## A questionnaire for eating-related distress in patients with cancer cachexia: Preliminary findings of reliability and validity analysis

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### Background
Cancer cachexia is one of causes of anorexia and reduced food intake in patients with advanced cancer. It is linked to deteriorations in quality of life and survival. A large number of patients have not only physical symptoms and disorders, but also psychosocial distress due to cancer cachexia. Eating-related distress is one type of psychosocial distress, and the alleviation of eating-related distress is a key issue in palliative and supportive care. However, there have been no validated tools for measuring eating-related distress among patients with advanced cancer. The aim of the present study is to investigate the potential measurement properties of a questionnaire for eating-related distress.

### Methods
A questionnaire survey was conducted. An exploratory factor analysis for factorial validity was performed, and Cronbach's α for internal consistency was calculated. Patients were categorized into two groups using the international consensus of cancer cachexia classification. The total scores of each factor and all items were calculated, and comparisons were performed for known-group validity. Statistical correlation analysis for concurrent validity, convergent validity, and discriminant validity was performed using Pearson's product moment correlation coefficient.

### Results
In the study, 140 out of 147 patients responded. Three factors were identified as follows: (factor 1) difficulties in coping with eating problems, (factor 2) eating-related distress, and (factor 3) conflict-related distress between patients and family members. The values of Cronbach's α were 0.90, 0.89, and 0.86, respectively. Patients were classified into two groups: Non-cachexia/Pre-cachexia ($n = 57$) and Cachexia/Refractory cachexia ($n = 83$). There were significant differences in the total scores of each factor and all items: (factor 1) 7.5 vs. 11.0, $p < 0.001$; (factor 2) 8.0 vs. 13.0, $p < 0.001$; (factor 3) 5.0 vs. 10.0, $p < 0.001$; (all items) 20.0 vs. 35.0, $p < 0.001$, respectively. The total scores of each factor and all items significantly correlated with the Edmonton Symptom Assessment System-revised and the nutrition impact symptoms: 0.62 ($p < 0.001$) and 0.63 ($p < 0.001$), respectively. Scaling success rates were 100% in factor 1, 2, and 3.

### Conclusion
The questionnaire appears to be useful in patients with cancer cachexia.

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## Colon carcinoma (C26) conditioned medium alters the β2-adrenergic response in skeletal muscle: possible role in cancer cachexia-induced muscle wasting

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Cancer-associated cachexia consists in a severe loss of skeletal muscle mass and functionality and affects most of the cancer patients. Tumor communicates with the host tissues through the induction of several cytokines, such as Tumor Necrosis Factor α (TNFα), Interferon γ (IFNγ), Leukemia Inhibitory Factor (LIF), and Interleukin 6 (IL-6), leading to increased energy expenditure and negative energy balance.

The Gαs-coupled β2-adrenergic receptor (β2AR) is a major regulator of skeletal muscle metabolism in both physiological and pathological conditions through the activation of the cAMP/PKA/CREB axis and consequent induction of several genes, including PGC-1α, thus counteracting protein degradation, regulating neuromuscular junction (NMJ) maintenance and mitochondrial biogenesis.

 

Skeletal muscles of cachectic mice display impairment of oxidative metabolism, defective neuromuscular junction integrity, and muscle atrophy, suggesting the presence of dysfunctional adrenergic responsiveness in this tissue. Indeed, the adrenergic-dependent PGC-1α induction is attenuated in C2C12 myotubes and myoblasts pre-treated with the colon carcinoma (C26) tumor conditioned medium (TCM). Consistently, C2C12 myoblast cell exposure to C26 TCM attenuates the adrenergic-dependent activation of the cAMP/PKA/CREB axis. Since the expression of PDE4B phosphodiesterase is increased in C26 TCM-treated C2C12 and in skeletal muscle of cachectic mice, we raise the hypothesis that it may mediate skeletal muscle adrenergic resistance. Indeed, treatment with the phosphodiesterase PDE4 inhibitor rolipram restores adrenergic signaling in C2C12 exposed to C26 TCM, suggesting that PDE4B could mediate an adrenergic resistance state in skeletal muscle during cancer cachexia.

## Dietary Walnuts Effect on the Gut Microbiome of Cachectic Tumor-Bearing Rats

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Cachexia is often associated with a cancer diagnosis however, to date, there are no medical interventions available to reverse cachexia. Alleviating cancer-induced cachexia could increase the likelihood of favorable outcomes, therefore our lab focuses on diet as a potential therapy to slow the development of cancer cachexia. Several studies have investigated the relationship between the gut microbiome and cancer-induced cachexia. We showed that walnuts promote probiotic gut bacteria in non-tumor-bearing rats. The goal of this study was to test the hypothesis that dietary walnut consumption could alter the gut microbiome of cachectic tumor-bearing animals. Fecal samples from Fisher 344 rats bearing one of two cachectic promoting transplantable tumor lines (Ward colon carcinoma and MCA sarcoma) were collected after the animals had developed cachexia. Two non-tumor-bearing, control groups were matched to the tumor-bearing animals: one group ad lib fed (n = 33) and the second group (n = 33) calorically paired to the tumor-bearing rats so they developed cachexia without the tumor. Both non-tumor-bearing groups were sham-operated. The tumor-bearing and non-tumor-bearing groups were fed one of two semi-purified diets: walnut diet (11% by weight ground walnuts) and control diet (protein, fat, carbohydrate, and fiber in the walnuts were matched). Diets were fed three weeks before subcutaneous tumor implantation and were continued until sacrifice. Fecal bacteria species were identified using 16sRNA microbiome analysis. Benjamini-Hochberg false detection rate was used to determine statistical differences. No difference in alpha diversity among the cachectic tumor-bearing and both non-tumor-bearing control groups was observed. The relative abundance of *R flavefaciens* and *T Turicibacter* were significantly increased in the non-tumor-bearing cachectic animals compared to the cachectic tumor-bearing animals. Adding walnuts to the diet promoted greater alpha diversity and significantly increased the relative abundance of *T Turicibacter* and *R Oscillospira*, but these changes were not conferred to the walnut-fed tumor-bearing animals. Also, tumor growth and development of cachexia were not altered by dietary walnuts. In summary, adding walnuts to the diet of the cachectic tumor-bearing rats did not significantly alter the gut microbiome or slow the development of cachexia, suggesting the effects of bearing a tumor are not easily overcome by diet.

## The acute myeloid leukemia '7+3' chemotherapy induction regimen induces cachexia and impairs physical activity in healthy mice

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Patients with haematological malignancies including acute myeloid leukemia (AML) are susceptible to cachexia, muscle wasting and reduced physical activity (1). The leading intervention against acute myeloid leukemia (AML) is the '7+3' chemotherapy induction regimen (CIR) using cytarabine and anthracycline, respectively, to induce remission. Although effective against AML, CIR also impacts healthy tissues such as skeletal muscle which may independently drive cachexia. Recently, clinical studies have highlighted the importance of maintaining body mass and composition in the AML setting, where positive treatment outcomes, patient suitability for curative allograft therapy and lower

mortality rates are associated with higher lean mass pre- and post-CIR (2). We aimed to investigate the effect of the AML '7+3' CIR on body composition indices and physical activity.

12-week old male Balb/C mice (n = 40) received the CIR via intraperitoneal injections with daunorubicin (1.7 mg/kg) administered from days 1–3 and cytarabine (33.2 mg/kg) administered from days 1–7. The control group (VEH) received a 0.9% saline vehicle. Animals were housed in Promethion® metabolic cages for the 7 day treatment period, where running wheels were either locked and animals were considered sedentary (SED) or were unlocked and animals were considered active (ACT).

CIR administration induced cachexia, irrespective of activity status, where body mass was reduced by ~12% from pre-treatment. Lean and fat mass were reduced by CIR administration, while fat mass loss was exacerbated in active VEH and CIR-treated compared to their sedentary controls. CIR administration reduced muscle and organ mass irrespective of activity status. Physical activity and energy expenditure were reduced in both sedentary and active CIR-treated mice.

Our study demonstrates that the AML '7+3' CIR induces cachexia, as observed through the loss of body mass and body composition indices, and voluntary physical activity. As such, further research is required to investigate the underlying mechanisms and evaluate potential therapeutic strategies to protect against cachexia induction from the AML '7+3' CIR.

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## Single-cell expression landscape of cachexia-inducing factors in pancreatic cancers

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Cancer cachexia is a syndrome characterized by skeletal muscle wasting, leading to a significant weight loss that impacts patient morbidity and survival. The action of cachexia-inducing factors (CIFs) secreted by cancer cells and cells within the tumor microenvironment contributes to muscle wasting. Pancreatic ductal adenocarcinoma (PDAC), the cancer type with the highest cachexia prevalence, demonstrates 5–20% neoplastic cellularity and high leukocyte infiltration. However, it remains unclear which cell types in PDAC (cancer cells or cells of the tumor microenvironment) produce these CIFs. Besides, the extent to which these cells express CIFs needs further analyses. Our objective was to profile tumor-infiltrating immune cells and comprehensively characterize the single-cell expression landscape of CIFs in PDAC. We first used gene expression profiles to assess immune cell type abundance from bulk PDAC and normal tissues (n = 150 and n = 165, respectively) using digital cytometry (CibersortX). We then determined the gene expression profile of 36 CIFs in 57,530 pancreatic cells using single-cell RNA sequencing (scRNA-Seq) data from PDAC and normal-like pancreatic tissues (n = 27 and n = 14, respectively). We also used scRNA-Seq data to analyze the expression profile of genes encoding secreted proteins. The reads were processed using the Cell Ranger pipeline, and the data from raw counts were processed using the Seurat package in R. Next, cell clusters and marker genes were visualized using tSNE and UMAP dimensional reduction plots. We found that low purity tumors present high infiltration rates of CD8+ T cells ($p < 0.0001$), which may contribute to cachexia development. The expression profiles of CIFs revealed IL8, IL18, and IL1B highly and specifically expressed by macrophages and CD40LG by T cells, whereas all cell types expressed CCL2, VGFA, and TGFB1. Except for fibroblasts, all cell types upregulate secretome genes involved in proteolysis, which may ultimately contribute to the worse outcomes in PDAC patients with cachexia. Our results represent a biological dimension of tumor-infiltrating immune cells and identify a cell-type-specific expression profile of secreted elements, including CIFs that are potentially useful to explain why PDAC patients are more prone to develop cachexia.

## Creatine supplementation attenuate muscle atrophy in Walker-256 tumor-bearing rats without change tumor microenvironment

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### Background

The creatine/creatine kinase system plays a key role in cellular energy buffering and transport in skeletal

 

muscle. Studies have demonstrated that creatine deficient muscle (creatine free diet or creatine transportation and synthesis knockout mice) are characterized by muscle atrophy, decreased force production, decreased ATP content and mitochondrial dysfunction. In contrast, creatine supplementation has demonstrated to reducing body mass loss in diseases characterized by muscle wasting disorders such as Duchenne dystrophy, myasthenia gravis, amyotrophic lateral sclerosis and immobilizations. However, the protective effects of creatine supplementation in cancer cachexia and its possible interaction with tumors are poorly investigated. The aim of this study was to analyze the effect of creatine supplementation on muscle and tumor microenvironment.

**Methods**
Thirty-two male Wistar rats were randomly allocated in one of three groups (n = 8): control (C), tumor-bearing (T) and tumor-bearing supplemented with creatine (TCr). Creatine (8 g/L) was added in drinking water for TCr group for 21 days. Tumor Walker-256 cells ($7.0 \times 10^7$ cells in 0.5 ml of PBS) were implanted in T and TCr groups after 11 days of experiment. At the end of 21 days, animals were weighed, anesthetized, euthanized for tissue analyses.

**Results**
Tumor growth promoted weight loss and muscle atrophy. In addition, tumor-bearing rats presented increased plasma inflammatory cytokines levels (1.5 TNF-α and 3.5 IL-6 fold higher than C group), muscle lipid peroxidation and increase protein expression of murf-1(∼49%) and atrogin-1(∼38%) compared to C group. Creatine supplementation protected tumor-bearing rats against body weight loss and skeletal muscle atrophy. This protection was accompanied by attenuated plasma inflammatory cytokine levels (∼39% TNF-α, ∼60% IL-6; TxTCr) and protein content of murf-1 and atrogin-1 (∼57% for both; TxTCr). Creatine supplementation did not accelerate tumor growth or increased tumor size. The Scarff-Bloom-Richardson histological grade analysis demonstrated that the tumors presented no changes in tubule formation, nuclear pleomorphism or in mitotic rates between creatine supplemented and non-supplemented groups. Also, creatine supplementation did not change apoptosis, cell proliferation, thickness of tumor capsule and collagen deposition.

**Conclusion**
Creatine supplementation mitigates cancer-induced cachexia characteristics such as body weight loss and muscle atrophy by attenuating persistent inflammation and decreasing proteolysis signaling. Importantly, creatine supplementation is safe because did not change tumor Walker-256 parameters of aggressiveness.

## SRF-mediated mechanotransduction is essential for the response to exercise in cancer patients and animal models

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Exercise training is employed as an anti-cachexia treatment in clinical trials, due to its capacity to regulate both the metabolic disturbances and the severe muscle wasting which characterize cachexia. Most of the molecular mechanisms underlying exercise beneficial effects are still unknown. Serum Response Factor (SRF) is a transcription factor of pivotal importance for muscle homeostasis, acting as a mechanosensor depending on muscle contraction. We show that cancer patients have reduced SRF activity associated to cachexia, but exercise increases SRF expression even in this condition, likely favoring the rescue of SRF transcriptional activity. To address this issue, we exploited a pre-clinical model of cancer cachexia (C26-bearing mice), with a subset of the mice exercised by wheel running. We found that exercise rescues SRF expression in a dose-dependent manner, while also counteracting cachexia. Furthermore, exercise has no longer effect in SRF-KO mice showing that SRF is necessary for exercise effectiveness. SRF transcriptional activity promotes the expression of several target genes, including pro-myogenic factors as well as IL-6 and IL-4. The latter is sufficient to counteract the negative effects of tumor-derived factors on muscle cells. We also show that exercise promotes the downregulation of Pax7 and, thus, the

incorporation of nuclei in muscle fibers. Finally, we show *in vitro* that the purely mechanical stimulation of myotubes mimics exercise (including SRF activation, IL-4 secretion and myoblast incorporation into myotubes) being sufficient to counteract the negative effects of tumor-derived factors. Our findings highlight a model whereby the mechanical stimulation of the musculature, through the essential activation of the SRF pathway, has paracrine/endocrine effects, consisting of IL-6 and IL-4 secretion by the muscle itself. These two cytokines have multiple, positive effects on both the muscle fibers (or myotubes, *in vitro*) and the activated myoblasts: protein degradation is diminished in the muscle fibers, whilst myoblast are recruited to the muscle fibers contributing to maintain muscle homeostasis in the presence of cachectic factors. We propose the importance of the mechanical stimulation, induced by exercise or other means, for the regulation of muscle homeostasis and we propose that IL-4 treatment may mimic the beneficial effects of exercise.

## Prevalence of Low Testosterone in Pancreatic Ductal Adenocarcinoma Patients

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### Background
Patients with pancreatic ductal adenocarcinoma (PDA) have a poor prognosis and commonly experience the cachexia wasting syndrome. In males, symptoms of testosterone deficiency are similar to those reported with cachexia, including fatigue, weakness, and decreased lean muscle mass. Despite the similarity in symptoms, a recent retrospective review of 1,566 male pancreatic cancer patients identified only 2.2% who had undergone serum testing for testosterone deficiency.

### Methods
Serum samples collected from 2012–2020 were obtained from a cohort of 89 male PDA patients enrolled in an institutional prospective biospecimen repository at a National Cancer Institute-designated comprehensive cancer center. Demographic and clinicopathologic variables were abstracted from the medical record and total testosterone was measured by ELISA. Per the American Urological Association guidelines, low testosterone was defined $<300$ ng/dL. Comparisons between groups were made by Chi-squared or Fisher's exact tests for categorical variables and Wilcoxon rank sum tests were used to compare continuous variables.

### Results
The majority of patients (69.7%) had documented complaints consistent with low testosterone in their medical records prior to their serum draw, with fatigue and/or weakness the most common symptom (61.8%). Forty-four of 89 patients (49.4%) were found to have testosterone levels $<300$ ng/dL.

Patients with low serum testosterone were not more likely to report any symptom of low testosterone (low: 77.3%, normal: 62.2%, $p=0.12$) or more likely to have documented fatigue or weakness (low: 65.9%, normal: 57.8%, $p=0.43$). Patients with low serum testosterone were of similar age (low median: 66, normal median: 65, $p=0.67$) and were no more likely to be obese (low: 43.2%, normal: 33.3%, $p=0.34$), have diabetes (low: 47.7%, normal: 37.8%, $p=0.34$), or have heart disease (low: 63.6%, normal: 77.8%, $p=0.14$). For the 49 patients with an Eastern Cooperative Oncology Group score, there was no difference between groups ($p=0.57$).

### Conclusion
Patients with PDA are rarely screened for low testosterone despite commonly reporting symptoms associated with testosterone deficiency. Because testosterone replacement therapy may improve symptom burden, male pancreatic cancer patients with symptoms consistent with low testosterone should be screened. Testing should be conducted without regard to age or other conditions, as increased likelihood of low testosterone was not evidenced.

## Modeling Clearance of Immune Checkpoint Inhibitors as a Potential Biomarker for Drug Resistance in Murine Models of Cancer Cachexia

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Immune checkpoint inhibitors (ICIs) made a dramatic entrance to the clinical scene, demonstrating remarkable activity in patients with a variety of solid tumor malignancies over the past decade. Despite these advances, only a subset of patients treated achieve durable responses from therapy. Clinical pharmacology data published over the past few years has revealed high baseline clearance of the ICI monoclonal antibody (mAb) therapies, though not necessarily decreased drug exposure, and stable or increasing ICI clearance over time are associated with shorter overall survival. Notably, high mAb clearance and shorter survival are also associated with classical clinical features of cancer cachexia. As these relationships linking ICI mAb clearance, cancer cachexia, and response represent a potential key to understanding resistance to ICI therapy, we sought to determine if the observed increase in ICI mAb clearance in patients could be replicated in immune competent murine models of cancer wasting. Our results demonstrate cachectic mice with both Lewis lung carcinoma (LLC) and colon-26 (C26) allografted tumors do in fact display altered pharmacokinetics of anti-PD1 mAbs. Furthermore, FcRn, the neonatal Fc receptor responsible for prolonging circulation of endogenous IgG species and therapeutic IgG mAbs, is downregulated in cachectic tissues. Population pharmacokinetic modelling supports FcRN suppression as a possible mechanism for increased mAb catabolic clearance in cachectic mice. Notably, proper FcRn function in specific immune cell subsets is required for robust anti-tumor T-cell response, supporting a possible mechanistic link between cachexia-mediated changes in mAb pharmacokinetics and reduced patient response to ICI therapy. These data demonstrate the potential utility of immune competent murine tumor models for studying mechanisms linking cachexia, ICI clearance and ICI response. Evaluation of the impact of cachexia burden and efficacy in these mouse models is ongoing.

## Lewis Lung Carcinoma Tumor Growth Rate Is Associated with Early Onset Metabolic and Activity Dysfunction in Male Mice

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### Background
Cachexia induces systemic metabolic disruptions involving the feeding and fasting response. While the Lewis lung carcinoma (LLC) model is widely used to study cancer cachexia, there is considerable heterogeneity in the cachexia exhibited, and many published studies report only pre- or mild cachexia. However, gaps remain in our understanding of what drives the LLC induced cachexia heterogeneity and how this is linked to disrupted whole body metabolism.

### Purpose
We examined the effect of tumor growth and size on cachexia development and associated metabolic dysfunction in a large cohort of LLC injected male mice.

### Methods
Male C57BL/6J (12 wks. age) were injected with $1 \times 10^6$ LLC cells (n = 28) or PBS (n = 23) subcutaneously in the right flank for 25–28 days. Tumor volume and body weight was measured every 5 days throughout the study to calculate growth rate. Fifteen days post tumor inoculation, a subset of mice (PBS n = 10, LLC n = 11) were individually housed in metabolic cages for 5 days.

### Results
We report a wide range of tumor mass at the end of the study (N = 28; 0.39–5.48 g), which coincided with a large variation in bodyweight loss (−20% to 6.2%). Mice with high growth rate large tumors (HLT) (n = 10) exhibited significantly greater bodyweight loss (−7.8%), decreased muscle mass (−17%) and fat mass (−42%) compared to low growth rate small tumors (LST) (n = 9). Tumor mass was associated with early (day 15) decreases in cage activity (r = −0.865). Metabolically, pre-cachectic mice demonstrated glucose intolerance, and dark cycle carbohydrate oxidation (r = 0.701) and respiratory exchange ratio (RER, r = 0.632) values that were significantly associated with body weight loss at the end of the study. Prior to differences in tumor growth, HLT mice exhibited decreased cage activity (−43%) and increased lipid oxidation (53%) compared to LST.

### Discussion
LLC-induced cachexia is linked to tumor growth rate and mass, which is associated with an early disruption to systemic metabolism and decreased physical activity. Low growth rate small tumors do not develop cachexia, thus the tumor growth rate is likely contributing to the cachectic environment.

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## TIMP1 is a biomarker for cachexia in mice with lung cancer

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The cancer-associated cachexia syndrome (CACS) is a systemic metabolic syndrome featuring body weight loss due to skeletal muscle and white adipose tissue wasting. It is suffered by up to 80% of advanced cancer patients, and it is directly responsible for 20% of cancer deaths. In non-small cell lung cancer (NSCLC), approximately 30% to 80% of patients with early and advanced-stage disease are affected. In spite of the large number of patients with cancer suffering from CACS, and all the morbidities associated with it, there is no FDA approved treatment nor early detection biomarker. We have previously identified *LSL-Kras*$^{G12D/+}$; *Lkb1*$^{f/f}$ (KL) genetically engineered mice as a suitable model for human CACS. KL mice spontaneously develop aggressive lung adenocarcinoma following intranasal administration of adenovirus containing Cre recombinase. In order to identify factors that predispose to CACS, we performed RNA sequencing on tumors removed from KL mice with or without CACS. We found that the expression of TIMP1 was greatly increased in CACS as compared to the non-CACS (NCACS) mice (CACS vs NCACS, p-value $<0.03$). We verified that this increase in gene expression corresponded to an increase in protein concentration in tumor lysates and serum at the time of euthanasia (CACS vs NCACS, $p=0.028$ and $p=0.0017$ for tumor and serum respectively). In the serum, the amount of TIMP1 significantly correlated with weight loss ($R^2=0.2$, $p=0.001$). Next, we collected serum from KL mice 5 weeks after induction, a time when tumors have developed but no weight loss has occurred in any mice. There was a significant linear relationship ($R2=0.56$, $p=0.01$) between the level of TIMP1 at this early time point and the degree of weight loss at endpoint. Lastly, we mined a publicly available human database (TCGA) and found that high expression of TIMP1 correlates with decreased survival in humans with NSCLC (5 year survival, high vs low expression, 29% vs 46% respectively, p-score $=0.0086$). We conclude that TIMP1 is a biomarker of CACS in KL mice and future studies are needed to validate this finding in humans with NSCLC.

## Resistance training's ability to prevent cancer-induced muscle atrophy extends beyond anabolic stimulus

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### Background

Among several therapeutic approaches, resistance training (RT) has emerging as a useful tool to counteract cancer-induced cachexia. We and other groups have previously demonstrated RT prevent the loss of muscle mass and function in rodents and humans with cancer cachexia. In healthy animals and humans, RT is a potent stimulator of protein synthesis for muscle hypertrophy because it can evoke the activation mTORC1 signaling, a primary regulator of muscle protein synthesis. Moreover, the mechanisms by which RT protects skeletal muscles to wasting during cancer are still poorly known. The aim of this study is to determine the contribution of RT-mediated mTORC1 signaling activation in cancer cachexia-induced muscle loss.

### Methods

Muscle atrophy was induced by Walker-256 tumor cell injection in rats that were exposed or not to a RT protocol (ladder climbing). The role of anabolic stimulation by RT was investigated by using a mTORC1 inhibitor (rapamycin) in tumor-bearing rats. Skeletal muscle cross sectional area was evaluated for atrophy/hypertrophy determination. Components of mTORC1 and ubiquitin-proteasome pathway were assessed by qRT-PCR or immunoblotting.

### Results

RT prevented muscle atrophy and impaired strength in tumor-bearing rats. In healthy rats, RT promoted anabolic stimulus by increasing phosphorylation of p70S6K (a downstream MTORC1 target) and muscle hypertrophy. However, RT promoted no changes in p70S6K/phospho-p70S6K ratio while prevented muscle atrophy in tumor-bearing rats. In addition, rapamycin treatment did not preclude preventive RT effect on muscle atrophy of tumor-bearing rats. Therefore, we hypothesized that the ability of RT to prevent cancer-induced muscle atrophy is independently of mTORC1/p70S6K activation. Indeed, our data demonstrated that the preventive effect of RT on cancer cachexia-induced muscle atrophy was associated with its capacity to attenuate persistent inflammation, muscle oxidative damage, and proteolysis signaling.

### Conclusion

The preventive role of RT on cancer-induced muscle atrophy extends beyond the anabolic stimulus. The attenuation of persistent inflammation, muscle

oxidative damage, and proteolysis play a key role in RT counteracting muscle atrophy during cancer.

## A Machine Learning study of cachectic and non-cachectic patients undergoing a physical exercise protocol

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Machine learning is a method of artificial intelligence in which the computer learns from experience, without explicit programming. The applications of this computational method are currently on the rise, especially in healthcare, due to the high predictive power. In this study, we applied an unsupervised learning algorithm, k-means, to a biochemical database of 443 patients with and without cancer-related cachexia. With only four features (hemoglobin, albumin, cholesterol and CRP serum levels) and no information about weight, this algorithm created two groups: one with the characteristics of cachexia (CC group) such as lower levels of hemoglobin ($11.5 \pm 2.5$ g/dL) and albumin ($3.13 \pm 0.76$ mg/dL), and higher CRP ($10.4 \pm 3.6$ mg/L); the other group mainly contained non-cachectic patients (CONTROL group). The program was able to identify cachectic patients with a 65.6% recall. With this well-established model, we characterized a different database of 36 patients who underwent a physical exercise protocol for six weeks; biochemical values were obtained three times at different moments during exercise training. We aimed at detecting clinical changes across protocol time. For that, we tracked down changes in group classification of those patients over time and changes in a numerical value, the score, which could show where the patient's biochemical levels fell between the CC and CONTROL groups. The more negative the score, nearer the patient was to the CC group; more positive scores meant being closer to CONTROL values. We concluded that this database contained few cachectic patients, which did not allow us to observe general trends: our program found only 5 to 7 patients who could fit in the CC group at any given time of the protocol. Scores per group (CC, CONTROL and also for cancer-bearing patients) did not show a clear trend change in time. There is, however, the possibility of conducting individual case studies of physical exercise protocols for those patients who have demonstrated some changes over these 6 weeks: three patients initially classified as cachectic changed to the CONTROL group over the course of the study.

## Investigating the role of FFA1 receptor in cancer-associated cachexia

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### Background

Free fatty acid (FFA) receptors FFA1 and FFA4 are promising pharmacological targets for metabolic diseases; nevertheless, their role in cancer cachexia (CC) remains unknown. Herein, the participation of FFA1 receptors in the Lewis lung carcinoma (LLC) mouse model of CC was assessed.

### Methods

LLC cells ($5 \times 10^6$/100 μL of phosphate-buffered saline; PBS), or the same volume of PBS, were injected subcutaneously into the right flank of C57BL/6jUnib male mice (20–15 g; 8–10 weeks old; n = 8/group). Tumor-free control + PBS; LLC + PBS; LLC + GW9508 8 mg/kg (FFA1 agonist; dosed every other day; subcutaneously) were evaluated for locomotor activity, motor coordination, and grip strength at 21 days. Afterward, mice were euthanized and epididymal (epWAT), retroperitoneal (rWAT), and interscapular (isWAT) adipose tissue, gastrocnemius, and tumor mass were collected for further analysis. The serum was collected to evaluate leptin levels. Immunohistochemistry assay was performed to assess FFA1 immunopositivity in isWAT and epWAT. Western blot was performed to detect UCP-1 protein expression in epWAT. Brain glucose uptake was assessed by microPET scanning analysis, through [$^{18}$F]-FDG intravenous administration. The local Ethics Committee (PUCRS/CEUA 7164) approved the experimental protocols.

**Results**
GW9508 treatment rescued cachexia-related behavioral impairments, tumor mass, and overall fat loss. The isWAT, epWAT, and rWAT adipocyte area did not differ among experimental groups; nevertheless, GW9508 increased the frequency of smaller adipocytes compared to the LLC-group. The treatment with GW9508 increased serum leptin levels in CC mice. LLC-cachectic mice displayed an increased FFA1 immunopositivity in isWAT, but not in epWAT. LLC implantation decreased UCP-1 expression in epWAT, but GW9508 failed to restore this parameter. Of note, LLC-cachectic mice displayed an impairment of brain glucose uptake, which was restored by GW9508 in the striatum, cortex, left hypothalamus, thalamus, superior colliculus, and right inferior colliculus.

**Conclusion**
The FFA1 receptors possibly participate in peripheral and central CC alterations. It is tempting to suggest that FFA1 receptor modulation is a promising pharmacological tool for CC management.
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## Tumor microenvironment metabolic regulation through physical training and modulation of antitumor immune response in patients

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A promising cancer treatment is immunotherapy, which stimulates the patient's immune system to fight the disease. However, the hostility of the tumor microenvironment, characterized by hypoxic regions and immunosuppression, proves to be a challenge for that therapy. Aerobic exercise exerts multiple l effects both upon the host's blood vessels and also in the heart and skeletal muscle of patients with ischemic disease. Thus, the hypothesis that exercise can significantly modulate vascular function a and the physiology of solid tumors as well as increase immunotherapy responsiveness is consistent. We investigated whether exercise training could lead to metabolic regulation of the tumor microenvironment, hence modulating antitumor immune response in cancer patients. Twenty-two treatmet naive gastrointestinal cancer patients were divided into sedentary and exercised groups. The exercised group was submitted to a six week, 150 min/week, progressive treadmill protocol. Tumor biopsies from sedentary and exercised patients were collected during surgery. Unpublished data shows that physical exercise modulate pro-inflammatory cytokine expression in the tumor microenvironment in cachetic cancer patients, as shown by IFNγ mRNA expression ($p = 0.0005$) and IL1β mRNA expression ($p = 0.0237$). IHC analyses in adenocarcinomas biopsies from sedentary and trained patients show that exercise increases angioneogenesis markers in peritumoral sites (healthy mucosa inside tumor microenvironment), demonstrated by increased α-smooth muscle actin, CD31, CD34 ($P = 0.007235$, $P = 0.008061$, and $P = 0.008854$, respectively), but not in intra-tumoral sites (tumor cells in the mucosa). These preliminary results indicate that physical training may affect the susceptibility to the antitumor immune response through modulation solid tumor microenvironment.

## Loss of REDD1 prevents chemotherapy-induced muscle atrophy and weakness in mice

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Carboplatin, a platinum-based chemotherapy used clinically to reduce solid tumors, causes an array of side-effects including muscle atrophy and muscle dysfunction. REDD1 (regulated in DNA damage and development 1) is a stress-response protein that represses signaling through the mTOR protein kinase in complex 1 (mTORC1) to block protein synthesis. Our previous work demonstrated that carboplatin-induced muscle wasting and muscle dysfunction occurred in as little as 7 days. In this study, we showed that REDD1 mRNA expression was increased in skeletal muscle of carboplatin-treated mice. To measure the direct effect of carboplatin on muscle cells, C2C12 myotubes were treated with 100 μM carboplatin. Carboplatin decreased myotube diameter in as little as 24 hours with progressive atrophy through 48 hours. REDD1 mRNA expression also increased significantly 12 hours after carboplatin treatment in C2C12 myotubes. Using genetically modified global REDD1 knockout mice ($REDD1^{-/-}$), we have shown that carboplatin-induced loss of muscle mass and muscle dysfunction were prevented in mice lacking REDD1. 8-week old female wild-type and $REDD1^{-/-}$ mice were injected with carboplatin (100 mg/kg) or vehicle and euthanized 7 days later. Wild-type mice lost significant body weight and muscle mass after carboplatin treatment compared to vehicle treated animals. Body weight and muscle mass were maintained in $REDD1^{-/-}$ mice treated with carboplatin. Additionally, carboplatin caused a decrease in forelimb grip strength and

skeletal muscle dysfunction (EDL specific force) which was prevented in REDD1$^{-/-}$ mice. Because REDD1 interferes with protein synthesis through mTORC1 signaling, we analyzed protein translation via puromycin uptake assay in skeletal muscle. Puromycin incorporation was decreased in muscles from REDD1$^{+/+}$ mice and not changed in muscle from REDD1$^{-/-}$ mice treated with carboplatin compared to vehicle. Our data provides in vivo evidence that REDD1 plays a vital role in chemotherapy-induced muscle atrophy and muscle dysfunction through the regulation of protein synthesis pathways.

## Lasting Effects of Folfox Chemotherapy on Whole Body Fatigue and Metabolic Function in Mice

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### Background
While chemotherapy is a first line treatment to mitigate cancer progression, it can also induce muscle atrophy weakness, and whole body metabolic dysfunction. There is growing evidence that adverse effects of chemotherapy extend beyond acute toxicities and can lead to lasting dysfunction, which serves to reduce patient health and life quality. Due to supra-physiological drug dosing, non-physiological treatment regimens and drug specific effects, gaps still remain in our mechanistic understanding of long-term metabolic and functional declines associated with chemotherapy.

### Purpose
We examined the lasting effects of Folfox chemotherapy after repeated cycles of treatment on whole body fatigue and metabolic function in mice.

### Methods
Male and female C57BL/6J mice (B6; N = 30), at 12 weeks of age, were injected with four cycles (1 cycle = 1 injection every other week) of Folfox (FOL; 5-Fluroracil 30 mg/kg, Oxaliplatin 6 mg/kg, and Leucovorin 90 mg/kg) or phosphate buffered saline (PBS; 100ul). Mice were placed in metabolic cages for 5 days, 4wks post the 4$^{th}$ cycle. To establish lasting effects of FOL (recovery period), mice were sacrificed 4wks (n = 14) or 10wks (n = 16) after the 4$^{th}$ cycle. Run to fatigue was examined pre and 4 days prior to sacrifice. Gastrocnemius muscle was used for protein analysis.

### Results
Four cycles of Folfox chemotherapy attenuated body weight gain (PBS:6.1%, FOL:−0.7%) during the treatment period, with no changes in food intake. However, 4 and 10 wks. post chemotherapy body weight changes were not different in PBS and FOL mice. FOL decreased treadmill run time to fatigue at 4 wks. (−23%) and 10wks (−39%) after the last chemotherapy treatment. Cage activity was reduced by FOL 4wks post chemotherapy. Metabolically, systemic carbohydrate oxidation was greater in FOL mice 4wks post, and glucose intolerance was present in FOL mice 10wks post chemotherapy. Muscle AMPK phosphorylation ($p = 0.038$) was induced in FOL mice 4wks post chemotherapy.

### Conclusion
Repeated cycles of FOL chemotherapy can induce lasting deficits in physical function and altered systemic and muscle metabolism in mice.

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## The Association of Cachexia with Head & Neck Cancer Burden and Pathologic Features

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### Background
Head and neck cancer (HNC) is frequently associated with cachexia, characterized by involuntary weight loss, sarcopenia, and malnutrition. In HNC patients, dysphagia and anorexia from obstructive aerodigestive tumors propagates cachexia even further. However, the impact of pathologic features and burden of HNC on cachexia has yet to be investigated. We therefore hypothesize that larger, more aggressive tumors impose greater cachexia severity in HNC patients.

### Methods
A single-institution, retrospective study of adult patients undergoing surgical resection of head and neck carcinoma from 2014–2017 was performed. Patients without 30-day preoperative abdominal CT imaging for skeletal muscle index (SMI, $cm^2/m^2$) measurements were excluded. Patient demographics, comorbidities, nutrition data, and cancer pathology reports were collected. Cachexia was defined as unintentional weight loss >5% over 6 months or >2% with BMI <20 $kg/m^2$. Statistical analyses were performed using 2-sided one-way

Welch's ANOVA or Pearson's $\chi^2$ tests. Significance was determined at $p < 0.05$.

**Results**
The cohort included 125 predominantly white (92.0%), male (75.2%) HNC patients age $59.9 \pm 11.5$ years. Sixty-seven (53.6%) patients had cachexia, twenty (16.0%) of whom were severe (weight loss $\geq$15%). Patients with severe cachexia had larger tumors ($5.5 \pm 2.1$ cm, $p = 0.021$) than patients with mild-to-moderate cachexia (weight loss 5–14.9%; $4.9 \pm 2.1$ cm) or no cachexia ($4.1 \pm 1.9$ cm). Worsening cachexia severity was also associated with lower SMI ($p = 0.004$), BMI ($p = 0.002$), and serum albumin ($p = 0.011$). There was no statistically significant difference between cachexia groups comparing patient age, comorbidities, tumor grade, depth of invasion, nodal metastases, extranodal extension, cancer stage, perineural invasion, or lymphovascular invasion.

**Conclusion**
Tumor burden of HNC patients, but not adverse pathologic features, is associated with greater cachexia severity. Identifying pro-cachectic markers produced by larger tumors could provide a molecular target for anti-cachexia therapies and improve cancer patient outcomes.

## Poor nutritional status and increased symptom burden is related to inflammation, but not hypogonadism, in cancer cachexia

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**Background**
Hypogonadism is common in men with advanced cancer. It is unclear if the hypogonadic condition in cancer cachexia leads to a greater negative impact on body composition, strength, function, symptoms, nutritional status and distress.

**Methods**
This is a cross-sectional, retrospective study of cachectic men referred to a cancer rehabilitation program. Calculated bioavailable testosterone and total testosterone were used to identify hypogonadism; patients were classified as hypogonadic or eugonadic based on previously reported cut-offs for each biomarker. Cancer cachexia was determined using a validated classification system, which includes assessment of c-reactive protein. Anthropometric data including height, weight, body mass index and body composition using dual-energy X-ray absorptiometry was collected. The revised Edmonton Symptom Assessment System, the abridged Patient-Generated Subjective Global Assessment and the Distress Thermometer were completed. Strength was measured via handgrip dynamometry and dynamic function assessed by the six-minute walk test and a sit-to-stand test. Cohen's kappa was used to determine agreement between the two biomarkers. Student's t-test and robust multiple regression analysis were used to determine relationships for each parameter between hypogonadic and eugonadic patients.

**Results**
Androgen deficiency was identified in 71% of the men based on total testosterone and 61% based on calculated bioavailable testosterone. Although a strong correlation was demonstrated ($r = 0.730$, $p < 0.01$), there was only moderate agreement between the two biomarkers in classifying eugonadal and hypogonadal patients ($\kappa$=0.5). There was no difference between hypogonadic and eugonadic men, in terms of body composition, strength, function, symptoms, nutritional status or measures of health-related quality of life, irrespective of biomarkers used to identify hypogonadism. Only fatigue was independently associated with calculated bioavailable testosterone ($B = -0.50$, $p < 0.05$). A direct and significant ($p < 0.05$) relationship between c-reactive protein and nausea, depression, appetite, wellbeing and shortness of breath, as well as nutritional status ($p < 0.01$) was found, irrespective of testosterone levels.

**Conclusion**
With the exception of fatigue, our results suggest that the presence of inflammation and not hypogonadism, is related to symptom burden and poor nutritional status in cancer cachexia. In this condition, testosterone replacement therapy may help primarily with relieving cancer-related fatigue.

## Low bone mass is associated with cachexia in advanced cancer patients

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### Background
Although decreases in body composition are hallmark features of cancer cachexia, less is known about skeletal bone health and its link with muscle mass in this population. This study was designed to determine if differences exist in femoral neck (FN) and total hip (TH) t-scores between cachectic and non-cachectic cancer patients. In addition, we explored the relationship between bone mineral density (FN and TH t-scores) and appendicular skeletal muscle index (ASMI).

### Methods
A retrospective sample ($n = 266$) of male and female cancer patients with a variety of different cancer primaries were placed into cachectic (C; $n = 97$) and non-cachectic (NC; $n = 169$) groups. Bone mineral density measurements (FN and TH t-scores) and ASMI were measured using dual energy x-ray absorptiometry. Comparisons were made between groups with respect to FN and TH t-scores and their relationship with ASMI.

### Results
Patients in the C group were older ($61.5 \pm 11.6$ vs $65.4 \pm 12.7$ years; $p = 0.012$), and had significantly lower body weight ($62.6 \pm 14.7$ vs $76.6 \pm 19.2$ kg, $p < 0.001$) and body mass index BMI ($22.2 \pm 4.4$ vs $28.0 \pm 6.0$ kg/m$^2$, $p < 0.001$) than the NC group. ASMI (C, $5.65 \pm 1.06$ vs NC, $6.44 \pm 1.17$ kg/m$^2$, $p < 0.001$), fat index (C, $6.04 \pm 3.38$ vs NC, $8.86 \pm 4.77$ kg/m$^2$, $p < 0.001$), and BMI (C, $21.7 \pm 4.1$ vs NC, $25.9 \pm 6.1$ kg/m$^2$, $p < 0.001$) were different between groups. The FN t-scores in the C group were significantly less than those values in the NC (C; $-1.376 \pm 1.201$ vs NC, $-0.984 \pm 1.122$, $p = 0.011$). A similar association was observed with the TH t-scores (C; $-1.097 \pm 1.201$vs NC, $-0.537 \pm 1.206$, $p < 0.003$). The FN t-scores in those below the ASMI cut-offs (7.26 kg/m$^2$ for males and 5.45 kg/m$^2$ for women) were significantly worse than in those with normal ASMI (FN above, $-0.953 \pm 1.082$ vs FN below, $-1.233 \pm 1.204$, $p = 0.05$). Multiple regression analyses showed that BMI was a significant independent predictor of FN ($p = 0.014$) and TH t-scores ($p = 0.0004$).

### Conclusion
Cachectic patients possess more compromised measures of bone health than non-cachectic patients. Patients with ASMI scores below the standard cut-offs also displayed poorer FN and TH t-scores than those above the cut-off. Weight bearing exercise and nutritional interventions should be targeted at maintaining not only bone mineral density but also muscle mass in cachectic cancer patients.

## Integrated tumor-cells secretome and wasting adipose-tissue membranome in LLC mice: A transcriptomic approach to explore ligand-receptor interactions in cancer cachexia

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Cancer-induced muscle and adipose tissue (AT) wasting, which commonly occurs in cancer cachexia, impairs patient quality of life and survival. Research on the underlying mechanism of AT remodeling in cancer cachexia is essential to identify mediators and treatment options for this syndrome. Animal models that simulate cachexia in humans have predominantly utilized implanted tumor cell lines, such as Lewis Lung Carcinoma (LLC). These cells are implanted subcutaneously into experimental animals and allowed to grow until overt symptoms of cachexia develop. However, the potential interactions between ligands secreted by LLC cells (secretome) with cellular membrane receptors (membranome) from major cachexia target tissues, such as AT, need further analysis. We built a pipeline to predict secretome-membranome interactions based on transcriptome data. We also investigate the ligand-receptor relationships between LLC cells and cachectic ATs using in silico predictions and public databases. The

expression of genes coding secreted proteins was compared between LLC and distal respiratory epithelial (MLE12) cell lines. SignalP 5.0, UniprotKB (term: ''secreted'') and Gene Ontology Cellular Compartments (term: ''extracellular region'') identified 350 deregulated genes predicted to encode secreted proteins. The expression of genes coding cellular membrane proteins was compared between cachectic and non-cachectic ATs. UniprotKB (term: ''cell membrane'') and Gene Ontology Cellular Compartments (term: ''plasma membrane'') identified 593 deregulated genes predicted to encode cellular membrane proteins. Using STRING DB 11.0 for protein-protein interaction network analysis (active interaction sources: experiments and databases), 1493 ligand-receptor interactions were mapped between 278 secreted proteins from LLC with 284 cellular membrane proteins from cachectic AT. Of these, the interaction of Parathyroid hormone-related protein (PTHLH) and the cell surface receptor PTH1R may trigger a pathway related to AT wasting in cachexia. Additional interactions were involved in the induction of positive chemotaxis, positive regulation of inflammatory response, and adipocytokine signaling pathways. In conclusion, our transcriptome-based pipeline identified ligand-receptor interactions in cancer cachexia, which may help to drive new experimental approaches and insights into the pathogenesis of the syndrome.

## The inflammatory potential of adipose mesenchymal stem cells in cancer cachexia

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### Background

Cachexia is a co-morbidity of cancer, which is associated with loss of lean mass, adipose tissue and inflammation. Cachectic patients show worsened prognosis, while no treatment to date exists that counteracts the many symptoms of this syndrome. Adipose tissue(AT) is a contributor to cachexia-related inflammation. Aim: To analyze the genes involved with inflammatory pathways and secretion of pro-inflammatory cytokines in adipose mesenchymal stem cells (MSCs) that may have a role in cancer cachexia.

### Methods

Colorectal cancer patients, with (CC), or without (WSC) cachexia, and without cancer-submitted to hernia operation (Ct). MSCs were isolated from biopsies of subcutaneous AT obtained in surgery. The MSCs were characterise by flow cytometry. The cells were plated in DMEM, supplemented with different stimuli: 15% fetal bovine serum (FBS), or 15% FBS plus 50 μL/mL IL6, or 15% FBS plus 100 μL/mL TNFα. The cells were maintained in culture for 24 hours. The cellular expression of the gene was analyzed by RT-PCR; the medium was collected and the cytokines quantified.

### Results

MSCs showed positive labelling for CD73, CD90 and CD105 surface antibodies and a negative result for CD11b. MSCs showed no difference in gene expression between the groups. Evaluation of different stimuli within the same group, those incubated with TNFα showed greater activation of the p50 gene involved in the inflammatory pathway, compared those stimulated with IL6 and FBS, in all groups studied Ct($p = 0.040$), WSC($p = 0.002$) and CC($p = 0.010$). Analysis of the incubation medium from the MSCs within the same group showed that the cells stimulated with TNFα exhibited greater secretion of the inflammatory cytokines MCP-1 and IL6, than the MSCs stimulated with IL6 and FBS, in the WSC(MCP-1, $p = 0.0004$; IL6, $p < 0.0001$) and CC(MCP-1, $p = 0.0002$; IL6, $p = 0.0014$). The different stimuli did not provoke changes in cytokine secretion in stem cells obtained from the adipose tissue of the control group.

### Conclusion

Our results demonstrate that MSCs display an inflammatory phenotype characterized by increased expression of p50 gene in all studied groups, and secretion of pro-inflammatory cytokines in WSC and CC, when stimulated with TNFα. This cytokine seems to contribute to the adipose tissue MSCs role in maintaining an inflammatory environment related to cachexia.

## Gemcitabine and nab-paclitaxel reduce tumor burden and preserve muscle and cardiac functions in murine model of PDAC cachexia

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More than 85% of patients with pancreatic ductal adenocarcinoma (PDAC) suffer from cachexia. Previous studies have shown that chemotherapy leads to

loss of muscle mass and function in different cancers. Gemcitabine combined with nab paclitaxel (GnP) are FDA-approved first-line treatment options for patients with locally advanced or metastatic PDAC. However, its effect on the musculoskeletal system has not been evaluated. Our objective was to test the effect of GnP in the progression of PDAC cachexia. 12-week old C57BL/6J male mice were either orthotopically implanted with KPC32908 tumor cells (KPC, 50,000 cells) or received a sham surgery. The mice were administered with either vehicle or GnP (KPC+GnP, 120 mg/kg of gemcitabine and 10 mg/kg of nab-paclitaxel on Day 4 and Day 10 post tumor cell implantation). To assess muscle and heart function, in vivo muscle force test and echocardiography were performed a day prior to euthanasia (Day 14). To determine muscle and cardiac gene expression changes, RNA sequencing was performed on the gastrocnemius and the heart. The tumor burden was reduced by 61% in KPC+GnP compared to KPC-vehicle. KPC-vehicle exhibited reduced gastrocnemius (−15%), quadriceps (−18%), and tibialis anterior (−20%) mass, while the muscles were preserved in the KPC+GnP group. While a significant decrease in plantarflexion force was observed in the KPC-vehicle group, it remained unchanged in KPC+GnP. Echocardiography showed a decrease in left ventricular mass, increased ejection fraction and fractional shortening in the KPC-vehicle group. However, no changes in cardiac function were observed in the KPC+GnP group. Skeletal muscle genes were enriched for muscle atrophy (Foxo1, Trim63, Fbxo32), hypoxia (Ednra, Lama4, Tfrc), inflammation (Il7, Tgfb1), and endoplasmic reticulum stress (Atf4, Ddit3). In cardiac muscle, the representative functions include cardiomyocyte differentiation (Bmp4, Bmp5, Gata4), and cardiac hypertrophy signaling (Gata4, Fgfr3, Atp2a3). The expression pattern of these genes was reversed in both the tissues in KPC+GnP vs KPC-vehicle comparison, suggesting that GnP preserved muscle and heart. To comprehensively understand the effect of GnP in the musculoskeletal system, bone analysis is currently in progress. Taken together, our data shows that GnP has a protective effect on muscle and heart in experimental PDAC cachexia.

## Unfavorable impact of decreased muscle quality on the efficacy of immunotherapy for advanced non-small cell lung cancer

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### Background

Quantitative skeletal muscle mass loss has the potential to predict the therapeutic effect of immune checkpoint inhibitor. This study aimed to assess the impact of muscular quality on the abovementioned outcomes.

### Methods

This study retrospectively reviewed the medical records of advanced non-small cell lung cancer (NSCLC) patients who had received PD-1/PD-L1 inhibitor monotherapy between March 2016 and February 2018. High muscle quality was stipulated as a skeletal muscle density $\geq$41 and $\geq$33 Hounsfield units in patients with a body mass index (BMI) $<$ 25 kg/m$^2$ and $\geq$25 kg/m$^2$, respectively, as assessed using lumbar computed tomography images. High muscle quantity was stipulated as a lumbar skeletal muscle index $\geq$41 cm$^2$/m$^2$ in women, $\geq$43 cm$^2$/m$^2$ in men with a BMI $<$25 kg/m$^2$, and $\geq$53 cm$^2$/m$^2$ in men with a BMI $\geq$25 kg/m$^2$. We evaluated the associations of these muscular parameters with the overall response rate (ORR) and progression-free survival (PFS).

### Results

Out of 156 patients, 80 (51.3%) and 47 (30.1%) showed low muscle quality and quantity, respectively. Patients with high muscle quality showed higher ORR (35.0 vs. 15.8%, $p < 0.05$) and longer PFS durations (median, 4.5 vs. 2.0 months, $p < 0.05$) than those with low muscle quality. There were no noted differences in the ORR or PFS between patients with high and low muscle quantities.

### Conclusion

Lumbar skeletal muscle quality has the potential to predict the therapeutic effect of PD-1/PD-L1 inhibitor monotherapy in advanced NSCLC patients.

## Lipocalin 2 as a mediator of cachexia-anorexia

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Illness behaviors and metabolic disturbances are commonly observed in patients with cancer and frequently lead to cachexia, a devastating metabolic syndrome that significantly reduces patients' quality of life, ability to tolerate treatment, and ultimate survival. Cancer cachexia imposes a significant caloric deficit to patients through a synergistic decrease in energy consumption and increase in resting energy expenditure. Thus, identification of molecular targets that improve caloric intake, or mitigate energy wasting, is paramount in improving outcomes for patients with cachexia. Recently, the pleotropic molecule Lipocalin 2 (LCN2) was identified as a physiologic mediator of appetite. However, it remains unknown if this molecule influences energy balance during cancer-associated cachexia. We identify LCN2 as a potent pathologic mediator of cachexia-anorexia through its recently identified actions on the melanocortin 4 receptor in the mediobasal hypothalamus. We show that LCN2 is robustly upregulated in numerous murine models of pancreatic cancer cachexia, and its expression is closely associated with reduced food consumption and lean mass loss. Lipocalin 2 knockout mice are protected from cachexia-anorexia, and melanocortin 4 receptor antagonism improves feeding behaviors during cachexia. Using pair-feeding paradigms, we demonstrate that LCN2 improves lean muscle mass through improved food consumption alone. Finally, we observe that LCN2 is upregulated in humans during pancreatic cancer and is associated lean mass wasting and increased mortality. Together, we show that LCN2 is robustly upregulated during pancreatic cancer cachexia, readily crosses the blood-brain barrier, and binds to its recently identified receptor to mediate appetite suppression and concomitant lean mass wasting.

## Cancer-associated cachexia impacts liver morphology

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### Background
Cachexia is highly prevalent among patients with cancer and associated with poor prognosis. This syndrome is characterized by continuous weight loss and systemic inflammation. The liver is a central organ in the control of metabolism and very likely to play a major role in this paraneoplastic syndrome. We aimed to characterize the morphological alterations in the liver of patients with cancer-associated cachexia.

### Methods
Colorectal cancer patients enrolled after signing the informed consent. Subjects submitted to cholecystectomy were included in the Control group ($n = 5–7$). Patients with cancer, without cachexia were included in the weigh-stable cancer group (WSC, $n = 4–9$) and patients with cancer cachexia, in the cachetic cancer group (CC, $n = 13$). Hepatic biopsies were obtained during surgery. Samples were fixed in paraformaldehyde and embedded into paraffin, followed by staining with hematoxylin and eosin, and Mallory Trichrome Connective Tissue dye. Samples were also submitted to transmission electron microscopy analysis, protein quantification of inflammatory markers by Multiplex technology and, gene expression assessment by qPCR. One-way analysis of variance and Kruskal-Wallis test were employed for parametric and non-parametric data, respectively. The significance level was set at $p < 0.05$.

### Results
CC presented higher hepatic lipid content demonstrated by the higher abundance of lipid droplets observed under light and electron microscopy, as compared to the Control and WSC groups. Moreover, higher collagen content was observed in the liver of CC patients. Protein content of IL-1α and IL-8 and gene expression of FABP1 were higher in WSC ($p < 0.05$). Liver FABP1 mRNA was higher in WSC, compared with the two other groups ($p < 0.05$).

### Conclusion
Cancer cachexia is characterized by hepatic lipid accumulation and fibrosis, possibly associated with the disruption of organ functions.

 

## Cancer cachexia associated muscle $NAD^+$ depletion can be rescued by blocking activin receptor ligands in a murine experimental model

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### Background

Cancer cachexia and muscle loss are associated with increased morbidity and mortality. In preclinical animal models, blocking the activin receptor (ACVR) ligands improved survival and prevented cancer cachexia without an effect on tumour growth. The underlying mechanisms are poorly understood. Our study aimed to identify cancer cachexia and soluble ACVR (sACVR) administration-evoked changes in muscle proteome and in $NAD^+$ metabolism.

### Methods

Healthy and C26 tumour-bearing (TB) mice were treated with recombinant sACVR. The sACVR or PBS control were administered either i) prophylactically prior to the tumour formation, ii) by continued administration before and after tumour inoculation, or iii) as a treatment modality after tumour formation. Muscles were analyzed by quantitative proteomics with further examination of mitochondria-related parameters.

### Results

Muscle proteomics from TB cachectic mice revealed increased acute phase response and a downregulated signature for mitochondrial oxidative phosphorylation (OXPHOS). Supporting these findings, histochemical in situ analysis revealed that the percentage of muscle fibres endowed with high mitochondrial complex II, succinate dehydrogenase, activity was significantly reduced in TB-mice. As nicotinamide adenine dinucleotide ($NAD^+$) is crucial for mitochondrial oxidative metabolism and biogenesis, we further examined $NAD^+$ metabolites and the expression of $NAD^+$ biosynthetic genes in muscle. Cachectic muscle showed $NAD^+$ deficiency and dysregulated $NAD^+$ biosynthesis possibly due to downregulation of the salvage pathway gene, nicotinamide riboside kinase 2. Interestingly, the continued sACVR administration before and after tumour formation rescued most of the observed disturbances in the OXPHOS proteome and $NAD^+$ metabolism. Importantly, sACVR treatment after cancer inoculation also displayed ability to normalize muscle $NAD^+$ homeostasis.

### Conclusion

We present evidence on disturbed muscle OXPHOS proteome and $NAD^+$ homeostasis in murine cancer cachexia, which are partially rescued upon treatment with a blocker of activin receptor ligands. These findings reveal a previously unknown connection between cancer-induced cachexia and $NAD^+$ metabolism and point out putative new treatment therapies, such as $NAD^+$ boosters, for cancer cachexia.

## The pancreatic tumor organoid secretome impairs the smooth muscle cell contractile phenotype

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### Background

Patients with pancreatic cancer often suffer from gastrointestinal symptoms which may be the consequence of underlying gastrointestinal motility problems. Although muscle loss in cachectic pancreatic cancer patients is most obvious in skeletal muscle, these clinical symptoms as well as our recent analysis of intestinal smooth muscle characteristics in cachectic patients suggest that cachexia manifests itself also in smooth muscle, a tissue responsible for contraction of the gastrointestinal tract. We hypothesized that tumor cells from pancreatic cancer patients secrete factors that directly affect the smooth muscle cell (SMC) contractile phenotype.

## Methods

Human visceral SMCs with a contractile phenotype were exposed to the tumor secretome of 3D pancreatic tumor organoid cultures. Markers of muscle atrophy, contractile proteins, and proliferation were analyzed by qPCR and Western blot. SMC proliferation and migration were monitored by live cell imaging. Furthermore, SMC proliferation was assessed by Ki-67 immunohistochemistry of the intestinal smooth musculature of twenty-two pancreatic cancer patients with either a low or high L3 skeletal muscle mass index (SMI) as assessed by CT-scan analysis.

## Results

CM from pancreatic tumor organoids of cachectic patients did not affect expression of Atrogin-1, a key E3-ubiquitin ligase that is involved in skeletal muscle atrophy. Nevertheless, exposure to organoid CM caused reduced protein levels of α-smooth muscle actin (1.7-fold; $p < 0.001$) and smooth muscle protein 22-α (2.8-fold; $p < 0.001$), two key proteins involved in SMC contraction. Moreover, γ-smooth muscle actin mRNA expression was significantly reduced (1.5-fold; $p < 0.001$). Concurrently, expression of S100A4, a protein associated with SMC proliferation, was increased (1.4-fold, $p < 0.001$). SMCs exposed to organoid CM showed a markedly reduced doubling time (control: 36.2 h vs. organoid CM: 29.9 h, $p < 0.001$). In line with this, a higher percentage of Ki67 positive nuclei was found in the intestinal smooth musculature of pancreatic cancer patients with a low L3-SMI compared to those with a high L3-SMI ($8.7 \pm 2.1\%$ vs. $6.4 \pm 3.2\%$ ($p = 0.047$), respectively).

## Conclusion

Pancreatic tumor organoids secrete factors that diminish the contractile SMC phenotype. In vivo, this may impair the contractile functionality of the intestinal smooth musculature and contribute to frequently reported gastrointestinal symptoms in cachectic cancer patients.

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# Heterogeneous gene expression of NLRP3 inflammasome pathway in the subcutaneous and peritumoral adipose tissue in patients with cancer cachexia

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## Background

Cachexia is a complex and highly debilitating metabolic syndrome that accompanies cancer and compromises several organs, including the adipose tissue. Inflammation is one of its hallmarks and in the presence of cancer cachexia, inflammatory pathways are activated. The NLRP3 inflammasome contributes to the maintenance of homeostasis and the organization of the immune response. Aim: To analyse the activation of genes of NLRP3 inflammasome in the subcutaneous (ScAT) and peritumoral visceral (mesenteric) adipose tissue (PAT) in patients with and without cancer cachexia.

## Methods

Surgical patients with colorectal cancer who signed the informed consent form were divided into weight stable (WSC) and cachectic cancer (CC) groups. After surgery, adipose tissue explants were distributed in 12-well culture plates, with approximately 500 mg per well and incubated in 500 μl of DMEM containing 10% SFB, supplemented or not with lipopolysaccharide (LPS, 1 μg/ml), in atmosphere of 10% CO2 at 37°C for 24 h. Total RNA was extracted, and the cDNA, obtained. The gene expression was analysed with RT-PCR.

## Results

For the 2 depots compared in WSC we found that mRNA expression of NLRP3, caspase-1 and IL-1β was not different disregard of previous incubation with LPS. IL-18 showed no significant difference in ScAT in the absence of LPS while exhibiting diminished expression in PAT, compared to ScAT. In the CC group, the expression of NLRP3 was reduced in PAT, as compared with ScAT, in both incubation conditions (absence/presence of LPS). The expression of caspase-1 was decreased in PAT, compared to ScAT in the absence of LPS and there was no significant difference between the 2 tissues in the presence of LPS. There was no significant difference regarding IL-1β between deposits in the absence of LPS. This cytokine expression was reduced in PAT, when compared to ScAT, after incubation with LPS. There was no significant difference of IL-18 expression between the 2 tissues in the absence of LPS stimulus and this parameter was diminished in PAT, compared to ScAT, after LPS stimulation.

## Conclusion

Our results demonstrate that the expression of NLRP3 inflammasome components is decreased in the peritumoral adipose tissue, when compared to subcutaneous tissue in cachectic patients, with possible consequences to adipose-immune-tumour interaction modulation.

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## Serum markers of muscle catabolism are associated with decreased functional recovery and independence in cancer patients requiring inpatient rehabilitation

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## Background

Cancer patients admitted to inpatient rehabilitation facilities (IRFs) using Centers for Medicare and Medicaid Services criteria, by definition, have had significant change in physical function leading to lack of independence in activities of daily living. However, the impact of cachexia or muscle wasting in this debilitated population of cancer patients has not been previously investigated in IRFs.

## Objective

To characterize the incidence of cancer-associated cachexia or muscle wasting syndrome in our IRF and determine its relationship with physical function. Secondarily, we investigated if any specific pre rehabilitation factors were associated with cachexia.

## Methods

This retrospective cohort study of 330 admissions to our IRF included subjects admitted to oncologic rehabilitation services and excluded pediatric subjects. 3 target cohorts were selected using weight- or serum based markers of cachexia syndrome/ muscle catabolism: chronic weight loss (>5% body weight loss in 6 months), serum creatinine <0.60 mg/dL, and serum albumin <3.5 g/dL. 30 pre rehabilitation factors were collected, including cancer type/stage/progression and acute care events. Rehabilitation outcomes included the 18 item Functional Independence Measure scale(FIM, including motor and cognitive sub scores), grip strength (GS), 6-minute walk test distance(6MWT), and post-rehabilitation discharge disposition.

## Results

Amongst cancer patients at our IRF, the incidence of chronic weight loss was 58%, low creatinine was 35%, and low albumin was 65%. In a multivariate analysis examining functional outcomes during rehabilitation, low creatinine and low albumin were independently associated with negative motor and cognitive FIM changes respectively ($p = 0.003$, $p < 0.001$). While no marker was significantly associated with changes in GS or 6MWT, low creatinine and low albumin were associated with increased odds of returning to acute care ($OR = 2.0$, $p = 0.02$) and requiring home-based services after discharge respectively ($OR = 14.5$, $p = 0.001$). A univariate screen of the 30 pre-rehabilitation factors found 11 significant associations with the target cohorts but detected no independent associations in a multivariate model, suggesting no specific sub-population was uniquely susceptible to cachexia.

## Conclusion

Cachexia and muscle wasting syndrome impacts a significant fraction of cancer patients requiring inpatient rehabilitation. In this functionally debilitated cancer population, serum markers of muscle catabolism, rather than weight loss, were independently associated with poor functional recovery and decreased independence at discharge.

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## Skeletal Muscle Regeneration is Impaired in Patients with Cancer-Associated Cachexia

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## Background

Cancer-associated cachexia is defined by loss of skeletal muscle mass with or without body fat loss. Skeletal muscle regenerative processes appear to be dysregulated in cancer cachexia, with increased muscle progenitor cell proliferation and hampered myogenic potential, resulting from systemic inflammation, as shown in both animal models and patients. Pax7 and myogenic factor 5 (Myf5) are transcription factors expressed by proliferating satellite cells. Increased expression of these genes indicates higher satellite cell

content. We aimed at evaluating skeletal muscle Pax7 and Myf5 mRNA expression and its correlation with markers of systemic inflammation in patients with cancer-associated cachexia.

**Methods**

Patients with gastric, colon and rectal cancer were recruited after signature of the informed consent form. Blood and anthropometric parameters were obtained before surgery for tumor resection, during which rectus abdominis muscle biopsies were collected. Patients with cancer cachexia were allocated in the Cachectic Cancer group (CC, $n = 16$) and compared to patients of the Weight-Stable Cancer group (WSC, $n = 10$). Serum C-reactive protein (CRP), interleukin (IL) -6, tumor necrosis factor (TNF) $\alpha$, and albumin concentration was measured using commercial kits. Hemoglobin was collected from the patients' medical records. Muscle mRNA was extracted and real time qPCR was performed, using specific primers for Pax7, Myf5 and GAPDH (housekeeping gene), and results were calculated by the comparative $2-\Delta\Delta CT$ method. Statistical analysis was performed using Student's T-test or Mann-Whitney test and Spearman correlation coefficient.

**Results**

Pax7 and Myf5 muscle gene expression was higher ($p = 0.02$ and $p = 0.0009$, respectively) in CC, who also presented weight loss ($p < 0.0001$), higher serum CPR ($p = 0.0009$), IL-6 ($p = 0.006$), TNF$\alpha$ ($p = 0.0183$) and lower hemoglobin ($p = 0.0063$), as well as higher CRP/albumin ratio compared to WSC. In CC, Pax7 gene expression was associated with weight loss ($rs = 0.58$, $p = 0.02$), CRP/albumin ratio ($rs = 0.67$, $p = 0.001$) and serum IL-6 ($rs = 0.65$, $p = 0.03$); Myf5 gene expression was correlated with serum IL-6 ($r = 0.67$, $p = 0.02$). No correlations among the same parameters were observed for WSC.

**Conclusion**

Muscle regeneration is dysregulated in the skeletal muscle of cachectic cancer patients and this is strongly associated with circulating inflammatory factors, which are increased in the weigh-losing patients. Our findings highlight the importance of controlling inflammation in cachexia.

## A new predictive model classifies cachexia in lung cancer patients for the characterization of the cellular composition in the tumor microenvironment

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Cachexia is a syndrome found in lung cancer patients. It is defined by a loss of muscle mass, with consequent involuntary weight loss, leading to a patient's functional impairment due to physiological, metabolic, and immunological alterations. Tumor-derived cytokines play a crucial role in the development of metabolic abnormalities that result in loss of muscle function and mass in cachexia. However, the cellular and molecular composition of the tumor microenvironment (TME) from cachectic patients with non-small cell lung cancer (NSCLC) is unknown. We classified patients with low muscle mass and decreased survival rates (cachectic patients) using machine learning to characterize the transcriptional profile of the secretome and the cellular immune fraction in the TME. We used computed tomographies from The Cancer Imaging Archive database (collection NSCLC-Radiogenomics, $N = 211$) to characterize NSCLC patients' muscularity. Cachexia predictive analyses based on machine learning were performed by the Classification and Regression Tree (CART) and Cutoff Finder packages to select the potentially cachectic patients based on the pectoralis muscle area (PMA), and clinical and survival data. RNA-seq data were analyzed using the BioJupies tool to identify tumor differentially expressed genes (DEG) between low- to high-muscularity patients. Genes encoding secreted proteins were filtered based on the Human Protein Atlas database. CART showed that lower PMA is more associated with high death-risk than tumor stage. Cutoff Finder selected a muscularity cutoff that separated low- and high-muscularity patients according to death risk (male: $<34\,mm^2$, female: $<22;mm^2$). We found 90 and 41 up- and down-regulated secretome genes in low-muscularity patients ($n = 9$), respectively, compared with high-muscularity patients ($n = 37$). Enrichment analysis of the DEG demonstrated leukocyte chemotaxis, cytokine/chemokine activity, and T cell activation as the most enriched terms in low-muscularity patients. We also found the cachexia-inducing factors IL6, IFNG, LIF, and CSF3 as highly expressed in low-muscularity patients. Digital cytometry based

 

on DEG (CIBERSORTx) revealed that these patients also presented high proportions of CD8+T cells. Taken together, the predictive machine learning model identified patients with lower muscle mass and worse survival, who showed a TME with an inflammatory profile and high expression of cachexia-inducing biomarkers.

## Neuronal morphology and neuroglia compromised in cancer cachexia: a post-mortem neuropathological study

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### Background

Cancer cachexia is a multifactorial syndrome whose aetiology remains elusive. Systemic inflammation which characterises the syndrome leads to central nervous system (CNS) dysregulation and neuroinflammation, with impact on neural circuits controlling feeding behaviour and body composition. The inflammatory markers sensed by the CNS activate and sensitise the microglia and the CNS-infiltrating immune cells mediating neuroinflammatory responses. However, little is known about how microglia activation and perpetuation of neuroinflammation act in CNS impairment.

Aim To elucidate neuronal morphology and central immune cell profile in the central nervous system of cachectic patients.

### Methods

Samples from autopsies of cancer patients were divided into: weight stable cancer (WSC, $n = 6$) and cachectic cancer (CC, $n = 10$), following the criteria proposed by Evans et al. 2008. Age- and sex-matched non-neurologic controls (CONTROL, $n = 10$) were available for study. Post-mortem sections of the hypothalamus, amygdala, and basal ganglia were stained with H&E and immunolabelled for microglia/macrophages counting (Iba1 and CD68) and with astrocyte (GFAP) markers. Basic regional neuropathological analyses and quantitative expression were performed for comparisons between cachexia and weight stable groups, using the Qupath Software.

### Results

Qualitatively, H&E sections from CC showed neurons with shrunken cytoplasm, and dark, sometimes pyknotic nuclei in the amygdala, hypothalamus and putamen. Quantitatively, cachectic patients showed increased neuronal density in the amygdala ($p = 0.024$) and hypothalamus ($p = 0.063$), while WSC patients demonstrated increased neuronal density in the hypothalamus ($p = 0.051$), both compared with controls. Moreover, the immune cell profile was also different in CC, with increased hypothalamic microglia (Iba1) ($p = 0.0465$) and higher CD68/Iba1 ratio in the caudate ($p = 0.036$) than shown by WSC, suggesting increased lysosomal and phagocytic activity. GFAP was increased in CC Caudate (vs. WSC, $p = 0.038$) and hypothalamus (vs. CONT, $p = 0.029$).

### Conclusion

Post-mortem cachectic patient brain tissue (hypothalamus, amygdala and basal ganglia) showed abnormal neuronal morphology, neuronal density, microglia/macrophage burden and astrocyte profile disruption. These affected brain areas play a key role in the development of metabolic and behavioural derangements. The novel findings add to the knowledge on the physiology of the cachectic brain and describe neuropathological alterations related with the syndrome, which may in the future contribute to possible cachexia treatment strategies.

## Dietary naringenin preserves muscle strength but not body weight in a mouse model of cancer cachexia

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Cancer cachexia is characterized by loss of skeletal muscle that results in functional impairment impacting quality of life and overall survival. There is a need to identify therapeutic treatments that can target skeletal muscle and improve quality of life in patients suffering from the disease. Naringenin is a flavonoid most commonly found in citrus fruits and tomatoes that has a wide range of positive effects in

pre-clinical models including improving insulin-mediated metabolism while reducing inflammatory and tumor growth. These observations suggest that naringenin supplementation during the progression of cancer cachexia may attenuate metabolic disturbances driving increased energy expenditure and body wasting. Therefore, we hypothesized that a diet supplemented with naringenin would prevent the progression of cancer cachexia by inhibiting body weight loss, improving insulin sensitivity, and decreasing inflammation in a mouse model of cancer cachexia. The Colon 26 (C-26) mouse model was used to study the effect of 2 wt% dietary naringenin on the pathogenesis of cancer cachexia. We examined the effects of dietary naringenin on changes of food intake, body weight, body composition, muscle function, insulin tolerance, and inflammatory status. Naringenin-fed tumor-bearing mice exhibited body weight loss and decreased food intake earlier than tumor-bearing mice fed control diets. Surprisingly, dietary naringenin was protective against loss of muscle strength and attenuated the onset of insulin resistance and markers of inflammation compared to tumor-bearing mice fed a control diet. Supplementation of diet with naringenin improved multiple aspects of metabolic disturbances and inflammation during cancer cachexia progression in [C-26 tumor-bearing] mice. These findings affirm a strong link between inflammation and insulin resistance as drivers of the progression of cancer cachexia and provide us with further understanding of how dysregulated metabolism impacts the loss of muscle strength and mass. Through its anti-inflammatory properties, dietary naringenin may be a useful phytotherapeutic tool to improve quality of life and attenuate the progression of cancer cachexia.

## Appetite-related distress experienced in the final 60 days of life: A prospective, consecutive cohort study of an Australian palliative care population

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**Background**

Despite the high prevalence of symptom burden throughout the illness trajectory of palliative care patients, reports on appetite-related distress as patients approach death have been limited. Improving our understanding of the patterns and longitudinal trajectory of appetite-related distress experienced in the last 60 days of life can help guide treatment recommendations and identify opportunities for improvement in symptom management.

**Methods**

A consecutive cohort of 116,604 patients who died in the care of specialist palliative care services participating in the Australian national Palliative Care Outcomes Collaboration (PCOC) was evaluated. Settings comprised of community-based (outpatient clinics and home, including residential aged care facilities) and inpatient (direct care, consultative) services. A total of 501,104 appetite distress data points were included in the analysis. Patient-reported distress from appetite was assessed using the Symptom Assessment Scale (SAS), and compared with patient's level of functioning using Australian Karnofsky Performance Scale (AKPS).

**Results**

Diagnostic cohorts included cancer (75%), end-stage organ failure (11%), neuro-degenerative disease (4%), dementia (3%) and other non-cancer (7%). The characteristics of patients in each cohort demonstrated expected differences in age with the cancer cohort the youngest ($\mu=71.8\pm13.4$) and the dementia cohort the oldest ($\mu=85.4\pm8.5$). Daily mean SAS appetite scores prior to death did not vary greatly by diagnosis. The reporting of mild to severe appetite problems ranged from 5% to 27% across levels of function (AKPS). While higher patients' functional status (AKPS) scores were associated with fewer appetite problems, the relationship was not clear when patients deteriorated. Moderate to severe appetite-related distress was associated with the presence of other symptom-related distress such as nausea-, bowel- and pain-related distress.

**Conclusion**

Appetite distress is prevalent in all cohorts and is relatively stable in patients receiving palliative care in the last 60 days of life. Early interventions targeting nausea and bowel-related distress for more

 

functional patients should be explored to investigate if it reduces appetite distress late in life.

## Ovarian cancer ascites-induced skeletal muscle cell wasting in vitro correlates to clinical muscle measures of the patient

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### Background

Cachexia-associated skeletal muscle wasting or 'sarcopenia' is highly prevalent in ovarian cancer, and contributes to poor outcome. Drivers of cachexia-associated sarcopenia in ovarian cancer remain elusive, underscoring the need for novel and better models to identify tumor factors inducing sarcopenia. We aimed to assess whether factors present in ascites of sarcopenic versus non-sarcopenic ovarian cancer patients differentially affect protein metabolism in skeletal muscle cells, and to determine if these effects are correlated to cachexia-related patient characteristics.

### Methods

Fifteen patients with an ovarian mass and ascites underwent extensive physical screening focusing on cachexia-related parameters. Patients were diagnosed with malignant ($n = 12$) or benign disease ($n = 3$). Based on CT-based body composition imaging, six cancer patients were classified as sarcopenic and six were not; the three patients with a benign condition served as an additional non-sarcopenic control group. Ascites was collected and used for *in vitro* exposure of C2C12 myotubes and direct measurements of protein synthesis and breakdown by radioactive isotope tracing, qPCR-based analysis of atrophy-related gene expression, and NF-kB activity reporter assays.

### Results

C2C12 protein synthesis was lower after exposure to ascites from sarcopenic patients (sarcopenia $3.1 \pm 0.1$ nmol/h/mg protein vs. non-sarcopenia $5.5 \pm 0.2$ nmol/h/mg protein, $p < 0.01$), and protein breakdown rates tended to be higher (sarcopenia $31.2 \pm 5.2\%$ vs. non-sarcopenia $20.9 \pm 1.9\%$, $p = 0.08$). Ascites did not affect MuRF-1, Atrogin-1, or REDD1 expression of C2C12 myotubes, but NF-κB activity was specifically increased in cells exposed to ascites from sarcopenic patients (sarcopenia $2.2 \pm 0.4$ vs. non-sarcopenia $1.2 \pm 0.2$, $p = 0.01$). Protein synthesis and breakdown correlated with NF-κB activity ($r_s = -0.60$, $p = 0.03$ and $r_s = 0.67$, $p = 0.01$, respectively). The skeletal muscle index of the ascites donors was correlated to both *in vitro* protein synthesis ($r_s = 0.70$, $p = 0.005$) and protein breakdown rates ($r_s = -0.57$, $p = 0.04$).

### Conclusion

Ascites of sarcopenic ovarian cancer patients induces pronounced skeletal muscle protein metabolism changes in C2C12 cells that correlate with clinical muscle measures of the patient and are characteristic of cachexia. The use of ascites offers a new experimental tool to study the impact of both tumor-derived and systemic factors in various cachexia model systems, enabling identification of novel drivers of tissue wasting in ovarian cancer.

## Evaluation of an interdisciplinary Cachexia and Nutrition Support Clinic - the patient and carers perspective

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### Background

The Barwon Health Cachexia & Nutrition Support Service is an outpatient clinic focused on improving

clinical outcomes and quality of life for patients with or at high risk of cancer cachexia. Patients see an interdisciplinary team, incorporating a physician, physiotherapist, dietician and nurse practitioner concurrently. This study aimed to evaluate the patient and carer perspective of the service.

**Methods**
In 2016/17, semi-structured interviews were conducted with 12 patients and 9 carers. Interviews focused on two broad themes: 1) recounting memories and experience of the Cachexia & Nutrition Support Clinic, and 2) describing their ideal experience or expectation of a cachexia-specific support service. All interviews were recorded and transcribed verbatim. Thematic analysis was supplemented with data from reviews of the patient and carer literature.

**Results**
Analysis generated four superordinate themes that reflected the complex dynamics of the clinic experience. Themes were the following: improved communication regarding health literacy/education for patients and carers, empowerment through person-centred care, evolution of perception of value, and importance of the interdisciplinary team-based approach. Generally, patients and carers reported overall positive experiences with the clinic, particularly with regard to improved communication and empowerment of the patient.

**Conclusion**
Findings confirmed that a cachexia-specific service was viewed as having a positive impact on quality of life and outcomes by patients and carers. A patient-centred and individualised approach by the interdisciplinary team in particular were of importance to those interviewed. These insights are a critical step in the development of recommendations for future clinical management of cancer cachexia.

## Effectiveness of Medical Cannabis to improve appetite in cancer patients: data from the Quebec Cannabis Registry

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**Background**
Through a pharmacovigilance and population-based study, the Quebec Cannabis Registry (QCR) was launched to evaluate the safety and effectiveness of medical cannabis (MC) in over 3000 patients followed between 2015 and 2019. Our aim was to determine MC's effectiveness to relieve anorexia in the 358 cancer patients included in the QCR.

**Methods**
Cancer patients in the QCR with anorexia (revised Edmonton Symptom Assessment System (ESAS-r) appetite score of $\geq 4$; 10 = worst appetite) prior to starting MC (visit 1), were included. For this cohort, changes in appetite scores were compared across follow-up visits 2 (3 months) and 3 (6 months), controlling for age, sex, route of administration (inhaled vs oral) and chemovars (THC-rich, THC:CBD-balanced, CBD-rich).

**Results**
Eighty-one cancer patients ($59.1 \pm 15.0$ y, 52% female) had a mean appetite score of $6.5 \pm 2.0$ at baseline (visit 1). Half of the patients (50.6%) were prescribed oral products (cannabis oil) and 44.4% received THC:CBD-balanced chemovars. Significant improvement ($p < 0.0001$) in appetite was found between visits 1, 2 and 3 ($6.5 \pm 2.0$, $3.8 \pm 3.4$, $3.4 \pm 3.2$) (Figure 1) and 74% of patients experienced a clinically significant improvement ($\geq 1$) in ESAS-r appetite score by visit 2. Inhaled and oral products, along with THC-rich and THC:CBD-balanced chemovars were associated with significant improvements in appetite.

**Conclusion**
MC appears to have a positive outcome in helping to reduce anorexia in cancer patients. This beneficial outcome be effective in reducing anorexia in cancer patients. Its effectiveness is consistent with both oral and inhaled routes of MC administration and primarily associated with products containing THC.

## Skeletal Muscle Resident Mast Cells as a Potential Novel Biomarker for Cancer-Associated Cachexia

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Roughly half of all cancer patients develop cancer-associated cachexia, leading to severe decreases in:

 

skeletal muscle mass, muscle function, and overall quality of life [1]. Inflammation is a key promoter in the characteristic muscle degradation of cachexia [2]. Consequently, inflammatory immune cells and their upregulation within the skeletal muscle may serve as useful biomarkers for cachexia development. This project examines skeletal muscle resident mast cells, a type of innate immune cell, as a potential novel biomarker for cancer-associated cachexia. Gene set enrichment analysis (GSEA) of immune cell signatures in a publicly available patient cohort (accession: GSE34111), showed enrichment of innate immunity and mast cell signatures in the skeletal muscle of cachectic upper gastrointestinal patients (patients losing 7% of their body weight) compared to healthy controls [3–6]. Conversely, gene signatures for adaptive immunity, macrophages, and neutrophils did not show enrichment. The Lewis Lung Carcinoma (LL/2) cell line was used to induce a cachexia phenotype in C57BL/6 mice following subcutaneous injection. Tumor bearing mice were stratified based on percentage of body mass lost at harvest into two groups: (i) mice losing less than 10% or (ii) mice losing 10% or more [LL/2 (10%≤)] of their baseline body mass. The gastrocnemius (GA), extensor digitorum longus (EDL), and tibialis anterior (TA) muscles from LL/2 (10% ≤) mice had higher mRNA levels of the TRIM63 and FBXO32 genes, commonly upregulated in cachexia. Likewise, muscle cross sections of the GA and TA showed shifts in fiber size distribution toward increased percentages of smaller muscle fibers compared to control. Toluidine blue staining revealed a higher number of activated degranulating mast cells in the TA and EDL of the LL/2 (10%≤) mice compared to control. LL/2 conditioned media (CM) added to cultures of murine bone marrow derived mast cells (BMMCs) *in vitro* induced activation of BMMCs to secrete IL-6, as measured with an ELISA at 24 h post treatment. Activated BMMC CM in turn decreased differentiated C2C12 murine myotube diameter respective to the control. Collectively, these data indicate that skeletal muscle resident mast cells may promote muscle atrophy and serve as a novel biomarker for cancer-associated cachexia.

## Increased sensitivity to systemic inflammation leads to elevated MMP-9 expression in mouse cachectic muscle

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### Background

Cancer cachexia is associated with skeletal muscle loss and function, which linked to patients' survival, quality of life, and the response to chemotherapy. The extracellular matrix (ECM) is the scaffolding framework for skeletal muscle and plays an essential role in tissue development and maintenance. Matrix metalloproteinase 9 (MMP-9) is a family of enzymes that can degrade type IV collagen, a component of the basal membrane in the ECM. While the change of MMP-9 expressions has been reported in muscle-atrophy conditions, the difference is not fully explored in cancer cachexia. The purpose of this study was to determine whether cachectic muscle shows increased levels of MMP-9 expression.

### Methods

Male wild-type (WT, n = 5) and $Apc^{Min/+}$ (Min, n = 6) mice were used in this study. At approximately 5-month of age, both groups of mice were sacrificed to collect quadriceps muscles. qRT-PCR and Western blot analyses were performed to examine the inflammatory status and MMP-9 expression in the tissues. Unpaired t-test was used for statistical analysis, and the level of significance was set at $p < 0.05$.

### Results

Min mice showed smaller quadriceps weights by 39% compared to WT mice. The mRNA levels of TNF-α and TWEAK were comparable, but those of TNFR1 (2.1-fold) and Fn14 (4.2-fold), the receptor for TNF-α and TWEAK, respectively, were higher in Min mice. The transcriptional level of CD68, indicative of M1 macrophages activation, was elevated in the cachectic muscle. Western blot analysis showed that Min mice had a trend towards increasing the level of p38 activation ($p = 0.073$) and significantly elevated the level of JNK activation by 2.1-fold. MMP-9 protein expression was increased by 2-fold in Min mice.

### Conclusion

These data suggest that cancer-induced systemic inflammation increased the sensitivity to proinflammatory cytokines, resulting in increased MMP-9 expression and subsequent ECM degradation in cachectic muscle. This project is supported by the Louisiana Board of Regents Support Fund (LEQSF(2017–20)-RD-A-22) to SS.

## Molecular mechanisms underlying the sex differences in pancreatic cancer-associated cachexia phenotypes

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**Background**
Cachexia frequently develops in patients with pancreatic ductal adenocarcinoma (PDAC) and contributes to cancer deaths. Sex differences have been observed in cancer cachexia with male patients generally having higher prevalence of cachexia, greater weight loss or muscle wasting, and worse outcomes compared to female patients. However, the underlying molecular mechanisms are far less addressed, while mechanistic understanding of the differences may improve treatment in both sexes.

**Objective**
We sought to assess sex differences in cachexia phenotypes and therapeutic effects as well as the molecular mechanisms using a genetic mouse model of PDAC. Design: Males and females were evaluated for tumor and cachexia initiation and progress and ACVR2B/Fc therapeutic effects. The collected muscles were subjected to molecular and transcriptome/proteome analyses.

**Results**
Males with PDAC experienced earlier cachexia and were more responsive to ACVR2B/Fc. PDAC induced muscle metabolic alterations at the protein level including increase in 4E-BP1 and STAT3 phosphorylation to a higher degree in males. RNAseq revealed dysregulation of many genes including upregulated muscle-specific E3 ligases, downregulated myosin heavy chains, and inhibited canonical pathways in male early PDAC-cachexia but fewer alterations in female counterpart. ACVR2B/Fc prevented many of these alterations in males but not females. IPA predicted sex-specific alterations of upstream transcription regulators and muscle function. Muscle proteome revealed inhibited mitochondrial function in males. TCGA query revealed high tumor INHBA correlating with mortality in men. Endogenous ACVR2B-binding competitor or inhibitors of the activin family ligand were upregulated in the muscle of female PDAC mice and the inhibitors were also more abundantly expressed in women, thus blocking the activin pathway and rendering females to display less responsive to ACVR2B/Fc.

**Conclusion**
PDAC-associated cachexia displays sex-specific phenotypes and molecular alterations. Early alterations in the metabolic pathways are responsible for the early cachexia-onset in males. Activin mediates early PDAC-induced cachexia in males while this pathway is blocked in females due to upregulation of the ACVR2B/Fc-binding competitor and inhibitors of the activin family ligand. This antagonizing mechanism in females partly accounts for the late cachexia-onset and less response to ACVR2B/Fc therapy. As such, anti-cachexia interventions might require sex-specific approaches.

 