Metastasis and cachexia: alongside in clinics, but not so in animal models

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Abstract

Cancer cachexia is a paraneoplastic syndrome characterized by lean mass wasting (with or without fat mass decrease), culminating in involuntary weight loss, which is the key clinical observation nowadays. There is a notable lack of studies involving animal models to mimic the clinical reality, which are mostly patients with cachexia and metastatic disease. This mismatch between the clinical reality and animal models could at least partly contribute to the poor translation observed in the field. In this paper, we retrieved and compared animal models used for cachexia research from 2017 and 10 years earlier (2007) and observed that very little has changed. Especially, clinically relevant models where cachexia is studied in an orthotopic or metastatic context were and still are very scarce. Finally, we described and supported the biological rationale behind why, despite technical challenges, these two phenomena—metastasis and cachexia—should be modelled in parallel, highlighting the overlapping pathways between them. To sum up, this review aims to contribute to rethinking and possibly switching the models currently used for cachexia research, to hopefully obtain better and more translational outcomes.

Keywords *In vivo* preclinical models; Advanced cancer; Weight loss; Wasting; Inflammation; Hypoxia; Metabolic reprogramming

Introduction

Cancer cachexia is a multifactorial host-wasting syndrome characterized by ongoing loss of skeletal muscle mass (associated or not with fat loss), which leads to progressive functional impairment,[1] contributing to morbidity, a decrease in chemotherapy tolerance/efficacy and mortality in cancer patients.[2,3] Another remarkable feature of this paraneoplastic syndrome is that conventional nutritional support cannot fully reverse it[1]. The extent and clinical presentation of cachexia varies according to several factors, including cancer type, age, presence of comorbidities, and genetic background.[4,5]

Diagnosis criteria for cancer-associated cachexia still seem to be a topic of debate.[6,7] Evans and collaborators[8] defined cachexia based on weight loss alongside other parameters, which might include some biochemical abnormalities (increased inflammatory markers, anaemia, and albuminaemia), whereas Fearon and collaborators[1] relied specially on lean mass content and proposed to stage cancer cachexia (pre-cachexia, cachexia, and refractory cachexia).

Cachexia's prevalence is estimated to be as high as 87% in pancreatic and gastric cancer patients, up to 61% in patients with colon, lung, prostate cancer, and non-Hodgkin lymphoma and around 40% in patients with breast cancer, sarcoma, leukaemia, or Hodgkin's lymphoma.[9] Overall, cachexia is believed to be directly responsible for up to 20% of all cancer-related deaths, which happen mostly due to cardiac and/or respiratory failure, when weight loss reaches 25–30%.[9–11] Especially, even though it might be present at relatively early disease stages,[12] cachexia could be considered a hallmark of metastatic cancer as these two parameters are remarkably correlated in clinics.[7,9,13–15] Severe cachexia is the picture of terminal metastatic cancer.[12,16]

Metastasis—the dissemination of tumour cells from the primary site and outgrowth of life-threatening lesions in distant organs—a complex multistep process, where every event is essential and limiting.[17–19] It is responsible for over 90% of all cancer-related deaths, and one of the mechanisms by which metastasis is so lethal is the development of cachexia.[9,16] Metastasis is still a poorly understood mechanism at the cellular and molecular levels; however, increasing evidence demonstrates that, beyond inherent characteristics of metastatic tumour cells themselves, the input of other elements within the tumour microenvironment (stromal cells and extracellular matrix components), as well as systemic metabolic and immune alterations, are mandatory for metastasis success.[17,20–25] Indeed, cancer progression seems to be driven by systemic responses to malignancy, and recent studies have indicated that paraneoplastic syndromes could benefit the tumour that gave rise to them.[21] This is particularly true for cachexia as inflammation and metabolic dysregulation are key factors in the pathophysiology of cancer-associated host wasting, tumour progression, and metastasis.[26]

Intriguingly, unlike what is seen in the clinical context, where cachexia and metastasis are often tightly in truncated,[7,9,13–15] there are very few studies involving animal models with the occurrence of metastasis for cachexia research.[27–31]

Of notice, the identification of early-disease stage biomarkers—which could be used to predict those patients who would evolve to cachexia and refractory cachexia, as well as the development of effective therapeutic interventions, could be considered as the ultimate desired outcomes for cachexia research. In this sense, the inclusion of animal models, in which both metastasis and cachexia evolve in parallel until terminal and refractory stage, as per often seen in patients, is of particular relevance and interest. Therefore, this review aims to support and motivate researchers to develop, validate, characterize, and use advanced disease or metastasis models for cachexia investigation.

The 'lack in translation'

The rate of success in oncologic clinical trials is low. Overall, only approximately 5% of the anti-neoplastic drugs that entered Phase III clinical trials were approved.[32] Unfortunately, considering specifically cancer cachexia, over a hundred clinical trials were conducted,[31,33] but none of them led to the approval of an agent or approach,[30,34–36] including the recent failure in Phase III clinical trials for anamorelin and enobosarm.[36]

Despite possible issues in clinical trial design, particularly the fact that, so far, they have been conducted in patients already at refractory cachexia stage, impinging the trials'

outcomes (reviewed elsewhere[33,36]), at the preclinical trial setting, there is an urge to improve the predictive power of the models used.[10,37] Many researchers question the translational value of broadly used cachexia models[4,16,30] regarding both drug efficacy assessment and molecular pathway elucidation. Along with disease heterogeneity, complexity, shortage of human data, and the aforementioned pitfalls in trials design, the lack of appropriate models are partially blamed for the failures in clinical trials.[10,16,38] Yet a complex, multifactorial and systemic syndrome such as cancer cachexia can only be modelled *in vivo*, showing the importance and need of these models for basic, preclinical, and translational research. Some suggestions, such as older age, presence of comorbidities, concurrent chemotherapy, multimodal approach, combination of models, and particularly the use of transgenic animals with spontaneous tumour development, as well as orthotopic transplantation (grafting the tumour in a natural position, the organ of its origin), and models with metastasis occurrence, have been pointed out as possible improvements for cancer cachexia modelling.[4,27,28,30,31,35,39,40]

Cachexia models: past, present, and hereafter

Historically, animal models of cancer cachexia consisted of rapidly growing, ectopically implanted (mostly subcutaneously - sc) tumour cells.[29] Interestingly, even though most of the progress on elucidating the underlying mechanisms of cancer cachexia was achieved using animals, these discoveries relayed in relatively few models,[26] which raises concerns regarding whether the observations are universal, or model specific.[31] Furthermore, current models are often designed to 'isolate' cachexia from other cancer-progression related events in order to understand whether reversing or minimizing the cachectic condition—as a distinct entity—would increase survival.[3] On the other hand, emerging cancer cachexia models include some genetically engineered mouse models (GEMMs),[27,31,35,41,42] cell line-based models comprising orthotopic injections[43–47] and/or metastatic colonization,[48,49] and some patient derived xenografts (PDXs) featuring both orthotopic and ectopic implantation.[38]

In order to investigate the evolution in cachexia modelling, we retrieved and reviewed manuscripts indexed on PubMed containing the words 'cancer' and 'cachexia' or 'wasting' or 'weight loss' and 'in vivo' or 'animal model' or 'mouse' that were published in 2017 and 10 years earlier (2007). Our search retrieved 95 studies published in 2007 and 233 studies in 2017. We then excluded reviews and other studies which did not meet the following criteria: (i) use of animal models; and (ii) occurrence of cancer-associated cachexia, which should be the focus of the study (Figure S1). The final list comprised 33 studies containing a total of 41 models for

2007 and 57 studies comprising a total of 73 models for 2017 (Figure S1, Tables S1 and S2). The number of excluded studies was high mostly due to the presence of a large amount of manuscripts just mentioning that a certain therapeutic intervention was not toxic and has not induced weight loss. For this final list, we carefully annotated details regarding which models were used in each study. These details included the type of cancer (organ of origin), whether it was an allograft or xenograft model, cell line (for transplantable models) or animal strain (for GEMMs) or drug used (for chemically induced), site of tumour inoculation or growth (orthotopic/ectopic), and metastasis occurrence (Tables S1 and S2).

Our search indicates that, over the last 10 years, very little has changed regarding the animal models used for cancer cachexia research. Overall, the types of cancer (original primary tumour site) used are pertinent with tumours that are known to trigger cachexia in humans, including pancreatic, gastrointestinal, and lung carcinomas (Figure 1, Tables S1 and S2). An improvement observed was the increase in studies focusing on pancreatic and lung cancer models, due to the high incidence and severity of cachexia in patients with these tumours.[9,10,40] However, as pointed out by others previously and confirmed by us, many of these studies relied on only a few classic models, such as C26, Mac16, and LLC.[27–29,31,35] These models have been extensively reviewed and discussed previously.[27–29,31,35] The vast majority of models used were and are still ectopically transplanted allografts, with subcutaneous implantation corresponding to more than half of the models used in both years reviewed (Figure 2A–2D).

The traditional C26 cancer cachexia model (allograft, ectopically transplanted colorectal cancer) was and still is the most frequently used (Tables S1 and S2). On the other hand, another traditional colorectal cancer model, Mac16 (also allograft, ectopically transplanted), was used in several

studies in 2007 but has not appeared in 2017 (Tables S1 and S2). Contrariwise, the number of studies involving the LLC model (Lewis Lung carcinoma, allograft, ectopically transplanted) more than doubled in 2017 compared to 10 years earlier (Tables S1 and S2). Considering this, even though the LLC lineage is known to metastasize in mouse models,[35,39] most cachexia studies using it and reviewed here have not acknowledged this fact—and unless metastasis was reported in the study, we did not include its occurrence in our data analysis. The number of studies involving xenografts dropped slightly in 2017 compared with 2007, and both datasets had only one register of PDX models each (Figure 2D, Tables S1 and S2). One possible explanation for this fact might be related to the essential role of the immune system for cancer cachexia, which is only fully present and compatible in allograft models.[50]

Despite the great similarities between the two time points analysed, a remarkable change was that the number of studies involving GEMMs (mostly the ApcMin/+ colorectal cancer model) more than doubled in 2017 compared with 2007 (Figure 2A–2C, Tables S1 and S2). This fact by itself also contributed to an increase in studies featuring orthotopic models in 2017 compared to 10 years earlier (Figure 2D). Moreover, we found one study in 2017 using a chemically induced colorectal carcinoma model (azoxymethane and dextran sodium sulfate models); these kinds of models were not found 10 years earlier (Figure 2A–2C). The use of GEMMs or chemically induced models is particularly interesting because both the tumour and metabolic and immune alterations arise and evolve concurrently in the host. However, their broad use might face a few challenges including long latency times for tumours to arise, variable burden, and especially limited metastasis occurrence.[32,50]

Moreover, orthotopic models (both GEMMs, transplanted or chemically induced) represent only a quarter of the models used in the studies conducted in 2017 (Figure 2D, Table S2). More importantly, our analysis also highlighted that, unlike what is often observed in the clinical setting, the vast majority of cancer cachexia studies was not conducted in a metastatic context (Figure 2E). Metastases were reported in about only 15% of the models used in cachexia studies in both 2007 and 2017. These are impressive concerning the low numbers when we consider the clinical relevance of cachexia and metastasis together. Furthermore, regarding the seven metastasis occurrences in our 2007 dataset (Figure 2E), two corresponded to model descriptions (Current Protocols series), and therefore, only the other five studies were actually research articles, in which four of them performed as experimental metastasis models—a setting that bypasses primary tumour formation. Three studies comprised lung colonization: lung cancer cells or bladder cancer cells inoculated into the tail vein (IV) and one featured colon cancer cells inoculated intrahepatically (Table S1).

Figure 1 Types of cancer used as models for cachexia research in 2007 and 2017, according to primary tumour site. Graphs were prepared in Microsoft Excel 2013. 'Others' include head and neck, kidney, gonads, cervix, and bladder cancer (2007) and lymphoma, neuroblastoma, and skin non-melanoma cancer (2017).
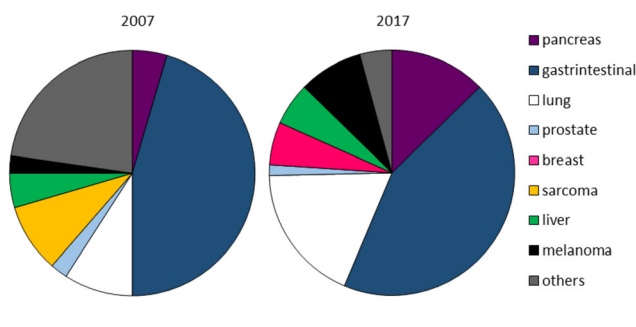
Figure 2 Cachexia models used in 2007 and 2017. (A) Types of animal models (regarding tumour induction) used for cancer cachexia research in 2007. (B) Number of different models in each category [transplantable, genetically engineered mouse models (GEMM), and chemically induced] in 2007 and 2017. (C) Types of animal models (regarding to tumour induction) used for cancer cachexia research in 2017. (D) Details of models used for cancer cachexia research in 2007 and 2017 regarding implantation site and type of graft (allograft, xenograft, or patient derived xenograft). (E) Venn diagrams showing the occurrence of metastasis in the models in 2007 and 2017. Graphs were designed in Microsoft Excel 2013 and Venn Diagrams in the Venn Diagram Plotter Software (PNNL, Richland, WA).
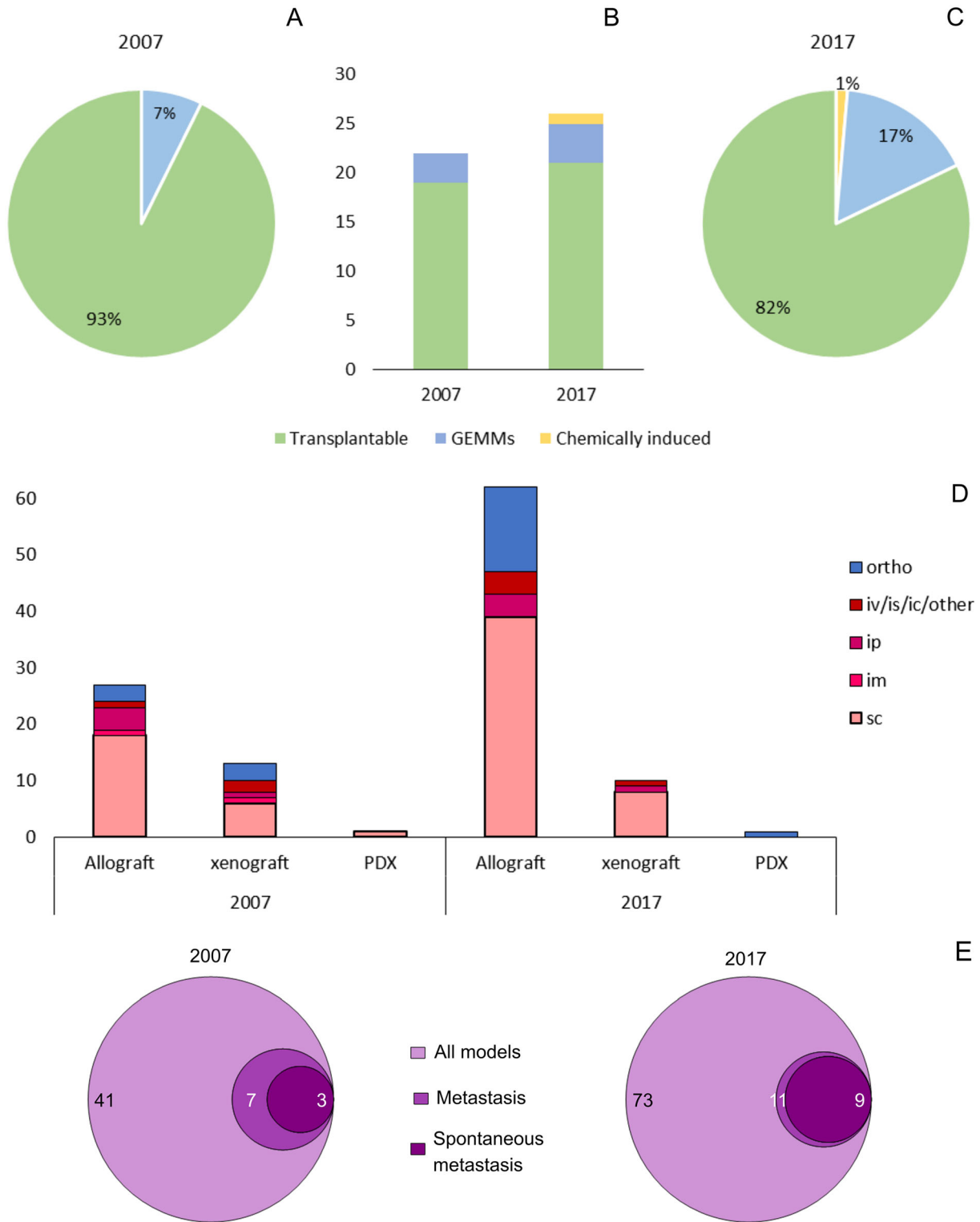
Similarly, in 2017 (Figure 2E), 11 metastasis occurrences were found; two of them used experimental metastasis approaches (breast cancer cells intrafemoral and lymphoma iv) and the other three featured orthotopic tumours (pancreas, breast, and melanoma). The remaining five had ectopic tumours: three lung cancer models (two of them LLC), one melanoma model implanted subcutaneously, and one intraperitoneal neuroblastoma model (Table S2). Moreover, in 2017, there was one entry of primary tumour resection in the database. This might be an interesting approach as it enables the animals to live longer, allowing suitable time for metastasis to arise and creating a clinically relevant early metastatic disease experimental setting.[19,51,52] Indeed, many authors point out that the lack of metastasis in cachexia models might be, in addition to the use of subcutaneous implants,[19,31,32,38,53] due to short experimental times.[31] Even though primary tumour resection in orthotopic models might only be feasible for breast cancer and melanoma,[53] orthotopic models for lung and pancreatic cancer are particularly relevant for cachexia research as these tumours trigger severe cachexia and are often inoperable.[38,54]

In recent years, substantial evidence has indicated that animals bearing orthotopic tumours better recapitulate a number of features relevant to human cancer when compared to models where tumours are implanted ectopically.[19] Ectopic tumour models do not reflect the human disease regarding tumour microenvironment, vascularization, metastasis occurrence, and chemotherapy response.[55] Therefore, there has been an increasing preference towards using orthotopic tumour models in the preclinical setting.[32] In addition, some studies have shown that drugs that were successful in preclinical trials conducted on ectopic/primary tumour models and that later on failed in clinical trials were also ineffective in animals with advanced metastatic disease, indicating that such models are more clinically relevant, considering that the vast majority of clinical trials are conducted in patients with advanced metastatic disease.[51,52]

This could also be the case for cachexia translational research, where several drugs, which have shown promising results in animal models, were unsuccessful in clinical trials.

Whether the lack in translation for cachexia research was due to inappropriate patient selection, or reflected the inaccuracy of the preclinical models used, remains uncertain. Most likely, it was a combination of both. Nevertheless, considering that cachexia is strongly correlated with advanced cancer, experiments involving animals with metastatic disease would better mimic this clinical scenario, providing relevant models not only to access treatment efficacy but also to elucidate the underlying mechanisms involved in the pathophysiology of cachexia, greatly improving the predictive power of preclinical research.

Supporting the use of these models specifically for cancer cachexia research, several studies have indicated that animals with both orthotopically implanted tumours and metastatic disease developed more severe cachexia symptoms than their ectopic counterparts.[38,45,47,48] Thus, there are some transplantable orthotopic and/or metastasis models available, which might be useful for cachexia research, including pancreatic, lung, colon, and breast cancer models[53] as tumours from these primary sites are known to induce host wasting and cachexia. On the other hand, at least three models that have been largely used in cachexia research (LLC, B16, and the KPC models) have also been used for metastasis research.[53] Notably, there are a number of studies, using the aforementioned approaches, in which weight loss was reported even though cachexia was not the research focus.[56–64] This further supports the existence of metastasis models, already in use, that are suitable for cancer cachexia research, broadening the experimental spectra.

Indeed, reinforcing our observations that the use of transgenic mouse models is rising, some emerging cancer cachexia studies have already used the PyMT breast cancer model[65] and at least two lung cancer GEMMs,[41,42] one of them reporting that lymph node metastasis was only found in cachectic animals.[42] Yet as pointed out here, studies where cachexia is modelled in a metastatic context are still lacking in the literature. Despite the challenges of modelling cachexia and metastasis together, this setting could be a great improvement in the field, bringing the models closer to the clinical context. The use of such models would not only be potentially more relevant for accessing anti-cachexia interventions but also to elucidate some key molecular mechanisms involved in the pathophysiology of cancer cachexia. It is also worth mentioning the fact that it is largely known and accepted that the best way to treat cancer-associated cachexia is to cure cancer,[66] which is clearly observed when efficient anti-cancer therapies lead to improvements in cachexia-related parameters as well as the tumour is the outstanding predictor of cachexia.[5,67] However, the debilitating effects inherent to cachexia increase the side effects and toxicity of anti-cancer therapies[28] and might ultimately preclude patients of achieving the full potential benefit of the therapy,[5] therefore creating a lethal loop where progressive tumours worsen cachexia, and increasingly severe cachexia worsens cancer progression and metastasis.

Considering this, in the following session, some of the major pathways where metastasis and cachexia overlap and nourish each other will be briefly highlighted, further supporting approaches encompassing the study of cachexia in a metastatic context in animal models.

Overlapping pathways in metastasis and cachexia

Many researchers have described cancer cachexia as an inflammatory and/or metabolic syndrome.[2,12,16,26,40,68,69]

Notably, tumour-promoting inflammation and metabolic deregulation appear as an enabling feature and an emerging hallmark of cancer, respectively.[17] The hallmarks of cancer consist of a number of key cellular and molecular steps in cancer development and progression, proposed by Hanahan and Weinberg[17,70] in two seminal publications. Indeed, during cancer progression, a number of crucial events observed within a tumour, such as inflammation and metabolic reprogramming, also occur systemically.[21,26,68,69,71,72] Intriguingly, many of these systemic events are also considered part of the cancer cachexia pathophysiology and further support cancer progression and metastasis by fuelling tumour growth,[3,10,26] establishing pre-metastatic niches[73,74] and promoting metastasis outgrowth.[54]

Figure 3 summarizes some of the overlapping pathways in metastasis and cachexia regarding inflammation. Systemic chronic inflammation is a common feature in advanced cancers, and it is linked to muscle and fat wasting in cachexia.[68] Increases in tumour or host derived pro-inflammatory cytokines have been pointed out as the most common correlation between cancer and cachexia.[3] Complementarily, inflammation corroborates for cancer progression by supplying the tumour or metastatic microenvironment with active molecules involved in key processes such as immune evasion, angiogenesis, pre-metastatic niche establishment, metastasis tropism, and metastatic outgrowth.[17,22] Cancer cells themselves, as well as tumour stromal cells, produce a number of cytokines and chemoattractants that recruit bone marrow derived cells to the tumour site.[73] This immune cell infiltration leads to tumour-promoting inflammatory responses, including cancer cell proliferation, invasion, migration, immune evasion, and angiogenesis.[22,73,75]

Accumulating evidences indicate that tumour cells secrete extracellular vesicles (EVs), particularly exosomes (30–150 nm in diameter) containing microRNA, nucleic acids, proteins, and different metabolites that reflect their original cell type and condition and that participate in the formation the pre-metastatic niche and regulate metastatic organotropism.[76,77] Some recent studies have also shown that EVs released by cachectic tumour cells could potentially induce muscle wasting,[78] lipolysis in murine, and human subcutaneous adipocytes[79] or have anti-adipogenic effects *in vitro*.[80] Besides tumour cells, the white adipose tissue also plays an important role secreting circulating inflammatory cytokines via EVs.[76,77,81] Therefore, in cancer-associated cachexia, EVs produced either by tumour cells or adipose tissue play a role in activating the inflammatory process in cancer; thus, they might be involved in both host-wasting processes, as well as metastatic dissemination, depending on the organ targeted.[76,77,82–84]

Complementary to inflammation, immune evasion is an important premise for metastatic dissemination and outgrowth,[20] and cachectic tumours seem to promote this phenotype.[69] Comparing the expression profile of cachectic

tumours vs. non-cachectic ones, Flint and collaborators[69] demonstrated that cachexia-promoting tumours presented remarkable downregulation in genes related to innate or adaptive immune responses, and the suppression in the adaptive antitumoral T response was through the increase of corticosterone levels mediated by IL-6.[69]

Indeed, maybe one of the most important players in this system is IL-6. This cytokine is broadly linked to both cachexia and metastasis events,[9,11,67,73,85] and interfering with its signalling impairs both tumour burden and cachexia.[41,67] Besides its pro-inflammatory and immune suppressive effects in the tumour,[22,67] systemically, IL-6 along with other cytokines—such as tumour necrosis factor alpha, interleukin-1 beta, and tumour growth factor beta—promote adipose tissue inflammation and lipid wasting.[23] Other roles attributed to IL-6 include stimulation of C-reactive protein production by the liver,[86] induction of muscle wasting and atrophy,[3,10,85] systemic autophagy for tissue wasting[87] and extracellular matrix degradation, promotion of invasion and surveillance of circulating tumour cells,[73] and cancer cell growth in a secondary site.[17,21]

As another example of the tight link between metastasis and cachexia, using breast cancer animal models, Waning and Guise[49] have shown the cooperation between muscle wasting and bone metastasis. They have demonstrated that while wasted muscles provide bone metastases with insulin-like growth factor-I, fibroblast growth factor 2 and IL-6, stimulating their growth, osteolytic metastatic lesions supply the muscle with tumour growth factor beta, activin-a, and bone morphogenic protein 2, which further promotes muscle wasting in a feedback loop manner.[49] In breast cancer, classical cachexia (dramatic weight loss) incidence is not as high as other cancer types (such as pancreatic, lung, and colorectal cancers)[9,40]; however, muscle weakness and dysfunction are present, which therefore underestimates cachexia in these patients.[13,49]

Finally, Hamilton and collaborators[54] have indicated that circulating tumour cells promote the differentiation of circulating macrophages into tumour associated macrophages and that they could already be involved in cachexia induction in small cell lung cancer by overexpressing complement factor D (CFD)/adipsin, further supporting the association of metastasis and cachexia at very early stages.

Inflammation has also been described as the driving force behind metabolic alterations in cancer.[40] Whereas at the tumour level metabolic reprogramming contributes to cancer cell proliferation, surveillance, and metastasis, at the systemic level, it culminates in cachexia, which in turn mobilizes metabolites for sustaining tumour growth.[26] Figure 4 brings some of the metabolic alterations triggered throughout cancer and cachexia progression and their interplay.

In this regard, another remarkable feature of aggressive tumours is hypoxia.[88] Hypoxia increases the expression of hypoxia-inducible transcription factors 1 and 2, which are

Figure 3 Overlapping pathways in metastasis and cachexia: inflammation. Abbreviations: BMDCs, bone marrow derived cells; DTC, disseminated tumour cells; CAF, cancer-associated fibroblast; OPN, osteopontin; VEGF-A, vascular endothelial growth factor A; COX-2, ciclo-oxigenase-2; IL, interleukin; TNF-a, tumour necrosis factor alpha; TGF-B, transforming growth factor beta; IFNG, interferon gamma; BMP-2, bone morphogenetic protein 2; IGF1, insulin growth factor 1; FGF2, fibroblast growth factor 2; aa, amino acid; CRP, C-reactive protein. Inflammation contributes to both metastasis and cachexia as pro-inflammatory cytokines, such as IL-6, IL-1, TGF-β, and tumour necrosis factor alpha, are believed to be involved in host wasting and metastatic progression.[3,9,11,17,22,67,68,73,75] Such cytokines are initially released by the primary tumour (either coming from tumour cells or from immune infiltrated cells previously attracted from the bone marrow), and later on by metastases and also by host tissues (such as muscle adipose tissue) under wasting.[20,22,69,73,75] Pro-inflammatory cytokines promote fat and lean mass degradation,[3,10,85,87] as well as a shift in liver protein synthesis towards the production of C-reactive protein instead of albumin—which contributes to sustaining chronic inflammation and for amino acid waste.[86] Conversely, this chronic inflammatory state also contributes to a metastasis-permissive environment,[17,21,73] probably including the establishment of pre-metastatic niches, which will ultimately increase tumour burden and thus inflammation in a loop. Additionally, bone metastases specifically lead to decreases in muscle function, and the affected muscle releases cytokines and factors that contribute to metastatic growth.[49] The artwork was prepared on Inkscape 0.92.2 (Software Freedom Conservancy, 137 Montague St. Ste 380, Brooklyn, NY).

clearly involved in the pathogenesis of cachexia and metastasis.[10,89] Hundreds of genes are transcribed in response to hypoxia-inducible transcription factors 1 and 2, allowing cancer cells to survive and adapt to low oxygen conditions, and also conferring changes in both the primary tumour and the host that promote metastasis[23,88,89] and cachexia.[10]

A lack of oxygen supply shifts cell metabolism towards a glycolytic phenotype, where from glucose, pyruvate is converted into lactate instead of entering the tricarboxylic cycle (oxidative metabolism). Intriguingly, tumour cells might exhibit a glycolytic metabolism even when oxygen is abundant, which characterizes the 'Warburg Effect'.[17,90]

Lactate can leave the tumour and reach the liver, where it is converted back into glucose via gluconeogenesis, which can then be uptaken again by cancer cells to be used for tumour growth in the so-called 'Oncogenic Cori Cycle'.[5,10,26] Nevertheless, the role of lactate goes much beyond being an energy source. Lactate is a powerful signalling molecule in the tumour, triggering angiogenesis, promoting inflammation, and inhibiting adaptive immune responses.[23,91] Due to these characteristics, lactate has been linked to metastasis occurrence and burden in animal models, and it is a poor prognostic marker in patients.[91,92] The liver can also use other sources for gluconeogenesis in the Oncogenic Cori Cycle, such as glycerol (from lipolysis) and aminoacids (from

Figure 4 Overlapping pathways in metastasis and cachexia: metabolic reprogramming. Abbreviations: WAT, white adipose tissue; BAT, brown adipose tissue; PTHrP, parathyroid hormone related protein; FA, fat acids; HIF, hypoxia-inducible factor; VEGF, vascular endothelial growth factor; EMT, epithelial-to-mesenchymal transition; NF-kB, nuclear factor Kappa beta; GCS-F, granulocyte colony-stimulating factor; LOX, lysil oxidase; MDSCs, myeloid-derived suppressor cells. Metabolic alterations also have a key role in both metastasis and cachexia.[10,17,26,89] Hypoxia is a common feature in tumours and leads to an increase in lactate production, which acts within the tumour as a signalling molecule that—among other functions—facilitates metastasis.[23,88,89,91,92] Through the circulation, lactate produced in hypoxic tumours also reaches the liver, where it is converted back into glucose that will be uptaken by the tumour cells, characterizing the 'Oncogenic Cori Cycle'.[5,10,26] Other sources for gluconeogenesis in the liver (which ultimately generated glucose for the tumour) comprise amino acids and glycerol (which also take part in metastasis to liver) from wasted muscle and adipose tissue, respectively.[10,40] Hypoxia also triggers the expression of HIFs, which further inhibit Krebs cycle contributing to an increase in lactate production.[10] HIFs also leads to the expression of a number of genes and other factors (such as Zeb1, Snail, Twist, Wnt, NF-kB, and LOX) which have roles in angiogenesis, EMT, pre-metastatic niche establishment, and therefore metastasis.[89] Additionally, non-essential FAs from lipolysis are uptaken by tumour cells, contributing for tumour growth,[40] complementarily glutamine and proline from wasted muscle contribute to liver metastasis.[24,74] The artwork was prepared on Inkscape 0.92.2 (Software Freedom Conservancy, 137 Montague St. Ste 380, Brooklyn, NY).
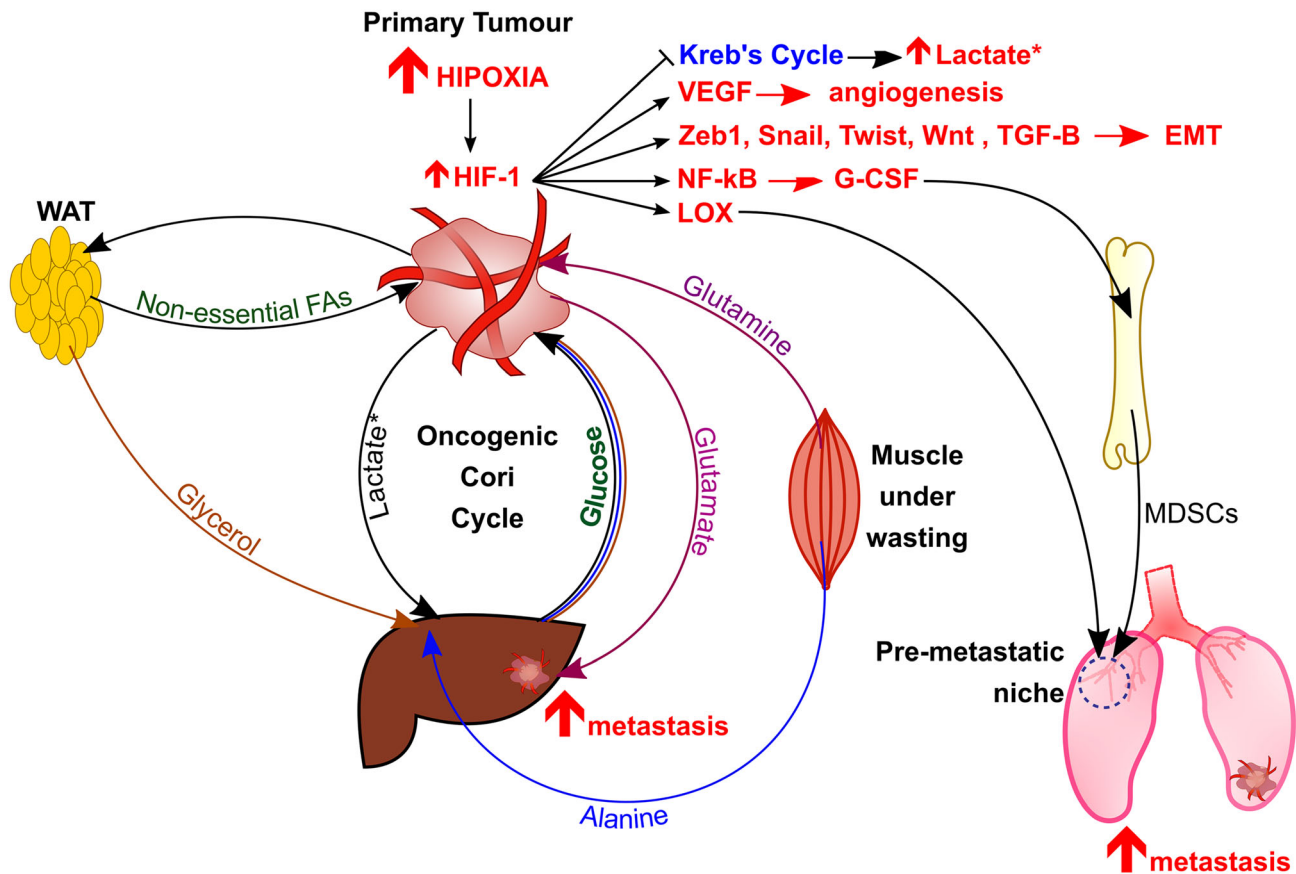
protein degradation in wasted muscles).[10,40] Altogether, these futile cycles contribute not only to the pathogenesis of cachexia but also to tumour growth and metastasis, representing up to 40% of the energy expenditure in patients with metastatic cancer.[3]

On the other hand, protein breakdown during muscle degradation provides aminoacids for acute phase protein synthesis in the liver under IL-6 stimulus. This process not only helps to sustain inflammation but also characterizes a source of aminoacid wasting.[5] Additionally, aminoacids from muscles under wasting can be uptaken by the tumour and participate on tumour growth and metastasis.[24,40,74] For

example, glutamine can be converted into glutamate in the tumour, which has been linked to liver metastasis promotion in colorectal cancer[74] and proline has also been reported to have pro metastatic effects.[24] Similarly, tumour-induced fat wasting provides non-essential fat acids for the cancer cells, and their oxidation promotes metastasis.[40]

Once again, systemic metabolic reprogramming, in association with systemic inflammation, both driven by the tumour, are major players in cancer cachexia and metastasis. The interplay between these two conditions favours their progress and is a great challenge for the development of efficient therapeutic interventions. Therefore, it is reasonable to use

animal models of advanced or metastatic disease for cachexia modelling.

Overcoming the challenges of modelling cachexia along with metastasis in animal models

There is not an animal model that fully recapitulates the complexity of either cancer cachexia syndrome or the metastatic process found in human beings.[32,50] Therefore, modelling cachexia in a metastasis model, even though biologically sound and significant, is certainly a great challenge. Additionally, behind the substantial lack in cachexia studies developed in animals bearing metastatic disease, there is also a concern that the survival time for animals, once advanced metastasis is reached, is relatively short. Furthermore, at this point, cachexia would already be in the refractory stage; hence, nowadays, not much could be performed besides palliative care.[1]

Some suggestions to overcome these issues, making it feasible to model cachexia in a metastatic context, would be (i) favouring the use of orthotopic models, which are generally more prone to metastasize spontaneously and evoke more severe cachexia[32,50,93], and (ii) using metastasis models at an early-disseminated disease stage—preferably in spontaneous metastasis models and also in experimental metastasis/organ colonization models.[32,50,94] Once in clinics metastasis and cachexia are often in a deadly loop, such as metastasis worsens cachexia and vice versa, the main point here is to take advantage of cachexia models which could be also able to form secondary tumours. This does not mean the experiments have to be conducted when animals are already bearing advanced metastatic disease and refractory cachexia. Rather, the interventions and analysis should begin much earlier, when metastatic burden is still negligible and the wasting disease is at pre-cachexia stage, but in a model with the ability to reach the terminal stage for both conditions.

Naturally, the timeframe for metastasis and cachexia onset varies depending on the model, but even for rapidly evolving metastasis models, such as those of organ colonization,[50,53,93] where, for example, tumour cells are inoculated into the tail vein (lung), spleen (liver), or left ventricle (whole body), animals survive very aggressive tumours for at least 10 to 15 days.[95–97] This lifespan post-tumour induction is not much different or shorter than what has been observed in past and current models traditionally used for cachexia research (such as C26, AH-130, Mac16, or LLC where the average experimental period usually ranges from 10 to 21 days[28,35]).

Indeed, there were cachexia studies that took advantage of such models. Mbalaviele and collaborators,[56] back in 1996, showed that the MDA-MB231 breast cancer cell line, when inoculated straight into the arterial circulation, leads to osteolytic bone metastasis and cachexia, which are attenuated when e-cadherin was re-expressed. This is one of the studies which led Waning and Guise[49] to propose a crosslinking mechanism between bone metastasis and muscle weakness. Another example is the work carried out by Murphy et al.,[48] which characterizes an experimental metastasis model to liver, using the C-26 colorectal cancer cell line, for cachexia studies. More recently, cachexia studies involving GEMMs for pancreatic (KPC) and breast cancer (PyMT), which are known to be able to metastasize, were conducted.[65,98] Finally, orthotopic PDXs for pancreatic cancer, which also formed secondary nodules, have also been characterized for cachexia studies.[38]

Therefore, by using well-developed and characterized models that are known to metastasize and evoke cachexia, it would be possible to study the mechanisms by which cachexia develops, as well as test therapies and interventions from very early stages of both disseminated disease and cachexia, before the metastatic burden is too wide and cachexia reaches the refractory stage.

Of note, metastasis models are not necessarily always the best choice for cachexia research. Another valid consideration underlying the choice of an appropriate model for a particular study permeates the biology of the specific tumour being modelled. For example, whereas non-small cell lung carcinomas are often inoperable[54] (and therefore the primary lesion is the 'cancerous' trigger of cachexia), breast cancer patients will most likely to present cachexia only at metastatic stage, once the primary tumour is relatively easily resected in a mastectomy. This way, orthotopic models seem to be the most appropriate for cachexia studies involving non-small cell lung carcinomas, while the use of metastatic models should be preferred when dealing with cachexia in breast cancer.

Hopefully, using a range of orthotopic and experimental metastasis models, both starting from early stages throughout disease progression (in terms of metastasis and cachexia), might shed some light on the field, improving the predictive power of preclinical models in terms of mechanisms and therapeutic response.

Concluding remarks

Even though cachexia is often closely associated with advanced metastatic disease in cancer patients, past and current animal models do not reflect this scenario. Despite the challenges of modelling cachexia in a metastatic context, considering the clinical relevance of these two phenomena in association, and the overlapping pathways between them, a shift towards their use and development could be a great step towards improving the predictive power of preclinical

models for both mechanism elucidation and therapy assessment.

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Flow chart showing criteria for manuscript selection. A. 2007. B. 2017.
Table S1. Models used for cachexia research in 2007
Table S2. Models used for cachexia research in 2017

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