Experimental cancer cachexia: Evolving strategies for getting closer to the human scenario

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Abstract

Cancer cachexia is a frequent syndrome that dramatically affects patient quality of life, anti-cancer treatment effectiveness, and overall survival. To date, no effective treatment is available and most of the studies are performed in experimental models in order to uncover the underlying mechanisms and to design prospective therapeutic strategies. This review summarizes the most relevant information regarding the use of animal models for studying cancer cachexia. Technical limitations and degree of recapitulation of the features of human cachexia are highlighted, in order to help investigators choose the most suitable model according to study-specific endpoints.

Introduction

Cancer cachexia (CC) is a multifactorial syndrome that affects more than 50% of cancer patients and is characterized by body weight loss, mainly due to skeletal muscle and fat depletion. Cachexia is not solely associated with neoplasms but frequently appears in patients affected by chronic inflammatory diseases. A consensus definition that lists the main features of the cachexia syndrome was given in 2008 [1]: *"cachexia, is a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass. The prominent clinical feature of cachexia is weight loss in adults (corrected for fluid retention) or growth failure in children (excluding endocrine disorders). Anorexia, inflammation, insulin resistance and increased muscle protein breakdown are frequently associated with cachexia. Cachexia is distinct from starvation, age-related loss of muscle mass, primary depression, malabsorption and hyperthyroidism and is associated with increased morbidity".* Later on, a specific definition of CC was given and included a classification with specific diagnostic criteria [2]. A relevant notion is that CC cannot be fully reversed by conventional nutritional support, thus requiring the adoption of mechanism-based therapeutic agents in order to prevent progressive wasting. Moreover, CC determines a reduction in anti-cancer treatment tolerance and effectiveness, further impairing both quality of life and survival. The massive loss of skeletal muscle is considered the main hallmark of CC, mainly because of its direct association with poor prognosis [3]. In addition to skeletal muscle (and fat) wasting, other tissues and their associated conditions have been proposed as targets in CC, including heart dysfunction, alterations in liver protein synthesis, changes in hypothalamic mediators, and brown adipose tissue hyperactivity [4].

To date, approximately one hundred clinical trials for CC have been performed that include active and concluded studies, as well as observational and interventional trials. The design of effective therapeutic strategies has been limited on the one side by the complexity of the syndrome, the difficulties in recruiting patients, and the hurdles at times in obtaining patient follow up information, and on the other side by the insufficient knowledge of the underlying pathogenetic mechanisms. A relevant point in CC research is the potential to improve patient status and overall survival by preventing cachexia in order to increase the effectiveness of the anti-cancer treatment. In this context the use of experimental pre-clinical systems for modeling human CC would be useful for many reasons, including attempts to circumvent the limitations of clinical trials, opportunities in expanding our understanding of the underlying mechanisms of wasting, and changes to explore the effectiveness of prospective treatments for translational purposes. This chapter of the series summarizes the most current information regarding the use of animal models for the study of CC, highlighting both the technical limitations and difficulties in recapitulating the features of human CC that influences the choice of the most suitable model.

Experimental models: a general view

Starting from the idea that a model is a simplified tool that allows the study of a reference system for the purpose of analyzing and conducting experiments, investigators have long sought to create the simplest system possible, in order to reduce cost and time required to achieve meaningful experimental results. An example of such a system is the use of cell culture, where murine C2C12 myotubes are exposed to pro-inflammatory cytokines or glucocorticoids, largely to investigate mechanisms driving protein catabolism and fiber atrophy [5]. Cell-based models are also useful to rapidly screen compounds for their anti-cachectic activity, prior to their use *in vivo*. In terms of multicellular organisms, the use of *Drosophila melanogaster* was recently described as a CC model [6]. The fly as a model is advantageous in having a relatively short generation time that can produce wild type or mutant animals, and can be used in drug screens. However, when the aim of the investigator is to reproduce a human pathology/syndrome by means of a model able to recapitulate the clinical features of the human disease, the system requires the use of mammals. In the case of CC, several murine tumors (either spontaneous or experimentally induced) have been used, but the search for more appropriate models is ongoing, given that a single tumor cannot reproduce the heterogeneity of human cancers.

The value of animal models of CC is supported by an available knowledge base for CC that was initially inferred from experimental models and subsequently validated or disproved in humans. The adoption of a CC model should be driven by the specific aim of the research based on the intrinsic characteristics of the model (reliability, reproducibility, availability, time required to induce cachexia, specific drug metabolism profiles for drug screening purposes, etc). In this regard, most of the research groups studying CC in the past relied on a single, thorough, standardized model, rarely comparing their datasets with those from other experimental systems. As a consequence, some observations reflected the particularities of a specific model, which may not be useful for the characterization of human CC and for the identification of therapeutic targets. On the same line, a consensus on the use of experimental models for CC is lacking, and the continual addition of new models only adds to this complexity, in particular for those researchers who are just starting out in the field and need to choose an adequate model to begin their studies.

Experimental cachexia from the human perspective

The accuracy of each model in comparison to human CC should be evaluated in terms of endpoints considered relevant to the clinical practice, in particular when anti-cachexia treatments are proposed. A useful hint is to design an experimental system that mimics conditions of a clinical trial [7]. Indeed, In the above mentioned CC definition, Fearon and co-workers included in the classification and clinical management the following characteristics: *'anorexia or reduced food intake, catabolic drive, muscle mass and strength, functional and psychosocial impairment' in order to 'aid clinical trial design, development of*

practice guidelines, and, eventually, routine clinical management' [2]. Using this definition as a guide for comparing human to experimental CC, the main CC hallmark is *'the loss of skeletal muscle mass (with or without loss of fat mass) leading to progressive functional impairment'*. These two characteristics (muscle mass and function) are important to include in original articles when accompanied with a description of a new mechanism or pre-clinical studies in cancer cachexia. Although common for investigators to focus on specific molecular alterations that might underlie the cachexia phenotype, efforts should be made to incorporate comparative data between muscle atrophy, reduced strength, and modulations of the specific pathway under investigation. In parallel to the reduction of muscle mass, many researchers measure muscle fiber cross sectional area (CSA) to provide evidence of reduced muscle size. Such an assay is fundamental when genetic manipulation is performed on the skeletal muscle, but it is also subject to potential errors due to the heterogeneity of fiber area and distributions of oxidative (small) and glycolytic (large) fibers. Functional (grip strength, tetanic force, treadmill test) and behavioral (activity cages) assays add value to morphological data and can be used as surrogate markers to satisfy the characteristics of 'functional status' and 'quality of life' in the CC definition.

At the molecular level, the CC definition refers to the importance of energy store depletion, since muscle wasting relies on a negative protein and energy balance. Several biochemical assays are available for analyzing such impairments. The most reliable method for measuring protein metabolism is the analysis of protein turnover rates by means of radioactive or stable isotopic tracers [8]; unfortunately, this technique is costly and not feasible to perform in most laboratories. However, it is currently possible to measure the protein synthetic rates without the use of radioactive isotopes, which reduces the costs and other practical limitations [9]. In the last decade, the practice of reporting mRNA levels corresponding to muscle specific ubiquitin ligases such as MuRF1 or atrogin-1, as a surrogate for muscle proteolysis has gained in popularity, even if the reliability of this method has been questioned [10]. As for the energetic state of skeletal muscle, intracellular ATP content using bioluminescence has proven to be a reliable assay. Results indicate that the reduction of ATP associates with mitochondrial abnormalities in experimental CC [11].

Additional important endpoints that appear in the CC definition are reduced food intake (anorexia) and body weight. Such measures are easily performed in experimental settings and should be included in any cachexia study, if not in the primary figures, at least as supplemental data. The kinetics of body weight change is an effective parameter for the detection of early *versus* late stages of cachexia. However, tumor burden in experimental CC is often substantial and masks the real body weight modification (see section on limitations below). Moreover, muscle mass is difficult to measure during the tumor growth phase, since tissue weight can only be assessed at the end of the experimental period, when the animal has been sacrificed. A time course analysis of body composition is a reasonable approach to monitor alterations of lean muscle and fat stores in tumor bearing animals. Indeed, DEXA (dual energy x-ray absorptiometry)

scanning was effectively used to detect fat and lean mass changes in rats with MCA sarcomas and the effectiveness of a ghrelin analog [12]. Unfortunately, DEXA is not widely used in pre-clinical CC studies, mainly due to the high cost of the instrument. An alternatively, less expensive technology to assess body composition in animal models is BIA (Body Impedance Assessment) [13]. However, from experience in our own laboratory with the Lewis lung carcinoma (LLC) model, we find that BIA contains its own technical challenges that limit the reliability of the data (unpublished observation).

Beyond these above-mentioned parameters used to define/diagnose CC in experimental conditions, it is common to focus on a single signaling pathway whose relevance in animals can be assessed by performing gain/loss of function experiments and/or by modulating the pathway with pharmacological agents. However, often conclusions are reached that have not been validated in humans [14]. Johns and co-workers emphasized this point in their study when they showed that muscle fiber CSA was lower in patients suffering from a reduction of lean mass, though not in those patients having body weight loss alone. When analyzing molecular alterations, they further showed that CC was directly correlated with SMAD and autophagy related protein expression, while no differences were found in activities of NF-κB or STAT3, likely suggesting that such signaling pathways described in preclinical models reflect only a subset of patients with CC.

Limitations of CC experimental systems

The lack of complete association between experimental and clinical data likely relates to the fact that not all tumors (either experimental or human) impinge their effects on the host in the same manner. Beyond the mere heterogeneity of cancer cells and individuals, the low translatability from experimental systems to clinical CC are likely to result from limitations intrinsic to the models themselves.

Most of the well-established experimental CC models require the injections of cancer cells previously obtained from donor animals, such as in the case of the Colon-26 (C-26) model, where tumors were generated by exposing mice to carcinogenic agents [15], and therefore lack the 'natural' development of the tumor and genetic characterization seen in patients [16]. However, developing genetically engineered models of CC require greater laboratory resources, and because of the tumor onset, generally more time to incur cachexia. In addition, such models will likely also exhibit greater variability in terms of the heterogeneity of the tumors and cachexia occurrence, together limiting the possibility to perform properly powered intervention studies.

Cancer cells from experimental tumors are commonly injected ectopically into rodents, either subcutaneously or intramuscularly, regardless of tumor origin. Orthotopic injection is normally excluded for technical reasons, since the surgical procedure required for injecting the cells complicates the system and adds variables reducing the reproducibility of the model. However, orthotopic mouse models are available [17] and are certainly useful for studying the natural progression of cancer, but it remains unclear whether

such systems will ultimately be useful for modeling CC. Ectopic cell injections themselves comes with their own caveats. A major limitation is that often, the growth of the tumor outpaces the development of cachexia. Tumor size is a relevant issue in the comparison between experimental and human CC. Indeed, tumor-host competition and metabolic adaptations associated with tumor burden become significant [18] and potentially represent the main cause of weight loss, distinct from other mechanisms characteristic of the human syndrome. Beyond mechanistic reasons, experimental animals bearing large tumors (in some cases tumor mass exceeds 30% of animal body weight, see [19]) generate ethical and practical concerns for animal care, limiting the duration of experiments for intervention studies.

As a basic rule, when choosing an experimental model, it is preferable to use cancer cells that induce cachexia with the smallest tumor burden. However, even with small tumors, the window for testing therapeutic compounds can be limited. As an example, in our experience, C-26 tumors induce 20 to 30% body weight loss in mice with a tumor mass that accounts for about 2% of body weight (see below for further details). However, body weight loss mainly occurs during the last 4-5 days, in parallel with severe anorexia, thus limiting the time for effective interventions. Such a short time frame is particularly limiting, as has been previously discussed [20], since the compounds being tested need to exert substantial and rapid effects to reach efficacy. Another drawback of short-term treatments is the inability to recognize drug tolerance effects or adverse events induced by chronic administration of the compound, or the drug effect on tumor growth. The rapid appearance and progression of CC in experimental models therefore limits intervention studies and complicates interpretations for whether the treatment is palliative or truly targeting the underlying pathogenetic mechanisms.

Another factor that is clearly distinct between experimental and human CC is the age of the hosts. Preclinical models make use of rodents generally aged from 4 to 12 weeks that approximately correspond to adolescent individuals, and not reflective of the majority of human cancers, which predominantly appear in more elderly subjects. Such discrepancy could generate potential confusion in the interpretation of drug effectiveness, since in young growing animals the contribution to homeostatic processes of synthetic and degradative pathways differs from older animals. However, a recent report argued otherwise, showing in a C-26 model that both phenotypical and molecular mechanisms underlying cachexia were similar in young (eight-week-old) and adult (12-month-old) mice [21]. Clearly, more studies will be required to ascertain whether similar results can be achieved in the other established models of CC.

As previously stated cachexia is a potential determinant of survival in cancer patients, as well as metastatic spreading, the latter being likely the most relevant factor. Unfortunately, the majority of cancer cells used to induce cachexia, distinctly from human tumors, do not metastasize significantly, possibly due to the ectopic injection and, more probably, to the relatively short experimental time period related to the model. A good example is the LLC tumor, where about 20% loss of carcass weight (body minus tumor weight) is typically reached in our laboratory by 15 days after intramuscular injection of tumor cells and 28

days after subcutaneous injection (see below for further details). Metastases are easily detectable in the latter setting and less frequent in the former (see Figure 1), suggesting that time is the limiting factor for LLC cell dissemination. LLC cells have also been used in specific studies aimed to investigate metastatic spreading, comparing the effect of subcutaneous versus intravenous injection [22]. This is an important consideration when performing intervention studies since the effectiveness of a therapy is often measured by animal survival. In the case of models where metastasis occurs, care must be taken to demonstrate that the efficacy of the CC intervention itself is not due to impaired cancer cell spreading.

The LLC example raises another notable issue in experimental CC, namely the lack of guidelines and consensus on the use of experimental models. On the one hand it is normal that each research laboratory should be capable of setting up their own system that meets their specific experimental needs, but on the other hand it is important to consider that the same cancer cells behave differently when conditions are not standardized from laboratory to laboratory. It is likely that cancer cells are similar among different laboratories, but it is equally probable that genetic drift, passage number, inoculation site, and injection procedures, together, affect the onset and degree of CC in animals. For example, intraperitoneal injection of C-26 tumor cells induced anorexia, whereas the subcutaneous injection of the same cells will not, although both lead to severe loss of muscle and adipose tissue mass [23]. To further exemplify the subtle features of the C-26 model, it was reported that even if experimental conditions are fixed in a laboratory, such as sex, age, source of cells, number of tumor cells injected, and injected site, simply changing the storage conditions of the cells or using the cells at advanced passage numbers is sufficient to alter IL-6 serum levels [24]. This again argues for some form of standardization of the model. Such differences will likely also impinge the ability to obtain reproducible results from laboratory to laboratory. An example of this disparity comes in the exploration of the proposed role of the transcription factor NF-κB, that in C-26 bearing mice have been shown to be required or not required for CC [25] [26]. Although the methodology to test the requirement of NF-κB was different in these studies (pharmacological inhibitor versus electroporation of dominant negative proteins), nevertheless, greater consistency from study to study using similar models will be improved by providing more details regarding the characteristics of the tumor cells and procedure used to induce cachexia, as well as the strain, sex, and age of recipient animals. Additional features specific to a particular model could also help, such as disclosing the serum levels of IL-6 in the C-26 model, which have been reported to vary from 250 [27] up to 5000 pg/ml [28]. Although one should keep in mind that heterogeneity is a characteristic of human neoplasm, there remains a benefit when working with experimental systems to duplicate experimental procedures as best as can be achieved from laboratory to laboratory.

Chemotherapy

Anti-tumor drugs in experimental models are seldom if ever used, as opposed to the majority of cancer patients that undergo chemotherapy as soon as the cancer is diagnosed, even if the treatment is palliative. The use of experimental models of CC that do not include anti-cancer treatments generates two main issues. Firstly, the results obtained experimentally is difficult to translate to humans since anti-cancer drug administration can interfere with CC, either worsening it, or, while arresting tumor growth, delaying its onset. Secondly, as described above, experimental CC is a rapid process, in some cases, within a few days, whereas in cancer patients it is often a more slowly progressive phenomenon, particularly when an effective chemotherapeutic protocol is adopted. An important consideration is that agents that exhibit efficacy in experimental CC might not necessarily succeed in patients given that standard of care often includes a regiment of chemotherapy, which can be a confounding variable especially since some chemotherapeutic compounds have themselves been found to induce wasting [29]. For example, our team has recently shown that in in rats bearing the Yoshida ascites hepatoma AH-130, the administration of Sorafenib, a drug approved for the treatment of kidney and liver tumors, increases animal survival by more than three times with respect to untreated rats, but cachexia persists even with a low tumor burden [30]. Although Sorafenib did not show anti-cachexia effect, by extending survival, it increases the therapeutic window for testing additional potential anti-cachexia compounds, allowing the evaluation of drug effectiveness in a reliable setting. As a proof of concept, the same anti-cachexia treatment (formoterol plus megestrol acetate) that proved effective in a three week treatment protocol in AH-130-bearing rats receiving Sorafenib obtained positive results in a phase I/II trial of eight week treatment in advanced cancer patients that were pre-treated with surgery, chemotherapy and/or radiotherapy [31].

Alternatively, care should be given in the interpretation of results when using agents whose primary therapeutic indication is not directly relevant to the tumor subtypes used in various CC models. For instance, Sorafenib was originally reported to worsen CC in humans [32]. However, when administered to mice bearing tumors lacking the treatment indication such as the C-26 (colon), the LLC (lung), or the B16 (skin), the anti-neoplastic activity is poor (while it is quite significant in the AH-130-bearing rats; see above), but cachexia is reduced, potentially due to inhibition of both ERK and STAT3 [33]. A parallel can be found in patients with cholangiocarcinoma where the MEK inhibitor Selumetinib stimulated muscle anabolism as a side effect [34]. By contrast, an opposite effect is obtained with chemotherapeutic agents having a high toxicity profile, such as cisplatin, that induces cachexia independently from the presence of the tumor [35]. In conclusion, the use of chemotherapy in any CC model is important since it is more likely to resemble the human scenario, but given the side effects of these agents, care should be taken when making any conclusion related to their anti-cachexia activities. Finding compounds or regiments, such as in the case of exercise training (reviewed in [36]), which was shown to exhibit a parallel anti-cachexia and anti-tumor

activity in rats bearing the Walker 256 carcinoma, will ultimately be the most effective treatments for CC patients.

Experimental tumors

Several cancer cell lines injected in syngeneic animals have been used in order to set up experimental CC models. The scope of this chapter is not to go into details in these models, which have been extensively discussed in several previous reviews that we recommend [37][20][38][39]. A rapid search on PubMed (http://www.ncbi.nlm.nih.gov/pubmed) including the term cachexia and the name of the particular model provided the following number of citations, shown here in parenthesis: C-26 (96), Walker 256 (88), MAC16 (77), AH-130 (69), LLC (61), B16 melanoma (28), Apc Min (16). An incomplete list of available, less used, models includes Yoshida Sarcoma, MCG-101 sarcoma, MCA-sarcoma, EHS chondrosarcomas and peritoneal carcinosis. In general, investigators using these models, which we admittedly include our own laboratory, describe each model as a CC experimental system, without emphasizing that such models are associated with the growth of a neoplasm from a specific tissue. For instance, in the above mentioned study from our group using colon, lung, and skin cancer cells [33], we mentioned the use of three distinct experimental CC models, but have not focused on the potential translational potential to human CC in patients afflicted with these particular tumors. A feature of each of these tumor cell lines that induce cachexia is the release of humoral mediators either from the cancer cells or by the host. Among these, pro-inflammatory cytokines (IL-1, IL-6 and TNF) have been shown to play critical roles in the onset of several CC alterations, including anorexia, and muscle atrophy [40], therefore mimicking the etiology of CC in humans. During the last few years there has been an emphasis to use cancer cells derived from patients that exhibited CC. Although such examples are rare, they include human JHU022 cells that give rise to a head and neck tumors in athymic mice [41] or the MKN-45 human cells, able to develop a gastric cancer in nude mice [42]. Using human cells is advantageous in getting closer to clinical CC features, but working with animals with a compromised immune system questions the appropriateness of the system, as both the innate and adaptive immune systems are likely to contribute to inflammation and immune responses relevant to the onset and progression of cachexia [40].

Comparisons of two established CC models: LLC vs C-26

LLC cells were isolated from a spontaneous tumor in a C57BL/6 mouse (by Margaret Lewis at the Wistar Institute in 1951) and are available for purchase at the American Type Culture Collection (ATCC) cell bank. C-26 tumor was originally chemically induced in BALB/c mice [15] and available at the National Cancer Institute Cell Repository. A detailed characterization of the C-26 model was previously reported [43]. However, the study was performed by subcutaneous grafting tumor fragments from donor mice, a

procedure that is not common among CC laboratories, mostly because as mentioned above, the tumor injection site will differentially affect the presence of CC and muscle wasting in C-26 bearing hosts [23].

Both C-26 and LLC models are easy to manage and cancer cells can be maintained and expanded either *in vitro* with standard adherent cell culture conditions or *in vivo* by means of mouse-to-mouse transplantation. By comparison, C-26 cells are subcutaneously injected into host mice while expanded LLC cells in culture are administered to syngeneic mice by intramuscular injection (Figures 2 and 3). Such conditions are likely to have been empirically chosen based on the cachectic phenotype resulting from these different injection methods within the window of tumor burden. As previously discussed, several molecular alterations serve as potential triggers of cachexia in both models. However, such alterations were not univocally related to a standardized method for scoring CC. As shown in Table 1, we advocate that similar data be provided in any article published with these two CC models to aid readers in the interpretation of the results.

Moreover, the LLC model is often used when CC is modelled in transgenic or knockout mice since the most frequent background used for producing transgenic animals is the C57BL/6 strain, which is syngeneic to LLC cells. In the case the researchers prefer to perform similar studies using the C-26 model, consideration must be given to the time needed to backcross mice into the BALB/c strain in order to obtain successful tumor implantation and a reproducible cachexia phenotype. However, based on our preliminary data, we do not believe extensive backcrossing is a necessity. Results show that even an F2 generation of C57/BL6 mice backcrossed to a BALB/c strain is permissive for C-26 growth, and induction of body weight loss, as compared to pure BALB/c mice in both male and female animals (Figure 4). Thus, it should be possible to obtain data from transgenic mice using both established CC models.

Genetic engineered mouse models (GEMMs)

As broadly discussed above, experimental CC in syngeneic mice has the main drawback that it is an 'acute' event that can be circumvented, partially at least, by adding a chemotherapy regimen (see above). In order to better resemble the natural history of the tumor and to be able to highlight the early alterations that drive the onset of cachexia, genetically engineered mouse cancer models originally generated with a focus on cancer growth have then been adopted to study CC. Such models overcome at least two weaknesses of transplantable tumors: the ectopic localization and the high growth rate. The resulting experimental tumors are more representative of human disease. In addition, the reduced growth rate allows longer prospective intervention studies.

The best characterized GEMM model of CC is the Apc Min/+ mouse, which was introduced by the Carson laboratory (18 papers support the use of the model with a detailed description included [44]). The acronym specifies that these mice bear a heterozygous mutation in the APC gene, sufficient to determine the appearance of intestinal polyps as soon as 4 weeks after birth. CC has an average onset 10 weeks,

which makes this model suitable for intervention studies. Another point of contact of the Apc Min/+ model with human CC is that, similarly to C-26 hosts, the wasting process depends on IL-6, one of the most promising cytokine to target in CC [45]. IL-6 plasma levels in Apc Min/+ mice are elevated (about 30 pg/ml, [44], however, C-26-bearing mice show 10 to 100 fold further increase (see above), likely accounting for the differences in the rapid cachexia progression. Less characterized GEMMS include inhibin, α-subunit knockout mice developing Sertoli and granulosa cell tumors [46], the very complex Pdx1-cre;LSL-$Kras^{G12D}$;INK4a/$arf^{fl/fl}$ mice bearing pancreatic cancers [47] and the SV40 large T induced hepatocellular carcinoma [48], have all been recently tested for their usefulness as CC models [49].

Although the Apc Min/+ mouse model seems to fit better with human CC than the systems using injected cancer cells, such a feature cannot be directly extended to the other afore-mentioned models before an in depth characterization is performed in these GEMMs. Further limitations to the use of genetic models arise from the considerable costs required for their maintenance and breeding efforts needed to generate sufficient numbers with the correct genotype for experimental analysis. This might be particularly cumbersome when setting up drug studies where large cohorts of animals are required. Lastly, in order to get closer to human CC, the adoption of a chemotherapy regimen is advisable even for GEMMs.

Conclusion

The described scenario of preclinical models for the study of CC is not static and is rapidly evolving as CC still represents an unmet clinical need. In the view of being able to define anti-CC effectiveness in experimental settings, we view that a consensus should be reached in order to standardize across laboratories the outcome measures for scoring CC, rather than defining the best cancer cells to use and the related procedures to perform. The inclusion of chemotherapy and the use of GEMMs will allow a more reliable evaluation of prospective drugs in terms of duration of the effective anti-cachexia activity, prognosis of the underlying disease, and survival. Several critical points have been discussed in this chapter, from the choice of the model to the types of experimental conditions adopted, and of main importance, the selection of appropriate outcome measures. Last but not least, mouse genetic backgrounds, as well as experimentation costs are frequently decisions that need to be made when considering the choice of models. We recognize that no one model is ideal, and the choice should be driven by the specific questions and endpoints the researcher desires to address. We also advocate that details of the experimental conditions should be clearly disclosed in any publications, which we feel will be useful to the scientific community in identifying therapeutic targets for future clinical trials.

Tables

*Table 1: effect of tumor growth on several cachexia related parameters. * p< 0,05 vs C; ** p< 0,01 vs C; *** p< 0,001 vs C;*

Group	**C (n=5)**	**LLC (n=8)**	**C (n=8)**	**C26 (n=9)**
Mouse strain, sex, age	C57BL/6 Male, 7 week	C57BL/6 Male, 7 week	BALB/c Male, 6 week	BALB/c Male, 6 week
Tumor mass (g)	-	5,42 ± 0,56	-	0,28 ± 0,06
Mean % Δ body weight (- tumor)	+3,73	-16,57	+ 9,46	- 14,74
Gastrocnemius (mg/g)	11,40 ± 0,64	8,35 ± 0,61 ***	11,36 ± 0,75	9,27 ± 0,73 **
IL-6 serum levels (pg/ml; or alternative inflammation marker)	8,77 ± 6,18	172,1 ± 41,79 *	34,1 ± 24,6	4388,1 ± 1943,5 **
Cumulative food intake (g)	44,65	30,14	47,75	37,22
Grip strength (N; or alternative functional assay)	1,19 ± 0,04	0,83 ± 0,16 **	1,32 ±0,11	1,09 ± 0,18 **

Figures

Figure 1

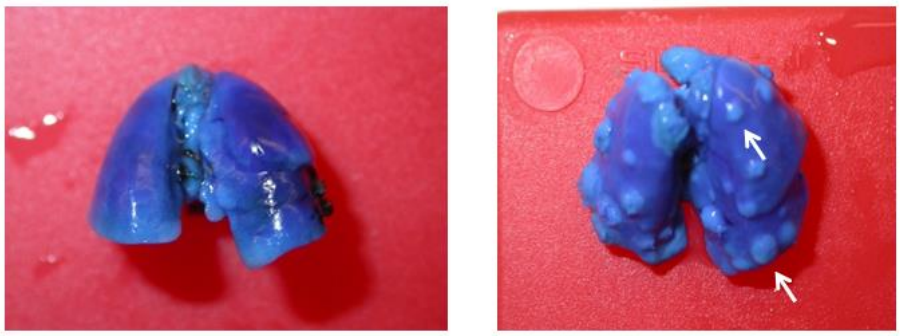

Figure 1: India ink stained lungs from LLC hosts 15 days after i.m. injection (left panel) or 28 days after s.c. injection (right panel). Metastases (indicated by arrows) are recognized by the pale staining.

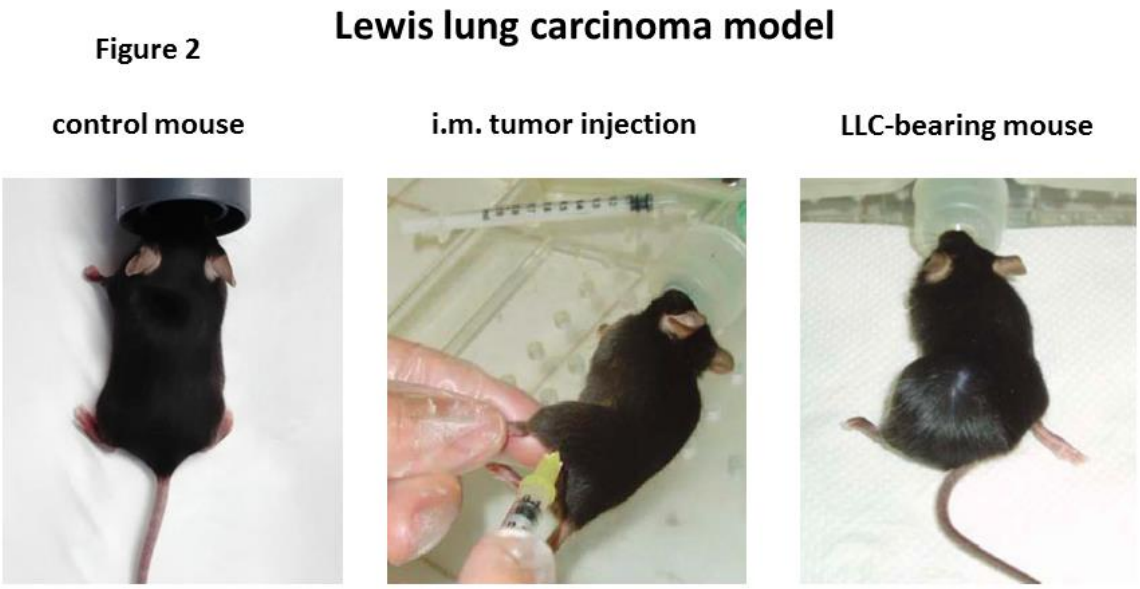

Figure 2: representative images of control and LLC-bearing mice and of tumor injection procedure. The large tumor obtained 14 days after intramuscularly injecting $5*10^5$ LLC cells is clearly detectable in the left hindlimb.

Figure 3

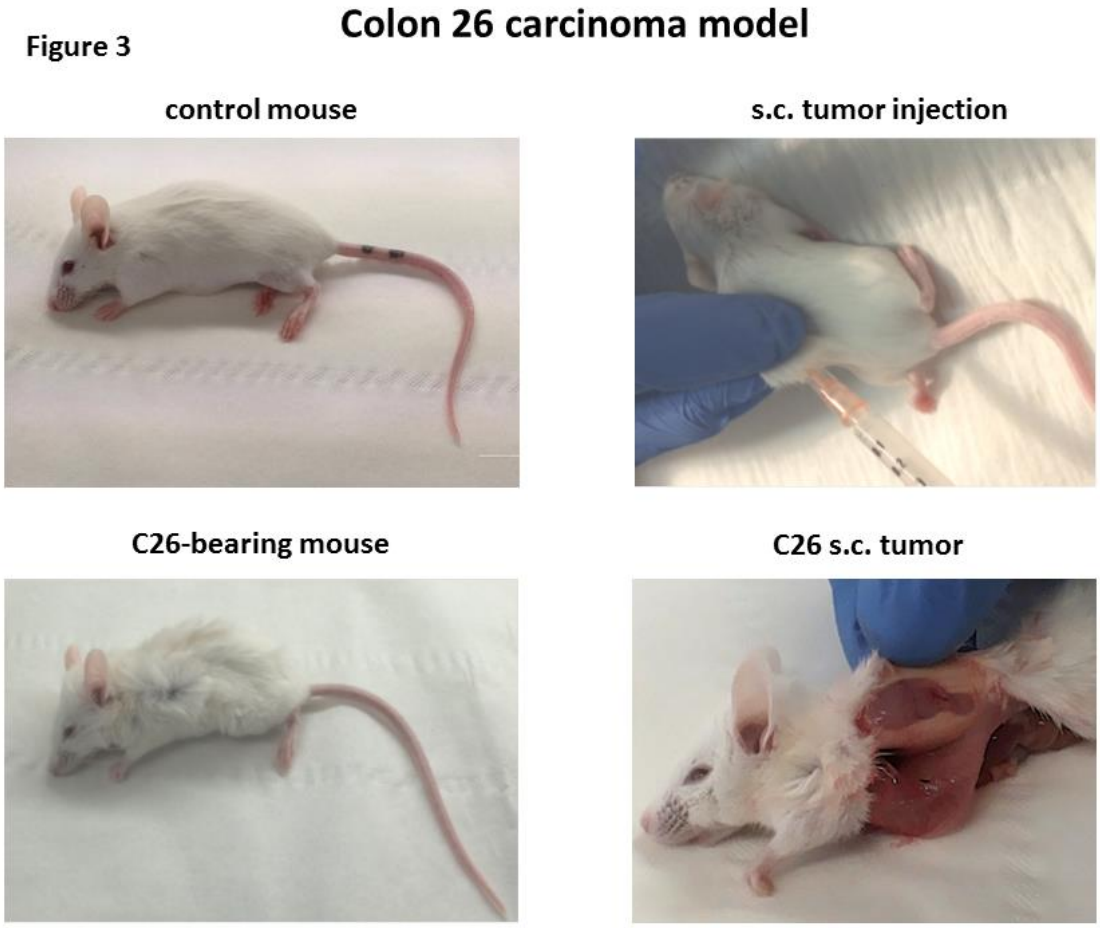

Figure 3: representative images of control and C26-bearing mice and of tumor injection procedure. The small tumor obtained 14 days after subcutaneously injecting $5*10^5$ C26 cells is not evident at visual inspection while is clearly detectable at palpation or necropsy.

Figure 4

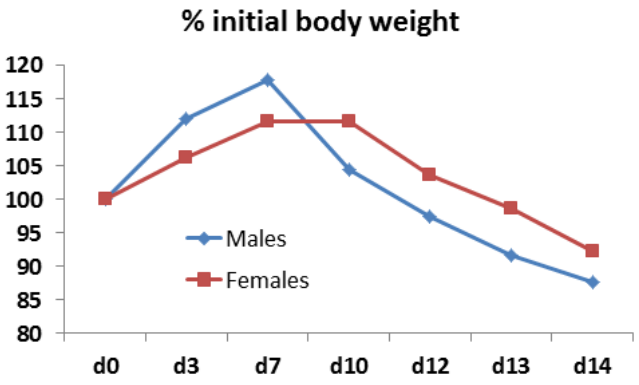

Figure 4: body weight change in F2 backcrossing generation of C57BL/6 to BALB/c mice injected with $5*10^5$ C26 cells.

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