents for the clinical management of this metabolic syndrome (31). Intriguingly, we now know that several of these effectors share the same or similar metabolic effects, and that most often they exhibit synergic effects when administered together. Moreover, most of them are simultaneously involved in both SM and AT depletion, though exerting a distinct role depending on the target tissue (see next section).

Tumor Necrosis Factors

Tumor necrosis factor alpha (TNFα, also known as cachectin) has long been shown to play a role in murine models of CC (32, 33). Albeit normally involved in acute phase reaction triggering and apoptosis, TNFα can also promote tumorigenesis and metastasis, and has been shown to act as an autocrine growth factor for various tumor types (34). Early studies showed that TNFα had the ability to inhibit differentiation of both skeletal myocytes and adipocytes (35, 36), while it caused reduced protein content and higher degradation of myofibrillar proteins in differentiated skeletal myocytes, in a time- and dose-dependent manner (37, 38). However, later experiments demonstrated that TNFα alone was not sufficient to cause a significant dysfunction of skeletal myofibers in differentiated myocytes, but a synergic action with other cytokines, such as interferon gamma (INFγ), was required to produce valuable effects [e.g., (35, 39)]. More recent studies have reported similar results for a structural homolog of TNFα, i.e., TNF-related weak inducer of apoptosis (TWEAK, also known as TNFSF12), which presents overlapping signaling functions with the former (40, 41).

Interleukins

Some of the cytokines belonging to the class of interleukins (ILs) have been shown to significantly contribute to tumor growth and CC. First and foremost, circulating interleukin-6 (IL-6) is recognized as one of the main factors leading to the outbreak of cachexia. For instance, significant concentrations of IL-6 were detected in the serum of cachectic mice transplanted with a cachexia-inducing colon-26 adenocarcinoma (C26) subtype, where serum level of IL-6—but not that of TNFα—correlated with severity of the pathological status (42). Yet, high constitutive levels of circulating IL-6 caused suppression of muscle protein synthesis at the initial stage of cachexia in a different murine model (43). On the contrary, attenuation of the IL-6 signaling was shown to abolish key features of CC (42, 44), although such inhibition was not sufficient to reverse the process (43). In a similar manner, it has been evidenced a role for IL-6 in induction of energy expenditure and loss of fat mass by promotion of WAT browning. In fact, the knock-out of IL-6R in cachectic mice transplanted with B16 melanoma cells showed partial though significant reduction of WAT browning when compared with control mice (11). At the same extent, the genetic blockade of IL-6 was found to be critical in a syngeneic graft model with C26 cancer cells, as it prevented WAT browning and cachexia (11).

Leukemia inhibitory factor (LIF) is another IL-6 family member, involved in a variety of distinct biological processes including inflammation, cell growth, differentiation, neural development, and hematopoiesis (45, 46). As in the case of IL-6, LIF was previously reported to induce muscle wasting in various animal models (47, 48). A recent study further demonstrated the involvement of tumor-secreted LIF in myotube atrophy in a C26 cancer-induced cachexia model (49). Here, elevated circulating levels of this interleukin induced expression of atrophy-related genes. On the contrary, immunological blockage of circulating LIF prevented the triggering of such phenomenon (49). LIF has recently been shown to be implicated in cachexia-associated lipolysis as well (50), whereby interaction between this interleukin and its cognate membrane receptor LIFR-α caused enhanced expression of genes involved in lipids catabolism. Interestingly, the same study revealed a double mechanism of action for LIF, since it sustained fat mass loss through an equal combination of peripheral (i.e., directly exerted on adipocytes) and central (i.e., exerted on the hypothalamus) contributions. In particular, the latter mechanism of action was counterbalanced by leptin signaling (50).

Similarly, to IL-6, IL-1 alpha (IL-1α) was initially evidenced as a factor capable to induce protein breakdown in isolated SM during systemic inflammation (51). Administration of IL-1α was found to induce cachexia together with anorexia by causing accelerated SM protein wasting in a rat model (52). Accordingly, the pharmacological blockade of IL-1α receptor reduced tumor growth and slowed down the development of CC in methylcholanthrene-induced sarcoma (MCG 101)-bearing mice (53).

A more recent study demonstrated how IL-1 beta (IL-1β) increased SM catabolism in a rodent model of CC by promoting a cachexia-associated gene expression pattern in the hypothalamic–pituitary–adrenal (HPA) axis, differently from IL-6 and IL-1α (54). Few other studies on experimental cachexia have raised the possibility for other interleukins to be involved in the onset of cachexia, as is the case of IL-10 and homodimeric IL-12 (55), although with a lesser extent of evidence.

Interferon Type II

INFγ, the only member of the type II class of interferons, has a critical role in innate and adaptive immunity against viral infections, promotes activation of macrophages and exerts mild antiproliferative effects on certain cell types (56). Nonetheless, several studies have reported a role for this cytokine in enhancement of tumor growth, metastasis (57, 58) and development of CC (59). Nude mice injected with genetically engineered ovary tumor cells (CHO) producing murine IFN-γ developed severe cachexia, contrary to those inoculated with the parental tumor cell line (60). Accordingly, when mice injected with CHO/INFγ cells were treated with anti-INFγ monoclonal antibodies, development of cachexia was prevented (60). In line with this, early or late treatment of Lewis lung carcinoma (LLC)-bearing mice with anti-INFγ monoclonal antibodies was shown to counteract progression of cachexia (61). Importantly, both of these studies reported that here AT, but not SM, was the main target of INFγ (60, 61).

MicroRNAs

Over the last decade, several proofs were gained about the involvement of non-coding RNAs, in particular microRNAs (miRNAs), in loss of lean and fat mass under cachectic conditions. In their mature form, miRNAs are ∼21–23 nucleotides in length and exert a well-recognized role in gene regulation. In particular, miRNAs have been extensively studied as biomarkers for histological classification, disease prognosis, clinical response to treatments and diagnosis of cancer (62, 63). Besides exerting their action within the cell, miRNAs can be released into extracellular fluids and are referred to as extracellular or circulating miRNAs. At the same extent of intracellular miRNAs, circulating miRNAs are capable to modulate gene expression in recipient cells, determining, adjusting or deregulating cells' physiological status (64). Moreover, secreted miRNAs can have a role in cell-to-cell communication [e.g., (65, 66)]. As CC mediators, miRNAs seem to exert their action by favoring cell apoptosis, protein degradation, or causing downregulation of anabolic processes. For instance, circulating miR-203a-3p secreted by metastatic human colorectal cancer contributed to exacerbation of myopenia in pre-operative cancer patients by targeting survivin (BIRC5), a negative regulator of caspase-dependent apoptosis (67). MiR-21-5p and−206, instead, played an important role in the onset of muscle atrophy in mice under distinct muscle-debilitating conditions, including CC. This was due to the miRNA-mediated inhibition of the translational initiator factor eIF4E3 and transcription factor Yin Yang 1 (YY1), involved in mitochondrial biogenesis (68). Yet, mir-155-5p secreted by human breast cancer cells was proven to promote WAT browning and increased BAT thermogenesis but not lean mass loss. This was due to the targeting of peroxisome proliferator-activated receptor gamma (PPARγ), a well-known regulator of glucose metabolism and fatty acid storage (69).

Toll-Like Receptors

Toll-like receptors (TLRs) are other essential components of the innate immune response. TLRs are present either on the cell surface or in endosomes, and are capable to interact with microbial components, danger-associated self molecules or non-self nucleic acids presenting well-defined patterns. Once stimulated by such interactions, TLRs activate two possible signaling pathway leading to downstream immunogenic gene expression (70). One recent study has evidenced differential expression of TLR genes in cachectic LLC-bearing mice compared to non cachechtic controls (71). Moreover, results from the same study suggested that increased expression of TLRs mRNA may depend on factors secreted by tumor cells, and that different tumors, such as LLC and C26, are likely to induce distinct pattern of TLR expression.

Among TLRs, TLR4 belongs to the cell-surface group and is well-known to possess high affinity for lipopolysaccharide (70). TLR4 was initially shown to act as a master regulator of inflammatory muscle catabolism, as its exposition to lipopolysaccharide caused significant loss of myofibrillar proteins and mitochondria in both cultured and *in vivo* murine muscle cells (72). Later, this receptor was demonstrated to be directly implicated in myotube atrophy in LLC-bearing mice, causing cachexia. In this murine model, activation of TLR4 was caused by cellular uptake of high levels of heat shock protein 70 (Hsp70) and Hsp90, secreted by tumor cells through extracellular vesicles (EVs) (73). In turn, activation of TLR4 correlated with evident muscle wasting and increased levels of circulating TNFα and IL-6 (73). TLR4 was also reported to be involved in remodeling of AT in LLC-bearing mice. In fact, it was demonstrated that either genetic ablation or pharmacological inhibition of TLR4 in cachectic mice caused suppression of adipocytes atrophy and also reduced macrophage infiltration into the AT (74).

TLR7/8 is mainly located at the endosomal level and recognizes viral single-stranded RNA molecules (70). TLR7/8 was found to have a significant impact in experimental CC after being activated by EV-derived cargo. Specifically, our group demonstrated that murine TLR7 was capable to interact with EV-derived miR-21-5p secreted by lung and pancreatic cancer cells. Once activated, TLR7 induced muscle loss by promoting apoptosis in cultured myocytes in a JNK-dependent manner. On the contrary, myocytes from $TLR7^{-/-}$ LLC-bearing mice showed significantly reduced cell death (66). Recent experiments confirmed these results (75) and demonstrated that also tumor-secreted miR-29a-3p was capable to interact with murine TLR7, inducing cell death even with higher effectiveness than miR-21-5p (75).

A role in development of cancer cachexia has been recently suggested for TLR5 as well (76). In fact, activation of this receptor, which is expressed on the plasmatic membrane of certain gastric cancer cells, seemed to contribute to the onset of CC, possibly due to TLR5-mediated overexpression of LIF (76).

Cytokines of the Transforming Growth Factor β Superfamily

Besides pro-inflammatory cytokines and TLRs, regulatory proteins belonging to the transforming growth factor β (TGFβ) superfamily of cytokines have been identified as potential actors exacerbating SM atrophy in cachexia. Among these, Myostatin

(Mstn) and Activin A (ActA) present a partial overlap in their signaling function, since both are capable to interact with either of the Activin type II receptors (ActRIIA or ActRIIB), expressed on the surface membrane of skeletal myocytes (77). Mstn (also known as GDF8) is primary synthesized by SM cells and functions as an autocrine factor causing a strong downregulation of myogenesis (78, 79), while inhibition of its expression/function leads to a dramatic increase in muscle mass (80, 81). Following this line, later studies demonstrated that Mstn mRNA expression levels increased up to ∼50% in experimental models of CC, while expression levels of MyoD, a myogenic transcription factor indispensable for myoblast fusion and SM development, decreased of ∼45% in comparison with controls (82, 83). In contrast, when synthetized antisense RNA oligonucleotides were used to target the Mstn mRNA *in vivo*, expression levels of both Mstn and MyoD tended to revert toward the normal phenotype, and led to a significant increase in muscle mass (83).

Differently from Mstn, ActA is expressed by a variety of cell types, has a wide range of regulatory functions and is negatively modulated by several factors including Inhibin (Inhb) and Follistatin (FS) (84). In the context of muscle wasting, concentrations of circulating ActA seem to be particularly increased during acute inflammation or in certain metastatic cancers (85, 86). Since ActA can reproduce the biological action of Mstn by binding to the ActRII receptors, knockdown experiments for Inhb caused dramatic loss of both lean and fat mass in mice, leading to death (87). Similarly, increase of local or circulating concentrations of ActA induced by direct methods caused muscle atrophy in treated mice (88). On the contrary, the pharmacological blockade of ActRIIB or administration of its soluble decoy forms (sActRIIB) in multiple rodent models of CC reversed atrophy of skeletal and cardiac muscles and prolonged survival of treated mice, albeit it had no effects on tumor growth or loss of AT (87, 89).

Parathyroid Hormone-Related Peptide

Two recent works highlighted the role of parathyroid hormone-related peptide (PTHrP) in WAT browning under cachectic conditions (10, 90). Besides being expressed in kidney and bone (91), PTHrP can be overexpressed by many tumor types and acts as an endocrine effector capable to induce thermogenic gene expression in adipocytes. Moreover, its presence in the circulation correlates with a greater degree of wasting in individuals with metastatic cancer (10). PTHrP induced thermogenesis and hypermetabolism of the AT in a murine model of LLC. The evidence was given by the fact that treatment with PTHrP antibodies strongly prevented the tumor-induced AT browning in comparison to the control group (10). In addition, treatment with PTHrP antibodies also impaired LLC tumor-induced muscle wasting, atrophy of muscle fibers, and atrophy-related gene expression in treated mice (10). In a later study, the same research group corroborated the previous results demonstrating a key role for the PTHrP receptor (PTHR) in triggering browning and thermogenesis following the binding to its ligand (90). Mice knockout for PTHR in their fat tissue were resistant to cachexia driven by the LLC tumor.

Adipokines

Zinc-α2-glycoprotein (ZAG) is an adipokine functioning as a lipid mobilizing factor (LMF). ZAG is usually expressed by differentiated adipocytes (92), albeit it was reported that other tissues express it as well (93), including several cancer cells (94). ZAG expression has been reported to be markedly elevated in AT of mice transplanted with cachexia-inducing tumor (93), whereby this phenomenon has been positively correlated with increased lipolysis and subsequent fat loss (95, 96). As a counterevidence, knockout of ZAG caused significant increases in bodyweight of mice fed with standard or lipid-rich diet when compared with wild-type controls. Similarly, the same group of ZAG-deficient mice showed significant decrease in adipocyte lipolysis in response to treatment with agents that increase cAMP (97). One study carried out on cachectic patients with gastrointestinal cancer showed that ZAG is primarily produced and secreted by WAT, and correlates with nutritional status in both malignant and nonmalignant conditions. However, it seems that neither WAT nor tumor cells secretory activity lead to significant increases of circulating levels of this adipokine. Thus, ZAG should be considered as a lipolysis-promoting factor locally produced (94).

PUTTING PIECES TOGETHER: PATHWAYS UNDERLYING CC

Dysregulated Pathways in SM

From a systematic standpoint, all the aberrant metabolic features characterizing CC are caused by activation of few distinct signaling pathways, triggered by the interaction between the aforementioned mediators and their cognate receptors.

Under normal conditions, myofibrils, the main structural components of myocytes, physiologically undergo to a balanced protein turnover. Under cachectic conditions, instead, excessive rates of myofibril breakdown and low rates of protein synthesis are typically observed in patients' SM, resulting in muscle weakness, fatigue, reduced tolerance to chemotherapy and low quality of life (QoL) (1, 3). Here, the ubiquitin-proteasome pathway, which takes part in breakdown of short-lived and regulatory proteins, is known to play a relevant role (98). As its name suggests, this proteolytic pathway is essentially dependent on the presence of active enzymes involved in protein ubiquitylation, which include the well-known family of E3 ubiquitin ligases. Among these, muscle RING finger-containing protein 1 (MURF1, also known as TRIM63) and muscle atrophy F box protein (MAFbx, also known as Atrogin-1) are defined as muscle-specific E3 ubiquitin ligases and are the most widely studied in the case of cachexia-associated muscle loss. Both these enzymes mediate myofibrillar protein breakdown by acting on several components of the sarcomeric thick filament, including myosin heavy chain (MHC), and are thought to interfere with processes related to protein synthesis (99). The proinflammatory cytokines TNFα, TWEAK and IL-1 have been shown to cause increased expression of both these E3 ubiquitin ligases via the nuclear transcription factor kappa B (NF-kB) signaling and the p38/CCAAT/enhancer-binding protein β

(p38/C/EBPβ) pathway, respectively (35, 41, 100). Activation of the NF-kB signaling was reported to cause overexpression of paired box 7 (PAX7) in myocytes (101). The latter is a transcription factor playing an essential regulatory role in myogenesis, being responsible for repression of MyoD and myogenin transcriptional activity. In line with this, it has been demonstrated that NF-κB–dependent upregulation of PAX7 impairs the regenerative ability of myogenic cells and drives toward muscle wasting under tumor conditions (101).

A different signaling pathway is instead activated by IL-6. In particular, this interleukin signals via its membrane receptor IL-6R, which in turn forms a heterodimer with its transducing subunit (gp130). This leads to activation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, with subsequent translocation of activated STAT3 into the nucleus. This event contributes to the regulation of expression of the E3 ubiquitin ligases and autophagy genes (102). STAT3 also induces expression of C/EBPδ, a transcription factor that promotes expression of Mstn under cachectic conditions (102). Indeed, a recent work revealed that, at the same extent of IL-6, TNFα, and INFγ synergistically activate STAT3 by promoting its JAK-mediated phosphorylation independently from IL-6 (103). This demonstrates that NF-kB and STAT3 both respond to the same upstream signaling, and thus cooperate amplifying the signal and promoting expression of pro-cachectic genes.

Although the precise mechanism have remained elusive, it is known that proinflammatory cytokines also suppress activity of the RAC serine/threonine-protein kinase (AKT), which is downstream of insulin signaling and central to many cellular processes. AKT negatively modulates the transcriptional activity of forkhead box protein O1 (FoxO1) and FoxO3, which control the expression of genes involved in metabolic homeostasis, including that of MURF1 and MAFbx (104, 105). In particular, under physiological conditions, AKT phosphorylates the FoxOs proteins, preventing their nuclear translocation. Moreover, AKT inhibits activity of tuberous sclerosis complex 2 (TSC2), a main inhibitor of the mammalian target of rapamycin complex 1 (mTORC1), which is notoriously involved in functions like protein synthesis, pentose anabolism and blockage of autophagy (106). To the contrary, cytokine-mediated suppression of AKT causes downregulation of mTORC1 activity and favors FoxOs dephosphorylation and nuclear location, thus promoting miofibrillar degradation (107, 108). Interestingly, it was recently reported that FoxOs also induce expression of a third muscle-specific E3 ubiquitin ligase, termed specific of muscle atrophy and regulated by transcription (SMART) (108).

One further negative regulator of FoxO3 is the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), a master regulator of mitochondrial biogenesis and transcriptional coactivator regulating expression of genes involved in energy metabolism (109). PGC1α was demonstrated to be downregulated in muscles of cachectic tumor-bearing mice, while its transgenic expression allowed muscle recovery *in vivo* (109).

Aberrant activation of signaling pathways downstream of TLRs is also known to contribute to cancer progression and overproduction of proinflammatory cytokines underlying cachexia (110). Some studies demonstrated that the notorious TLR/myeloid differentiation factor 88 (TLR/MyD88) signaling (70) mediates skeletal muscle wasting during CC (71, 111). Previous studies suggested that the mechanism involved in such phenomenon is the TLR/MyD88-mediated activation of NF-kB signaling (112–114). Indeed, *in vivo* experiments carried in a recent work partially confirmed such hypothesis by demonstrating that ablation of MyD88 in LLC-bearing mice inhibited tumor-induced activation of NF-kB in SMs (71). However, the authors showed that most of muscle mass loss in this murine model was due to activation of the TLR4/MyD88/inositol-requiring protein 1α (IRE1α)/X-box-binding protein 1 (XBP1) axis. Both IRE1α and XBP1 are linked to the unfolded protein response signaling, which is activated following accumulation of misfolded proteins or dysregulations in calcium levels within the SM. In particular, the spliced form of the transcription factor XBP1 was demonstrated to be a potent promotor of expression of proinflammatory cytokines and autophagy-related genes, while its ablation resulted in significant attenuation of loss of lean mass both *in vitro* and *in vivo*. Importantly, the authors showed that this mechanism is likely to involve not only TLR4, but also some other TLRs, including TLR7 (71).

Through their binding to ActRII receptors, TGFβ-family members like Mstn and ActA promote protein degradation via p38/JAK-mediated phosphorylations and activation of small mother against decapentaplegic (SMAD) signaling (115). In particular, it is known that, in the case of myoblasts and myocytes, Mstn specifically interacts with the ActRIIB receptor, subsequently inducing the assembly of type-I receptor ALK4. This in turn leads to activation of the SMAD2/3/4 complex (115, 116). Once assembled, the SMAD complex is translocated to the nucleus and acts as a transcription factor favoring expression of genes related to protein degradation and inhibiting that of genes involved in protein synthesis and proliferation (115).

As previously stated, other important pathways are involved in cachexia-associated muscle loss. Among these, pathways of autophagy certainly represent other important and widely activated mechanisms that underlie protein breakdown in CC. However, such pathways are far less characterized than those related to ubiquitin-proteasome pathway. Some studies showed that markers of autophagy like Beclin-1, (an indicator of autophagy induction), p62 (a marker of lysosomal degradation) and the two forms of microtubule-associated proteins 1A/1B light chain 3B protein (LC3B-I and LC3B-II, used to measure autophagosome abundance) are overexpressed in SM of tumor-bearing animals and cancer patients (117–120). However, in spite of the increased autophagic production and activity, the findings gained so far showed impairment of autophagosome clearance in the muscle of cancer hosts, suggesting that the process of lysosomal degradation does not come to complete cargo degradation (117, 119, 120). A further interesting finding was the phosphorylation and subsequent activation of Unc-51 like autophagy activating kinase 1 (ULK1), a driver of autophagy, in SMs of cachectic mice (121). Such an event was suggested to be mediated by upstream activation of p38, which was previously
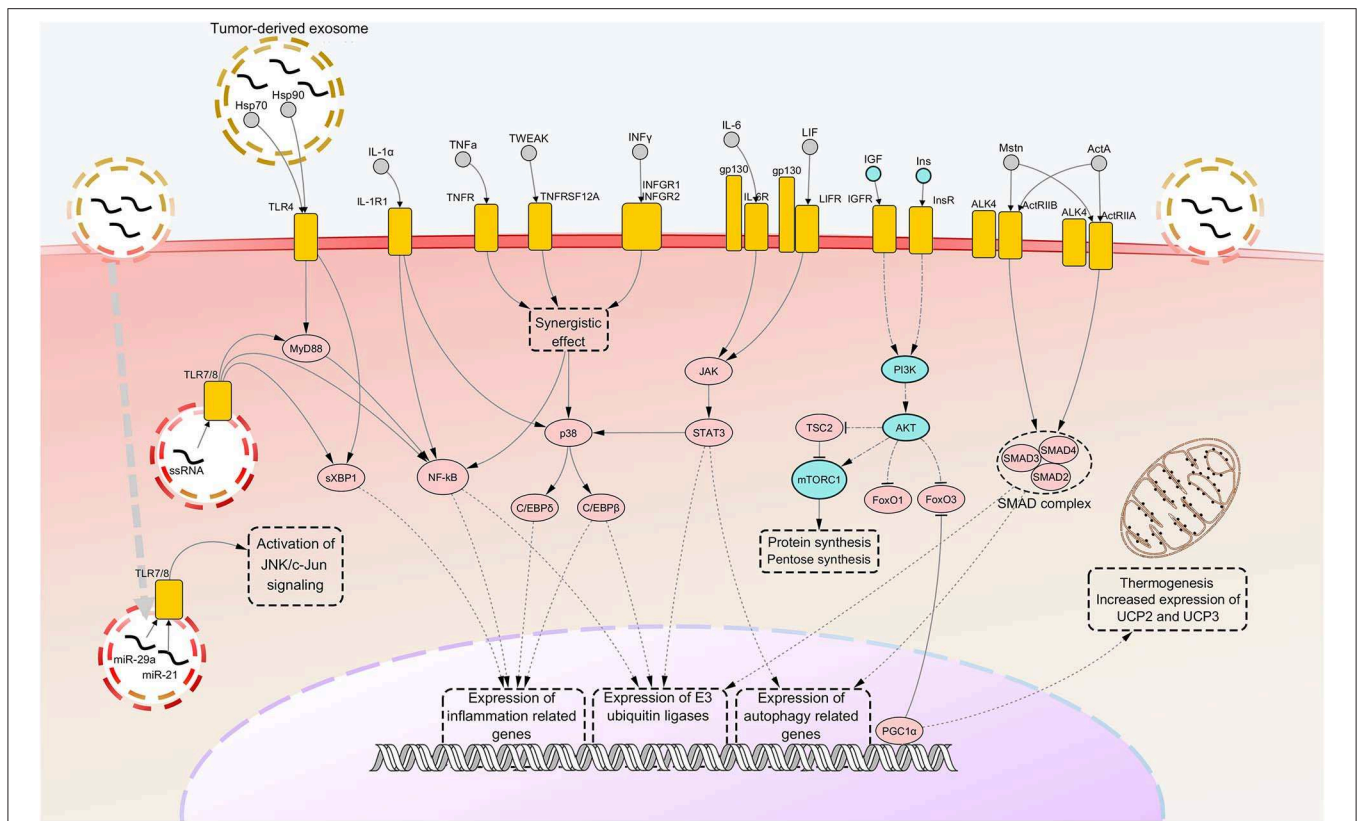
FIGURE 1 | Notorious molecular mechanisms underlying skeletal muscle wasting during cancer cachexia. Atrophy of skeletal muscle in cancer cachexia is due to aberrant activation of specific signaling pathways, consequent to the binding of factors secreted by the tumor, the stroma or the immune system to their cognate receptors. Most of such signaling pathways converge toward activation of selective transcription factors, causing their nuclear translocation and binding to promoters of cachexigenic genes. These include genes encoding cytokines [e.g., tumor necrosis factor alpha (TNFα) and interleukin 6 (IL-6)], inflammation-related receptors [e.g., toll-like receptor 4 (TLR4) and TLR7], myokines [e.g., myostatin (Mstn)], muscle-specific E3 ubiquitin ligases and autophagy-related proteins. Such events potentiate inflammatory processes at the local level and cause the breakdown of myofibrillar proteins, impairing the contractile function of skeletal muscles. Several cachexia-inducing factors are known to exert their cachectigenic effect by acting synergistically, as in the case of TNFα and interferon gamma (INFγ). To the contrary, downregulation occurring in the insulin (Ins) and insulin growth-like factor (IGF) signaling determines a decrease in mTOR-dependent protein synthesis, due to upstream downregulation of RAC serine/threonine-protein kinase (AKT) activity. In normal conditions, AKT functions also as a negative regulator of forkhead box protein O1 (FoxO1) and FoxO3 transcription factors, preventing their nuclear translocation. Thus, consequent to AKT downregulation under cachectic conditions, both these FoxOs localize into the myonucleus and induce transcription of autophagy components and muscle-specific E3 ubiquitin ligases. Peroxisome-proliferator-activated receptor-gamma co-activator 1-alpha (PGC1α), overexpressed during cancer cachexia, is known to inhibit FoxO3 binding to the DNA and cause enhance expression of genes involved in energy metabolism and mitochondrial biogenesis. Upregulated nodes are colored in pink and connected with other nodes through continuous edges. Downregulated nodes are colored in azure and connected with other nodes through dash-dot edges. Dashed edges represent connections between transcriptional/co-transcriptional factors and gene expression. ActA, activin A; ActIIR, activin type 2 receptor; ALK4, activin receptor type-1B; C/EBP, CCAAT/enhancer binding protein; c-Jun, proto-oncogene c-Jun; Gp130, glycoprotein 130; Hsp, heat shock protein; IL-1a, interleukin 1 alpha; IL-1R1, interleukin 1 receptor 1; IL-6R, IL-6 receptor; INFGR, INFγ receptor; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; LIF, leukemia inhibitory factor; LIFR, LIF receptor; miR, microRNA; mTORC1, mammalian target of rapamycin complex 1; MyD88, myeloid differentiation factor 88; NF-kB, nuclear transcription factor kappa B; p38, p38 mitogen-activated protein kinase; PI3K, Phosphoinositide 3-kinase; SMAD, small mother against decapentaplegic; STAT3, signal transducer and activator of transcription 3; sXBP1, spliced isoform X-box-binding protein 1; TNFR, tumor necrosis factor receptor; TNFRSF12A, TNF Receptor Superfamily Member 12A; TSC2, tuberous sclerosis complex 2; TWEAK, TNF-related weak inducer of apoptosis; UCP, uncoupling protein.

reported to cause activation of C/EBPβ. The molecular pathways involved in SM atrophy are depicted in **Figure 1**.

Dysregulated Pathways in AT

To date, knowledge about pathways involved in loss of AT under cachectic conditions is markedly less detailed than that available for loss of SM. However, it is now widely recognized that excessive degradation of fatty acids and energetic imbalance in AT can contribute to the destructive impact of cachexia on tumor hosts.

Under physiological conditions, AT lipolysis is an essential catabolic process that provides lipids and energy to tissues and organs following opportune stimulations. In particular, this process occurs after interaction between certain molecules (including β-adrenergic neurotransmitters) and their cognate G-protein-coupled receptors (GPCRs) expressed in adipocytes. Downstream of this event, a number of adenosine

triphosphate (ATP) molecules are converted to cyclic adenosine monophosphate (cAMP) by GPCRs-stimulated adenylyl cyclases. Increased levels of cytosolic cAMP eventually lead to activation of protein kinase A (PKA) by phosphorylation, which in turn leads to phosphorylation of both HSL and perilipin-1, a protein acting as a protective coat for lipid droplets. This implies activation of HSL and its translocation to the surface of lipid droplets, resulting in greater access to triglycerides and enhanced lipolysis (12, 13). ATGL represents another key point of the lipolytic process, since its enzyme kinetics determines the rate limiting of triglycerides catabolism. Specifically, ATGL is mainly responsible for the first step of triglycerides hydrolyzation, which leads to the release of a single fatty acid and diacylglycerol (DAG). The other two steps of lipolysis are then completed by HSL and monoacylglycerol lipase (MGL), respectively (12).

Under conditions of chronic systemic inflammation, several events are known to occur affecting the AT metabolism, including suppression of appetite, enhancement of lipolysis and inhibition of LPL (122). The latter, in particular, has a dual function, being involved in receptor-mediated lipoprotein uptake and degradation, and hydrolysis of serum triglycerides in non-esterified fatty acids and 2-monoacylglycerol for tissue utilization. LPL deficiency leads to hypertriglyceridemia, while its upregulation causes insulin resistance and can promote obesity (123). Nonetheless, results from some studies clearly indicate that depletion of triglycerides in cachectic individuals is not due to impairments in LPL activity nor in lipogenesis, but depends on an increase in lipolysis, confirmed by high expression rates of HSL (124, 125).

Among procachectic cytokines, TNFα was shown to cause increased levels of phosphorylated HSL in adipocytes through activation of ERK, downstream of the MAPK signaling. Importantly, ERK-mediated activation occurred by phosphorylation on Ser 600 of HSL, which corresponds to the phosphosite targeted by cAMP-activated PKA (126). Instead, TNFα was not reported to induce any change in expression levels of HSL. Similarly, to TNFα, ZAG was demonstrated to cause hyperactivation of HSL by leading to increases in cAMP levels and subsequent activation of adenylyl cyclase in a dose dependent manner (93, 127). Moreover, differently from TNFα, ZAG was reported to induce upregulation of expression of Gαs (a G-protein subunit involved in activation of adenylate cyclase pathway) and HSL, and downregulation of expression of Gαi (a G-protein subunit involved in inhibition of adenylate cyclase pathway) (128). Interestingly, some experimental data suggested that ZAG expression in adipocytes might be negatively regulated by TNFα (92).

Further evidence about involvement of procachectic cytokines in fat loss during cachexia was found for IL-6. High levels of this cytokine in the plasma of cachectic mice correlated with enhanced AT lipolysis and increased levels of circulating free fatty acids. These changes were shown to be associated with activation of IL-6 signaling in WAT, which caused downstream activation of STAT3 and p38 (11, 129). All the above mentioned pathways are represented in **Figure 2**.

Enhanced Thermogenesis During CC

As outlined above, most of cachectic cancer patients experience involuntary excessive energy expenditure. A considerable fraction of this phenomenon has been attributed to aberrant activation of thermogenesis, which, in general, involves the interplay of two main actors, i.e., SM and the brown adipose tissue (BAT) (130). In both cases, this event seems to depend on expression of high amounts of uncoupling proteins (UCPs). UCPs are located on the inner membrane of mitochondria and redirect them toward heat generation instead of ATP synthesis (130). In particular, UCP1 seems to be prevalent in BAT, while UCP2 and UCP3 would be primarily expressed in SM (131, 132).

Browning of white adipocytes is the process by which adipocytes of the WAT are transformed into beige adipocytes, consequently to endocrine, paracrine, or autocrine stimulation. This phenotypic transition seems to occur during the initial stages of CC, preceding SM atrophy (133). Several reports have argued that WAT browning mainly relies on increased expression of UCP1 in mitochondria of white adipocytes [e.g., (134)]. Nonetheless, some recent studies have remarked the non-consistency of these results for certain models of experimental cachexia and questioned the real importance of UCP1 in such a context. A possible key role was suggested for the transcriptional coactivator cell death inducing DFFA-like effector A (CIDEA), which is highly expressed in WAT and has a role in lipid metabolism (2). However, this topic has remained controversial.

Both PTHrP and IL-6 were reported to drive the expression of thermogenic genes *in vivo*, including UCP1, CIDEA, and PGC1α (10, 90). Consistent with this, neutralization of PTHrP or IL-6 in cachectic mice prevented WAT browning, rescued the cachectic phenotype and blocked the increased expression of UCP1 in subcutaneous AT of C26-bearing mice (10, 11). Moreover, silencing of IL-6 reversed the enhanced mitochondrial respiration in WAT of tumor-bearing mice toward the normal condition (11). However, it remained to understand whether these two factors induced thermogenesis by direct or indirect action on WAT/BAT.

Concerning PTHrP, the evidence that knockout of PTHR in AT blocked adipose browning and wasting provided a strong validation of direct action of PTHrP on both WAT and BAT (90). Surprisingly, the same study demonstrated that knockdown of PTHR in AT also prevented lean mass depletion and improved muscle strength. This raised the question whether PTHrP exerts a direct action even toward SM or not. Indeed, besides the well-known cross-talk between AT and SM (2, 135), a direct effect of PTHrP on myocytes could be also speculated. In fact, a previous report demonstrated that PTH1R expression is required for myocyte differentiation and is highly expressed by CD34- or PAX7-positive myosatellite cells in both mice and humans (136). In the same study, it was shown that myotubes also express PTH1R, albeit to a much lesser extent than differentiating myoblasts (136).

Regarding the role of IL-6 in WAT browning, elegant experiments carried on an *in vitro* model of pre-brown adipocytes demonstrated a direct, though modest, effect of IL-6 on UCP1 expression (11). Nonetheless, mechanistic
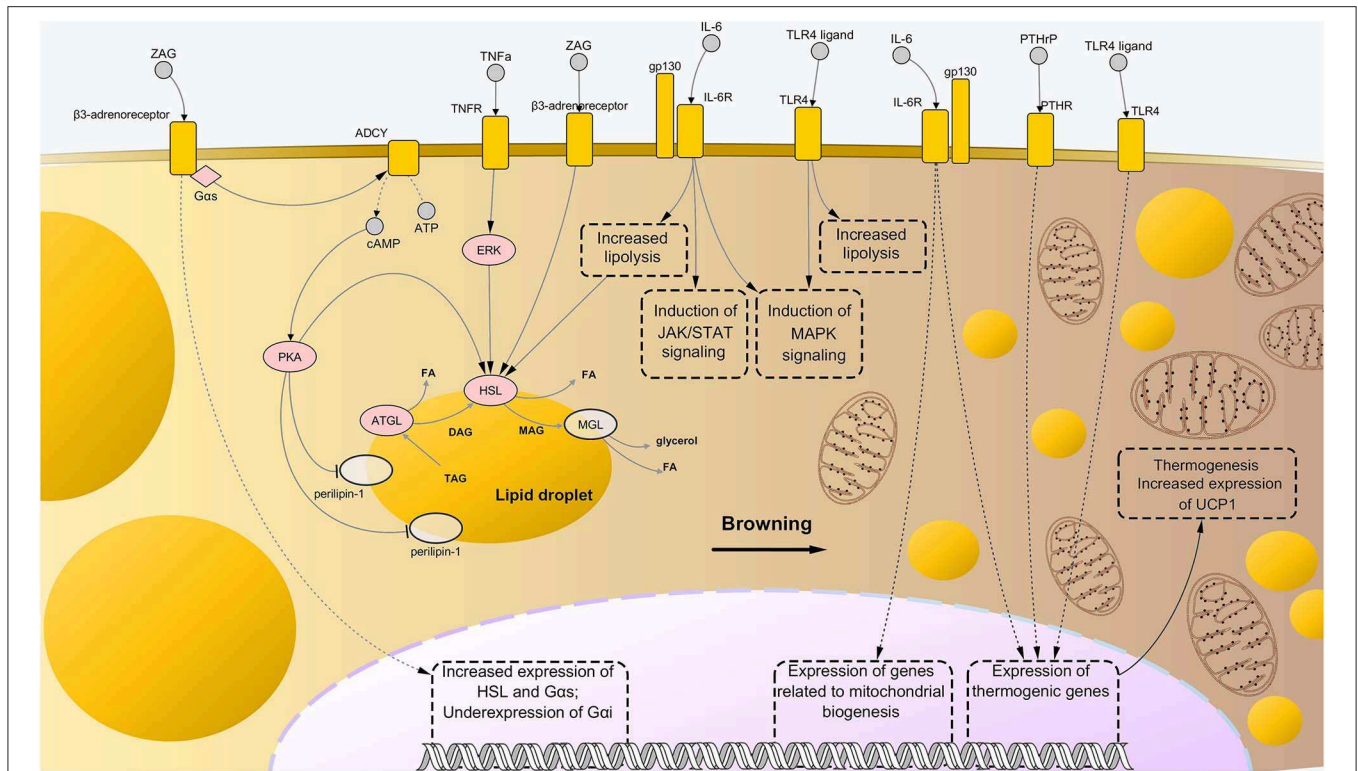
FIGURE 2 | Molecular mechanisms driving adipose tissue loss and remodeling during cancer cachexia. Similarly to the case of skeletal muscle wasting, the combination of abnormally activated pathways including β-adrenergic signaling, cytokine- and toll-like receptor (TLR)-mediated inflammation, and parathyroid-related peptide (PTHrP) stimulation, leads to enhanced lipolysis and thermogenesis of the AT in cancer cachexia. In particular, binding of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) to their respective membrane receptors induce high phosphorylation levels of enzymes involved in catabolism of triglycerides, i.e., adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), implying higher rates of their enzyme activity. Interaction between zinc-α2-glycoprotein (ZAG) and beta-3 adrenergic receptor is known to induce even stronger lipolytic effects than those mediated by IL-6 and TNFα, partly due to induction of expression of lipolytic genes (including G proteins of the type Gαs) and suppression of expression of Gαs, an inhibitor of β-adrenergic signaling. Moreover, ZAG promotes phosphorylation of perilipin-1, which results in wider exposition of lipid droplets to the catabolic action of ATGL, HSL, and monoglyceride lipase (MGL). A role in enhanced lipolysis during cancer cachexia has been demonstrated also for TLR4, although precise mechanisms remained largely elusive. Meanwhile, such pattern recognition receptor seems to exert an important role during cachexia-driven white adipose tissue browning, along with IL-6 and PTHrP, promoting expression of thermogenic genes, such as uncoupling protein 1 (UCP1), and favoring mitochondrial biogenesis. In the present illustration, the process of browning proceeds from the left to the right side of the figure, with the left side presenting bigger lipid droplets and almost mitochondria, while the right side presenting a large number of small, sparse lipid droplets and mitochondria. Upregulated nodes are colored in pink and connected with other nodes through continuous edges. Non-deregulated nodes are represented as semi-transparent nodes. Dashed edges represent connections between transcriptional/co-transcriptional factors and gene expression. ADCY, adenylate cyclases; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; DAG, diglyceride; FA, fatty acid; gp130, glycoprotein-130 IL-6R, IL-6 receptor; JAK, Janus kinase; MAG, monoglyceride; MAPK, mitogen-activated protein kinase; PTHR, parathyroid receptor; STAT, signal transducer and activator of transcription; TAG, triglyceride; TNFR, tumor necrosis factor receptor.

insights regarding this aspect remained elusive. Indeed, IL-6 was suggested to mediate thermogenic effects on BAT through activation of p38, which is known to induce increased mitochondrial biogenesis and expression of thermogenic genes (137, 138). Another possible mechanism would involve the IL-6-mediated activation of AMP-activated protein kinase (AMPK), which in turn would cause an increase in hydrolase activity of ATGL and inactivation of mTORC1 (129). However, the dominant idea is that IL-6-mediated enhancement of thermogenesis and lipolysis is the outcome of both direct and indirect actions of this cytokine on BAT and WAT (139–142).

A role in increased lipolysis and WAT browning was recently demonstrated for TLR4 as well (74). Precisely, LLC-bearing mice showed enhanced lipogenic activity and AT remodeling,

while cachectic mice knockout for TLR4 showed reduced thermogenic activity BAT, underexpression of UCP1, and lower phosphorylation levels of p38 compared to the former group (74). This may suggest a potential connection between TLR4, MAPK signaling (70) and transcription of UCP1. Pathways related to WAT browning are represented in **Figure 2**.

Discrepancies: From the Laboratory to the Clinical Practice

In spite of the considerable knowledge we have lastly gained regarding CC, the clinical practice still lacks standard diagnostic criteria and treatment guidelines. One of the main reason for such difficulties certainly reside in the multifactorial nature of this syndrome, and thus in its intrinsic complexity. On the other

hand, however, the scarceness of experimental models of CC capable to accurately recapitulate the features of human CC in various tumor types has represented a major limitation in relation to this issue.

Most preclinical studies conducted so far have been mainly based on exploitation of few ascertained animal models of cachexia-inducing tumors, including LLC, C26 and Yoshida ascites hepatoma (YAH 130) (143). These were chosen and maintained as general models of CC owing to the broad documentation in their regard. However, a lot of skepticism have been lastly raised about their actual potential to be employed as reliable surrogates of human CC. In particular, it is now becoming predominant the opinion that the failure of most anti-cachexia therapeutics to reach primary endpoints during clinical trials (see the next section) is at least in part attributable to the limitations imposed by the use of inappropriate experimental models (143). In line with this reasoning, a number of recent studies have demonstrated the relative inconsistency of such models, since their molecular characterization at the tissue level revealed quite different patterns of gene expression between animal models and human patients [e.g., (144, 145)].

In this context, genetically engineered mouse (GEM) models of CC are being evaluated as a relevant solution to overcome such restrictions. GEM models currently tested for preclinical studies avail of the tamoxifen-inducible conditional genetic mutation system Cre-ER (146). Adenovirus-containing Cre-ER encodes for a fusion protein in which a mutated hormone-binding domain of the estrogen receptor (ER^T or ER^{T2}) is fused to Cre, a bacterial site-specific recombinases that catalyzes recombination between well-defined "*loxP*" sites flanking the gene of interest (146–148). As this system relies upon the presence of tamoxifen-inducible promoters, it allows the spatio-temporal control of Cre expression, with the subsequent deletion of genes flanked by *loxP* sites. In contrast with tumors developed by traditional CC models, tumors arising in GEM models closely mimic the histopathological and molecular features of their human counterparts and spontaneously progress toward metastases and cachexia.

A valuable GEM model of CC is that engineered by Talbert et al. (144), which was proved to closely resemble the typical features reported for cachexia induced by pancreatic ductal adenocarcinoma (PDA) in human patients. The mouse model, named KPP (Kras$^{+/G12D}$, Ptf1a$^{+/ER−Cre}$, Pten$^{f/f}$), exhibited progressive wasting of SM, heart and AT mass, similar to that experienced by humans, and gene ontology of its SM-derived mRNAs resembled that of PDA patients (144). By contrast, this model displayed loss of normal pancreatic parenchyma, which could significantly contribute to weight loss in KPP mice. Furthermore, it is likely that such a model is selective for PDA, and thus not suitable for studying cachexia associated to other tumor types (144). In the same study, the GEM model KPC (Kras$^{+/LSL−G12D}$, Trp53$^{+/R270H}$, Pdx1$^{+/Cre}$) was reevaluated. Indeed, KPC is a GEM model of PDA (149) that has been recently considered a reliable model of cachexia by a number of studies [e.g., (11, 150, 151)]. Nonetheless, Talbert et al. demonstrated that changes in size and tissue mass of KPC mice did not correlate with PDA progression, and that patterns of gene expression in

their SM did not resemble that of PDA patients (144). Thus, the KPP genotype appears to be more robust for research on CC compared with the KPC model. Further examples of potentially useful GEM for research on CC are the KL model (KRAS$^{G12D/+}$; LKB1$^{f/f}$), recently used to study the metabolic profile of advanced non-small cell lung cancer (NSCLC) (152), and the lately developed KPC:APC model (APC$^{f/f}$; KRAS$^{+/f}$; CDX2-Cre-ER^{T2}), which aims to recapitulate features of human colorectal carcinoma (CRC) with mutated KRAS and leads to development of CC (153).

Orthotopic models of patient-derived cancer cells (PDCC) may represent another valuable option for research on CC. Here, tumor cells derived from human patients are seeded into the corresponding tissue of immunocompromised animal models. This has the obvious advantage of allowing the study of organ-specific tumor microenvironment with increased accuracy and higher genetic heterogeneity and stability compared to the traditional xenograft models, similarly to GEM models. In particular, this was demonstrated for both orthotopic and subcutaneous engraftment of patient-derived pancreatic tumor (154, 155). However, some data suggested that orthotopic engraftment of pancreatic tumor cells within mice's pancreas could induce systemic inflammation distinct from that observed in subcutaneous engraftments, leading toward more severe SM wasting [e.g., (154)].

One limitation of orthotopic models of CC is that here engrafted PDCC maintains their metastatic potential (154, 156), thus preventing researchers to study stages related to development and progression of cachexia. Moreover, lean mass loss in these models may be the result of post-surgical inflammation instead of tumor-induced tissue loss. Yet, the exploitation of immunocompromised mice for generating orthotopic models may fail in reproducing the immune profile of CC (144). The latter limitation, however, can be overcome by employing syngeneic mouse models of GEM-derived tumors, i.e., models in which immune-competent mice are implanted with tumor cells derived from GEM presenting the same genetic background [e.g., (157)].

CURRENT UNIMODAL TREATMENT OPTIONS FOR CC

There is a wide range of treatment options currently available for CC. Nutritional support is very important in cancer patients, as their food intake is often reduced due to symptoms like anorexia caused by systemic inflammation, nausea, vomiting, and mucositis (158). For these reasons, as soon as patients are diagnosed with cancer, they should be nutritionally monitored and receive both nutritional and metabolic support (159). Meanwhile, since systemic inflammation is a hallmark of CC, the pharmacological targeting of individual proinflammatory cytokines or that of their cognate receptors has been considered as a potential powerful approach. Also, several hormones and hormone-derived compounds known to stimulate appetite and to induce weight gain have been tested. Nonetheless, most of the current trialed agents has not received the FDA (Food and Drug

Administration) approval since either no substantial benefits or manifestation of important side effects were observed throughout Phase I/III of clinical trials (143, 160) (**Table 1**).

TNFα, IL-6, and IL-1 Inhibitors

Because of their widely recognized role in induction of cachectigenic effects, TNFα, IL-6, and IL-1α had represented the ideal target of several anti-cachectic compounds. However, in the case of anti-TNFα therapy, neither the TNFα receptor–blocker Etanercept (161) nor the chimeric IgG1 kappa monoclonal antibody Infliximab (167) were able to prevent muscle atrophy or improve appetite in two randomized controlled trials of terminal cachectic patients. Moreover, Infliximab was found to increase fatigue and treatment-related mortality in administered patients (167). Likewise, slight recovery of muscle mass accompanied by worsening of life or no significant beneficial effects were reported by five randomized clinical trials testing two pharmacological agents capable to downregulate expression of TNFα, namely Thalidomide (162–164) and Pentoxifylline (165, 166), respectively.

Anti-IL-6 and anti-IL-1α pharmacological agents showed promising results over the course of clinical trials; nonetheless, they were inconclusive in terms of clinical management of the disease. Specifically, in a Phase I clinical trial involving patients with advanced cancer, the humanized anti-IL-6 monoclonal antibody Clazakizumab was well tolerated, increased hemoglobin and albumin levels and reversed fatigue in treated patients (168). During the subsequent Phase II randomized controlled trial, administration of Clazakizumab to patients with NSCLC resulted in a slight lower degree of lean mass depletion (169). However, effects on SM mass were not considered satisfactory according to the acceptance criteria and further studies were required (188). Similarly, in Phase I and III clinical trials, the IL-1α-specific humanized monoclonal antibody Xilonix was found to prevent alteration of body composition and improve control of thrombocytosis in refractory cancer patients (171) and advanced CRC patients with cachexia (172), respectively. The most common adverse events observed at the end of these studies were proteinuria (171), anemia and increased concentration of both alkaline phosphatase and aspartate aminotransferase (172). A second phase III clinical trial attempting to further demonstrate the efficacy of Xilonix in advanced CRC patients (NCT01767857) was stopped at its early stage as the study crossed the prospective futility boundary of primary endpoint.

Indirect blockage of IL-6 action by pharmacological inhibition of the JAK/STAT3 signaling may represent another valid strategy to be persued. In preclinical studies, the allosteric inhibitor of MAPK/ERK kinase 1 (MEK1) and 2 (MEK2) Selumetinib was proved to exert tumor suppressive activity and prevent cancer-induced IL-6 production [e.g., (189)]. Yet, results from a Phase II trial conducted in patients with metastatic biliary cancers suggested that Selumetinib induced non-fluid weight gain in administrated patients (170). Thus, this drug was tested in a Phase II trial in patients with advanced cholangiocarcinoma, aiming to study its potential as modulator of IL-6/JAK/STAT3 signaling and mediator of SM anabolism (22). Despite results showed that Selumetinib effectively promoted SM gain in patients

with cholangiocarcinoma, its actual relevance for CC remained to be demonstrated (22).

Similarly, the selective JAK1/2 inhibitor Ruxolitinib was trialed on patients with myelofibrosis and was found to reduce splenomegaly and disease-related symptoms, meanwhile inducing a significant increase in body weight (190). An open-label Phase II trial was then started in 2014 aiming to examine both safety and efficacy of Ruxolitinib as well as its influence on overall survival. However, enrollment of participants for this study did not succeed and the trial was unsuccessfully terminated at the beginning of 2019 due to poor recruiting (NCT02072057).

Non-steroidal Anti-inflammatory Drugs

Non-steroidal anti-inflammatory drugs (NSAID) have been considered to counteract chronic inflammation in CC as well. In particular, Celecoxib, a cyclooxygenase-2 (COX-2) inhibitor, significantly improved QoL and BMI in cachectic patients vs. control (173). Another study reported significant increases of lean mass and improvements in handgrip strength and QoL in patients with advanced cancers administrated with Celecoxib (174). In addition, a significant decrease in TNFα levels was observed, and no particular toxic effects were reported (174). More recently, several trials have evaluated the effects of a combination of Celecoxib with other drugs on cachectic patients, including combination with L-carnitine [a nutritional supplement that improves the cachectic condition (191)] and combination of these two compounds plus Megestrol acetate (see below). These studies led to more effective improvements in lean mass, total physical activity and decreased sense of fatigue, without toxic effects (192).

Myostatin Inhibitors

In recent times, the treatment of cachexia has been focused on myokines, which include cytokines and other proteins produced and secreted by SM cells (193, 194). Among these, Mstn can act as autocrine, paracrine or endocrine effector along with cytokines like IL-6, IL-8, and IL-15, and is responsible for immune and metabolic changes associated to exercise or stress (193–195). Thus, Mstn inhibitors have been considered promising tools for the treatment of cachexia. In 2012, two Phase I clinical trials under the same study evaluated the safety and tolerability of the humanized monoclonal antibody to myostatin LY2495655 in healthy subjects and patients with advanced cancer (175). According to the results obtained, the efficacy of LY2495655 was well demonstrated in the case of healthy volunteers, in which it caused an increase in thigh muscle volume. Increases in muscle volume were observed also in patients with advanced cancer, though only when the drug was administrated at relatively low doses (21 and 70-mg) without unusual safety concerns (175). In 2018, a Phase II trial was performed on a group of inoperable patients or with metastatic pancreatic cancer, who suffered from cachexia. Here, patients were treated with different doses of LY2495655, and the authors reported that pre-cachectic patients were more responsive to the treatment than cachectic patients (176). These results may suggest that treatment with LY2495655 should be better considered for prevention muscle loss rather

TABLE 1 | Table summarizing unimodal treatment options mentioned in this review.

Drug name	Drug class	Molecule type	Disease	Status	Positive outcomes	Adverse effects/ Difficulties	References
Etanercept	Anti-TNFα therapy	TNFα receptor-blocker	Cancer (other than brain cancer)	Phase III RCT (placebo)	–	Induced negligible weight gain; did not improve median survival; caused higher rates of neurotoxicity.	NCT00046904 (161)
Thalidomide	Anti-TNFα therapy	Synthetic derivative of glutamic acid	Pancreatic cancer	RCT (placebo)	Attenuated weight and lean body mass losses	No significant improvements of QoL	(162)
Thalidomide	Anti-TNFα therapy	Synthetic derivative of glutamic acid	Cancer	Phase III RCT	Significant decrease in levels of circulating IL-6; did not cause worsening of life	Lack of satisfactory documentation	(163)
Thalidomide	Anti-TNFα therapy	Synthetic derivative of glutamic acid	Cancer	RCT (placebo)	Slight decrease in levels of circulating IL-6 and TNFα	No significant benefits compared with placebo; possible risk of treatment-related mortality	(164)
Pentoxifylline	Anti-TNFα therapy	Methylxanthine derivative	Cancer (other than brain cancer)	RCT (placebo)	–	No differences in QoL and possible worsening of life after 4 weeks of treatment	(165)
Pentoxifylline	Anti-TNFα therapy	Methylxanthine derivative	Cancer	RCT (placebo)	No toxicity	Did not improve appetite nor significant weight gain	(166)
Infliximab	Anti-TNFα therapy	Chimeric IgG1k monoclonal antibody	NSCLC	Phase III RCT (placebo)/ docetaxel	–	Did not induced weight gaining; caused increased fatigue and inferior global QoL	NCT00040885 (167)
Clazakizumab	Anti-IL6 therapy	Humanized anti-IL-6 monoclonal antibody	Cancer	Phase I CT	No apparent toxicity; increased hemoglobin and albumin levels; reduced fatigue	Lack of satisfactory documentation	(168)
Clazakizumab	Anti-IL6 therapy	Humanized anti-IL-6 monoclonal antibody	NSCLC	Phase II RCT	Generally well tolerated; improved the lung symptom score; attenuated lean mass loss; reversed fatigue	May cause rectal hemorrhage or treatment-related mortality in a minority of patients; inconclusive in terms of clinical management	NCT00866970 (169)
Selumetinib	Anti-IL6 therapy	MEK1/2 inhibitor	Biliary cancer	Phase II CT	Overall acceptable tolerability; induced significant weight gaining	Induced low-grade adverse events including rush and xerostomia; may worsen fatigue in a minority of patients	NCT00553332 (170)
Selumetinib	Anti-IL6 therapy	MEK1/2 inhibitor	Cholangiocarcinoma	Phase II CT	Induced significant gaining of lean body mass	Lack of satisfactory documentation	(22)
Ruxolitinib	Anti-IL6 therapy	JAK1/2 inhibitor	Cancer	Phase II CT	–	The study was terminated due to poor recruiting	NCT02072057
Xilonix	Anti-IL-1α therapy	IL-1α-specific humanized monoclonal antibody	Cancer	Phase I CT	Well tolerated by all participants, no dose-limiting toxicities were reported	Caused proteinuria, anemia, nausea and fatigue in a minor fraction of patients	NCT01021072 (171)
Xilonix	Anti-IL-1α therapy	IL-1α-specific humanized monoclonal antibody	CRC	Phase III RCT (placebo)	Prevented alteration of body composition and improved control of thrombocytosis	Caused proteinuria, anemia, increased concentration of alkaline phosphatase and aspartate aminotransferase and fatigue in a minor fraction of patients	NCT01767857 (172)
Xilonix	Anti-IL-1α therapy	IL-1α-specific humanized monoclonal antibody	CRC	Phase III CT	–	The study was stopped as it crossed the prospective futility boundary of primary endpoint	NCT01767857
Celecoxib	NSAID	Cyclooxygenase-2 (COX-2) inhibitor	Cancer	Phase II RCT (placebo)	Significantly improved BMI and QoL; moderate decrease of IL-6 levels after 3 weeks of treatment	–	(173)

(Continued)

TABLE 1 | Continued

Drug name	Drug class	Molecule type	Disease	Status	Positive outcomes	Adverse effects/ Difficulties	References
Celecoxib	NSAID	Cyclooxygenase-2 (COX-2) inhibitor	Cancer	Phase II CT	Significantly improved BMI and QoL; moderate decrease of TNFα levels; increased handgrip strength and improved performance status	–	(174)
LY2495655	Mstn inhibition	Monoclonal antibody to Mstn	(1) Cancer (2) Healthy	(1) Phase I RCT (placebo) (2) Phase I CT	Well tolerated; no dose-limiting toxicities were reported; increase in thigh muscle volume; consistent increases in handgrip strength observed at doses ≥21 mg; improvement in functional measures	No clear trend in dose-dependent efficacy	NCT01524224 (175)
LY2495655	Mstn inhibition	Monoclonal antibody to Mstn	Pancreatic cancer	Phase II RCT (placebo) + standard chemotherapy	–	No significant improvements in muscle volume; pre-cachectic patients were more responsive than cachectic patients; trend toward poorer overall survival in treated patients vs. placebo	NCT01505530 (176)
Bimagrumab	Mstn inhibition	Human monoclonal anti-ActRII antibody	NSCLC and Pancreatic adenocarcinoma	Phase II RCT (placebo)	Significant increase in lean body mass and thigh muscle volume	Significant decrease in total body weight	NCT01433263
AMG 745	Mstn inhibition	Fc fusion peptibody	Prostate cancer	Phase I RCT (placebo)	Generally well tolerated; increased lean body mass	Slight decrease in fat mass; may cause adverse events including diarrhea and fatigue	NCT00975104 (177)
Megestrol acetate (FDA approved)	Appetite stimulant	Progesteron derivative	Several cancer types	Summary of 35 CT	Improvement of appetite and increased caloric intake, weight gain and nutritional status; improvement of QoL; downregulation of proinflammatory cytokines or that of their cognate receptors	More than 40 side effects including edema, thromboembolitic episodes and treatment-related death	Summary of 35 clinical trials (178)
Medroxyprogesterone acetate (FDA approved)	Appetite stimulant	Progesteron derivative	Several cancer types	Summary of most relevant CT	Improved anorexia and QoL parameters; impaired synthesis and release of IL-6, IL-1, and TNFα	Weight gain was due to increased body fat mass rather than lean body mass	Summary of most relevant clinical trials (179)
Ghrelin	Orexigenic mediator	Hormone	Esophageal cancer	Phase II RCT (placebo) + cisplatin-based neoadjuvant chemotherapy	Increased food consumption and weight gain; reduced nausea and anorexia related to chemotherapy	–	(180)
Anamorelin HCl	Orexigenic mediator	Ghrelin Receptor agonist	NSCLC	Phase III	Generally well tolerated; improved appetite; increased food intake, body weight and lean body mass	Caused hyperglycemia, nausea and gastrointestinal disorders in a minority of patients; no significant improvement of handgrip strength	NCT01387269 (181)
Anamorelin HCl	Orexigenic mediator	Ghrelin Receptor agonist	NSCLC	Phase III	Generally well tolerated; improved appetite; increased food intake, body weight and lean body mass	Caused hyperglycemia, nausea and gastrointestinal disorders in a minority of patients; no significant improvement of handgrip strength	NCT01387282 (181)

(Continued)

TABLE 1 | Continued

Drug name	Drug class	Molecule type	Disease	Status	Positive outcomes	Adverse effects/ Difficulties	References
Anamorelin HCl	Orexigenic mediator	Ghrelin Receptor agonist	NSCLC	Phase III	Generally well tolerated; improved appetite; increased food intake, body weight and lean body mass	Caused hyperglycemia, nausea and gastrointestinal disorders in a minority of patients; no significant improvement of handgrip strength	NCT01395914 (182)
THC	Appetite stimulant and metabolism modulator	Endogenous agonist of CB1 and CB2 receptors	Cancer	Phase III CT	Well tolerated, no adverse effects	No significant improvements in appetite or QoL	(183)
THC	Appetite stimulant and metabolism modulator	Endogenous agonist of CB1 and CB2 receptors	Cancer	Phase III CT	Well tolerated, no adverse effects; significant increase in appetite and caloric intake; improved chemosensory perception and QoL	–	NCT00316563 (184)
THC	Appetite stimulant and metabolism modulator	Endogenous agonist of CB1 and CB2 receptors	Cancer	Pilot study	Well tolerated; significant increase in appetite and caloric intake; improved QoL; reversed fatigue	May induce dizziness or anxiety in a small fraction of patients	NCT02359123 (185)
Nabilone	Appetite stimulant and metabolism modulator	Synthetic analog of THC	NSCLC	Phase II RCT (placebo)	Increased appetite and caloric intake; improvement of QoL; attenuated pain and insomnia	–	NCT02802540 (186)
Erythropoietin	Anemia reversal	Hormone	Cancer	Randomized study	Reversed anemia; improved exercise ability and sense of well-being	No significant improvements in QoL; discrepancies between objective and subjective self-reported measures	(187)

CT, clinical trial; RCT, randomized clinical trial.

than for reverting the process, although deeper investigation and additional functional readouts are required.

In 2014, one study described the properties of the human anti-ActRII antibody Bimagrumab (BYM338) (196). BYM338 prevented the binding of ligands to the receptors and hence the activation of signaling pathway downstream of ActRII. *In vivo*, administration of bimagrumab resulted in significant SM hypertrophy and increased muscle fiber diameter. Furthermore, it protected muscles from glucocorticoid-induced atrophy and weakness through the impairment of muscle and tetanic force losses (196). Lastly, Novartis Pharmaceuticals completed a randomized control trial of BYM338 for treatment of CC associated with pancreatic adenocarcinoma and NSCLC. Patients administrated with Bimagrumab displayed significant increases in lean body mass and thigh muscle volume, yet also had decrement in total body weight (NCT01433263).

Another myostatin inhibitor is AMG 745, a peptibody resulted from the fusion of a human N-terminal Fc region of an immunoglobulin and the C-terminus of a myostatin-neutralizing peptide. AMG 745 has been successfully tested in various murine models including C26 tumor-bearing mice, where it increased SM mass, body weight and strength with respect to the control group (177). In patients undergoing androgen deprivation as a

consequence of non-metastatic prostate cancer, AMG 745 was reported to improve the lower-extremity muscle mass (177).

Metabolism and Appetite Modulators

One of the first pharmacological options proposed for the treatment of CC include Megestrol acetate, an appetite stimulant derived from progesterone capable to improve caloric intake and nutritional status. Albeit its precise mechanism of action still remains undetermined, it has been proven that this hormone-derivated drug exerts an anti-inflammatory action, as it has the ability to downregulate proinflammatory cytokines levels or that of their cognate receptors (197, 198).

After being tested in several trials, Megestrol acetate was reported to improve cancer patients' appetite, QoL and weight gain compared to placebo group, but not when compared to patients treated with other drugs (178). For this reason, the FDA approved it in 1993 for the treatment of anorexia, cachexia and unexplained weight loss in patients with AIDS (178). Nonetheless, Megestrol acetate was found to be responsible of more than 40 side effects, including odema, thromboembolic episodes and death (178). Yet, a case study about a 65 year-old man who suffered from metastatic renal cell carcinoma revealed that Megestrol acetate can induce adrenal insufficiency (199).

More recently, the efficacy of the combination of Megestrol acetate plus Thalidomide was tested in cachectic cancer patients compared to the treatment with Megestrol acetate alone (200). At the end of the study, results revealed that the combination of these two drugs was more effective than the treatment with Megestrol acetate alone, as patients showed statistically significant increase in body weight, appetite, QoL and grip strength, as well as significant decrease of IL-6 and TNFα levels (200).

Medroxyprogesterone acetate is another progesterone derivative taken into consideration for the clinical treatment of cancer-related anorexia/cachexia syndrome. At the same extent of Megestrol acecate, Medroxyprogesterone has been shown to impair the synthesis and release of several procachectic cytokines, in particular IL-6, TNFα, and IL-1 (179, 197). In placebo-controlled studies, it was reported that Medroxyprogesterone generally improved anorexia, QoL and body weight gain, although the latter was caused by an increase in AT rather than lean mass.

Due to his orexigenic properties, ghrelin has been evaluated as palliative remedy in CC. Ghrelin is mainly produced and secreted by the stomach and, to a lesser extent, by other organs such as pancreas, lung, liver, muscle, and AT (201). Ghrelin mediates multiple pathways involved in the regulation of body weight, body composition, energy storage and appetite, and regulates energy expenditure (202). In particular, it has been demonstrated that this hormone causes an increase in food consumption in both rodents and human patients with cancer, leading to significant weight gain [e.g., (180, 202)]. Moreover, patients who received ghrelin manifested less adverse effects during chemotherapy in terms of nausea and anorexia in respect to the control group (180). Thus, ghrelin is considered a potential remedy for the management of CC. However, an apparent contradictory observation was reported by some studies. In fact, cachectic murine models and cancer patients with cachexia both usually presented hyper-ghrelinemia, though paradoxically showing loss of appetite. This is probably due to grelin's short half-life, which is nearly 30 min (201, 203). In order to solve this issue, more stable ghrelin analogs have been developed as potential therapeutic agents. Among them, Anamorelin HCl has a half-life corresponding to nearly 7 h and is considered a valid agonist of the ghrelin receptor, as it displays significant appetite-enhancing activity and increases food intake and body weight in rats in a dose-dependent manner (204).

Anamorelin was tested on a group of volunteers in a randomized Phase I clinical trial. At the end of the trial, subjects who underwent the treatment displayed a significant dose-related weight gaining compared to the placebo group, with no particular adverse effects (205). Anamorelin has also been tested in several Phase II trials, with patients suffering from different types of solid and blood tumors, showing safety and efficacy (206). Notoriously, Anamorelin has been widely tested on patients with NSCLC during the ROMANA international clinical trials. ROMANA 1 and ROMANA 2 are two randomized Phase III trials performed on cachectic patients with incurable stage III/IV NSCLC for 12 weeks (181). In both studies, the Functional

Assessment of Anorexia/Cachexia Therapy (FAACT) and the fatigue domain of the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) showed an improvement in patient conditions, with an increase in body weight compared to the placebo group (181). Across the groups, Anamorelin treatment was well tolerated and caused general improvement of anorexia-cachexia symptoms, with the exception of some low incidence adverse events such as grade 1-2 hyperglycemia, nausea and gastrointestinal disorders (181).

A subgroup of patients who completed both trials were successively recruited for the ROMANA 3 study. Patients continued to receive Anamorelin at 100 mg a day or placebo for additional 12 weeks (182). Overall, the treatment was still well tolerated and improvement in body weight and anorexia-cachexia symptoms were significantly increased from baseline of original trials at all time points vs. placebo (182). Nonetheless, the drug has not been approved by FDA since the ROMANA trial did not demonstrated significant benefit handgrip strength, which is one primary outcome required for drug approval.

Further palliative care may be provided by endocannabinoids, i.e., endogenous agonists of the cannabinoid receptors CB1 and CB2. These two receptors are expressed at different levels in both neuronal and non-neuronal tissues, including the gastrointestinal system, SM and AT (207). To date, the endocannabinoid system is known to regulate appetite by differential activation of two brain sites, namely the limbic system and hypothalamus; moreover, it exerts an influence on intestinal and AT functions (207). Among endocannabinoids, Δ-9-tetrahydrocannabinol (THC) was tested as appetite stimulant in a Phase III clinical trial. Although the drug was well tolerated throughout the study, no significant differences were observed in terms of appetite or improvement in QoL between treated patients and the placebo group (183). However, more promising results were obtained by a later trial (184) and a recent pilot study (185) for the same drug. More recently, the synthetic analog of THC nabilone was reported to significantly increase appetite in cachectic NSCLC patients during a Phase II trial, with subsequent increase in patients' caloric intake. The same study reported a significant improvement in patients' QoL and no adverse effects (186).

Erythropoietin

Anemia is an additional feature displayed by cancer patients who suffer from cachexia, and contributes to weight loss and various metabolic alterations (187, 208). Thus, erythropoietin was tested in unselected cancer patients on palliative care. Patients treated with erythropoietin manifested a number of clinical benefits including improved exercise ability and sense of well-being (187). In mouse models of CC, it was shown that treatment with erythropoietin can counteract AT wasting and increase the lipogenic rate through the activation of erythropoietin receptor (EpoR) (208). Moreover, erythropoietin administration improved the survival of cachectic mice along with their exercise capacity, the latter being a consequence of increased erythrocyte count (209).

New Upcoming Potential Approaches

Results from latest experimental studies let emerge new potential therapeutics for the clinical management of CC, relying on different strategies than those previously followed (**Table 2**). For instance, the targeting of endogenous AT and energy metabolism modulators underlying CC may represent a valuable therapeutic option, as in the case of anti-PTHrP antibody (10). Another example is represented by the pharmacological inhibition of fatty acid oxidation by Etoxomir, a carnitine palmitoyltransferase-1-selective inhibitor. In a preclinical setting, Etoxomir was shown to effectively rescue human myotubes *in vitro* as well as muscle mass and body weight of cachectic mice (210). Yet, Metformin was shown to prevent WAT browning by increasing PP2A activity, with subsequent dephosphorylation of acetyl-CoA carboxylase and HSL (211). Targeting of hepatic peroxisome proliferator-activated receptor-α (PPARα) by PPARα agonists has also show a considerable potential. In particular, administration of the PPARα agonist fenofibrate prevented loss of SM mass and body weight in KL mice (152). Moreover, this treatment was capable to reduce elevated glucocorticoid levels in the serum of KL mice, which result from IL-6-induced suppression of hepatic ketogenesis and cause impairment of intratumoral immunity (213).

Further potential drugs that have recently been considered for clinical management of CC belong to the family of histone deacetylase (HDAC) inhibitors. Experimental evidences demonstrated that HDAC1, a class I HDACs family member, is required for muscle atrophy and contractile dysfunction caused by SM disuse and nutrient deprivation, although such mechanism can occur also independently from loss of SM (214). In line with this, a comprehensive study was performed in 2015 showing that AR-42, a novel class I/IIB HDAC inhibitor, displayed anti-cachectic activity in two independent murine models of CC (212). In both models, AR-42 reduced expression of Atrogin-1 and MuRF1 and production of certain proinflammatory cytokines, while significantly improved typical CC features such as body weight loss, reduced survival, muscle and AT loss, reduction of muscle strength, and muscle fiber size (212).

Finally, results obtained from preclinical studies investigating the effects of TLR inhibitors in CC experimental models have raised expectations for the future employment of these drugs in clinical trials (see below), although further investigations on more reliable models of CC are certainly required.

TLR Inhibitors: Novel Therapeutics for Efficient CC Management?

Involvement of TLRs in muscle atrophy (TLR4 and TLR7/8) and AT remodeling (TLR4) has been highlighted by several *in vitro* and *in vivo* studies. These findings led to the development of efficient TLR inhibitors and antagonists, which are currently being considered as part of a new therapeutic strategy against CC. Nonetheless, to date, only a small number of such pharmacological agents is available for clinical use (215).

3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitor, also known as atorvastatin, is a pharmacological inhibitor of TLR4 (74). Atorvastatin impairs the inflammatory pathways through the inhibition of NF-κB activation and downregulation of TLR4 and MyD88 gene expression (215, 216). This drug resulted to be a good candidate as potential anti-cachectic tool since in LLC-bearing mice it significantly prolonged animal survival, increased body weight, impaired AT atrophy and browning and tumor mass compared to untreated animals. Moreover, atorvastatin administration resulted in a decreased concentration of pro-inflammatory cytokines at the plasma level (74).

Several oligonucleotide-based antagonists have been developed to prevent endosomal TLR overactivation in patients with autoimmune diseases (215). Among these, IRS-954 and DV-1179 represent the first two oligonucleotide-based TLR antagonists tested throughout preclinical studies and Phase I/II clinical trials, respectively. However, in spite of the safety shown by these two drugs, none of them were approved for the clinical use due to the lack of adequate documentation (IRS-954) or failure to comply with primary endpoints (215). Few years later, other immune modulatory oligonucleotide (IMO) were developed by Idera Pharmaceuticals with the same goal. Among these, IMO-9200 and IMO-8400, two TLR7, 8 and 9-specific antagonists, were tested in clinical trials and the outcomes were more successful than those obtained for DV-1179 (215). However, none of these drugs had yet been considered for clinical management of CC.

In 2018, our group carried out an *in vitro* and *in vivo* study in order to test the ability of IMO-8503 to impair miR-21-5p-induced myoblast cell death (75). Results were very encouraging as IMO-8503 significantly inhibited apoptosis induced by lung and pancreatic cell-secreted miR-21-5p and miR-29a-3p on both immortalized and primary myocytes. The same results were obtained with myocytes exposed to cancer patients-derived microvesicles. *In vivo*, IMO-8503 strongly impaired several cachexia features, including the loss of lean mass in tumor-bearing mice (75).

Despite the potential of TLR inhibitors in the management of CC, it should be recalled that also TLR7 agonists have been explored as antitumor agents for treatment of advanced cancers, alone or as vaccine adjuvants. In particular, Imiquimod (R837), a topical TLR7 agonist, was approved by FDA for the treatment of patients with basal cell carcinoma and skin metastases [e.g., (217)]. Imiquimod is generally well-tolerated, with only a minority of patients reporting grade I or II side effects, and lead to significant increases in the activity of both CD8+ and CD4+ T cells. INFα levels were markedly increased in tumor samples from treated patients, while only slight increases were reported for INFγ levels (217). Similarly, recent studies have suggested that Resiquimod (R848), a more potent counterpart of Imiquimod, may be beneficial for cancer patients suffering of cachexia (157). R848 is a TLR7 agonist that was proved to cause lower expression of programmed cell death protein 1 (PD-1) on T cells and enhancement of CD8+ T-cell cytotoxic response in cancer through an IL-12-mediated mechanism (218). R848 was

TABLE 2 | Table summarizing emergent unimodal treatment options for CC recently tested in preclinical studies.

Drug name	Drug class	Molecule type	Disease	Status	Positive outcomes	Adverse effects/ Difficulties	References
anti-PTHrP	PTHrP inhibitor	Monoclonal neutralizing antibody against PTHrP	LLC murine model	Preclinical study	Prevented weight loss, AT browning and preserved lean body mass; improved physical activity; suppressed thermogenic gene expression	–	(10)
Etoxomir	Fatty acid oxidation blocker	Carnitine palmitoyltransferase-1-selective inhibitor	Stable murine models of human cancer-induced CC (by injection)	Preclinical study	Rescued muscle mass and body weight of cachectic mice	–	(210)
Metformin	Inhibitor of complex I of the electron transport chain	Biguanide compound	Murine burn model	Preclinical study	Prevented WAT browning by increasing PP2A activity	–	(211)
Fenofibrate	PPARα agonist	Fibric acid derivative	GEM model of NSCLC	Preclinical study	Prevented loss of SM mass and body weight; reduced glucocorticoid levels in mice serum	–	(152)
AR-42	Epigenetic modulator of gene expression	I/IIB HDAC inhibitor	LLC and C26 murine model	Preclinical study	Preserved muscle and fat mass; prolonged survival, reduced splenomegaly, reduced levels of Atrogin-1, MuRF1 and pro-inflammatory cytokines	The treatment showed distinct efficacy between the two models of CC	(212)
Atorvastatin	TLR inhibitor	HMG-CoA reductase enzyme inhibitor	LLC murine model	Preclinical study	Increased body weight; decreased tumor mass; attenuated AT remodeling; decreased levels of pro-inflammatory cytokines; prolonged survival	–	(74)
IMO-8503	TLR inhibitor	Immune modulatory oligonucleotide	LLC murine model	Preclinical study	Attenuated loss of lean mass; decreased levels of Pax7; prevented caspase-3 and PARP cleavage in SM	Caused toxic effects when administered at a concentration $\geq$15 mmol/L	(75)
Resiquimod (R848)	Topical TLR7/8 agonist	Imidazoquinoline compound	KPC-derived PDA syngeneic murine model	Preclinical study	Impaired expression of PD-1 on T cells; enhancement of CD8+ T-cell cytotoxic response; decreased tumor burden and to improvement of CC features	Initial or prolonged hypophagia and weight loss; may cause initial treatment-related decreases in locomotion	(157)

reported to be able to lower tumor burden and improve cachexia manifestations in experimental models of pancreatic ductal adenocarcinoma (157).

CONCLUSIONS

Over the last two decades, the study of CC has provided new insights that allowed a better understanding of the multiple mechanisms regulating the onset and progression of this metabolic disorder, and paved the way to the development of novel therapeutic strategies. Nonetheless, CC still remains a poorly defined syndrome, especially from the clinical standpoint. The main limitation probably lies in the fact that the precise role of individual CC mediators has remained undefined or controversial in several circumstances, thus complicating the process of establishing their clinical relevance. For instance, not all murine models of CC have shown involvement of the

same set of signaling molecules during the disease development. For this reason, the idea of defining distinctive diagnostic and prognostic signatures of CC represents a considerable challenge for researchers and clinicians. In this scenario, the role of miRNAs as cell-to-cell mediators between the tumor cells and the surrounding microenvironment represents an interesting topic that deserves deeper investigation. Overall, these considerations highlight the urgent need to generate and validate new experimental models of CC, capable to accurately recapitulate molecular and histopathological aspects of human CC in a tumor type-dependent manner.

As systemic chronic inflammation and alternate metabolism are hallmarks of CC, the pharmacological targeting of individual underlying biomolecules has been considered as one possible effective approach to clinically manage this syndrome. Thus, several unimodal treatments have been developed in the attempt to properly manage this metabolic syndrome. Nonetheless, to

date scarce or no effectiveness/safety has been reported for each of them. Given the complexity of CC, and considering the different patients' response to treatments through the many different clinical trials, it appears clear the impossibility to develop a general, universal therapy. On the contrary, it is crucial to define the cachectic patient's profile at both the molecular and clinical levels in order to reach a point where it is possible to provide the best customized nutritional support and treatment. Meanwhile, the emergence of further mediators of CC such as TLRs, PTHrP, and glucocorticoids, suggests the possibility to develop further safety pharmacological agents capable to prevent, or at least limit, the excessive triggering of catabolic processes and inflammation-related signaling.

Considering the multiplicity of metabolic and molecular aberrations, an important goal would be the development of appropriate multimodal approaches—intended as a balanced combination of pharmacological and non-pharmacological interventions—as recently suggested by several influential authors.

AUTHOR CONTRIBUTIONS

GM and FC wrote the manuscript. GM, PL, and FC contributed to the editing of the manuscript as needed.

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