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1-01

Body composition and prognostication in cancer

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Cancer cachexia contributes to poor prognosis through progressive depletion of the body's energy and protein reserves; research is revealing the impact of the quantity of these reserves on survival. Our group has exploited computed tomography (CT) images to study body composition in cancer patients. We argue that CT taken for the purposes of diagnosis and routine follow-up can be used to derive clinically useful information on skeletal muscle and fat amount and distribution. Population-based data sets have been analyzed, revealing wide variation in individual proportions of fat and muscle (Prado et al. *Lancet Oncology* 2008;9:629–35; Martin et al. *J. Clin. Oncol.* 2013: 31:1539–47). Muscle loss during aging is well known and is prognostic of frailty, falls, fractures, loss of independence, increased length of hospital stay, infectious complications in hospital and mortality. Muscle depletion is not limited to people who appear underweight and it may be a hidden condition in normal weight, overweight or obese people (*i.e.* sarcopenic obesity). Disparate behaviour of skeletal muscle and fat was acknowledged by an international consensus of experts on cancer cachexia, defined as being characterized by loss of skeletal muscle *with or without* loss of fat mass. Within the large inter-individual variation of body composition in cancer patients, several consistent themes are emerging. Skeletal muscle depletion is a powerful predictor of cancer related mortality as well as of severe toxicity during systemic chemotherapy. Distinct from skeletal muscle, the fat mass is an important reserve of energy. High fat mass (*i.e.* obesity) appears to confer a survival advantage in patients with diseases associated with wasting, including cancer, rather than a disadvantage as understood from studies of all-cause mortality. The larger energy reserve of obese persons is thought to confer this advantage. Obesity predicted higher survival especially strongly when sarcopenia is absent. To specifically understand the relationships between body composition and cancer outcomes, we have reviewed several thousand clinical CT images. We used statistical methods (*i.e.* optimal stratification) to define muscle mass cutpoints that relate significantly to increased mortality and evaluated them in survival models alongside conventional covariates including cancer site, stage and performance status. Muscle depletion is associated with mortality in diverse tumor groups including patients with cancers of the pancreas, lung, breast and gastrointestinal tract, liver, bladder and kidney. Cancer patients who are cachexic by conventional criteria (involuntary weight loss) and by the additional criterion of severe muscle depletion share a very poor prognosis, regardless of overall body weight. Severe muscle depletion was identified in patients with cancers of the breast, colon, lung, kidney, liver, head &

neck and lymphoma and these consistently had worse toxicity resulting in dose reductions or definitive termination of therapy when treated with 5-FU, capecitabine, sorafenib, sunitinib, carboplatin, cisplatin or a regimen (5FU with epirubicin & cyclophosphamide; 5FU with oxaliplatin or CPT 11). Reduced treatment may explain excess early mortality in patients affected by severe muscle depletion. Survival models including cachexia and body weight/composition characteristics showed excellent fit (*i.e.* concordance statistics >0.9) and outperformed prediction models using only conventional cancer related covariates (C-statistics 0.75–0.8). In renal cell carcinoma muscle depletion was independent of the frequently used Memorial Sloan Kettering Cancer Center prognostic score and similar results were seen for muscle depletion in lymphoma independent of the FLIPI prognostic score.

1-02

Myostatin as a marker of cachexia in gastric cancer

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Myostatin, also known as growth and differentiation factor-8 (GDF-8), is a negative regulator of muscle mass, belonging to the TGF- β superfamily. Myostatin is secreted as an inactive propeptide that is cleaved to generate a mature ligand, whose activity may be regulated *in vivo* by association and dissociation with binding proteins, including propeptide itself as well as follistatin or related molecules. Active myostatin binds the activin type II B receptor (ActRIIB) and, to a lesser extent, the related ActRIIA, resulting in the phosphorylation and consequent recruitment of the low-affinity type I receptor ALK (activin receptor like-kinase)-4 or ALK-5. This binding induces phosphorylation and activation of the transcription factors SMAD2 and 3 [mammalian homologue of *Drosophila* MAD (Mothers-Against-Decapentaplegic gene)], which translocate into the nucleus and together with SMAD 4 regulate the expression of target genes. In addition, myostatin has been suggested to exert its action through different pathways, such as the extracellular signal-regulated kinase (ERK)/mitogen activated protein kinase (MAPK) cascade. Moreover, cross-talking between myostatin pathway and the IGF-1 axis has been postulated. Inactivating mutations of myostatin gene have been found in the "double-muscling cattle phenotype" as well as in humans. Myostatin null mice are characterized by marked muscle enlargement (~100 to 200% more than controls), exhibiting both fiber hypertrophy and hyperplasia, whereas systemic administration of myostatin in adult mice induces profound muscle and fat loss. Moreover, high myostatin protein levels have been reported in conditions associated with muscle depletion, such as aging, denervation atrophy, or mechanical unloading. Results from our laboratory have shown that myostatin signaling is enhanced in skeletal muscle of tumor-bearing rats and mice. Similarly, others have shown that myostatin inhibition, either by antisense oligonucleotides or by

administration of an Actin Receptor II B/Fragment-crystallizable (ActRIIB/Fc) fusion protein or ActRIIB-soluble form, prevent muscle wasting in tumor-bearing mice. When myostatin signaling was studied in muscle biopsies obtained during surgical procedure from non-weight losing gastric cancer patients, we found that protein expression of both myostatin and phosphorylated GSK-3 β were significantly increased, while phosphorylated-SMAD 2/3 did not significantly change with respect to controls. Although the reason of this result is not known at present, a possible explanation could be that myostatin increase is paralleled by a concomitant rise in the expression of follistatin, a physiological inhibitor of myostatin. This would result in a myostatin/follistatin ratio similar to controls, thereby maintaining the myostatin signaling in basal conditions. In addition, unchanged levels of p-Smad 2/3, despite increased myostatin protein expression, also may reflect a modulation of other molecules acting through the activin receptor type IIB, such as activin A. Interestingly enough, we found that the expression levels of muscle myostatin mRNA are significantly reduced in gastric cancer patients. Although the reason for these apparently contradictory results is not known at present, it is conceivable that the differences may at least in part be due to posttranscriptional mechanisms, such as increased myostatin synthesis secondary to increased translational efficiency or reduced degradation of myostatin. Based on the available data, it may be concluded that myostatin signaling is perturbed in the skeletal muscle of patients with gastric cancer. Changes occur even in early disease stage and in the absence of significant weight loss, supporting the view that the molecular changes contributing to muscle wasting and cancer cachexia are operating since the early phases of cancer. Myostatin signaling is complex and may be affected by the interplay of inhibitors such as follistatin and/or other members of the TGF- β superfamily. Myostatin may represent a suitable target for future pharmacological interventions aimed at the prevention and treatment of cancer-related muscle loss.

1-03

Role of Activin A in human cancer cachexia (ACTICA study)

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Cachexia is a complex metabolic syndrome associated with underlying illness, characterized by loss of skeletal muscle and not reversible by nutritional support. Recent animal observations suggest that the production of Activin A (ActA), a member of the TGF β superfamily, by some tumors might contribute to cancer cachexia. This hypothesis seems attractive since inhibitors of ActA have been developed. Nevertheless, the role of ActA in the development of cancer cachexia has never been investigated in humans.

Our goal was to demonstrate the role of ActA as a mediator of the human cancer cachexia and to assess its potential use as a biomarker of cachexia.

Patients with colorectal or lung cancer were prospectively evaluated. All patients had clinical, nutritional and functional assessment. The skeletal muscle mass was measured by bioimpedance (BIA) and abdomen CT-scan (CT). Blood samples were collected in standardized conditions to measure circulating levels of ActA.

One-hundred fifty-two patients were recruited (59 lung and 93 colorectal cancer patients), at the time of diagnosis (n=125) or at relapse (n=27). The prevalence of cachexia, as defined by K. Fearon *et al.* (2011), reached 43%. As expected, cachexia was associated with a reduced lean body mass (−9% by BIA and −11% by CT, $p < 0.001$), but also a reduced fat mass (−23% by BIA and −32% by CT, $p < 0.001$). Cachexia was associated with reduced physical function (ECOG and QLQC30;

$p < 0.0001$ and $p < 0.001$), reduced quality of life (QLQC30; $p < 0.001$) and increased symptoms (QLQC30; $p < 0.001$). Anorexia, defined by a SNAQ score < 14 , was more common in cachectic patients than in non-cachectic patients (46% vs 9%; $p < 0.001$). Interestingly, ActA levels in cachectic patients were two-fold higher than in non-cachectic patients (1308 vs 495 pg/ml; $p < 0.001$) and were positively correlated to the weight loss ($R = 0.439$; $p < 0.001$) and negatively correlated with SNAQ score ($R = -0.237$; $p = 0.003$).

In conclusion, these results demonstrate an association between circulating concentrations of ActA and the presence of the anorexia/cachexia syndrome in cancer patients. Given the muscle atrophic effect of ActA, our study suggests that increased circulating concentrations of ActA in cancer cachectic patients may contribute to the development of this syndrome.

1-04

Prehabilitation vs rehabilitation, a randomized control trial in patients undergoing colorectal resection for cancer

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Background: The preoperative period (prehabilitation) may represent a more appropriate time than the postoperative period to implement a program to facilitate recovery of functional exercise capacity in patients undergoing colorectal resection for cancer.

Methods: A parallel-arm single-blind superiority randomized controlled trial (RCT) was conducted. Seventy-seven patients were randomized to receive either prehabilitation (n=38) or rehabilitation (n=39). Both groups received a home-based intervention of moderate aerobic and resistance exercises, nutritional counselling with protein supplementation, and relaxation exercises initiated either 4 weeks before surgery (prehabilitation) or immediately after surgery (rehabilitation), and continued for 8 weeks after surgery. Patients were managed with an enhanced recovery pathway. Primary outcome was functional exercise capacity measured using the validated six-minute walk test.

Results: Median duration of prehabilitation was 24.5 days. While awaiting surgery, functional walking capacity increased (≥ 20 m) in a higher proportion of the prehabilitation group compared to the rehabilitation group (53% vs 15%, adjusted $p = 0.006$). Complication rates and duration of hospital stay were similar. The difference between baseline and 8-week six-minute walking test was significantly higher in the prehabilitation compared to the rehabilitation group (+23.7 m (SD 54.8) vs. -21.8 m (SD 80.7); mean difference 45.4 m (95% CI 13.9, 77.0). A higher proportion of the prehabilitation group were also recovered to or above baseline exercise capacity at 8-weeks compared to the rehabilitation group (84% vs 62%, adjusted $p = 0.049$).

Conclusions: Meaningful changes in postoperative functional exercise capacity can be achieved with a prehabilitation program. (ClinicalTrials.gov registration: NCT01356264).

1-05

NEO1940: a novel cannabinoid receptor agonist with reduced brain exposure as a multimodal supportive care therapy in cancer patients with anorexia and weight loss

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Cancer is the leading cause of death in developed countries. In addition to the efforts towards tumor regression, a critical need in the management of

cancer patients is supportive care to improve quality of life and survival. Anorexia, weight loss and sarcopenia are critical issues in the management of cancer patients, with essentially no effective therapy approved by Regulatory Agencies. Cannabinoids offer distinct benefits to address many such conditions in cancer patients (e.g. loss of appetite, diminished food intake, weight loss, pain, anxiety, depression etc.) The use of Cannabinoid agonists however, is limited due to the accompanying central side effects.

NEO1940 is a non-cannabis and non-THC based phase II ready, orally available, mixed cannabinoid (CB1/CB2) receptor agonist new chemical entity with reduced brain penetration and excellent pharmacokinetic properties. NEO1940 offers potential of therapeutic benefits for a number of conditions impacting quality of life and survival in cancer patients along with a better central side effect profile compared to other available cannabinoids.

NEO1940 has completed five Phase I (SAD, MAD) clinical studies. In human, NEO1940 demonstrated excellent pharmacokinetic properties, long half-life of >80 hours, and safe dose levels for the future clinical studies have been determined. NEO1940 displays an improved safety margin versus central side effects typically observed with known cannabinoids.

In a 2-week multiple ascending dose study, subjects on NEO1940 attained significant dose and exposure dependent gain in body weight which was not associated with fluid retention, including at doses where profile of adverse event was similar to placebo. This effect on body weight is likely mediated by increase appetite and caloric intake. Weight gain and several other potential benefits of NEO1940, such as quality of life, increase appetite, food palatability and intake, anti-emetic effects, analgesia and opioid sparing effects will be evaluated in a planned proof-of-concept Phase II study in cancer patients with anorexia and weight loss.

1-06

Cachexia evolution in renal cell carcinoma patients and its relation with cardiac ejection fraction evaluated by MUGA scan

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Introduction: Cancer cachexia is a continuum, progressing from absent, to early stage (pre-cachexia) to cachexia, which can then go on to be moderate, severe or refractory. Cachexia is characterized by skeletal muscle loss, and it has recently been suggested that cardiac muscle also atrophies and shows functional loss such as reduced left ventricular ejection fraction (LVEF) in cachexia.

Material and Methods: In this retrospective chart review of renal cell carcinoma (RCC) patients, lumbar L3 CT scan images were used to evaluate skeletal muscle (SM) and adipose tissue (AT) loss over time. Multi gated acquisition (MUGA) scan-defined LVEF were abstracted from medical charts.

Results: Representing the early disease trajectory, 13 patients (55.9±9.6 y, 8 males) from a randomized phase III trial of adjuvant therapy (sunitinib vs sorafenib vs placebo) in resected RCC were evaluated. During 11.0 ±2.2 months on treatment only 2 / 13 patients (15%) showed L3 muscle loss [>6cm² (~1kg total muscle)] on CT and also only 2 out of 13 patients showed over all tissue loss (SM+ total AT). All MUGA scans (n=4 / patient) were within a normal range during the same time. Representing more advanced disease, 11 patients (61.8±8.9 y; 9 males)

with metastatic disease treated with sunitinib on different clinical trials were evaluated. During 10.4±2.5 months on treatment, 6 / 11 patients (54%) developed muscle loss > 6 cm² (17.2± 9.3 cm²) and 4/11 had an abnormal MUGA (EF <50% or fell by >10%). Muscle loss and abnormal MUGA findings were overlapping in 3 patients.

Conclusion: RCC patients eligible for clinical trial participation in an adjuvant setting had a low likelihood of cachexia, muscle loss (15%) or altered cardiac EF (0%). In a metastatic setting, muscle loss (54%) and LVEF impairment (36%) were more prevalent and were overlapping phenomena. Further studies are needed to verify these findings and to probe the relationship between muscle loss and LVEF impairment.

1-07

The role of p38β MAPK in cachexia

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Systemic inflammation has been recognized as a major stimulus of accelerated muscle protein degradation in cachexia. However, the underlying mechanisms are poorly defined. Over the last ten years our lab has studied the role of p38 MAPK, a family of protein kinase activated by various inflammatory mediators, in the development of cachexia. Utilizing pharmacological p38α/β MAPK inhibitors, we observed that p38 activation in muscle by TNFα, lipopolysaccharide or diverse types of cancer is responsible for their stimulation of muscle proteolysis and mass loss. Conversely, p38 activation by ectopic expression of constitutively active MKK6 causes muscle catabolism. Thus, p38 activation appears necessary and sufficient for the development of cachexia. Mechanistically, p38 is capable of coordinately activating the ubiquitin-proteasome and the autophagy-lysosome pathways in muscle by upregulating some important genes in the two pathways through the activation of transcription factor C/EBPβ. Consequently, C/EBPβ knockout effectively blocks the development of cancer-induced muscle wasting similar to p38 inhibition. Further, the catabolic actions of p38 is attributable to the p38β isoform only, due to its unique capability in phosphorylating T188 of C/EBPβ, which is critical to the activation of C/EBPβ binding to DNA. Therefore, p38β appears a key mediator and a therapeutic target of cachectic muscle wasting.

1-08

Uncovering IL-6-independent mechanisms involved in STAT3-induced cachexia

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Cachexia, an end of life syndrome, is characterized by excessive weight loss and skeletal muscle deterioration. This disorder often develops at the late stages of deadly diseases such as cancer. Patients with this condition experience a loss of skeletal muscle mass due to a decreased synthesis rate and enhanced degradation of muscle proteins. Although 20 to 50% of all cancer deaths are due to the consequences of this condition, no effective treatment is available at this time. The molecular mechanisms underlying cancer-induced muscle wasting are multifactorial and involve multiple cell signaling pathways, many of which are ill defined. Thus, in order to design effective therapeutic strategies to combat muscle wasting, it is important to elucidate the molecular mechanisms and identify the players involved in this process. One of the well-known causes of cancer induced muscle wasting is the tumour-related increase

in proinflammatory cytokines that trigger a decreased synthesis rate and enhanced degradation of muscle proteins. Cytokines such as IL-6, IFN γ and TNF α have been associated with the development of cancer-induced muscle wasting. Others and we have shown that IFN γ and TNF α trigger muscle wasting by activating the nuclear accumulation of the transcription factor NF- κ B resulting in the increased expression of factors that either inhibit general translation (such as iNOS) or mediate the activation of the ubiquitin-proteasome pathway. IL-6, on the other hand, is thought to trigger cancer induced-muscle wasting by activating the transcription factor STAT3. However, the molecular mechanism(s) through which STAT3 promotes cancer-induced muscle wasting remains elusive. Here we report data suggesting that STAT3 plays a key role in cytokine-mediated muscle atrophy in a mechanism that is independent of IL-6. We have observed that in response to IFN γ and TNF α STAT3 associates with NF- κ B and that STAT3/NF- κ B complex is required to promote iNOS transcription. In addition, we also show that IFN γ and TNF α modulate STAT3 expression at the posttranscriptional level through a mechanism involving the RNA-Binding protein HuR. Therefore, together these observations suggest that the modulation of STAT3 expression and STAT3 function by IFN γ and TNF α is an important contributor to cancer induced cachexia.

1-09

BMP axis in experimental cancer cachexia

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Cancer cachexia is a syndrome characterized by loss of skeletal muscle protein, depletion of lipid stores, inflammation, anorexia, weakness, and perturbations of the hormonal homeostasis. Muscle wasting, mainly due to increased protein breakdown rates, is one of the most prominent features of cachexia. Recent observations showed that bone morphogenetic protein (BMP) signaling, acting through Smad1, Smad5 and Smad8 (Smad1/5/8), is a master regulator of muscle homeostasis. BMP-Smad1/5/8 axis negatively regulates a novel ubiquitin ligase (MUSA1) required for muscle loss induced by denervation (1).

Aim of the present work was: 1) to test if alterations of the BMP signaling pathway occur in cancer-induced muscle wasting; 2) to verify if modulations of this pathway could prevent the wasting process in tumor-bearing mice. Colon26 (C26)-bearing mice received 5×10^5 tumor cells s.c. in the back. The day after the *tibialis anterior* muscle was transfected by electroporation with a plasmid coding for a constitutively active (ca) form of ALK3 and sacrificed 14 days after tumor implantation. In a separate set of experiments, shRNA directed against muscle specific ubiquitin ligases were delivered 7 days before tumor injection.

Gene expression of BMPs 13 and 14 and of the receptor ALK6 progressively increased in the muscle of the C26 hosts. Also Noggin levels, a BMP endogenous inhibitor, markedly increased with respect to controls. As a consequence, Smad1/5/8 phosphorylation, readout of the state of activation of the BMP pathway, was nearly completely abrogated. Reactivation of the BMP-dependent signaling by means of caALK3 overexpression was sufficient to prevent tumor-induced muscle atrophy. Downstream Smad1/5/8, MUSA1 was strongly induced in the muscle of C26 hosts and its suppression was able to counteract the wasting process. Similar sparing effects were observed silencing the ubiquitin ligase MuRF-1, though not atrogin-1. Overall, the present data suggest

that the BMP axis can be an effective target for therapeutic approaches to reverse cancer cachexia.

(1) Sartori R *et al.*, Nat Genet. 2013 Nov;45(11):1309-18

1-10

Exploring new targets within the TGF β signaling network as interventions for cachexia

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Transforming growth factor- β (TGF- β) superfamily proteins that signal via activin type II receptors (ActRII), including activin A, activin B, myostatin and GDF11, are central regulators of tissue homeostasis. More recently, increased circulating levels of key ActRII ligands have been implicated in promoting the loss of lean and fat mass associated with conditions in which cachexia is observed. Blocking the activity of ActRII ligands via administration of soluble forms of ActRII has been shown to protect against muscle wasting in cachexia models, but can exert effects in other tissues and organ systems due to the pleiotropic nature of ActRII ligands. Thus, while these results are exciting, the application of soluble ActRII interventions has been limited, and highlights a need to explore the development of new reagents that target the effects of ActRII ligands. Here, we present data on new strategies for targeting the TGF- β signalling network, as prospective interventions for cachexia. Firstly, as activin A and activin B can induce a cachectic phenotype, we have developed modified activin A and activin B prodomains that function as specific activin antagonists. We have established that administration of these novel reagents via gene delivery or protein treatment is protective in mouse models of activin-induced wasting, and can increase muscle mass in healthy mice, whilst avoiding the comparatively broad ligand tropism of soluble ActRII. Secondly, we have developed gene-delivery based approaches to limit ActRII-mediated signalling within skeletal and cardiac musculature that avoid targeting ligands at the extra cellular level. Local and systemic administration of interventions that inhibit ActRII-mediated signalling via enhanced expression of inhibitory Smad proteins achieved marked conservation of skeletal and cardiac muscle mass in mice bearing cachexia-inducing tumors. Finally, we have found that increasing the expression of specific TGF- β family ligands can antagonise ActRII-mediated signalling, and afford protection from features of cachexia caused by ActRII ligands, or implantation of cachexia-inducing tumors. Combined, these studies introduce new strategies for preventing and reversing features of frailty associated with cachectic conditions. Our communication will explore the mechanisms of action underlying these intervention strategies, and consider therapeutic utility in relation to the prevention and treatment of cachexia.

1-11

Myofilament adaptations to cancer and its treatment in humans: mediators of reductions in muscle size and function

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Myofilament proteins are the primary structural component of skeletal muscle and the end effectors of muscle contraction. Pre-clinical and clinical studies suggest that cancer and its treatment are associated with selective reductions in myosin and decreased myofilament protein function, which may contribute to cachexia and cancer-related physical disability, respectively. Considering the negative impact of muscle

atrophy and disability on quality of life and prognosis, knowledge of the adaptations in myofilament protein content and function with cancer and its treatment is important for guiding both basic science studies of the mechanisms underlying muscle atrophy/dysfunction, as well as preventative/corrective clinical interventions. Recent work in pre-clinical models has challenged the notion that cancer cachexia is associated with a selective reduction in myosin protein content and studies in humans have found no evidence for selective myosin reduction or alterations in myosin isoform expression using a range of biochemical and anatomical measurements. In contrast, myofilament protein dysfunction is observed in human cancer patients regardless of cancer site or cachectic status. Perhaps most importantly, deficits in myofilament protein function scale to the tissue and whole body levels to contribute to reduced functionality, suggesting that myofilament dysfunction may contribute to muscle weakness and physical disability in cancer patients. Although there are similarities in myofilament contractile phenotypes across different cancer sites, there are also differences that have unique relationships to pathophysiological sequelae, such as altered mitochondrial biology. These results will be discussed in the context of how they may inform our broader understanding of the skeletal muscle atrophy and dysfunction in human cancer

1–12

Myostatin function in cancer cachexia

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Myostatin, a TGF-beta family member is a negative regulator of muscle growth. Genetic ablation of Myostatin leads to hyper muscularity while ectopic expression of Myostatin results in cachexia/muscle atrophy. Myostatin is predominantly expressed in skeletal muscle and thus is a primary source of myostatin in circulation. Our recent findings show that Myostatin is also expressed in colon carcinoma C26 tumors, and is secreted in the tumor conditioned medium. Consistent with Myostatin activity, the tumor-conditioned medium is able to induce cachexia in differentiated myotubes indicating that tumor secreted Myostatin would further contribute to cancer cachexia. Our results also show that Myostatin induced cachexia through several different mechanisms. One of the mechanisms is through a feed forward mechanism by which Myostatin induces reactive oxygen species through NFκB and TNF-α that in turn can increase myostatin expression. Furthermore, Myostatin is also able to induce DNA damage in type I diabetic muscle. Hence, Myostatin is an important inducer of cachexia and its role in muscle wasting will be discussed.

1–13

Cytokine regulation of muscle wasting in cachexia

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The cachexia syndrome in cancer patients is characterized by a marked weight loss, anorexia, asthenia and anemia and leads to a malnutrition status due to both the induction of anorexia and an accelerated catabolic state, which promotes severe metabolic disturbances in the patient, including hypermetabolism --that generates an increased energetic inefficiency. The key mechanisms leading to cancer cachexia, in which nutritional impairment is a major clinical issue, appear to be primarily immune reactions caused by chronic inflammation. Anti-inflammatory treatments may be effective in clinically improving various symptoms associated with these mechanisms. Although exploration of the role that cytokines play in the host response to invasive stimuli is an endeavour that has been

underway for many years, considerable controversy still exists over the mechanisms of lean tissue and body fat dissolution that occur in the patient with either cancer or inflammation, and whether humoral factors regulate this process. A better understanding of the role of cytokines, both host and tumour-derived, interfering with the molecular mechanisms accounting for protein wasting in skeletal muscle is essential for the design of future effective therapeutic strategies. Because metabolic alterations often appear soon after the onset of tumour growth, the scope of appropriate treatment could influence the course of the patient's clinical state, and would, no doubt, contribute to improving the patient's quality of life and, possibly, prolong survival. In any case, understanding the humoral response to cancer and modifying cytokine actions pharmacologically may prove very suitable and, future research will concentrate on this interesting field. In addition, understanding the intracellular signaling mechanisms, particularly transcriptional factors, may also be very important in the near future for the designing of effective therapeutic approaches.

1–14

Role of the USP19 deubiquitylating enzyme in muscle wasting and myogenesis

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Although enzymes involved in ubiquitin conjugation have been implicated in atrophying muscle, little is known about the role of deubiquitylating enzymes. We previously showed that the USP19 deubiquitylating enzyme is induced in various conditions of muscle atrophy including cancer and that silencing of USP19 can promote expression of myofibrillar proteins in muscle cells. We therefore inactivated USP19 in mice. In response to denervation or dexamethasone, USP19 KO mice showed 26-39% less muscle wasting than WT mice. This sparing of atrophy was associated with improved muscle grip strength and increased cross sectional areas in both Type I and Type IIb fibers as well as decreased expression of atrogenes in both the UPS and autophagic pathways. Electroporation of USP19 shRNAs into tibialis anterior muscles resulted in blunting of denervation induced atrophy. Since impaired myogenesis may also play a role in cachexia, we explored whether USP19 modulates muscle cell differentiation. USP19 is expressed as cytoplasmic and ER localized isoforms. Silencing and over-expression in rodent muscle cells of specifically the ER localized isoform resulted respectively in enhanced or inhibited fusion of muscle cells and expression of myosin heavy chain. This appears to be due to the ability of USP19 to modulate the ER stress response that occurs during differentiation as overexpressing USP19 inhibited the induction of CHOP that occurs during differentiation whilst reinitiating ER stress in these cells with thapsigargin could reverse the inhibition of fusion. These effects appear relevant in vivo as USP19 KO mice subjected to cardiotoxin induced muscle injury regenerated muscles with increased myofiber cross sectional area associated with increased expression of myogenin, myosin heavy chain as well as CHOP. Finally, to explore whether USP19 plays a role in human muscle wasting, we verified its expression in muscle samples of patients with cancer (20 with non-small cell lung cancer and 95 with abdominal cancers) and found that expression of USP19 correlated with expression of the MURF1 and MAFbx ligases. Thus, inhibition of USP19 may prevent muscle atrophy by suppressing proteolytic pathways as well as by promoting myoblast fusion.

1–15

Persistent C/EBP β expression in muscle satellite cells contributes to the pathogenesis of cancer cachexiaFrançois Marchildon¹, Neena Lala¹, Catherine St-Louis², Nadine Wiper-Bergeron²¹Graduate Program in Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada; ²Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada

Normally in response to muscle injury, skeletal muscle stem cells, called satellite cells, are activated to express myogenic regulatory factors and to differentiate to promote regeneration. In cachectic patients, muscle catabolism is increased and the repair mechanism is insufficient, leading to progressive muscle wasting. Using an animal model of cancer cachexia, we have demonstrated that muscle regeneration after acute injury is impaired, suggesting that satellite cell dysfunction contributes to the pathogenesis of cancer cachexia. We have identified a novel transcriptional regulator of myogenesis, CCAAT/Enhancer Binding Protein beta (C/EBP β), which is expressed in satellite cells and is rapidly downregulated when cells are activated to differentiate. In cancer cachexia, persistent expression of C/EBP β in myoblasts potentially inhibits differentiation at least in part through the inhibition of MyoD protein expression and function. Using a conditional knockout model, we demonstrate that loss of *Cebpb* expression results in precocious differentiation of myoblasts in growth conditions and greater cell fusion upon differentiation. In *in vivo*, the absence of *Cebpb* expression in Pax7+ cells resulted in an increase in muscle fiber cross-sectional area and improved repair after muscle injury. However, loss of C/EBP β expression also sensitized satellite cells to apoptosis such that loss of C/EBP β in the context of cancer cachexia worsened the wasting phenotype. These data place C/EBP β as an important regulator in satellite cells acting to promote the undifferentiated state and to protect stem cells in the context of inflammation.

1–16

Inhibiting Stat3 activation suppresses cancer-induced loss of muscle massKleiton Augusto Santos Silva^{1,3}, Jiangling Dong^{1,4}, David J Tweardy², William E Mitch¹, Liping Zhang¹¹Nephrology Division, Department of Medicine, Baylor College of Medicine, Houston, TX, USA; ²Section of Infectious Diseases, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA; ³Nephrology Division, Department of Medicine, Federal University of Sao Paulo, Sao Paulo, Brazil; ⁴College of Life Sciences, Sichuan University, Chengdu, China

Cachexia affects up to 80% of patients with advanced cancers and accounts for ~30% of cancer-related deaths. Unfortunately, mechanisms causing cancer-induced cachexia are not fully understood and there are virtually no successful therapies. We examined how cancers can stimulate muscle wasting by treating C2C12 myotubes with conditioned media from cultures of C26 colon carcinoma or Lewis lung carcinoma (LLC) cells. There was increased activation of Stat3 (p-Stat3) followed by sequential increases in the expression of C/EBP δ and myostatin resulting in decreased sizes of myotubes. When C2C12 myotubes were treated with C188-9, a small molecule inhibitor of Stat3, catabolic responses of the C2C12 myotubes were eliminated despite the addition of conditioned media from C26 or LLC cancer cells. To identify whether Stat3 activation is required for cancer-induced cachexia in mice, we injected LLC into the right flank of control mice and mice with muscle specific Stat3 KO. The muscle-specific KO of Stat3 suppressed the loss of muscle mass that is induced by LLC cancers. In agreement with the muscle wasting pathway of p-Stat3/C/EBP δ /myostatin that we identified in a model of chronic uremia, we found that C/EBP δ KO in mice also blocked LLC-induced muscle wasting

independently of p-Stat3. Next, we treated CD2F1 mice bearing C26 tumor with the Stat3 inhibitor, C188-9. C188-9 significantly ($p < 0.05$) suppressed tumor-induced loss of muscle mass associated with an increase in protein synthesis and a decrease in protein degradation. The responses led to an improvement in muscle strength. We conclude that cancer activates Stat3 in muscle which increases C/EBP δ and myostatin sequentially, causing muscle wasting. In evaluating changes in muscle metabolism, we found that activated p-Stat3 or the presence of C26 or LLC tumors raised the expression of pro- and active caspase-3 and the activity of the ubiquitin-proteasome system in muscles of mice. Evidence for proteolytic activity of caspase-3 in muscles of mice with cancer cachexia was detected by an increase in the 14kD actin fragment (a marker of caspase3 activity). To determine how caspase-3 is activated, we found 3 putative Stat3 binding sites in the promoter of caspase-3 and with CHIP assays; we confirmed that Stat3 binds to the caspase-3 promoter. In C2C12 muscle cells, we examined caspase-3 promoter activity following addition of IL-6 or in response to transfecting cells with constitutively active Stat3. Both IL-6 and constitutively active Stat3 stimulated caspase-3 promoter activity. We conclude that Stat3 activation stimulates caspase-3-mediated proteolysis in muscle. In addition to the action of caspase-3 to cleave the complex structure of muscle proteins, it also stimulate proteolytic activity of the proteasome, cancer cells stimulate both caspase-3 expression and activity in muscle. Thus, inhibiting Stat3 genetically and chemically can suppress a Stat3/C/EBP δ /myostatin pathway; it also suppressed the expression of caspase-3 and its activity, thereby preventing the loss of muscle that occurs in response to certain cancers. Strategies that block p-Stat3 could lead to novel therapeutic strategies against cancer-induced muscle atrophy.

1-17

What is the impact of AMPK activation on cytokine-induced muscle atrophy?Derek T. Hall^{1,2}, Jennifer F. Ma^{1,2}, Takla Griss^{2,3}, Russell G. Jones^{2,3}, Sergio Di Marco^{1,2}, Imed-Eddine Gallouzi^{1,2}¹Department of Biochemistry, McGill University, Montreal, QC, Canada,²Rosalind and Morris Goodman Cancer Centre, Montreal, QC, Canada,³Department of Physiology, McGill University, Montreal, QC, Canada

Cachexia is a disease-associated severe muscle wasting syndrome that dramatically impacts patient morbidity and mortality. As it is well known that chronic inflammation acts as a primary trigger for cachexia, the downstream pathways induced by inflammation have been of particular interest for the development of novel anti-cachectic therapies. However, few effective anti-cachectic therapeutic avenues have been identified. One aspect of muscle physiology that appears to be severely altered by inflammatory stimulus is cellular metabolism, especially at the level of mitochondria. Indeed, we have found that inflammatory cytokine-treated C2C12 muscle fibers undergo a metabolic shift towards aerobic glycolysis and have severely impaired oxidative respiration capacity. Thus, we wished to explore the possibility of targeting cellular metabolism as a potential therapeutic avenue for cachexia. Importantly, the energy-stress response kinase AMPK has previously been shown to down-regulate inflammatory processes, as well as promote an oxidative metabolism. Given this, we hypothesized that AMPK activation could oppose cytokine-driven processes and prevent muscle atrophy.

In order to test the effects of AMPK activation on cytokine-driven wasting, we co-treated muscle fibers with inflammatory cytokines (TNF α and IFN γ) and several AMPK agonist compounds (AICAR, metformin, and A-769662). Intriguingly, we found that, despite the fact that AMPK was already activated upon cytokine treatment, the compounds AICAR and A-769662 prevented cytokine-driven wasting, while metformin had no effect. This prevention correlated with an inhibition of inducible nitric oxide

synthase (iNOS), recovery of myogenic regulatory factor (MRF) expression, and restoration of protein synthesis. Initial experiments in the C26 cancer cell driven mouse model of cachexia show that AICAR treatment is also able to partially recover muscle mass in an *in vivo* model of muscle wasting. Further investigations will attempt to characterize how these compounds impact muscle fiber metabolism during wasting, whether these changes depend on AMPK activation, and if they represent the therapeutic mechanism underlying the ability of AICAR and A-769662 to impair cytokine-driven muscle atrophy.

1–18

The neuroendocrine response in cancer cachexia, and its relationship to neuroinflammatory signaling in the hypothalamus

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Sickness behavior and metabolic adaptation is a highly evolved adaptive host defense against acutely life-threatening events including infection and trauma. Anorexia, fatigue, pain, fever, and muscle catabolism all provide immediate survival benefits, but are unsustainable for extended periods of time. We view cachexia as a manifestation of this unsustainability, and recognize that the core features of this disorder are driven by the interaction of the immune surveillance system with brain centers regulating behavior and metabolism. The idea that cachexia is the result of chronic inflammation is not new. However, new insights into the critical role of localized hypothalamic inflammation and microglial activation in body weight regulation provide strong support for neuroinflammation as a fundamental initiating event in cachexia. While the brain and the periphery share pro-inflammatory ligands and receptors, key features distinguish these two compartments and provide an avenue for novel therapeutic approaches. The elucidation of neuron-specific pathways for interleukin-1 signal transduction, and the demonstration that the developmental pathway for microglia is distinct from peripheral monocytes are examples of recent fundamental discoveries highlighting the unique features of the central immune surveillance system. This talk will address the unique features of hypothalamic inflammation during cancer cachexia and describe how these inflammatory signals elicit neuroendocrine responses that ultimately control activity, feeding and metabolism. The focus will be on understanding the neural basis of cardinal features of cancer cachexia, including anorexia, lethargy, and muscle catabolism. The potential role of these same neuroendocrine responses in generating pathological behavioral and metabolic responses to cancer chemotherapy will also be discussed.

1–19

The role of the muscle stem cell niche in regulating regenerative myogenesis

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Satellite cells in adult skeletal muscle are a heterogeneous population composed of stem cells and committed progenitors. Wnt7a signalling dramatically stimulates the symmetric expansion of satellite stem cells and this expansion requires Fzd7 and Vangl2, both components of the planar cell polarity (PCP) signaling pathway. Importantly, Wnt7a over-expression results in a large expansion of the satellite stem cell population, and Wnt7a deficiency in impaired maintenance of the satellite cell compartment. Therefore, Wnt7a signaling through the planar cell polarity pathway controls the homeostatic level of satellite stem cells and hence regulates the regenerative potential of muscle. In differentiated myofibers, Wnt7a

binding to Fzd7 directly activates the Akt/mTOR growth pathway thereby inducing myofibre hypertrophy. Notably, the Fzd7 receptor complex is associated with GNAS1 and PI3kinase in differentiated myofibres but not in myoblasts, and are required for Wnt7a to activate the Akt/mTOR growth pathway. Wnt7a/Fzd7 activation of this pathway was completely independent of IGF-receptor activation. We found that Syndecan-4 (Sdc4) and Frizzled-7 (Fzd7) form a co-receptor complex in satellite cells and that binding of the glycoprotein Fibronectin (FN) to Sdc4 stimulates the ability of Wnt7a to induce the symmetric expansion of satellite stem cells. Newly activated satellite cells dynamically remodel their niche by transient high-level expression of FN. Knockdown of FN in prospectively isolated satellite cells severely impairs their ability to repopulate the satellite cell niche following transplantation into regenerating muscle. Conversely, *in-vivo* over-expression of FN with Wnt7a dramatically stimulates the expansion of satellite stem cells in regenerating muscle. Therefore, activating satellite cells remodel their niche through autologous expression of FN that provides feedback to stimulate Wnt7a signaling through the Fzd7/Sdc4 co-receptor complex. Thus, FN and Wnt7a together regulate the homeostatic levels of satellite stem cells and satellite myogenic cells during regenerative myogenesis. We generated a truncated Wnt7a variant, consisting of the C-terminal 137 amino acids lacking the conserved palmitoylation sites, which retain full biological activity in skeletal muscle. This includes binding to and signaling through its receptor Fzd7 to stimulate symmetric expansion of satellite stem cells by activating the planar cell polarity pathway, and inducing myofibre hypertrophy by signaling through the AKT/mTOR pathway. Furthermore, this truncated Wnt7a shows enhanced secretion and dispersion compared to the full-length protein. Together, these findings open important new avenues for the development of a Wnt7a as a treatment for muscle wasting diseases and have broad implications for the therapeutic use of Wnts as biologics. M.A.R. is a founding scientist in Fate Therapeutics who have licensed the Wnt7a technology.

1–20

Tumor-derived GM-CSF enhances cancer-associated cachexia by promoting increased adipocyte lipolysis

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Cancer-associated cachexia (CAC) is a wasting syndrome manifested by muscle and adipose loss with no durable interventions. As part of a screen for cachexia relevant serum markers, we found that granulocyte-macrophage colony stimulation factor (GM-CSF) is elevated in the cachectic state. Given the potentially novel connection between GM-CSF and cachexia and in light of other data suggesting a tumor regulatory role for GM-CSF, we sought to understand the molecules fundamental effect on CAC. Knock-down of GM-CSF in a cachexia-inducing tumor (CIT) attenuated adipose tissue loss and tumor growth in murine models. Over-expression of GM-CSF in a non-expressing CIT cell line greatly enhanced cachexia onset, severity, tumor growth and led to decreased survival. However, over-expression of GM-CSF in a non-cachexia inducing tumor (NCIT) did not promote cachexia development and rather promoted tumor regression. The importance of GM-CSF signaling was subsequently investigated in GM-CSF receptor null mice. A CIT implanted into GM-CSFR $-/-$ mice no longer was able to promote cachexia and adipose loss and tumor growth was attenuated compared to wild type mice. Furthermore, a collection of human non-small cell lung cancer (NSCLC) cell lines with elevated expression of GM-CSF had significant cachexia inducing potential *in vivo*. As validation, human serum samples from

patients with NSCLC that displayed clinical signs of cachexia showed elevated levels of GM-CSF compared to non-cachectic NSCLC patients. To determine the biologic role of GM-CSF activity on cachexia induction, we found that GM-CSF promotes the accumulation of myeloid-derived suppressor cells (MDSCs) in adipose tissue, having a high correlation with severity of cachexia symptoms *in vivo*. Attenuation of GM-CSF signaling or depletion of MDSCs *in vivo*, both of which prevented MDSC accumulation and adipose loss driven by CITs, led to a frank limitation on cachexia development. *In vitro* lipolysis assays verified the ability of GM-CSF to enhance lipolysis in the presence of MDSCs and CIT conditioned media, through an arginase mediated mechanism. This study provides the first mechanistic evidence connecting GM-CSF amplification with cachexia-inducing potential through an adipocyte specific lipolytic pathway. Furthermore, it suggests that GM-CSF has dual, but competing roles in the cachectic and non-cachectic settings.

1–21

Cancer-associated cachexia: a switch from white to brown fat due to systemic inflammation leads to organ wasting

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Cancer-associated cachexia (CAC) is well recognized as a severe wasting syndrome, characterized by body weight loss, atrophy of white adipose tissue (WAT) and skeletal muscle, which is accompanied by systemic inflammation. Limited therapeutic options are available and the underlying molecular mechanisms are poorly defined. Using a variety of genetically engineered mouse models (GEMMs) of cancer, such as murine transgenic, chemically-induced, syngeneic and patient-derived xenografts, we were able to show that a phenotypic switch from WAT to brown fat, a phenomenon termed WAT browning, takes place at the initial stages of CAC, before skeletal muscle atrophy (1). WAT browning is associated with increased expression of Uncoupling Protein 1 (UCP1) that uncouples mitochondrial respiration towards thermogenesis instead of ATP synthesis, leading to increased lipid mobilization and energy expenditure in cachectic mice. Chronic inflammation and increased expression of the cytokine IL-6 likely lead to increased UCP1 expression in WAT. Moreover, treatments that reduce inflammation/IL-6 or β -adrenergic blockade reduce WAT browning and ameliorate the severity of cachexia. Importantly, increased UCP1 staining is observed in WAT from cachectic cancer patients compared to weight stable patients. Thus, inhibition of WAT browning represents a promising approach to manage cachexia in cancer patients.

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1–22

Muscle wasting and impaired myogenesis in cachexia

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One of the most relevant features of cancer cachexia is the progressive loss of skeletal muscle protein. Increased protein degradation rates,

resulting from hyperactivation of both the ubiquitin-proteasome system and autophagy play a crucial, though not exclusive, role. Impaired myogenesis also contributes to cancer-induced muscle depletion, as shown by increased expression of Pax7 and by reduced levels of myogenin, markers of activated satellite cells and ongoing differentiation, respectively (1,2). Specific ERK inhibition partially prevents muscle wasting by restoring normal levels of Pax7 and myogenin, demonstrating that this MAPK contributes to the impaired myogenesis (1). By contrast, treatment with dorsomorphin, a molecule known to promote satellite cell differentiation, does not improve muscle mass.

Reduced number of muscle precursor cells could explain the low myogenic potential observed in tumor hosts. However, both satellite cells and mesoangioblasts (MABs) in the muscle of tumor-bearing mice are significantly more abundant than in controls. Not only, in appropriate culture conditions both fully differentiate, excluding the occurrence of a cell autonomous defect. Of interest, during MAB isolation, a conspicuous population of Red Oil-positive cells, absent in controls, becomes evident in tumor-bearing muscle explants, suggesting a shift towards the adipogenic lineage.

Muscle wasting induced by the LLC carcinoma is improved by exercise (3) or by overexpression of the peroxisome proliferator-activated receptor gamma coactivator (PGC)-1 α . Such protection does not occur in C26-bearing mice. This is also reflected by Pax7 expression, normalized in the LLC bearers though not in the C26 hosts. However, the number of central nuclei, indicative of ongoing myogenesis, is higher in PGC-1 α transgenic mice (tg) than in wild-type (wt) animals, irrespective of the presence of the tumor. Indeed, tg muscles on their own contain more CD34+/Sca1+ cells than wt ones; not only, cells from tg mice differentiate better than those from wt animals.

These results suggest that, depending on the tumor, the environment in which myofibers of tumor hosts are embedded may generate a so marked wasting drive that cannot be bypassed by stimulation of the myogenic potential alone, rendering therapeutic strategies such as exercise suitable only for selected cancer patients.

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3. Penna F. *et al.*, *J Cachexia Sarcopenia and Muscle*, **2**: 95–104, 2011.

1–23

Cachexia and the role of the muscle microenvironment

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Cachexia is a debilitating condition characterized by extreme skeletal muscle wasting that contributes significantly to morbidity and mortality. The wasting of skeletal muscle largely derives from aberrant signaling of pathways that maintain a balance between the anabolism and catabolism of muscle protein. In cachexia, this balance is tipped towards a catabolic state resulting from the activation of the ubiquitin proteasome and autophagy systems that regulate protein breakdown, and reduced Akt and mTOR activities that decrease protein synthesis. Whereas these events are firmly established to reside within the myofiber, less regard has been given to potential contributory factors that might act outside the myofiber within the muscle microenvironment. Recently, our laboratory discovered that cachectic muscle derived from either tumor bearing mice or pancreatic cancer patients with weight loss, undergoes a damage response, which signals the activation of both satellite and nonsatellite muscle progenitor cells. These cells commit to a myogenic program, but are inhibited from completing differentiation by an event linked with

persistent expression of the self-renewing factor Pax7. Genetic evidence support that Pax7 is sufficient to drive muscle wasting in cancer by impairing the regenerative capacity of myogenic cells. Thus, Pax7 impairs the regenerative capacity of myogenic cells in the muscle microenvironment to drive muscle wasting in cancer. We have also discovered that NF- κ B signals upstream of Pax7 to mediate the block in differentiation. Additional discussion will focus on the fate of myoblasts whose differentiation is blocked in the presence of tumor factors and the newly identified role of tumor associated microvesicles that may contribute to this process.

1–24

Cancer derived MIC-1/GDF15 induced starvation leads to cachexia

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MIC-1/GDF15 is a TGF- β superfamily cytokine that is overexpressed and secreted by many common cancers. It causes anorexia/cachexia in mice and is linked to this syndrome in humans by causing anorexia and progressive weight loss leading to cachexia.

Our initial observations regarding the biology of MIC-1/GDF15 indicated that its overexpression by cancers lead to a marked decrease in appetite and food intake which could be reproduced by administration of recombinant MIC-1/GDF15 and inhibited by monoclonal antibodies to it. In human diseases associated with increased MIC-1/GDF15 expression, as in mice, weight loss was related to increasing serum levels of MIC-1/GDF15.

In mice, MIC-1/GDF15 decreases appetite and consequently food intake, which led to a loss of both fat and lean mass. This change in body weight could not be explained by altered energy expenditure and pair feeding experiments indicated that loss of body weight as well as fat and lean mass, was commensurate with decreased food intake. A body of data now indicates these effects are mediated by direct actions of MIC-1/GDF15 on feeding centres in the brain. A single IP injection of recombinant protein induces rapid activation of neurons in the hypothalamus and brainstem and modifies expression of neuronal peptides important in appetite regulation such as NPY and POMC. Further, viral expression of MIC-1/GDF15 in the hypothalamus, or direct injection of tiny amounts of recombinant MIC-1/GDF15 into the brain's lateral ventricle has the same anorexigenic effects as systemically administration of MIC-1/GDF15.

To determine if MIC-1/GDF15 might participate in physiological regulation of appetite, we have also studied germline MIC-1/GDF15 gene deleted (MIC-1^{-/-}) mice. Throughout their first year of life, these mice eat more, weigh more and have greater adiposity than their syngeneic controls, an effect that is more marked in female than male mice. Further, this phenotype can be corrected by infusing MIC-1/GDF15 by osmotic minipump, in amounts sufficient to raise their serum levels into the human normal range. To further investigate the major sites of action of MIC-1/GDF15 in the brain, we have undertaken lesioning experiments. Mice which had undergone lesioning of brainstem nuclei which are activated by MIC-1/GDF15, were completely resistant to the anorexigenic actions of MIC-1/GDF15 and as a consequence did not reduce their food intake or lose any body weight. Thus lesioning these brainstem nuclei makes mice resistant to the anorexigenic actions of MIC-1/GDF15.

The available data suggests that overproduction of MIC-1/GDF15 in advanced cancer subverts a physiological pathway of energy homeostasis, leading to anorexia and as a consequence, reduction of fat and lean mass

which eventually results in cachexia. Further, monoclonal antibodies to this cytokine may be an effective therapy for those patients with cancer anorexia/cachexia who have significant elevation in MIC-1/GDF15 serum levels. Conversely, recombinant MIC-1/GDF15 might be effective therapy for patients with severe obesity.

1–25

Ghrelin action in cachexia

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A growing body of evidence indicate that Ghrelin, an acylated peptide hormone released by the stomach upon fasting, counteracts muscle wasting in cachectic patients. Ghrelin stimulates appetite and GH release through acylation-selective binding to GHSR1, its receptor highly expressed in pituitary and hypothalamus. However, the contribution of ghrelin-induced increased appetite and of activation of GH/IGF1 axis in ghrelin anti-cachectic activity have never been established in its anti-cachectic activity.

We recently showed that both acylated and unacylated ghrelin act on the skeletal muscle, independently of GHSR1a expression. Namely we showed that acylated and unacylated ghrelin i) promote muscle differentiation of C2C12 myoblasts in vitro, by inducing cell cycle exit of proliferating C2C12 myoblasts in growing medium, muscle protein expression and myoblasts fusion into myotubes; ii) prevent skeletal muscle atrophy by acting directly in the skeletal muscle to inhibit muscle protein degradation without inducing protein synthesis and hypertrophy; iii) rapidly activate cAMP/PKA, p38 and mTORC2 pathways thus leading to inhibition of muscle atrophy, without inducing mTORC1-mediated phosphorylation of S6 Kinase and S6.

Although the receptor mediating ghrelin anti-atrophic activities in the skeletal muscle is still elusive, we provide preliminary evidence that cachectic skeletal muscles feature reduced sensitivity to ghrelin anti-atrophic activity. In addition, Ghrelin stimulates muscle regeneration through enhancing satellite cells proliferation. Although the role of muscle regeneration in protecting from muscle wasting still remains to be fully elucidated, these data, along with the finding that cachexia is associated to hyper-ghrelinemia, suggest that ghrelin, by acting through a yet unidentified muscle receptor, may play an important role in the stress-induced adaptive response to maintain muscle mass.

1-26

Thermogenesis and altered lipid metabolism in cancer cachexia

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While muscle wasting is the predominant manifestation of cancer cachexia/anorexia, the loss of fat reserves and general metabolic imbalance associated with inflammatory signaling in cancer also contribute to the devastating impact of cachexia. Therefore a broader consideration of the disruption to integrative physiology and homeostatic energy balance due to cancer cachexia is required for the development of treatments for cachexia.

Rapid mobilization of lipids from adipose tissue during cancer cachexia primarily involves Adipose Triglyceride Lipase (ATGL) indicating a tightly regulated process that predominates over lipolysis via Hormone Sensitive Lipase that is normally stimulated in response to calorie restriction^{1,2}. While circulating triglycerides are reduced, plasma free fatty acids increase during cachexia, reflecting active lipolysis of fat alongside anorectic calorie restriction. To understand the role of the liver in the aberrant metabolism of cachexia we carried out transcript, proteomic and MS-based lipid profiling of livers from cachectic C26 tumour-bearing mice. Extensive disruption of lipid metabolic pathways in the liver was evident including repression of fatty acid β -oxidation, while cholesterol and ceramide synthesis were enhanced. An alternative fate for fatty acids mobilized from adipose tissue during cachexia may be as high-energy substrates for thermogenesis in Brown Adipose Tissue (BAT). We previously showed increased uncoupling protein 1 s(UCP1) together with key regulators of thermogenesis in BAT of cachectic C26 mice³. Circadian studies revealed tight diurnal regulation of thermogenesis, with increased UCP1 and BAT temperature during the dark cycle. However these changes in BAT were not evident in mice with a non-cachectic variant of the C26 tumour that doesn't produce IL6, implicating tumour-derived factors in enhanced thermogenesis in cancer cachexia. Silencing IL6 production by C26 tumours using RNAi prevented activation of thermogenesis and development of cachexia. In addition to providing direct evidence for the role of IL6 as a tumorkine in disrupting integrative physiology, these studies indicate potential therapeutic strategies for cancer cachexia by targeting inflammatory mediators.

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1–27

TGF β mediates muscle weakness in bone metastases via oxidation-induced calcium leak

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Cancer-associated muscle weakness is an important paraneoplastic syndrome for which there is currently no treatment. Using a murine model of human breast cancer bone metastases due to MDA-MB-231, we show muscle dysfunction and skeletal muscle oxidation in mice with bone metastases, but not in mice in which the tumor is confined to the primary site. In mice with bone metastases, there was a strong correlation between increased muscle weakness and increased bone destruction. Collectively, these data indicate a critical role of the tumor-bone microenvironment to cause muscle weakness, especially since there was no direct involvement of tumor cells in muscle.

To investigate the role of the bone microenvironment in inducing oxidative stress, we performed proteomic analysis for oxidized proteins of muscle from mice with bone metastases compared with control muscle. The ryanodine receptor/calcium release channel (RyR1) on the sarcoplasmic reticulum (SR), a key protein involved in skeletal muscle E-C coupling was oxidized in muscle from mice with bone metastases. Tetanic Ca²⁺, which directly determines the force of muscle contraction, was reduced

in mice with bone metastases (4.91 ± 0.21 v. 2.28 ± 0.28 ; $p < 0.0001$) and RyR1 was depleted of the stabilizing subunit, calstabin1. *Ex vivo* contractility of the extensor digitorum longus (EDL) muscle showed a significant reduction in specific force in tumor mice ($213.2 \text{ kN/m}^2 \pm 16.6$ v. $361.1 \text{ kN/m}^2 \pm 9.6$; $p < 0.001$). Inhibiting RyR1 mediated SR Ca²⁺ leak with a Rycal (S107) restored muscle force production ($431.0 \text{ kN/m}^2 \pm 19.4$ v. $362.8 \text{ kN/m}^2 \pm 7.2$; $p < 0.0001$) without affecting tumor burden.

TGF β is stored in mineralized bone matrix and released as a consequence of tumor-mediated osteoclastic bone destruction. Since increased bone destruction correlated with increased muscle weakness, we hypothesized that bone-derived TGF β could mediate muscle dysfunction and oxidation. Indeed, muscle from mice and humans with breast cancer bone metastases showed increased SMAD3 phosphorylation compared to normal muscle. We next blocked TGF β signaling in mice with bone metastases either directly, using an anti-TGF β antibody (1D11), or indirectly by blocking osteoclastic bone resorption using a bisphosphonate, zoledronic acid (ZA). Both treatments restored muscle function ($427.2 \text{ kN/m}^2 \pm 12.0$, ZA; $403.1 \text{ kN/m}^2 \pm 11.9$, 1D11 v. $345.6 \text{ kN/m}^2 \pm 12.1$, vehicle; $p < 0.001$), prevented oxidation of RyR1 and maintained calstabin1 binding. Muscle from mice with bone metastases had increased NADPH oxidase 4 (Nox4) levels, a constitutive source of reactive oxygen species (ROS). There was also increased association of Nox4 with RyR1 channels in muscle from mice and humans with breast cancer bone metastases. TGF β induced Nox4 expression, oxidation of RyR1, reduction of calstabin1 binding to the RyR1 complex and SR Ca²⁺ leak (0.93 ± 0.8 sparks/ $100 \mu\text{m}^2 \text{ s}^{-1}$ v. 1.44 ± 1.3 sparks/ $100 \mu\text{m}^2 \text{ s}^{-1}$; $p < 0.05$) in C2C12 myotubes.

Our data show that bone-derived TGF β leads to oxidative stress in skeletal muscle, which causes SR Ca²⁺ leak and contributes to cancer-associated muscle weakness in the setting of bone metastases. These findings indicate an important role for the bone microenvironment to mediate systemic and complex effects on muscle to significantly impair function and suggest multiple targets to treat muscle dysfunction associated with bone metastases.

1–28

T cell function in diabetes and cancer-associated cachexia

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Cachexia is seen in patients with a variety of primary disorders including cancer, diabetes and chronic infectious disease. These chronic disorders are often associated with immune deficiency, also known as lymphopenia. Cachexia is the result of active protein degradation via the ubiquitin proteasome pathway, a decrease in protein synthesis, and a deficiency in the capacity for skeletal muscle regeneration. Despite progress in understanding the mechanisms of cachexia treatment options remain limited.

We have previously discovered a novel CD4+ T cell subset identified by its very low density of the cell surface protein CD44 (CD44v.low cells). CD44v.low cells are absent in diabetic mice that are cachexic. They are also absent in mice with cancer cachexia, but present in mice that have cancer without cachexia. Moreover, infusion of highly purified CD44v.low cells, but not other CD4+ T cell subsets, inhibits both cancer- and diabetes-associated cachexia, as well as cancer-associated lymphopenia. Protection from cachexia is associated with an increase in both skeletal muscle protein and DNA suggesting that the mechanism of protection is not simply to prevent protein degradation. Importantly, CD4+ T cells that express a very low density of CD44 are present in human blood.

CD44v.low cells are precursor cells for a variety of CD4+ T cell subsets, including naïve and memory T cells. They also generate Foxp3+ regulatory T cells. Further characterization of the cells generated by CD44v.low cells

shows that they secrete significantly more non-inflammatory cytokines (IL-4 and IL-6), and less pro-inflammatory cytokines (IL-17A and IL-22) than cells generated by their naïve cell counterparts. Whether the non-inflammatory cytokine profile generated plays a role in the capacity of CD44v.low cells to inhibit cachexia, or whether CD44v.low cells inhibit cachexia by promoting skeletal muscle regeneration remains to be determined. Additional studies are aimed at providing insight into the mechanism by which the CD44v.low cells protect from cachexia.

1–29

IL-6 and mitochondrial dysfunction in cachexia

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Our lab and others have demonstrated that inflammatory cytokine IL-6 and muscle Janus Kinase (JAK) - Signal Transducer and Activator of Transcription (STAT) signaling can regulate muscle mass in some types of tumor-bearing mice. IL-6 activates gp130/STAT signaling in a variety of tissues through either classical or trans IL-6 signaling. In addition, our work and the work of others have established that skeletal muscle mitochondria content is suppressed in cachectic tumor-bearing mice. However, significant gaps remain in our understanding of altered skeletal muscle oxidative capacity with cancer cachexia. The cellular mechanisms causing the cancer cachexia-induced mitochondrial loss and the ramifications of the loss are not well understood. Although skeletal muscle mitochondrial loss coincides with the progression of cachexia in the Apc Min/+ (Min) mouse, IL-6 regulation of these events has not been established. Additionally, the role of mitochondrial loss in the muscle wasting process has not been clearly defined. Since muscle oxidative capacity is modulated by activity level and contraction, it is of further interest to determine if cachectic skeletal muscle maintains metabolic plasticity related to contraction induced mitochondria biogenesis. The purpose of this presentation is to examine the relationship between IL-6 signaling and mitochondrial dysfunction during the development of cachexia in MIN mice. First, the role of IL-6 signaling for the induction of mitochondrial dysfunction will be examined through IL-6 systemic over-expression, IL-6 systemic inhibition, and muscle fiber specific loss of gp130 signaling. Data will then be presented related to the importance of mitochondrial function for the maintenance of skeletal muscle mass in MIN mice, and the role of muscle phenotype in the response. Lastly, we will examine if cancer cachexia interferes with contraction-induced skeletal muscle biogenesis. The presentation will point to the need for future research examining the differential roles of IL-6 trans signaling and muscle gp130 signaling for the regulation of skeletal muscle mitochondrial function during cancer cachexia.

1–30

Metabolic rearrangement in cancer cachexia

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Metabolic derangements are a hallmark of cancer cachexia. The use of Metabolomics has allowed investigators to simultaneously study 1600 – 2500 metabolites in animal models, before and after therapeutic interventions. Hyperlipidemia, hyperglycemia, and reduced branched-chain amino acids are consistently seen in animal models and this profile distinguishes mice with cachexia from other groups such as those with caloric restriction or those with tumor burdens. These metabolic biosignatures or fingerprints may help to distinguish those cancer patients with cachexia or even pre-cachexia which would help with

accurately enrolling patients in clinical trials. Finally, novel therapeutic targets using enzymes in altered metabolic pathways may be possible.

1–31

Malignant inflammation and its role in disease progression and HPA mediated cancer symptoms

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Refractory cancer patients experience symptoms related to their underlying disease, such as anorexia and muscle wasting, which do not directly correspond to the size or location of their lesions. These symptoms are likely the result of a tumor related inflammatory response that stimulates neurons in the hypothalamus responsible for metabolic homeostasis. This inflammation also paradoxically suppresses immune surveillance in the tumor microenvironment and promotes tumor growth.

Interleukin 1 alpha (IL-1 α) is a potent pro-inflammatory cytokine that is thought to play a key role in the initiation and maintenance of tumor related inflammation. Specific blockade of IL-1 α is expected to deprive the tumor of its ability to grow, spread, and evade immune surveillance. In the context of this therapy, the improvement in key cancer symptoms, including reversal of lean body mass loss, is the direct result of an anti-neoplastic effect and should thus correlate with an overall survival benefit. To test this hypothesis, two Phase 3 trials in advanced colorectal cancer are currently underway.

This presentation will highlight current knowledge of IL-1 α involvement in tumor biology, as well as review clinical experience using a true human anti-IL-1 α antibody (Xilonix).

1–32

Emerging approaches to treating muscle atrophy and weakness

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Muscle atrophy and weakness have been observed in many conditions including advanced cancer, where it is known as cancer cachexia. Cachexia confers worse prognosis, results in loss of independence, and has been tightly associated with inflammation. One hypothesis is the switch from white adipose tissue (WAT) to brown fat, a phenomenon termed WAT browning that takes place in the initial stages of cancer cachexia, before skeletal muscle atrophy. Blocking all agents promoting inflammation or β -adrenergic blockade could reduce the fat transformation and subsequently cachexia. One of the challenges is to predict which cancer patients will develop cachexia in order to prevent it. Currently, there is no approved treatment for preserving muscle mass or function. A number of agents, both biologics and small molecules, are in various development stages. One approach is to target muscle anabolism / catabolism pathways including activin, myostatin, GDF-11, and GDF-2/BMP-9. Among those approaches that reached the clinical phase are the anti-myostatin mAb, virus based gene therapy, and activin receptor inhibitors. Two additional targets in the management of muscle wasting are the androgen receptor (SARM) and Ghrelin receptor. It is believed a better understanding of the complex interaction and cross-talk between the negative controllers of muscle mass represented by the myostatin family and the recently reported positive role for bone morphogenetic proteins (BMP's) mediated signaling in muscle would allow the development of new therapies for muscle atrophy and weakness. Safety and efficacy of these various approaches continue to be open questions. In addition, extrapolation of an increase in muscle mass to improved patient outcomes remains to be proven.

1–33

Preclinical investigation of the novel histone deacetylase inhibitor AR-42 in the treatment of cancer-induced cachexia

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Cachexia is a pathophysiological condition characterized by extreme loss of skeletal muscle mass and adipose tissue that cannot be reversed by nutritional support, and leads to pronounced weight loss and weakness that contributes significantly to morbidity and mortality. Cancer cachexia occurs in more than 50% of cancer patients, and is particularly prevalent in those suffering from pancreatic, gastric or esophageal cancer. Cachectic cancer patients are often weak and fatigued, respond poorly to therapy, and have a lower tolerance to therapy and surgery. Thus, the development of effective therapies for cancer cachexia, which could provide tangible clinical benefits to patients, is clearly warranted. AR-42 is a novel class I/II histone deacetylase (HDAC) inhibitor that was developed in our laboratory and is currently in Phase I/II trials in both hematological malignancies and solid tumors at The Ohio State University James Cancer Hospital. Here, we report the anti-cachectic activity of AR-42 in two murine models of cancer cachexia. In the colon-26 (C-26) adenocarcinoma model, oral AR-42 attenuated cachexia-induced losses of skeletal muscle mass, adipose tissue, and body weight, with minimal effects on C-26 tumor growth, and prolonged survival time relative to mice treated with vehicle or other HDAC inhibitors (vorinostat and romidepsin). Metabolomic and gene expression analyses revealed that these anti-cachectic effects of AR-42 were associated with its ability to maintain metabolic and gene expression profiles in skeletal muscle comparable to those in non-cachectic muscle from tumor-free mice. Importantly, this AR-42-induced abrogation of cachexia and rescue of muscle weight was confirmed in the Lewis lung carcinoma model of cancer cachexia. Together, these results support further evaluation of AR-42 as a potential treatment for cancer cachexia.

1–34

Next generation sequencing of the muscle and fat transcriptome in patients with cancer cachexia reveals novel mechanisms

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Cachexia is responsible for one third of cancer deaths and more than 80% of cancer patients are cachectic towards the end of life. To shed light on the mechanisms involved in the major cachexia target tissues, we investigated the entire muscle and fat transcriptome from GI cancer patients with and without early cachexia.

Samples of rectus abdominis muscle and visceral fat were collected at surgery from patients exhibiting 5-10% weight loss prior to surgery,

compared with stable weight patients. Analysis of all expressed genes was carried out using next generation sequencing (Illumina HiSeq 2500).

In muscle, 30 genes showed highly significant change in expression (25 downregulated and 5 upregulated: $P < 0.0005$ - $P < 0.00001$, FDR 0.2). Analysis of the 25 downregulated genes involved included 7 that are involved with metabolism (5 of which are mitochondrial); 4 with signaling; 4 with ubiquitination; and 3 with intracellular trafficking. There was marked downregulation of multiple genes involved in glycogen metabolism which correlates with the lack of glycogen, muscle weakness, and fatigue; characteristic of cachexia. The 5 upregulated genes include 2 involved with calcium signaling and 2 with cell matrix interactions.

Expression of genes previously thought to be important in cachexia, including several inflammatory cytokines, was not significantly different. FBXO32, which encodes atrogen-1, upregulated in an *in vitro* cachexia model, was actually downregulated. No transcripts for the dermicidin gene, which contains the sequence that codes for the backbone peptide of proteolysis-inducing factor, were detected. Expression of myostatin was significantly decreased as was its receptor (ACTR2B), possibly reflecting end organ adaptation to tumor produced myostatin.

In visceral fat, expression of 6 genes were downregulated and 10 upregulated with high statistical significance ($P < 0.001$ - 0.0002). Several of these encode metabolic enzymes. Of genes in fat previously implicated with cachexia, such as hormone sensitive lipase and adipose tissue triglyceride lipase, were unchanged. In contrast, leptin was significantly downregulated and the zinc- α -2-glycoprotein (lipid mobilizing factor) was significantly upregulated as expected.

These studies explain some documented evidence in cachexia pathogenesis, highlight ambiguous data from animal models, and reveal unexpected changes in gene expression that underlie the pathophysiology of the cachectic state in cancer.

1–35

Baseline characteristics of anamorelin phase III clinical trial program (ROMANA), a novel treatment for anorexia-cachexia in Non-Small Cell Lung Cancer (NSCLC) patients

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Introduction: Cancer anorexia-cachexia, a frequent multifactorial syndrome involving altered metabolism and loss of lean body mass (LBM), is associated with increased morbidity and mortality. Anamorelin HCl (ANAM) is a new, oral, selective ghrelin receptor agonist.

Objectives: ROMANA 1 and ROMANA 2 are double-blind, placebo-controlled, randomized (2:1 ANAM vs. placebo) phase III trials assessing ANAM safety/efficacy in Stage III/IV NSCLC patients with cachexia ($\geq 5\%$ weight loss within prior 6 months or $BMI < 20 \text{ kg/m}^2$). ROMANA 3 is their safety extension study.

Methods: In ROMANA 1 and 2, patients receive 100mg ANAM or placebo once daily for 12 weeks. Co-primary endpoints are the change from baseline in LBM (measured by DXA) and in muscle strength (measured by handgrip strength [HGS]). Secondary endpoints include change in body weight, overall survival, and quality of life (FACIT-F, FAACT). ROMANA 1 only additionally assesses population pharmacokinetics. Patients who complete ROMANA 1 or 2 with $ECOG \leq 2$ are eligible for additional 12-week treatment in ROMANA 3.

Results: ROMANA 1 and ROMANA 2 enrolled 484 and 495 patients, respectively. These are mainly Stage IV NSCLC patients (76.1%; 70.5%) with ECOG=1 (69.8%; 66.5%), weight loss $\leq 10\%$ (61.8%; 52.3%), baseline LBM and HGS lower than values commonly observed in healthy adults or patients without weight loss. ROMANA 3 enrolled 513 patients mainly with ECOG=1 (71.9%).

Conclusions: After completing 12-week treatment in ROMANA 1 or 2, the majority of patients maintained an eligible performance score and chose to continue in the optional extension study. Efficacy and safety results are awaited.

1–36

Ghrelin therapy in cancer cachexia

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Ghrelin and its mimetics, also known as growth hormone secretagogues (GHS), increase appetite, lean and fat mass and GH secretion. Furthermore, recent animal studies suggest that ghrelin may also decrease inflammation. Based on these properties, ghrelin and GHS are currently under development as therapeutic options for cancer anorexia/cachexia syndrome (CACS).

Preclinical and clinical data available to date will be reviewed in this presentation. Clinical trials recently have shown that administration of ghrelin or GHS to patients suffering from CACS improves appetite and quality of life, and increases body weight and food intake. Better tolerance to chemotherapy has also been reported and this treatment appears to be safe and well-tolerated. Phase II studies for the GHS anamorelin have been completed and results from phase III studies are expected to be released later this year.

In summary, ghrelin and GHS have the potential to effectively prevent or reverse CACS. Preliminary studies show improvements in weight stabilization and appetite with short-term usage. Further studies are required to fully characterize the role of ghrelin and GHS for the treatment of CACS and to establish the safety of this approach.

1–37

Promotes TNF α - & IFN γ -induced Muscle Wasting in an NF- κ B – Dependent and IL6-Independent Manner

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Cachexia is an end of life syndrome that often develops in patients with cancer. It is characterized by progressive weight loss and skeletal muscle deterioration. The wasting of skeletal muscle mass is due to a decreased synthesis rate and enhanced degradation of muscle proteins. Although 20 – 50% of all cancer deaths are due to consequences of this disorder, no effective treatment is available. The trigger of muscle wasting is thought to be the inflammation associated with cancer. Pro-inflammatory cytokines such as Tumor Necrosis Factor alpha (TNF α) and Interferon gamma (IFN γ) have been shown to mediate muscle wasting through the activation of the transcription factor NF- κ B. Our lab has previously demonstrated that iNOS is a key effector of NF- κ B-mediated muscle wasting. Recently, the Signal Transducer and Activator of Transcription 3 protein (STAT3) was shown to mediate IL-6-induced muscle wasting. Interestingly, studies in non-muscle cells have shown that phosphorylation of the Y705 STAT3

residue mediates gene expression by interacting with NF- κ B. Based on these observations we investigated whether phospho-Y705-STAT3 can mediate IFN γ /TNF α -induced muscle wasting by collaborating with the NF- κ B-signaling pathway.

Our data indicate that the phosphorylation of the STAT3 Tyr705 rapidly increases in C2C12 myotubes treated with IFN γ /TNF α . Surprisingly, this STAT3 phosphorylation occurs in an IL-6-independent manner. Using a phospho-STAT3 inhibitor, we show that STAT3 is a key component of TNF/IFN-induced muscle wasting. Additionally, we observed that STAT3 promotes muscle wasting by activating the transcription of the iNOS mRNA in TNF/IFN-treated muscle fibers. Our data also indicates that the TNF/IFN-induced phosphorylation promotes the nuclear translocation of STAT3. In the nucleus, STAT3 interacts with the p65 subunit of NF- κ B resulting in the induction of iNOS transcription. Using chromatin IP (ChIP), we show that STAT3 can bind to the iNOS promoter. Moreover, preliminary data suggests that this binding is dependent on NF- κ B.

Together, our data provides evidence that STAT3 can be activated by TNF/IFN in an IL-6-independent manner leading to muscle wasting.

1–38

SAA1 in limb muscle of patients with COPD during stable state and acute exacerbation

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Background: Limb muscle dysfunction is a major consequence of chronic obstructive pulmonary disease (COPD). The inflammatory response is believed to contribute to the development of muscle atrophy related to chronic diseases such as COPD. Serum amyloid A1 (SAA1) is an acute phase protein which is overexpressed in lungs of patients with COPD. We hypothesized that SAA1 could trigger a cascade of inflammatory events contributing to limb muscle alteration in COPD.

Objectives: To investigate the pattern of systemic and muscle SAA1 expression in COPD, we 1) determined SAA1 plasma protein level, 2) evaluated quadriceps SAA1 mRNA and protein expression levels during stable state and acute COPD exacerbation, and 3) studied whether SAA1 could be produced by cultured primary skeletal limb muscle cells from healthy people.

Methods: We recruited 10 age-matched healthy subjects, 11 patients with stable COPD and 11 patients experiencing an acute COPD exacerbation. Blood samples were collected and quadriceps biopsies were performed. Primary skeletal muscle cells were cultured. Blood and cell supernatant SAA1 levels were assayed by ELISA. SAA1 mRNA and protein levels were respectively evaluated using RT-qPCR and western blot.

Results: A 32-fold ($p = 0.04$) increase in SAA1 plasma level was seen during acute COPD exacerbation while no difference between patients with stable COPD and healthy subjects was observed. Eight-fold ($p = 0.01$) and 15-fold ($p = 0.015$) increases in muscle SAA1 mRNA expression were observed in stable condition and during acute COPD exacerbation, respectively. Although there was no detectable SAA1 protein in muscle biopsies, it was measurable in the supernatant of IL-1 β -stimulated skeletal muscle cells.

Conclusions: We observed a sharp increase in SAA1 plasma protein and muscle mRNA levels in patients with COPD during an acute exacerbation of their disease. Although there was no evidence of SAA1 protein in muscle, IL-1 β -stimulated human skeletal muscle cells released SAA1 protein in supernatant. Our results demonstrate that SAA1 is not confined to muscle but is secreted in the blood flow, therefore behaving like a myokine.

1–39

Increased mitochondrial reactive oxygen species emission is required for doxorubicin-induced cardiac and skeletal muscle myopathy

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Doxorubicin (DOX) is a highly effective antitumor agent widely used in cancer chemotherapy. Unfortunately, the clinical application of DOX is limited by its adverse effects on several tissues and organs, including cardiac and skeletal muscle. Indeed, one of the most serious side effects of DOX is life-threatening cardiac injury, including the development of cardiomyopathy and ultimately congestive heart failure. Further, recent reports indicate that systemic DOX administration at clinical doses depresses skeletal muscle specific force production. Therefore, the development of a countermeasure to prevent DOX-induced myopathy requires a detailed understanding of the mechanism(s) responsible for DOX-induced damage to both cardiac and skeletal muscles. It is widely suggested that DOX-induced cellular damage and dysfunction occurs due to increased mitochondrial production of reactive oxygen species (ROS) leading to oxidative stress, activation of key proteases and ultimately damage to key cellular components. Mitochondria appear to be a significant source of oxidant production in cardiac and skeletal muscles following DOX administration, in part, because DOX binds with high affinity to cardiolipin, a phospholipid that is uniquely expressed on the inner mitochondrial membrane. Indeed, DOX treatment significantly increases mitochondrial ROS production leading to mitochondrial dysfunction. However, it is experimentally unproven if prevention of DOX-induced mitochondrial ROS emission can protect against DOX-induced cellular damage. Therefore, the current experiments tested whether increased mitochondrial ROS emission is required for DOX-induced myopathy of cardiac and skeletal muscles. Cause and effect was determined by using a novel mitochondrial-targeted peptide (SS-31) to prevent DOX-induced increases in mitochondrial ROS emission. Our findings reveal that mitochondria are the major site of DOX-mediated ROS production in cardiac and skeletal muscle fibers and that prevention of DOX-induced mitochondrial ROS emission protects against atrophy and dysfunction in both cardiac and skeletal muscles.

1-40

Weight, weight change, and colorectal cancer mortality in the Kaiser Permanente Northern California population

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Objective: Higher body weight has been associated with a higher risk of colorectal cancer (CRC). However, little evidence shows that overweight/obesity predicts worse survival. We evaluated whether weight prior to, at the time of, and following a CRC diagnosis, was related to post-diagnosis mortality. Since weight loss is a hallmark of cancer cachexia, we also evaluated whether weight change after diagnosis, adjusted for at-diagnosis weight, was related to mortality.

Methods: We identified 3,409 men and women from Kaiser Permanente Northern California diagnosed with stages I-III CRC between 2006–2011 with data on weight and height at diagnosis. Using Cox models, we evaluated associations between categories of body mass index (BMI) approximately nine months prior to diagnosis, at diagnosis, and one year

following diagnosis, and all-cause and CRC-specific mortality, omitting recurrences within the first six months, adjusted for sociodemographic characteristics, disease severity, and treatment. We also evaluated associations between categories of weight change between diagnosis and follow up, and mortality, simultaneously adjusted for at-diagnosis BMI.

Results: At-diagnosis BMI was associated with all-cause and CRC-specific mortality in a U-shaped fashion, with underweight (BMI < 18.5 kg/m², hazard ratio (HR)=2.27, 95% confidence interval (CI):1.34-3.84) and morbidly obese (BMI ≥ 35 kg/m², HR=1.31, 95% CI:0.94-1.83) patients exhibiting elevated mortality risks, compared with low normal-weight (BMI 18.5-<23 kg/m²) patients. By contrast, overweight (BMI 25-<28 kg/m², HR=0.78, 95% CI:0.58-1.06 and BMI 28-<30 kg/m², HR=0.43, 95% CI:0.28-0.66) and high normal-weight (BMI 23-<25 kg/m², HR=0.68, 95% CI:0.48-0.96) patients showed reduced mortality risks. Associations of BMI with CRC-specific mortality were similar, but morbidly obese patients showed no higher risk. Additionally, associations of pre-diagnosis and post-diagnosis BMI and all-cause mortality were similar to at-diagnosis associations, but morbid obesity was not associated with survival. Independent of at-diagnosis weight, weight loss > 10% between diagnosis and follow-up was uniquely associated with elevated all-cause (HR=3.10, 95% CI:2.40-4.00) and CRC-specific (HR=3.42, 95% CI:2.34-5.00) mortality compared to those who maintained weight within 5% of at-diagnosis weight.

Conclusion: High normal-weight and overweight were associated with reduced mortality after a CRC diagnosis. Extremes in weight at diagnosis, and large subsequent weight loss independent of weight at diagnosis, were associated with higher mortality.

1–41

A systematic review of features of cancer cachexia in commonly used experimental models

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We performed a review of literature on experimental models of cancer cachexia prior to July 2012. Of 632 articles containing original experimental data, 485 articles focused on the 7 commonest rodent cancer models or human xenograft experiments. Data from 397 articles with full-length manuscript accessible were analyzed.

A significant minority (18%) of reports included data on more than one tumour. In 397 articles we identified 494 separate tumour-host experiments and report data for 448 of these experiments. Experiments used either mice (57%) or rats (43%) and tumours derived from colon (32%), liver (17%), lung (11%), breast (13%) and chemically-induced sarcoma (20%). The commonest mode of tumour implantation was subcutaneous injection (75%) and most (66%) experiments lasted 2–4 weeks after implantation. Where possible we calculated the whole body rate of weight change (including tumour). Weight data from pair-fed controls, to correct for the effect of changes in food intake, were used but this was only available in 16% of reports. The majority of articles (>72%) reported weight loss, with a median rate for all models of 6% loss per week.

Reporting of the experimental protocols was often incomplete and we identified several potential sources of variance in reported results. In nearly half the reports (46%) the source of tumour cells was unclear, and there was wide variation in the conditions for pre-experimental culture and expansion of cells: 39% cultured in vitro, 51% grown in vivo in other animals. In all cachexia models a majority of experiments were

performed in males, with the exception of the xenograft group that had the inverse pattern (61% female). Confirmation of basic dietary macronutrient content was provided in only 22% of reports.

The evolution and frequency of features of cancer cachexia varies between different human cancers and in different individuals with the cancer type. The causes for this variation may have important implications for anti-cachexia treatments. A systematic comparison of preclinical models of cancer cachexia has potential to identify key determinants of cachexia in humans. For this to succeed improvements including a comprehensive, standardized approach to performance and reporting of experimental cachexia studies are needed.

1-42

USP19 deubiquitinating enzyme inhibits muscle cell differentiation by suppressing the unfolded protein response

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Muscle wasting is a common complication of aging and many chronic diseases, including cancer. The protein catabolism in muscle during wasting is largely induced by the activation of the ubiquitin proteasome system (UPS). We have shown that the deubiquitinating enzyme USP19 is upregulated in rat skeletal muscle under various catabolic conditions and that its inactivation in mice results in less muscle wasting. In addition, USP19 modulates the expression of myogenin and myofibrillar proteins in L6 muscle cells. This raised the possibility that USP19 might regulate muscle cell differentiation. We therefore tested the effects of adenoviral mediated over expression or siRNA mediated silencing of either the cytoplasmic or ER localized isoforms of USP19. Only the ER localized isoform of USP19 (USP19-ER) modulated myoblast fusion as well as the expression of myogenin and myofibrillar proteins and these effects were also dependent on USP19 catalytic activity. USP19-ER inhibited both muscle cell differentiation and the unfolded protein response gene CHOP that occurs during differentiation. Inducing mild ER stress with thapsigargin was able to reverse the defect in myoblast fusion caused by the overexpression of USP19-ER suggesting strongly that USP19 exerts its effects on fusion through its effects on ER stress signaling. USP19 also functions similarly *in vivo*, as USP19^{-/-} mice display improved muscle regeneration concomitant with enhanced expression of CHOP. Collectively, these results implicate for the first time a deubiquitinating enzyme as a regulator of the unfolded protein response. They also suggest that inhibition of USP19 may be a therapeutic approach for the enhancement of muscle growth following injury or during recovery from wasting.

1-43

The role of activin C in cancer cachexia

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Muscle wasting and cachexia have long been considered key determinants of cancer-related death and reduction in the quality of life of cancer patients. A signaling pathway recognized to affect cancer cachexia and skeletal muscle atrophy-hypertrophy balance is the activin signaling pathway, which comprises extracellular ligands (inhibin, activin, myostatin) trans-membrane receptors (IIA/IIIB) and intracellular signal transducers (Smad-2/3).

A mouse genetic model of gonadal tumorigenesis in which the normal balance of inhibin/activin signaling has been disrupted by a target mutation

in the *Inha* gene (inhibin α -KO mouse), exhibits a cancer related muscle wasting associated with high levels of activin-A and represents an excellent model for the study of cancer-associated cachexia.

A study conducted by our group provided the first evidence that activin-C is an antagonist of activin-A both *in vivo* and *in vitro*.

Based on this evidence, we hypothesized that overexpression of activin-C could modulate the cachexia phenotype in the inhibin α -KO mouse antagonizing the activin signaling pathway and repressing the ubiquitin-proteasome catabolic system.

Therefore, we used mice overexpressing activin-C (ActC++) crossed with inhibin α -KO mice (α -KO/actC++) to assess the biological effect of activin-C overexpression on the onset of cancer-associated cachexia and muscle metabolism.

Male and female ActC++, α -KO and α -KO/ActC++ mice and littermate WT controls were studied. Western blot analysis for the specific E3 ubiquitin ligases, atrogin-1 and MuRF1, effectors Smad-2/Smad-3 and myostatin were performed in the gastrocnemius of age-matched mice. Histopathology of the gastrocnemius and survival analysis was also conducted in animals from the same breeding cohort. Serum levels of activin-A and inflammatory cytokines TNF- α , IL-6 and IF- γ were also assessed.

Results revealed increased levels of atrogin-1, MuRF-1, Smad-2 and serum levels of activin-A in the α -KO mice. These mice developed gonadal cancers followed by severe weight loss, reduced muscle weight and reduced survival. Overexpression of activin-C antagonized the activin signaling cascade, reducing the levels of atrogin-1, MuRF1 and serum levels of activin-A. α -KO/ActC++ mice displayed a less aggressive cachectic phenotype, reduced tumor weight and prolonged survival.

Our findings clearly demonstrate the importance of the activin signaling pathway in cancer-associated cachexia and indicate that activin-C may be a novel anti-activin therapy to combat cancer associated weight loss and prolong survival.

1-44

Cancer cachexia stimulates the expression of CCAAT/Enhancer Binding Protein beta (C/EBP β) to protect muscle satellite cells from apoptosis **François Marchildon, Nadine Wiper-Bergeron**

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Cancer cachexia is a syndrome affecting nearly half of cancer patients and is believed to be the cause of death for more than 20%. These patients suffer from colossal muscle and adipose wasting and this excessive muscle loss impairs muscle function, promotes frailty and increases morbidity. Understanding the mechanisms of muscle wasting and developing strategies to block this loss is of most importance to promote survival and quality of life, and to increase the efficacy to cancer treatment strategies. While much work has been done on the fiber-intrinsic mechanisms driving myofiber atrophy, we concentrated on the effects of cachexia on the capacity of muscle to regenerate. Satellite cells (SCs) are adult stem cells found on each muscle fibers that act to repair damaged muscle. In cancer cachexia, SCs malfunction and cripple the regeneration process. Here, we investigate the function of CCAAT/Enhancer Binding Protein beta (C/EBP β), a bzip transcription factor expressed in SCs in this process. C/EBP β is a negative regulator of myogenesis that is downregulated upon induction to differentiate. C/EBP β can stimulate Pax7 expression and can inhibit MyoD expression and function. C/EBP β expression is stimulated by a number of cytokines and can inhibit apoptosis. By using a conditional knockout mouse model in which C/EBP β is deleted in Pax7+ cells, we found that loss of C/EBP β increased myoblasts apoptosis and that cancer cachexia stimulates persistent C/EBP β expression in SCs, protecting these from apoptosis. As such, loss of C/EBP β *in vivo* exacerbated cachexia by increasing apoptosis of SCs needed for regeneration. Our findings support the notion that the inhibition of

regeneration, through the expression of C/EBP β contributes to the pathogenesis of cancer cachexia, but that this persistent expression is necessary to protect the stem cell population from apoptosis in a cachectic environment.

1–45

The Bromodomain inhibitor JQ1 prevents skeletal muscle loss during cancer cachexia

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Skeletal muscle wasting is a hallmark of cancer cachexia. This metabolic syndrome is responsible for about 25% of cancer deaths. In particular, muscle loss in cachectic patients often leads to increased morbidity and mortality, decreased beneficial effects from chemotherapeutic treatment, and poorer quality of life. Therefore, the development of therapeutic avenues addressed at preventing muscle wasting during cancer cachexia is attracting increasing clinical interest. To date, no effective therapies for cachectic muscle are available. Recently, our research group reported that the small bromodomain inhibitor JQ1 enhances muscle fiber size and protects from dexamethasone-induced muscle atrophy in C2C12 myotubes, by blocking skeletal muscle pro-atrophic pathways, triggered by myostatin. In the present work we evaluated the effect of JQ1 treatment in skeletal muscle wasting during cancer cachexia. To this aim, C26 (adenocarcinoma cell line) bearing mice were chronically treated with JQ1 or vehicle. After 12 days, body weight, skeletal muscle weight and the anabolic/catabolic pathways involved in skeletal muscle homeostasis were analyzed. The results show that JQ1 treatment blocks muscle-specific ubiquitin ligases expression, and protects tumor-bearing mice from body weight loss and muscle wasting. Furthermore, JQ1 administration prevents adipose tissue loss and restores lipids levels in the blood. These results suggest that the epigenetic modulation mediated by bromodomain inhibitors may represent a promising therapeutic approach in the management of muscle wasting during cancer cachexia.

1–46

The regulation of satellite cell function and myogenesis by isoforms of CCAAT/enhancer binding protein beta

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Adult skeletal muscles have remarkable regenerative capacity. Muscle regeneration occurs when muscle tissue experiences injury, causing a population of normally quiescent muscle-resident stem cells, called satellite cells to become activated. The CCAAT/enhancer binding proteins known as C/EBPs are transcription factors belonging to the bZIP family. Previous study from our lab has identified that C/EBP β is an important negative regulator of myogenesis. C/EBP β expression was shown to be localized to satellite cells of healthy muscle and was downregulated upon induction to differentiate which mirrored the loss of Pax7 expression and negatively regulated the expression of MyoD. Leaky ribosomal scanning of the *Cebpb* mRNA produces three C/EBP β isoforms: LAP*, LAP and LIP. This study focuses on the role of each of the three C/EBP β isoforms in skeletal muscle differentiation. We demonstrate that C/EBP β -LIP can control cell proliferation possibly by impinging upon the cell cycle machinery or signaling mechanisms that regulate cell growth and division. Upregulation of C/EBP β -LIP expression in myoblasts led to the loss of Myf5, MyoD, and myogenin expression under differentiation conditions. Since LIP expression is controlled by numerous signalling pathways and is regulated in pathological conditions such as cachexia, study involving the mechanisms and regulation

underlying C/EBP β isoforms is an essential to advance our knowledge into the pathogenesis of skeletal muscle wasting and cancer cachexia.

1–47

Hypothalamic modulation of neuropeptide Y by serotonin in cancer anorexia

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Anorexia is a common symptom among cancer patients and contributes to malnutrition and strongly impinges on quality of life. Cancer-induced anorexia is thought to be caused by an inability of food intake-regulating systems in the hypothalamus to respond adequately to negative energy balance during tumour growth. We show that this impaired response of food-intake control is likely to originate from failure in posttranscriptional neuropeptide Y (NPY) regulation mediated by altered serotonin signalling.

Two tumour cachectic mouse models with different food intake behaviours were used: a C26-colon adenocarcinoma model with increased food intake and a Lewis lung carcinoma model with decreased food intake. This contrast in food intake behaviour between tumour-bearing (TB) mice in response to growth of the two different tumours was used to distinguish between processes involved in cachexia and mechanisms that might be important in food intake regulation. The hypothalamus was used for transcriptomics (Affymetrix chips).

In both models, hypothalamic expression of orexigenic neuropeptide NPY was significantly higher compared to controls, suggesting that this change does not directly reflect food intake status. Expression of genes involved in serotonin signalling showed to be different between C26-TB mice and LLC-TB mice and were inversely associated with food intake. Plasma levels of TNF α were significantly increased in LLC-TB mice, but not in C26-TB mice. In addition, levels of IL-6 were higher in LLC-TB mice than in C26-TB mice, suggesting that inflammatory response is different between the two models. *In vitro*, using hypothalamic cell lines, serotonin repressed neuronal hypothalamic NPY secretion, while not affecting messenger NPY expression, suggesting that serotonin signalling can interfere with NPY synthesis, transport or secretion.

Overall, our results underline the importance of serotonin in food intake regulation in cancer cachexia, due to its ability to inhibit food intake likely via affecting neuropeptide Y. Serotonin regulation might therefore be a therapeutic target to prevent the development of cancer-induced eating disorders. Furthermore the results indicate that TNF α and IL-6 might play a crucial role in cancer anorexia as well, however this still has to be further elucidated.

1–48

Role of the USP19 deubiquitinating enzyme in the activation of the ubiquitin proteasome system in skeletal muscle upon stimulation with dexamethasone

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Loss of skeletal muscle is a prominent feature of cancer cachexia. The muscle atrophy results from decreased protein synthesis and increased

degradation. The increase in protein degradation is dependent on the activation of the ubiquitin proteasome system (UPS), a pathway in which selected proteins are labeled with ubiquitin that targets them for degradation. Many studies have revealed the role of ubiquitin ligases in muscle wasting conditions but little is known about the role of deubiquitinating enzymes. Our laboratory has previously shown that the deubiquitinating enzyme USP19 is induced under different conditions of muscle atrophy including fasting, glucocorticoid treatment, denervation and cancer bearing animals. To further evaluate the role of USP19, we have tested the loss of USP19 in dexamethasone-induced atrophy in muscle cells and mouse muscles using both gene knockout and silencing methods. USP19 WT and KO mice were injected twice per day with dexamethasone 5mg/kg for 7 days. USP19 KO mice lost 64% less tibialis anterior (TA) muscle mass than WT mice. Similarly, upon dexamethasone treatment, average cross sectional area of USP19 KO TA fibers was larger than the WT. Rates of muscle protein synthesis were decreased in dexamethasone treated mice, but similarly so in both WT and KO. However, the induction of ubiquitin ligases MuRF1 and Atrogin1 and autophagy genes Atg4, LC3b, Bnip3 seen in muscles in dexamethasone treated WT mice were significantly attenuated in muscles of the KO mice. To test whether these muscle protective effects are likely due to direct action of USP19 in muscle, we tested the effect of silencing USP19 in differentiating L6 muscle cells exposed to dexamethasone. Depletion of USP19 in L6 myotubes attenuated the dexamethasone induction of Atrogin1. Electroporation of shRNAs targeting USP19 into TA muscle of mice protected them from denervation atrophy. Inactivation of USP19 also protects mice from muscle wasting caused by fasting or denervation. Expression of USP19 correlates with that of MuRF1 and Atrogin1 in muscle samples of patients with either lung cancer or gastrointestinal tumors. Taken together, these results suggest that the inhibition of USP19 may be useful for the prevention of muscle atrophy caused by a broad spectrum of common conditions.

1–49

Self-renewal and differentiation of skeletal muscle satellite cells are regulated by C/EBP β

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Post-natal growth and repair of skeletal muscle relies upon a population of quiescent muscle precursor cells, called satellite cells (SCs). These cells are capable of being activated to proliferate and differentiate into new myofibers, as well as self-renew to re-populate the SC niche. Our lab is interested in examining the role of CCAAT/Enhancer Binding Protein beta (C/EBP β), a bZIP transcription factor, in myogenesis. We found that overexpression of C/EBP β in C2C12 myoblasts and primary myoblasts reduced MyoD and myogenic protein levels during differentiation, in addition to reducing fusogenic ability. Furthermore, C/EBP β increased Pax7 protein expression, *in vitro*, and was localized to Pax7⁺ SCs, *in vivo*. C2C12 myoblasts overexpressing C/EBP β were phenotypically similar to slowly proliferating satellite cells as demonstrated by their decreased BrdU incorporation and increased expression of the quiescent satellite cell marker, Caveolin-1. Using genetic tools to conditionally abrogate C/EBP β expression in SCs, we found that loss of C/EBP β led to increased differentiation and decreased self-renewal of SCs in myofiber cultures. Furthermore, loss of C/EBP β in satellite cells led to a decrease in the number of Pax7⁺ cells in the satellite cell niche. Further, C/EBP β is regulated during

muscle wasting and sarcopenia placing it as a novel regulator of satellite cell function in health and disease and is a promising new therapeutic target to enhance differentiation or self-renewal of satellite cells.

1–50

Muscle Volume (MV) is correlated with body mass index (BMI) in cancer patients

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Background: Cachexia is a common problem in patients with advanced cancer. While biomarkers of appetite and inflammation are readily available, the study of cachexia is limited by the difficulty of easily measuring lean body mass (LBM) in the clinic. Advances in radiology software have enabled LBM estimates from muscle area measured on CT scans. More recently, the capability to measure muscle volume (MV, cm³) radiologically has been developed, potentially improving the estimation of LBM.

Aims: to describe the MV of patients diagnosed with cancer and correlate MV with various clinical and demographic variables.

Methods: Retrospective chart review of the electronic medical record including images on the picture archiving and communication system (PACS) of 141 consecutive patients seen in a Pain & Palliative Care clinic at an NIH-designated comprehensive cancer center. Cancer patients and disease-free survivors who had a CT scan within 30 days of being seen were included. MV was measured manually over 5 CT scan slices at the L3 level and calculated using commercially available radiology software (TeraRecon Inc., Foster City, CA 94404). Clinical and demographic variables at the time of the clinic encounter were recorded.

Results: 42% cases (46/112 cancer patients and 14/29 cancer survivors) had had a CT within 30 days of a clinic visit. Mean MV at L3 was 389 \pm 11 cm³. MV was significantly associated with BMI ($r^2=0.099$, $p=0.033$). While more of the patients lost >10% body weight in the prior 6 months than the survivors (30% vs. 0%), there was no difference in BMI (27.3 vs 29.9, $p=0.28$) or MV (396 cm³ vs. 378 cm³, $p=0.58$) between the two groups, respectively.

Discussion: To our knowledge, this is the first study to measure MV radiologically in patients with advanced cancer. No normative data exists for healthy adults. With practice, measurement of MV can be completed in 5–10 minutes in most cases. Larger validation studies are needed, as is the development of a formula to derive LBM from MV.

1–51

A standardized and mature human myotube cellular model to detect myotoxicity and hypertrophic compounds

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Progressive muscle wasting is a well described syndrome of cancer associated cachexia. This usual complication of tumor progression is known to contribute to 20% of cancer death. Besides decreasing survival, cachexia also leads to worsen tolerance to chemotherapy and alters patients quality of life, drastically reducing their autonomy. Research on compounds able to renew muscle mass is therefore crucial to compensate tissue wasting during neoplasia progression. In this context, CYTOO has developed a physiological muscle model allowing the detection of compounds inducing atrophy and hypertrophy. When cultured on CYTOO micropatterns, primary human myoblasts faster differentiate into myotubes displaying a higher level of sarcomere striation and nuclei

alignment compared to standard culture conditions. Moreover, the use of micropatterns greatly standardized myotube formation and morphogenesis. Thanks to the development of new image analysis algorithms and the reduced variability of myotube morphology, the achieved cellular model enabled accessing new parameters for myotube characterization upon drug treatment. To demonstrate the benefits of our model, reference drugs inducing hypertrophy or atrophy were tested, and a library of 56 myotoxic compounds was screened using an atrophy readout. Our results showed that this model is highly robust and compatible with High Content Screening with increased Z' factors compared to standard assays for atrophy and hypertrophy. Enhanced image analysis capacities also allowed the simultaneous screening of compounds inducing cytotoxicity, apoptosis and globally affecting myogenesis.

1–52

Microvesicles containing miRNAs promote muscle cell death in cancer cachexia via TLR7

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MicroRNAs (miRNAs) are small, noncoding RNAs that regulate gene expression and, in cancers, are often packaged within secreted microvesicles. The cachexia syndrome is a debilitating state of cancer that predominantly results from the loss of skeletal muscle mass, which is in part associated with apoptosis. How tumors promote apoptosis in distally located skeletal muscles has not been explored.

Using both tumor cell lines and patient samples, we show that tumor derived microvesicles induce apoptosis of skeletal muscle cells. This proapoptotic activity is mediated by a microRNA cargo, miR-21, which signals through the Toll-like 7 receptor (TLR7) on murine myoblasts to promote cell death. Furthermore, tumor microvesicles and miR-21 require c-Jun N-terminal kinase activity to regulate this apoptotic response. Together, these results describe a unique pathway by which tumor cells promote muscle loss, which might provide a great insight into elucidating the causes and treatment options of cancer cachexia.

1–53

Improvements in overall survival and tumor control in cancer cachexia through modulation of a novel FABP8-FABP4-PPAR gamma axis that regulates adipocyte lipolysis

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Cancer cachexia (CC), a debilitating syndrome associated with muscle and fat loss and reduced survival, has no established interventions targeting adipose. In a candidate screening approach, we showed that the anti-diabetic pioglitazone (PGZ) limited CC in murine models by 1) promoting adipose tissue health, 2) reducing tumor burden, 3) dampening muscle atrophy, and 4) increasing overall survival. Use of tissue specific, inducible models of PPAR gamma ablation demonstrated that PGZ's sentinel system-wide effect in CC is on adipocyte lipolysis, with tumor control a subsequent event. In adipocytes exposed to cachectic

factors, there was a partial relocalization of PPAR gamma to lipid droplet surfaces, inhibiting its pro-lipogenic transcriptional capacity. With block of PPAR gamma nuclear export through PGZ and other agonist action, cachexia induced lipolysis was abrogated. FABP8, a fatty acid binding protein previously thought to be restricted to neuronal tissues, had its expression upregulated in adipocytes during CC states and suppressed with PGZ co-administration. In adipocytes, it directly interacted with FABP4 and PPAR gamma around lipid droplets during cachexia-induced lipolysis, promoting PPAR gamma's partial extranuclear localization. FABP8 was only induced if either FABP4 was saturated with free fatty acids (FFAs) or inhibited, i.e. during states of adipocyte lipolytic stress. Both *in vitro* and with novel *in vivo* models, fat specific FABP4 or FABP8 ablation significantly suppressed CC, controlled tumor volumes, and improved survival similarly to PGZ, concomitant with reduced extranuclear PPAR gamma. Human fat from cachectic cancer patients uniquely demonstrated up-regulation of FABP8 but stable FABP4 and PPAR gamma expression, suggesting a regulatory role for FABP8. Control of adipocyte lipolysis through novel fatty acid binding protein relevant pathways appears to alter distant tumor kinetics, helping to explain why cachexia correlates with survival in cancer patients.

1–54

Myostatin and mitophagy

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Skeletal muscle atrophy is thought to result from increased protein degradation pathways, including autophagy and the ubiquitin-proteasome system. However, the precise contributions of these pathways to muscle atrophy are unclear. Specifically, the dysfunction and subsequent loss of mitochondria termed, mitophagy, has been shown to be a potent inducer of skeletal muscle wasting. However, the molecular mechanisms that govern the deregulation of mitochondrial function during muscle wasting are unclear. Using a molecular screen we have identified that muscle-wasting stimuli upregulated mitochondrial E3 ubiquitin protein ligase 1 (Mul1), through a mechanism involving FoxO1/3 transcription factors. We observed that overexpression of Mul1 in skeletal muscles and myoblast cultures were sufficient for the induction of mitophagy. Consistent with these results we find that Mul1 expression not only protected against mitophagy but also partially rescued the muscle wasting observed in response to muscle-wasting stimuli. In addition, we also find that upregulation of Mul1, while increases mitochondrial fission, resulted in ubiquitination and degradation of the mitochondrial fusion protein Mfn2. Therefore, we conclude that Mul1 is a potent inducer of mitophagy and muscle wasting in mice models

1–55

Dissecting the possible role of CXCR4 pathway in muscle loss induced by cancer

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Cancer cachexia is a life-threatening syndrome characterized by severe body weight loss, due to depletion of adipose tissue and skeletal muscle, and affects up to 80% of patients with advanced cancers. The rapid loss of muscle mass is the main cause of function impairment, fatigue and respiratory complications, leading to death in 20-48% of cases. To date, no effective treatment is available.

Interestingly, previous microarray analysis has identified a subset of genes whose expression is specifically altered in cachectic muscles of hepatoma-bearing rats. The recent development of novel softwares to analyze long list of genes, like Ingenuity Pathways Analysis software, enabled us to find a gene signature suppressed specifically in rat muscles atrophying because of cancer (and not because of diabetes or fasting or disuse): CXCR4 pathway.

To test if suppressing this pathway is sufficient to drive muscle atrophy, we treated fully differentiated C2C12 myotubes with the inverse agonist AMD3100 and measured fiber diameter as index of muscle atrophy. Importantly, neither 48h-treatment with AMD3100 (up to 1 ug/ml) nor with the agonist SDF1 (up to 200 ng/ml) of CXCR4 receptor causes evident myotube toxicity. Interestingly, myotubes treated for 24 or 48h with 0.25, 0.5 or 1 ug/ml AMD3100, but not with SDF1, displayed decreased diameter in a dose- and time-dependent way, supporting its action through a saturable pathway (i.e. CXCR4). Ongoing experiments in cultured myotubes and *in vivo* adult mouse muscles aim at dissecting the possible role of this pathway in muscles during cancer cachexia.

In conclusion, our preliminary data show that attenuating CXCR4 pathway recapitulates muscle atrophy at least in cell culture.

1-56

Cachexia index (CI) in advanced non-small cell lung cancer (NSCLC) patients

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Introduction: Cancer cachexia affects mainly advanced NSCLC patients. Clinically it presents with weight loss and sarcopenia. In order to assess degree of cachexia in advanced NSCLC patients we developed cachexia index and correlated it with patient outcome.

Methods: Patients with metastatic NSCLC at LSU Health between Jan 1, 2000 to June 30, 2011 were retrospectively studied. Abdominal CT scan done within one month of diagnosis was reviewed to determine the skeletal muscle area (SMA) using MIPAV (Medical Image Processing, Analysis, and Visualization) software at L3 level. Skeletal muscle index (SMI) was calculated as (SMA/height (m²)). Cachexia index was developed as followed:

$$CI = SMI \times Alb / NLR$$

Where CI = cachexia index, SMI=Skeletal muscle index, Alb=Serum albumin, NLR= Neutrophil to lymphocyte ratio (A marker of systemic inflammation).Kaplan-Meier method was used to estimate progression free survival and overall survival. Log-rank test were used to compare the survivals among various factors. Multivariate Cox regression was used to perform survival analysis in order to estimate the hazards ratio for various factors.

Results: 112 patients were included in the analysis. CI range was from 1.08-248. Patients were divided into two groups around median into stage I cachexia (CI>35, n=56) and stage II cachexia (≤35, n=56). There was no difference in age, gender, ethnicity and histology of cancer between the two groups. Patients with stage II cachexia were significantly more likely to have more than two sites of metastatic disease and have poor response to chemotherapy. Patients with stage II cachexia had significantly poor PFS (2.45 vs 5.43 months, P=< 0.0001) and OS (3.45 vs 8.8 months, P=0.0001) as compared to stage I cachexia. CI correlated with outcome in both men and women independently. On multi-variate analysis adjusting for gender, race and histology patients with stage II

cachexia were found to have adverse PFS (Hazard ratio(HR), 95%CI 1.94 (1.27-2.95) and OS (HR=1.53 (1.0009-2.34)). Low weight (BMI <20) and sarcopenia (SMI <55cm²/m² for men and <39cm²/m² for women) alone did not correlate with outcome.

Conclusion: Cachexia index is a novel index for estimating cachexia which correlates with prognosis in both men and women with advanced NSCLC patients.

1-57

Analysis of body composition and sarcopenia prevalence using computed tomography imaging: a comparison of 1-year survivors and non-survivors in lung cancer

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Background: Despite the recognition of the clinical importance of sarcopenia (severe muscle depletion) in lung cancer, body composition in this disease has been mostly assessed using methods that do not allow differentiation between muscle and other tissues. Moreover, there is growing awareness of the relationship between sarcopenia and outcomes in cancer.

Aim: To compare body composition and sarcopenia prevalence in patients surviving or not lung cancer during a 1-year follow-up.

Method: Thoraco-abdominal computed tomography (CT)-scan of 318 patients newly diagnosed with lung cancer and separated in two groups according to their survival status at one year were retrospectively analysed. Following the collection of clinical data, muscle, visceral fat and subcutaneous fat areas were quantified from a single abdominal cross-sectional image at the level of the third lumbar vertebra. Sarcopenia was assessed using CT-based criteria and defined as having a lumbar skeletal muscle index ≤ 55 cm²/m² for men and ≤ 39 cm²/m² for women.

Results: CT scans from 42 patients with stage I-II non-small lung carcinoma (NSCLC), 130 patients with stage III-IV NSCLC and 39 patients with small cell lung carcinoma (SCLC) were analysed. Patients had a mean age of 64 ± 9 years and a body mass index of 25.5 ± 5 Kg/m². The overall one-year survival rate was 50%. At diagnosis, muscle, visceral fat and subcutaneous fat areas were 131 ± 35 cm², 155 ± 116 cm² and 106 ± 90 cm², respectively. Fifty-four percent of patients reached the definition of sarcopenia at diagnosis. The prevalence of sarcopenia was higher in non-survivors compared to survivors (65% vs 44 %, respectively; p<0.001) even when considering the extent (staging) of lung cancer.

Conclusion: Sarcopenia was prevalent at the time of diagnosis in this group of patients with lung cancer and its prevalence was higher in patients who did not survive at one year compared to those who survived. Considering that sarcopenia may influence survival, that it could be improved with appropriate intervention, screening for sarcopenia may be clinically relevant at the time of diagnosis to enhance patient management.

1-58

Staging sarcopenia in advanced cancer patients: does it matter clinically?

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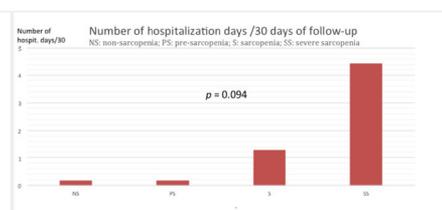
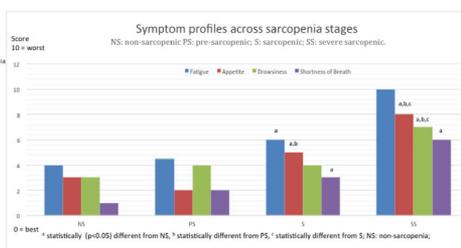
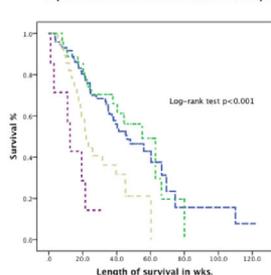
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Background: Sarcopenia, defined only by low muscle mass (LMM) and measured via different research methods, has been extensively studied in cancer patients. However, the European Working Group on Sarcopenia in Older People (EWGSOP) have recently proposed specific “stages” for sarcopenia. We wanted to determine if these stages have any clinical relevance in advanced cancer patients.

Methods: From the Human Cancer Cachexia Database, we selected patients for whom DXA scans were available. According to the criteria proposed by the EWGSOP, patients were classified as: pre-sarcopenic (PS) if they presented only with a low appendicular skeleton muscle index (LASMI) by DXA (Men <7.26 kg/m²; Women <5.45 kg/m²); sarcopenic (S) if they had LASMI *plus* low muscle strength (LMS) (Men <30 kg; Women <20 kg by Hand Grip Strength) or low physical performance (LPP) (Eastern European Oncology Group Performance Status > 2/4) and severely sarcopenic (SS) if they presented with LASMI, LMS and LPP. Covariates of interests included quality of life, survival and administrative outcomes.

Results: One-hundred and thirty-six patients (60% males) with advanced cancer (68.4% NSCLC, 19.1% GI and 12.5% others) with 60.3% having metastases and median survival of 40.4 wks (95 % CI: 32.4-48.4) were included in our study. Patients were classified as: NS n=78 (53.7%); PS = 28 (20.6%); S = 28 (20.6%); SS = 7 (5.1%).

Kaplan-Meier survival curves related to sarcopenia stages



Discussion: LASMI could not differentiate NS from PS, whereas the presence of LMS or/and LPP in addition to LASMI clearly distinguished both S and SS from other stages for several clinical outcomes. Our data support the notion that the diagnosis of sarcopenia should be based on both body composition and functional parameters in advanced cancer patients. The staging of sarcopenia appears to be both feasible and clinical relevant in this patient population.

1-59

Short and long term effect of EPA and DHA on Mechanisms Underlying myosteatosis in an animal model of colorectal cancer receiving CPT-11 / 5-FU

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Myosteatosis (triglyceride accumulation in muscle) is associated with wasting syndrome in cancer. We have previously shown in an animal model that myosteatosis develops during Irinotecan (CPT-11) plus 5-fluorouracil (5-FU) treatment but is prevented by a diet containing fish oil (FO) [eicosapentaenoic (EPA) and docosahexaenoic (DHA)]. Mechanisms underlying these observations are not known. This study aimed to measure expression of adipogenesis transcriptional-factors within gastrocnemius muscle of Ward colorectal tumor bearing rats receiving two

cycles of CPT-11/5-FU that received isonitrogenous, isocaloric, semi-purified diets with or without FO (2.3 g FO/100 g containing 0.64% EPA and 0.16% DHA). CPT-11(50 mg/kg/day) was initiated on Day 0, and 5-FU (50 mg/kg/day) on Day 1. Rats were euthanized on Day 7 (one cycle). A second group received another cycle of chemotherapy starting on Day 7 and rats were euthanized on Day 14 (two cycles). Tumor bearing rats received control diet served as a reference group. Diets containing FO started either prior to tumor implantation (long-term) or on the same day of the first dose of CPT-11 (short-term). Muscles were isolated prior to starting chemotherapy and after each cycle of CPT-11/5-FU. qRT-PCR used to measure expression of mRNA for CCAAT/enhancer binding proteins (C/EBPs), peroxisome proliferator-activated receptor (PPARs), and sterol regulatory element binding protein 1c isoform (SREBP-1c). CPT-11/5-FU treatment significantly increased PPAR- γ (~2.5-fold) after 2 cycles compared to the reference group. No genes were significantly altered between rats fed a short-term FO and rats fed control diet following either one or two cycles of CPT-11/5-FU. Rats fed a long-term FO diet exhibited the lowest expression of PPAR- γ (0.4-fold), C/EBP- β (~0.3-fold), C/EBP- σ (~0.8-fold), and SREBP-1c (~0.7-fold) compared to the other groups ($P < 0.05$) following one cycle of CPT-11/5-FU. mRNA of SREBP-1c (~0.04-fold) and C/EBP- β (~0.2-fold)

were significantly reduced ($P < 0.05$) in rats fed long-term FO diet compared to rats fed a control diet or short-term FO diet following two cycles of CPT-11/5-FU. Only long-term feeding of EPA and DHA reduced the expression of adipogenic transcriptional factors in this animal model of colorectal cancer providing one mechanism to explain reduced fat content of muscles in rats provided FO during CPT-11/5-FU treatment.

1-60

Treatment and underlying mechanisms of pancreatic cancer cachexia (PANCAx Study)

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Background: Advanced pancreatic adenocarcinoma is characterized by progressive weight loss and nutritional deterioration. This wasting has been linked not only to survival, but also to alterations in host defenses, functional ability, and quality of life. Inflammatory cytokines, gut hormones, and gut barrier dysfunction are implicated in the pathogenesis of pancreatic cancer cachexia. However these mechanisms are not fully understood and there are no effective therapies for this condition. We propose the first prospective study of peptide based enteral nutritional support in advanced pancreatic adenocarcinoma patients undergoing chemotherapy. Our aims are to establish the feasibility and efficacy of enteral nutrition as measured by changes in weight and lean body mass. The anorexic component of this syndrome will be assessed by caloric

intake and taste and smell alterations. We will also explore the relationship between anorexia-cachexia and altered levels of circulating cytokines and gut hormones accounting for decreased food intake and muscle loss. Finally, the fecal microbiome will be analyzed in this cohort as well as the changes resulting from our intervention.

Methods: Eligible patients will have a diagnosis of both pancreatic adenocarcinoma and cachexia defined as greater than 5% unintentional weight loss within 6 months prior to screening. Peptamen will be administered through a jejunal tube and dosing will be calculated using the Mifflin St. Jeor equation for 12 weeks. Subject's lean body mass will be evaluated by DEXA, will be checked at predefined intervals. To determine the significance of anorexia and associated taste alterations we will measure 24 hour food recall and a validated taste and smell survey. Secondary outcomes including, response to chemotherapy, survival, and quality of life will be measured using recist 1.1, measuring ECOG performance status, and EORTC quality of life survey (EORTC QLQ-C30). Gut hormones including ghrelin, leptin, and GLP-1 will also be measured. Fecal microbiome changes as measured from analysis of fecal sample will also be performed. We have received funding from NIH/NCATS Grant# UL1TR000124 and will be open to enrollment in September of 2014.

1-61

Germline copy number variations as genetic susceptibility determinants for cancer cachexia

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Background: Cancer Cachexia (CC) is a multifactorial syndrome characterized by skeletal muscle depletion with or without fat loss and net loss of weight. Copy number variants (CNVs) are structural changes in the genome. Amplifications and deletions of more than 1 kb and extending up to several million base pairs comprise structural aberrations; CNVs may regulate gene expression through gene dosage or through intergenic cis-regulatory elements. CNVs have gained considerable prominence as susceptibility determinants for various diseases such as Parkinson disease, Autism and several cancer types. Although genetic basis for CC susceptibility were attempted using candidate gene Single Nucleotide Polymorphisms (SNPs), interrogation for determinants at whole genome level remain unexplored. Hypothesis: CC as a phenotype has a heritable component and that the germline common DNA variants explain individual's overall genetic susceptibility.

Objective: To identify germline CNVs as genetic markers for CC using a Genome-Wide Association Study (GWAS) design.

Methods: Stratification of patients was based on weight loss and C-Reactive Protein (CRP); CRP, an indicator of systemic inflammation positively correlates with weight loss. From a CC genetic study cohort of 1500 cases with documented weight loss and CRP data, we selected a subset of cases for this feasibility study. Oesophagogastric and lung cancer cases with weight loss $\geq 10\%$ and CRP $> 10\text{mg/L}$ (n=16, cachectic) and controls with no weight loss and CRP levels $< 10\text{mg/L}$ (n=25, weight stable) satisfied the criteria of the extremes of phenotype of CC in the current study design. Affymetrix Genome-wide Human SNP array 6.0 served as a genotyping platform. Partek Genomics suite 6.6 was used for analysis. Functional annotations of genes proximal to CNVs were identified using DAVID Bioinformatics v6.7.

Results: We identified 7,688 CNVs that were statistically significant ($p < 0.05$). We initially examined only those CNVs showing copy gains or copy loss regions of $> 1\text{ kb}$ (n= 496), of which 171 CNVs were

proximal to genes. Gene Ontology identified clusters associated with amino acid catabolic process, muscle development and proteolysis. Further genotyping of larger cohorts confer statistical rigor and confidence in study findings. This is the first genome-wide study targeted towards identifying genetic determinants for CC.

1-62

The role of an adiponectin mimetic in treatment of mice with cancer cachexia

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About one-third of cancer-related deaths may be attributed to cachexia. The loss of skeletal muscle in cancer ultimately contributes to death due to cancer cachexia; yet, recent studies suggest the loss of adipose tissue may be a better predictor of mortality. A mechanism of how adipose loss contributes to muscle atrophy during cachexia is poorly understood. Adipose mass declines early in the pathogenesis of cancer cachexia, and occurs coincident with loss of adipocyte function resulting in an inability to secrete adipokines. Adiponectin is an abundant adipokine that decreases systemic inflammation and modulates metabolism. These two properties are important in cachexia, which is characterized by inflammation and dysregulated metabolism. Our lab found that adiponectin is significantly decreased in two different murine cancer cachexia models (C26 adenocarcinoma and LLC). Surprisingly, this decrease of adiponectin occurred prior to weight loss and the onset of anorexia in the C-26 model. Additionally, the depletion of adiponectin was inversely related to levels of IL-6, a pro-inflammatory cytokine. Moreover, plasma adiponectin of cachectic mice was inversely associated with muscle loss, suggesting a link between adipose and skeletal muscle in the pathogenesis of cachexia. We hypothesize that the loss of muscle during cachexia is mediated by loss of adiponectin resulting in changes in metabolism and inflammation in skeletal muscle. To test this, we conducted a pilot study in the C-26 mouse model of cancer cachexia that were administered AdipoRon (N=5), an adiponectin mimetic, or vehicle (N=5), daily. Mice treated with AdipoRon tended to have higher muscle strength in hind limbs 18 days after tumor inoculation ($P = 0.14$) compared to mice treated with vehicle. We will next determine whether activation of the adiponectin receptor, AdipoR-1, is associated with activation of AMP-activated protein kinase (AMPK) and p-38 mitogen activated protein kinase (MAPK), two downstream targets of the receptor. Identifying the relationship of adiponectin and its receptors with markers of atrophy and muscle regeneration in skeletal muscle of mice with cancer cachexia could lead to therapeutic targets for attenuating muscle loss during cancer cachexia.

1-63

Short term body composition changes after a colorectal cancer diagnosis - a study of Sarcopenia, Colorectal Cancer and Near-term Survival (SCANS) within the Kaiser Permanente Health Plan of Northern California

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Objective: The SCANS study will measure the impact of body composition on colorectal (CRC) mortality. We present descriptive results of body composition at diagnosis and changes approximately one year post-diagnosis.

Methods: We established a retrospective cohort of 3,409 men and women from Kaiser Permanente of Northern California diagnosed with Stage I-III CRC. Body composition was assessed using computed tomography (CT) scans. Skeletal muscle (SM), subcutaneous adipose tissue (SAT), and visceral adipose tissue (VAT) were quantified at the third lumbar vertebra (L3) using Slice-O-Matic software. Preliminary data from 872 CT scans were available. Means and medians were examined individually by sex across race, age, stage and body mass index (BMI) categories. Sarcopenia was defined using cutpoints in cm^2/m^2 from two previously published papers: Prado¹ <55 for men and <39 for women and Martin² <43 for men and <41 for women for BMI <25 and <53 for men and <41 for women for BMI \geq 25.

Results: 53% and 33% of men and 31% and 41% of women were categorized as sarcopenic at diagnosis using the Prado and Martin cutoffs, respectively. SM remained stable but VAT and SAT increased between baseline and Year 1. Differences in change were not seen by age or stage but were seen in men by race and in both sexes by initial BMI. SM remained stable across ethnic groups. However, Black and White men had an approximately 8% gain in both SAT and VAT while Hispanic men gained almost twice as much (14% and 18%) and Asian men half as much (5% and 3%) in SAT and VAT respectively. Those with BMI <25 kg/m² had large increases in VAT (46% and 37% in men and women respectively). All other BMI categories had decreases in VAT; those with a BMI >35 kg/m² had the largest decrease (-12% and -17% in men and women respectively).

Conclusion: The prevalence of sarcopenia in early-stage newly diagnosed CRC patients is high and likely to be occult. Short-term changes in body composition can lead to a less favorable ratio of VAT to SM mass in normal weight individuals but not in the overweight or obese.

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1–64

Dose limiting toxicity by body weight and composition in patients receiving a hydrophobic agent for the treatment of ovarian cancer

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Objectives: To investigate the relationship between adipose tissue (AT), lean body mass (LBM) and the toxicity profile of patients receiving pegylated liposomal doxorubicin (PLD) and trabectedin for the treatment of advanced ovarian cancer.

Methods: Toxicity profile after cycle 1 was obtained from a previously conducted phase III trial and graded according to the National Cancer Institute Common Toxicity Criteria, version 3.0. Adipose tissue and LBM were assessed by analysis of computerized tomography images at the 3rd lumbar vertebrae, as standard for body composition analysis.

Results: Dose limiting toxicity was more prevalent in patients with lower body mass index ($p=0.03$) and lower AT ($p=0.03$). For patients with excess body weight, the ratio between AT/LBM was the most significant variable associated with toxicity, with a lower ratio predicting higher

drug exposure and consequently, higher risk for toxicity (OR = 0.006, 95%CI = 0.00–0.15).

Conclusion: PLD and trabectedin toxicity may be partially explained by differences in AT and LBM suggesting a synergistic importance of these compartments in explaining toxicity.

This study has been published: Prado CM et al. Appl Physiol Nutr Metab. Jun;39(6):693–8, 2014.

1–65

Disrupting cytokine signaling in pancreatic cancer: a phase I/II study of etanercept in combination with gemcitabine in patients with advanced disease

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Objectives: Etanercept blocks tumor necrosis factor- α , a proinflammatory cytokine that plays a role in cancer-related cachexia and tumor growth. A phase I/II study was conducted to assess the tolerability and efficacy of gemcitabine and etanercept in advanced pancreatic cancer.

Methods: Twenty-five patients received etanercept 25mg subcutaneously twice-weekly in combination with gemcitabine. Eight additional patients received gemcitabine alone as a control cohort. The primary end-point was progression-free survival (PFS) at 6 months. Blood specimens were analyzed for serum levels of TNF, IL-1b, IL-6, interferon- γ , IL-10, and NF- κ B activation.

Results: Thirty-eight patients participated in this study. In the gemcitabine-etanercept cohort, grade 3/4 drug-related toxicities included leucopenia (3) and neutropenia (6). There were 3 (12%) patients with partial response and 8 (32%) patients with stable disease. The rate of PFS at 6 months was 28% ($n=7$; 95% CI 20–36%). Median time to progression was 2.23 months (95% CI, 1.86–4.36 months) and median overall survival was 5.43 months (95% CI, 3.30–10.23 months). Clinical benefit rate was observed in 33% of the evaluable patients. A correlation was seen between IL-10 levels and clinical benefit.

Conclusions: Etanercept added to gemcitabine is safe but did not show significant enhancement of gemcitabine in patients with advanced pancreatic cancer, and did not improve the clinical benefit rate or weight loss.

1–66

Is it possible to classify cancer cachexia in clinical practice?

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Introduction: A definition and classification of cancer cachexia has been proposed including different stages along a continuum of cachexia and implying various criteria. Our aim was to validate a simplified classification system measuring survival, nutritional and functional clinical outcomes.

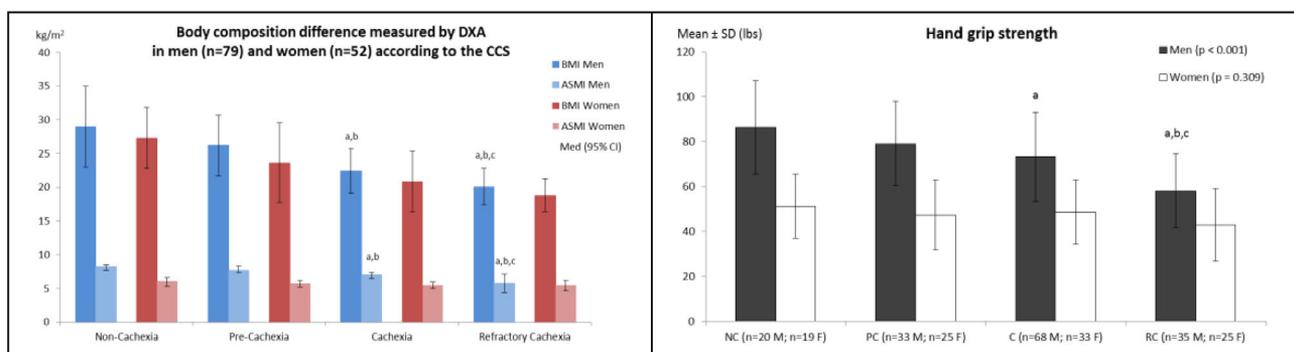
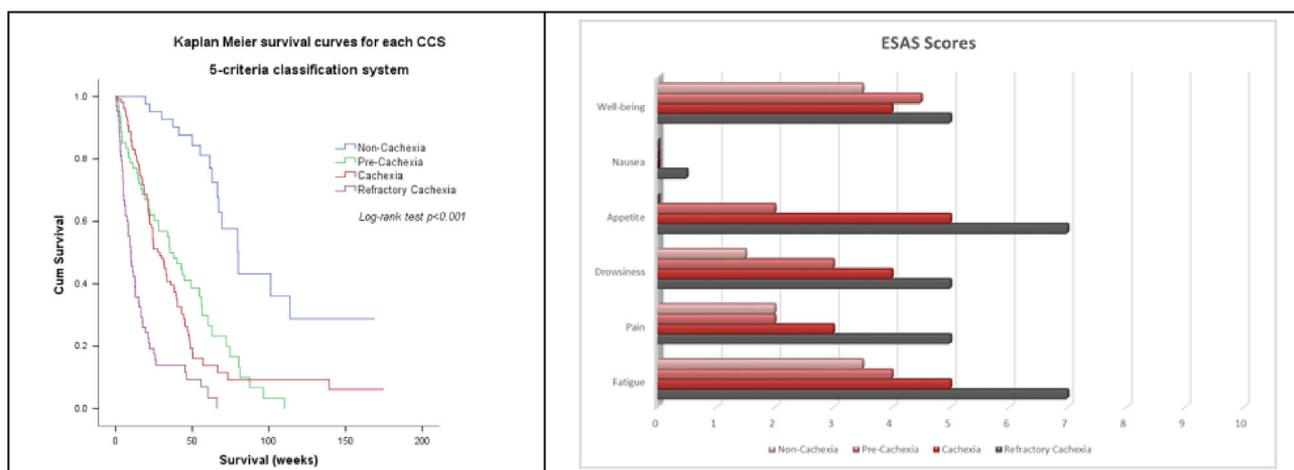
Methods: Abnormal biochemistry (C-reactive protein >10 mg/L, white blood cells >11,000/L, serum albumin <32 g/L, haemoglobin <120 g/L in men

and <110 g/L in women); decreased food intake; weight loss 0-5% or $>5\%$ in 6 months; decreased performance (ECOG >2) were used to classify patients into the following cancer cachexia stages (CCS): non-cachexia (NC), pre-cachexia (PC), cachexia (C) and refractory cachexia (RC).

Results: From the Human Cancer Cachexia Database, 297 patients were included (60% males) and 277 classified into NC (16%), PC (23%), C (38%) and RC (23%). Primary tumors were gastrointestinal (59%) and pulmonary (31%) with 65% metastasis. Survival, body composition (muscle and fat mass, body mass index), function (hand grip strength) and several symptoms (fatigue, pain, drowsiness, appetite, nausea and well-being) were significantly different across CCS ($p < 0.05$).

Conclusion: Cancer cachexia can be staged via routinely available measures. These stages can provide significant advantages for both clinical and research purposes.

during this differentiation process is the upregulation of the myogenic regulatory factor MyoD, whose expression promotes the terminal differentiation of satellite cells into myocytes and whose absence has been shown to promote the self-renewal of satellite cells. We have shown that CCAAT/Enhancer Binding Protein beta (C/EBP β), a bzip transcription factor that inhibits myogenesis and is highly expressed in satellite cells and downregulated during their differentiation inhibits MyoD protein expression without affecting its mRNA levels. We report that C/EBP β can interact with MyoD protein indirectly. We further show that MyoD protein levels are not controlled by the ubiquitin pathway as treatment with the proteasome inhibitor MG132 fails to rescue MyoD protein in the presence of C/EBP β . We identify novel MyoD interacting proteins that may act to control MyoD protein levels in satellite cells and myoblasts.



1-67

The role of CCAAT/enhancer binding protein beta in the regulation of MyoD levels during myogenesis

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During muscle regeneration, muscle stem cells, called satellite cells are activated to differentiate into myocytes and to fuse to one another or to damaged muscle fibers to ensure repair. One of the early events

1-68

Endurance training improves cancer-cachexia

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Introduction: Cachectic patients show profound wasting, reduced physical function, metabolic disorder and chronic systemic inflammation. Recently an international consensus proposed specific criteria for

cancer-cachexia definition based on the generic definition of Evans and co-workers^{1,2} which includes, beyond weight loss, biochemical markers, such as augmented serum C-reactive protein (CRP > 5.0 mg/l), low serum albumin (< 3.2 g/dl) and haemoglobin (< 12 g/dl). Exercise training has been suggested as an effective anti-inflammatory strategy to counteract the symptoms of cachexia³. Therefore, the goal of the study was to test the effectiveness of a protocol of endurance training in reducing specific symptoms of cachexia in cancer patients.

Methods: patients with gastrointestinal carcinoma – non-cachectic (NcC, n = 12), and cachectic (cC, n = 7), and a control group of patients with umbilical hernia, Control group (CG, n = 10), were submitted to an endurance training protocol (Dimeo and co-workers, adapted⁴), consisting of 6 weeks of walking on a treadmill with increasing volume and intensity, assessed individually after a submaximal test⁵, to estimate each volunteer's VO_{2max}. The submaximal test, resting heart rate, body mass and serum CRP, albumin and HDL levels evaluations were carried out in the first, third and sixth week of the experimental protocol as to assess the effectiveness of exercise training in modulating markers of cachexia. None of the patients received anticancer drugs.

Results: Patients showed significant difference in body mass (CG: 82.4 ± 5.26 Kg, NcC: 78.05 ± 3.70 Kg and cC: 57.73 ± 3.44 Kg) (p = 0.0258) and the cachectic group presented a discreet gain in body mass after six weeks of training (cC: 58.56 ± 4.09 Kg). At the end of the six weeks, patients of all groups increased significantly their estimated VO_{2max} (CG: 24.49 ± 1.71 ml.kg.min, NcC: 16.23 ± 5.91 ml.kg.min and cC: 21.10 ± 4.96 ml.kg.min) when compared with the first week (CG: 8.25 ± 2.43 ml.kg.min, NcC: 7.23 ± 3.37 ml.kg.min and cC: 5.28 ± 3.22 ml.kg.min), and decreased significantly the resting heart rate (CG: 76.4 ± 5.78 bpm, NcC: 74 ± 2.94 bpm and cC: 70.33 ± 5.17 bpm) compared to the first week (CG: 80.8 ± 3.59 bpm, NcC: 83 ± 2.52 bpm and cC: 81.60 ± 4.44 bpm), proving the effectiveness of exercise training protocol. Cancer cachectic patients presented higher CRP levels than the other sedentary groups (CG: 4.16 ± 4.02 mg/L, NcC: 1.83 ± 1.27 mg/L and cC: 10.18 ± 2.46 mg/L) and at end of the sixth week of the protocol, CRP concentration was significantly reduced in cC (CG: 1.63 ± 1.56 mg/L, NcC: 1.26 ± 0.81 mg/L and cC: 5.20 ± 4.42 mg/L). Serum albumin levels of the cachectic patients was significantly different compared with the controls and non-cachectic cancer patients in week 0 (CG: 4.44 ± 0.11 g/dL, NcC: 4.07 ± 0.09 g/dL and cC: 3.30 ± 0.34 g/dL) and continuously increased towards the 6th week, when finally, the values of circulating albumin were similar to those of the other groups (CG: 4.40 ± 0.18 g/dL, NcC: 4.16 ± 0.11 g/dL and cC: 4.07 ± 0.58 g/dL). Serum HDL levels were significantly lower in cachectic cancer patients in relation to the other groups, at the beginning of the exercise protocol (CG: 38.17 ± 7.10 mg/dL, NcC: 45.10 ± 10.70 mg/dL and cC: 28.50 ± 15.24 mg/dL), which caused the parameter to reach control values in the 6th week (CG: 53.50 ± 16.29 mg/dL, NcC: 51.40 ± 11.72 mg/dL and cC: 48.00 ± 9.93 mg/dL).

Discussion: Reduction of cardiorespiratory fitness and metabolic disorders are a common consequence of cancer-cachexia¹. Several studies in experimental models reported that exercise training can improve symptoms of cachexia^{6,7}. Our results show that endurance training is effective to improve the physical condition and the biochemical markers of cachexia in human patients.

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1–69

Regulation of Smad3 expression in myoblasts promote myogenesis

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Adult skeletal myogenesis is orchestrated by a well established cascade of transcriptional events, governed by the myogenic regulatory factors family of proteins. In adult skeletal myogenesis, TGFβ signalling is known to inhibit the differentiation of myoblasts. Smad3, an effector protein in TGFβ signalling, is activated by phosphorylation events and shuttles to the nucleus to act on target genes. Using a myoblast cell model, we demonstrate that Smad3 expression is transiently upregulated by low dose retinoic acid treatment (RA) and this treatment can partially rescue inhibition of myogenesis imposed by both TGFβ and overexpression of CCAAT/Enhancer Binding Protein beta (C/EBPβ). Our results suggest that Smad3 exerts a promyogenic role in muscle and that RA can promote myogenic differentiation at least in part by antagonizing TGFβ-signalling and disrupting C/EBPβ actions. Given that both TGFβ signalling and C/EBPβ expression are increased in pathological muscle wasting, these findings could lead to new insights into the pathogenesis and treatment of cancer cachexia.

1–70

Selumetinib attenuated tumor growth and exacerbated muscle wasting in LLC cancer cachexia

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Cachexia, or skeletal muscle and fat wasting, afflicts many patients with cancer. Muscle wasting reduces quality of life and response to therapy. Cachexia is caused partly by elevated inflammatory cytokines, including interleukin-6 (IL-6). Others and we have shown that IL-6 is sufficient to induce cachexia both *in vitro* and *in vivo*. The mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK) inhibitor Selumetinib has been used in clinical trials against various cancers. Moreover Selumetinib has also been shown to inhibit inflammation and the production of IL-6. In a retrospective analysis of a phase II clinical trial of patients with advanced cholangiocarcinoma, Selumetinib treatment was associated with significant gain of skeletal muscle as compared standard therapy.

Here we sought to investigate Selumetinib in experimental skeletal muscle wasting. Selumetinib induced modest hypertrophy of C2C12 myotubes *in vitro* (+7.88% P=0.0015). Next we tested Selumetinib *in vivo* against cancer cachexia. Lewis lung carcinoma (LLC) cells were injected subcutaneously into C57BL/6J mice. Selumetinib treatment began 24 hours after tumor implantation, and was administered twice daily by gavage for 17 days. Selumetinib treatment reduced tumor size (–43.18% versus vehicle P=0.002). Western blotting analysis revealed that IL-6 levels were

significantly reduced in tumors from mice with Selumetinib treatment versus carrier. Similar levels of IL-6 were observed in skeletal muscle among all treatment groups. Protein levels of phosphorylated ERK1/2 were significantly reduced in skeletal muscle of the Selumetinib treated group, thus confirming inhibition of the MEK pathway. However, Selumetinib was not able to preserve skeletal muscle or fat in these mice. Mice treated with Selumetinib showed the same level of wasting in the tibialis anterior, quadriceps, and gastrocnemius muscles as the LLC vehicle treated mice. Gonadal fat was also equivalently reduced in both groups. Given that experimental muscle wasting normally correlates with tumor size, these data indicate that Selumetinib was not protective and actually exacerbated muscle wasting. It is possible that the LLC model may not exert the same effects on the host as cholangiocarcinoma, or that the MEK pathway is not similarly conserved between murine and human cancer cachexia.

1-71

Role of tripartite motif family of E3 ubiquitin ligases in muscle atrophy **Heather Manring¹, Wei He², Denis C Guttridge², Noah Weisleder¹**

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Cachexia, similar to other skeletal muscle wasting conditions, involves progressive skeletal muscle wasting induced by many cancers. Muscle atrophy associated with cachexia impairs the quality of life and mortality of cancer patients ultimately due to loss of skeletal muscle function. Atrophy of skeletal muscle includes decreased muscle mass, smaller muscle fibers, weight loss and decreased muscle strength. Proteomic studies have focused on identifying proteins that are upregulated in cachexia and mediate the atrophy process in an attempt to determine targets for reducing this detrimental loss of muscle functionality. MURF1, also known as TRIM63, is observed to be upregulated in mouse models of cachexia. MURF1 is a member of the tripartite motif family (TRIM) of E3 ubiquitin ligases. Ubiquitination of proteins and degradation by the proteasome is a key process in the recycling of protein in cells, and excessive ubiquitination can lead to break down of skeletal muscle proteins and progression of atrophy. With upregulation of several ubiquitination and proteasomal proteins in mouse models of cachexia, it is suspected that these classes of proteins play a vital role in skeletal muscle atrophy. Another member of this large family of E3 ubiquitin ligases, mitsugumin 53 (MG53), also known as TRIM72, has been linked to changes in muscle fiber size and is known to have a role in membrane repair in striated muscle cells. We hypothesize that expression of TRIM72 and other TRIM family E3 ubiquitin ligases would be altered during development of cachexia. The expression of TRIM72, TRIM41, and TRIM38 were evaluated in muscle samples from a model of cancer cachexia where mice were injected with either colon-26 (C-26) tumor cells or saline. Muscles were collected 21 days after injection and protein levels were assessed using immunoblotting approaches. Preliminary data shows increased TRIM72 protein in cachectic tibialis anterior and soleus muscles compared to non-cachectic muscles. An upward trend was also observed for TRIM38 and TRIM41 expression in several hind limb muscles of cachectic mice. Resolving new ubiquitin ligases associated with cachexia will increase the understanding of the basic atrophy process and provide alternative clinical interventions for cachexia and other muscle wasting conditions.

1-72

Lack of canonical SMAD2 pathway activation by recombinant GDF15 in vitro **Heidi Okamura**

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Growth differentiation factor 15 (GDF15) is a divergent member of the TGF β superfamily that has been implicated in the induction of cancer cachexia. Mice bearing tumors overexpressing GDF15 become cachectic, and recombinant GDF15 injected subcutaneously into mice causes significant weight loss.

The GDF15 receptor and associated downstream effectors are currently unknown. In order to elucidate the pathways that are activated downstream of GDF15 signaling, we developed *in vitro* cell based assays to assess the effects of recombinant GDF15 on tissue culture cells.

Among the different pathways investigated, the SMAD signal transduction pathway was closely examined since it is the canonical pathway for TGF β signaling. When recombinant hGDF15 from commercial sources was used, we detected activation and phosphorylation of SMAD2 in several different assays, using several different cell lines. Curiously, when biologically active, recombinant GDF15 produced in our laboratories was used in these same assays, no activation of SMAD2 signaling was observed. Inhibitory antibodies against GDF15 had no effect on the SMAD2 activation induced by the commercially available recombinant proteins. In stark contrast, however, inhibitory antibodies against TGF β 1 blocked all SMAD2 activation induced by the commercially available GDF15 preparations. ELISA assays confirmed the presence of low levels of TGF β 1 in all batches of recombinant GDF15 tested.

The signaling pathway downstream of GDF15 has been a subject of controversy. Throughout the years, numerous publications have found links between GDF15 and activation of various pathways, including the SMAD signaling pathway. In our hands, SMAD2 activation could only be achieved when using commercially available preparations of GDF15 that apparently contain low levels of TGF β 1, which is most likely responsible for the observed SMAD signaling. In light of these findings, appropriate controls to rule out TGF β 1 must be used when designing and interpreting results of experiments using recombinant GDF15

1-73

Preclinical investigation of the HDAC inhibitor AR-42 for the treatment of cancer-induced cachexia

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Cachexia is characterized by extreme loss of skeletal muscle mass and adipose tissue that cannot be reversed by nutritional support, and leads to pronounced weight loss and weakness that contributes significantly to morbidity and mortality. Cachexia occurs in more than 50% of cancer patients, and is particularly prevalent in those suffering from pancreatic, gastric, or esophageal cancer. Cachectic cancer patients are often weak and fatigued, respond poorly to therapy, and have a lower tolerance to therapy and surgery. Thus, the development of effective therapies for cancer cachexia, which could provide tangible clinical benefits to patients, is clearly warranted. AR-42 is a novel class I/II histone deacetylase (HDAC) inhibitor that was developed in our laboratory and is currently in Phase I/II trials in both hematological malignancies and solid tumors at The Ohio State University James Cancer Hospital. Here, we report the anti-cachectic activity of AR-42 in two murine models of cancer cachexia. In the colon-26 (C-26) adenocarcinoma model, oral AR-42 attenuated cachexia-induced losses of skeletal muscle mass, adipose tissue, and body weight, with minimal effects on C-26 tumor growth,

and prolonged survival time relative to mice treated with vehicle or other HDAC inhibitors (vorinostat and romidepsin). Metabolomic and gene expression analyses revealed that these anti-cachectic effects of AR-42 were associated with its ability to maintain metabolic and gene expression profiles in skeletal muscle comparable to those in non-cachectic muscle from tumor-free mice. Consistent with the protective effect on muscle mass, handgrip dynamometry and histological cross-sectional area of muscle fiber measurement showed that AR-42 was able to preserve muscle function and morphology in drug treatment groups comparing to vehicle control. Importantly, this AR-42-induced abrogation of cachexia and rescue of muscle weight was confirmed in the Lewis lung carcinoma (LLC) model of cancer cachexia. Moreover, we proved that in a delay treatment experiment, AR-42 still able to prevent part of muscle atrophy in C26 model. Together, these results support further evaluation of AR-42 as a potential treatment for cancer cachexia.

1-74

Surveys of American community oncologists highlight discrepancies between goals of care and patient-reported outcomes

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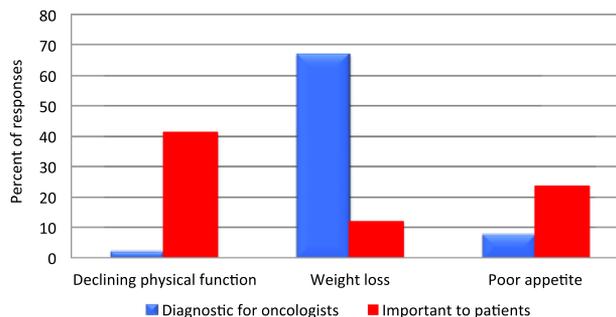
Background: A consensus definition has been developed to identify patients with cancer cachexia. However, of 275 international oncology societies, only six (2.2%) provide guidelines for physicians, not all of which are up-to-date [Mauri D, et al. *BMJ Support Palliat Care*. 2013;3:155–60]. The objective of these pilot surveys is to assess how community oncologists diagnose and treat cancer cachexia in general cancer patients, and in more-difficult-to-manage non-small cell lung cancer (NSCLC) patients.

Methods: Three surveys were conducted electronically between July and December 2013. Community oncologists from the Sermo research database were surveyed using a Sermo Pulse Survey to obtain geographic randomization within the United States. A total of 101 community oncologists responded across all three surveys.

Results: Community oncologists (n=51) reported weight loss as the prominent criterion for the diagnosis of cancer cachexia (67%), and 94% reported that albumin levels are helpful in further evaluating weight loss. Weight maintenance/gain was the primary treatment objective of oncologists (56%), followed by improving tolerance to chemotherapy (20%). Conversely, oncologists reported that patients and their families placed a more balanced emphasis on declining functional status (41.2%), poor appetite (23.5%), and weight loss (12%) (Figure). For NSCLC patients, the likelihood of developing cachexia during treatment was ranked as very likely (56%) or somewhat likely (36%) by 25 oncologists. For general oncology populations, use of appetite stimulants plus nutrition support for treatment of cachexia was reported by 64% of community oncologists; an additional 24% add exercise to the regimen. Only 16% reported having an in-practice cachexia management team, while 8% refer patients to an outside palliative care specialist. While 10% of oncologists currently use a standardized questionnaire to assess patient-reported outcomes, 72% were willing to implement an appropriate tool in their practice.

Conclusion: This pilot survey confirmed the importance and prevalence of cachexia in cancer patients, and identified important gaps in physician and patient criteria for assessing cachexia. Surprisingly, few physicians systematically reviewed patient-reported outcomes, despite their reported willingness to use a standardized tool to help direct goals of care.

Important Criteria for the Diagnosis of Cachexia, and Important Patient Concern.



1-75

Skeletal muscle fat infiltration is reversed by an EPA and DHA intervention in an animal model of colorectal cancer receiving irinotecan/5-FU

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Background: Pathological accumulation of fat into skeletal muscle (myosteatosis) has been identified in cancer patients and independently correlates with survival and treatment outcomes. Fatty muscle development is associated with insulin resistance, a tumour-bearing state, and chemotherapy. Our lab has shown that 1) Fish oil (FO), a concentrated source of EPA and DHA, prevents fat deposition in skeletal muscle during chemotherapy in an animal model for colorectal cancer (CRC) and 2) FO supplementation in cancer patients undergoing chemotherapy reduced intramuscular adipose tissue content upon treatment completion compared to a control group. The ability for FO to reverse fat accumulation once it has occurred has not been investigated.

Objective: To determine if FO intervention may reverse tumour-associated fat infiltration and attenuate fat infiltration that occurs during treatment with 5-FU/irinotecan in an animal model of CRC.

Methods: Fischer-344 rats bearing the Ward colorectal carcinoma were fed a semi-purified control diet and either received chemotherapy (n=32), or no chemotherapy (n=8). Upon chemotherapy initiation, rats remained on control diet (n=16), or began FO diet (n=16) (2.0% w/w). Diets were isocaloric with equivalent fat content, differing only in fatty acid (FA) composition (FO diet contained n-3 FAs, EPA/DHA). Rats were killed before chemotherapy, after 1-cycle, or 2-cycles; gastrocnemius muscles were isolated. Lipids were extracted, and triglyceride (TG) fractions separated by thin layer chromatography. FAs were identified and quantified with gas liquid chromatography.

Results: Compared to healthy rats (847.3 µg/g), tumour-bearing rats exhibited ~3-fold greater concentration of total-TG in muscle (2504.9 µg/g; $p < 0.01$). After 1-cycle, muscle TGs in control-fed animals were 2-fold greater than FO-fed animals and TG content in FO-fed animals resembled that of control animals receiving no chemotherapy. After 2-cycles, control-fed rats exhibited a greater concentration of total-TG in muscle compared to FO-fed rats ($p < 0.01$). Overall, TG content increased with succeeding chemotherapy cycles. FO-fed animals had a greater proportion and concentration of EPA and DHA in TG-FA after both 1- and 2-cycles ($p < 0.01$), which occurred concurrent with reduced TG-FA content.

Conclusions: Fish oil intervention during chemotherapy reverses fat accumulation in skeletal muscle of tumour-bearing rats. FO supplementation during chemotherapy may attenuate tumour and chemotherapy-associated myosteatosis.

1-76

Transcription factors network reprogramming hepatic gene expression under cachexia

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Weight loss is a common and serious complication in cancer patient that adversely effect the prognosis. Severe tissue wasting, called cancer cachexia is characterized by loss of fat and lean muscle. These effects are caused by the soluble factors released by both tumor cells, such as proteolysis-inducing factors, and host tissues, including cytokines IL-1, IL-6, TFNa. However, anti-cytokine therapies are ineffective, which suggests there may be other players involved in the process of cachexia.

In contrast to starvation, cachexia results in increased liver mass as a result of induction of biosynthetic pathways including acute phase response. Liver converts amino acids released by muscle proteolysis into glucose, which supply tumor's energy needs and recycles the lactate produced by tumor into glucose.

Changes in the hepatic transcriptional network are caused by delivery of amino acids, lipids and soluble factors from tumor. Although extensive literatures have shown the effects of transcription factors (TF) including HNF4a, Onecut1, SREBP1, SREBP2 on hepatic metabolism, little is known about their contribution to gene expression in cancer cachexia.

Since the liver plays a important role in energy metabolism in cachexia, we use an established Colon-26(C26)-adenocarcinomas model to study changes in liver gene expression. This model has successfully identify the molecular effects of PIF, PTHRP in cachexia. Our preliminary result from microarray analysis has shown a list of TF may be involved in rewiring hepatic gene expression. By profiling the active genomic regions of cachectic liver, it showed several novel TFs may be involved including ETS family transcription factors.

1-77

Carnosine supplementation prevents cachexia associated hypothalamic inflammation

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Cachexia is associated with increased morbidity and mortality in cancer. Chronic systemic inflammation is a common feature of this syndrome and hypothalamic inflammation concomitant to anorexia is also reported. Carnosine (β -alanil-L-histidine) supplementation has been show to modulate inflammation and oxidative stress in chronic disease, as well as to reduce tumour proliferation. Therefore, it was our aim to examine the effect of carnosine supplementation on hypothalamic inflammation in cachectic rats. 40 male Wistar rats (~250g) received water and food *ad libitum* and randomly divided into 4 groups (n=10) as follows: Control (CG), Tumour-bearing (TB), Supplemented Control (SC) and Supplemented Tumour-bearing (ST). All experimental procedures were approved by the Ethics Committee on Animal Experimentation of the

Institute of Biomedical Sciences (protocol N°050/2011). Supplementation followed the protocol of Aydin et al. (2010 - DOI 10.1007/s10522-009-9232-4): 250 mg/kg/day of carnosine (Sigma-Aldrich®) for 28 days, by intragastric administration. On the 14 day of the supplementation protocol, Walker 256 carcinosarcoma cells were injected subcutaneous in groups ST and TB. Food and Water intake and animal weight decreased significantly in TB, compared to CG. Supplementation rendered water and food consumption of TB animals to be similar to the control. The analysis of gene expression (RT-PCR) of pro and anti-inflammatory cytokines in the hypothalamus of the animals, showed that TNF- α and IL-6 in TB and ST were higher than in CG and SC. A significant decrease in IL-1 β (ST) compared to the CT group was found. Furthermore, the expression of IL-10 was higher in ST, compared to the other groups. Protein expression (ELISA) of IL-10, IL-6 and TNF- α did not differ among groups. IL-1 β protein content was decreased in ST compared to TB. Tumour mass was reduced in ST (20%, p<0.05), compared with TB. In conclusion, carnosine supplementation was effective in promoting weight maintenance, increased water and food intake, decreased tumour growth and inflammatory cytokine (IL-1 β) expression in the hypothalamus of the tumour-bearing rats, suggesting a potential therapeutic action in cancer-cachexia.

1-78

Pattern of change in visceral and subcutaneous adipose tissue mass in advanced cancer patients

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The majority of advanced cancer patients experience adipose tissue loss during cancer progression. Differences between visceral and subcutaneous patterns of change have not been consistently demonstrated. The objective of this study was to assess the intensity and time course of changes in visceral and subcutaneous adipose tissue of advanced cancer patients in the year preceding death. To capture patients at a similar time in disease trajectory, we focused on date of death rather than date of diagnosis in this analysis. Longitudinal quantitative analysis of computed tomography (CT) images for loss or gain of adipose tissue depots were conducted in advanced colorectal and cholangiocarcinoma cancer patients (n=57) at intervals spanning 9, 6, 3 and 1 month before death. Changes in visceral (VAT), subcutaneous (SAT) and total adipose tissue (TAT) were calculated as the absolute loss or gain of the tissue (change in cross sectional area) and as the rate (change/100d) for each time interval. On average, fat loss is occurring at all time intervals but the intensity of loss increases as patients approach death. Stratification of patients into fat stable, losing and gaining groups showed that fat gain or stability of tissue occurs in some patients mainly at 9 and 6 months prior to death. Nine months from death 42% of patients were losing fat (TAT mean rate of loss= $-6.5 \pm 8.4 \text{ cm}^2/100\text{d}$) whereas within one month of death, fat wasting was observed in 78% of patients ($-58.6 \pm 9.5 \text{ cm}^2/100\text{d}$). To further elucidate the change occurring in each depot, patients were also classified into VAT and SAT losing, gaining and stable groups. Interestingly, 9 months before death, VAT loss and SAT gain were predominant whereas, within one month of death loss of VAT was accompanied by SAT loss. In conclusion, further away from death, VAT and SAT behave differently whereas close to death, the largest and the most accelerated loss occur for both depots. Identifying the time course of changes and the intensity of VAT and SAT change over the disease trajectory may help to define the onset of wasting.

1–79

Computational design of novel peptide inhibitors of myostatin**Nikita Bhatia¹, Neerav D Padliya¹, Volker Stadler², Maghsoud Dariani¹, Robert J Hariri¹**¹Biotherapeutics Research & Development, MYOS Corporation, Cedar Knolls, NJ, USA 07927, ²Peptide Microarray Technologies, PEPperPRINT GmbH, Heidelberg, Germany 69123

Over the last several years, myostatin inhibition has emerged as a potential strategy for the treatment of cancer cachexia along with other muscle wasting disorders. Myostatin, a member of the TGF- β superfamily, inhibits the process of myogenesis, the development of muscle tissue. First-generation myostatin-directed monoclonal antibodies exhibited poor specificity towards myostatin with respect to other TGF- β ligands, giving rise to many undesired side effects such as the inhibition of wound healing. Rather than focus on antibodies, we have turned our attention towards the protein follistatin, a natural antagonist of myostatin which exists as three alternative splice variants, FS-288, FS-300 and FS-315 with the highest concentration found in the female ovaries followed by the skin. Specifically, we predicted peptides that the action of the proteases, pepsin followed by chymotrypsin on follistatin would give rise to and then modeled the binding of these follistatin-biomimetic peptides with myostatin. The secondary and tertiary structure of myostatin was modeled using the program, PHYRE2 (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>).

The three dimensional structure of the follistatin-biomimetic peptides was determined using the program, CORINA (<http://www.molecular-networks.com/products/corina>) in conjunction with the program, JMOL (<http://jmol.sourceforge.net/>). The program, DockingServer (www.dockingserver.com) was used to compute the best fit of each follistatin-biomimetic peptide with the active site of myostatin calculating a binding energy (kcal/mol) along with a binding constant (μ M). Three peptides, gave rise to binding energies of -6.28 , -5.35 , and -4.10 kcal/mol and binding constants of 24.79 μ M, 120.26 μ M and 985.49 μ M, respectively. This suggests that the 12-mer peptide with a 24.79 μ M binding constant may have a favorable binding profile with myostatin. Therefore, future work will focus on screening permutations of this 12-mer peptide with respect to myostatin binding by applying peptide microarray technology. Computational design in conjunction with peptide microarray technology is an approach that offers considerable promise with respect to the development of next generation myostatin inhibitors.

1–80

Varying severity of bone loss in mouse models of colorectal cancer cachexia**Valorie A Parker¹, Ryan R Matthews¹, Andrea Bonetto¹, Melissa Puppa², Kyung Shin Kang³, James Carson², Theresa A Guise^{4,5}, Khalid S Mohammed⁴, Alexander G Robling^{3,4}, Leonidas G Koniaris^{1,5}, Teresa A Zimmers^{1,3,5}**¹Department of Surgery, Indiana University School of Medicine, Indianapolis, IN, ²Department of Exercise Science, Arnold School of Public Health, University of South Carolina, ³Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, ⁴Department of Anatomy & Cell Biology, Indiana University School of Medicine, Indianapolis, IN, ⁵Indiana University Simon Cancer Center, Indianapolis, IN

Research on cachexia has focused largely on loss of muscle and fat. Given the increasing recognition of the physiological and molecular crosstalk between bone and muscle, bone loss might also be of concern in cancer cachexia. Here, we sought to study the bone phenotype in colon cancer cachexia. Three models were used: mice with multiple intestinal

neoplasia due to mutation of the APC gene (APCmin), athymic nude mice with s.c. implanted HT29 human colorectal adenocarcinoma tumors (HT29), and immunocompetent CD2F1 mice with s.c. implanted C26 murine colon carcinoma tumors (C26). APCmin, HT29, and C26 mice all showed substantial muscle loss (-52% , -20% , -24% for quadriceps weight, respectively; $p < 0.001$) versus non-tumor strain controls. PIXImus imaging of APCmin mice showed significantly decreased bone mineral density (BMD), including total (t), vertebral (v), femoral (f), and humeral (h) (-19% , -22% , -30% , and -25% respectively; $p < 0.001$). MicroCT of APCmin mouse femurs showed greatly decreased bone volume fraction, trabecular number, and trabecular thickness (-58% , -24% , and -39% respectively; $p < 0.001$). Three-point bending/mechanical testing of APCmin femurs revealed significantly decreased ultimate force, stiffness, and energy to failure (-44% , -43% , and -58% respectively; $p < 0.01$), while X-ray revealed abnormalities in the femurs and humeri. These results demonstrate that severe muscle wasting in APCmin mice is accompanied by severe bone loss and decreased bone quality. HT29 mice also showed significantly decreased BMD (5% (t), 11% (v), 15% (f), and 11% (h); $p < 0.05$). MicroCT of HT29 femurs showed significantly decreased BV/TV, trabecular number, and trabecular thickness (-14% , -35% , -15% , respectively; $p < 0.05$). HT29 mice also showed anomalous regions in femurs and humeri on X-ray. Thus muscle loss, bone loss, and decreased bone quality were correlated in HT29 mice. However, although they exhibited considerable muscle loss, C26 mice did not show significantly decreased BMD. Differences in bone loss in these models could be due to specific effects of strain, tumor type, and duration of cancer exposure. However, these data show that bone loss is not required for muscle wasting in these models, though future study is warranted.

1–81

A multifactorial anticachectic approach for cancer cachexia: the role of chemotherapy**Míriam Toledo¹, Fabio Penna¹, Francesc Oliva³, Melania Luque¹, Angelica Betancourt¹, Enrica Marmonti¹, Francisco J López-Soriano^{1,2}, Josep M Argilés^{1,2}, Sílvia Busquets^{1,2}**¹Cancer Research Group, Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain, ²Institut de Biomedicina de la Universitat de Barcelona, Barcelona, Spain, ³Departament d'Estadística, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

Treatment of rats bearing the Yoshida AH-130—a highly cachectic tumour—with sorafenib (90mg/kg) causes an important decrease in tumour cell content. This decrease, which is associated with both reduced cell proliferation and apoptosis results in a significant improvement in survival. Concomitant treatment of the tumour-bearing animals with formoterol, promoted a recovery of muscle wasting, as measured taking into consideration muscle weights and physical performance. Multifactorial treatment using both formoterol and megestrol acetate has even more powerful effects on the cachectic response associated with tumour growth. Indeed the animals show muscle weights similar to control non-tumour-bearing animals. In addition both physical activity and grip strength are significantly improved as compared with the untreated tumour-bearing animals. The effects of the multifactorial treatment rely on increased food intake (due to the administration of megestrol acetate) and possibly a decrease in protein degradation, as shown by the gene expression of proteins associated with both the ubiquitin-dependent system and the calcium-dependent one. It can be concluded that the combination of the two drugs is a good strategy for treating cancer cachexia; further studies involving human subjects are required.

1-82

Isotopic dilution of plasma deuterated N-tau-methylhistidine: an alternative method to measure muscle protein breakdown for use in frail and cachectic patients.

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Muscle wasting in cancer cachexia could be due to impaired action of insulin in stimulating protein synthesis and suppressing breakdown (PB). Current in vivo techniques to measure muscle PB are too invasive to use in such frail subjects. N-tau-methylhistidine (N τ -MH) is an irreversible metabolite of actin and myosin released during PB. We tested the suppressing action of insulin on myofibrillar PB by measuring the release of N τ -MH in the plasma using a stable isotopic tracer dilution approach with corroboration from skeletal muscle biopsies.

Methods: Nine healthy men (age 24 \pm 1y; BMI 22.6 \pm 0.6 kg/m²) received primed constant intravenous infusions of [²H₃]N τ -MH, [1-C¹³]leucine for whole-body PB, and [²H₅]phenylalanine for protein fractional synthesis rate (FSR). Protein kinetics were measured postabsorptively followed by a 3h-hyperinsulinemic (1.25 mU/kg lean body mass (LBM).min), euglycemic (5.5mmol/L), isoaminoacidemic clamp. Tracer enrichment and amino acid concentrations were determined by LC-MS/MS, gene expression by RT-PCR and autophagosome lipidation by immunoblotting.

Results: During clamp, whole-body PB was reduced by 27.4 \pm 4.0% (p=0.003). Plasma N τ -MH concentrations did not change (2.29 \pm 0.19 vs. 2.48 \pm 0.20 μ mol/L, NS). Endogenous N τ -MH release decreased from 0.62 \pm 0.05 to 0.45 \pm 0.03 μ mol/kg LBM/h (p<0.0001) translating in a 27.9 \pm 1.3% decrease in myofibrillar PB. It represented 48-63% of whole-body PB during both fasting and hyperinsulinemia. Skeletal muscle N τ -MH enrichment increased by 67.8 \pm 15.0%, corroborating a decrease in skeletal muscle PB. Concurrently, muscle MuRF-1 and Atrogin1 gene expression decreased by 41.1 \pm 1.0% and 44.8 \pm 3.0% respectively (n=8, p<0.0001); USP19, GABARAP1, and LC3 gene expression did not change. Muscle LC3B-II/LC3B-I protein expression was also lower following the 3h-clamp (by 68.7 \pm 6.7%, n=4, p=0.002). Lastly, synthesis of myofibrillar and sarcoplasmic proteins increased by 90.4 \pm 16.6% and 90.2 \pm 15.1% (p<0.001).

Conclusion: Physiological postprandial insulin levels at maintained postabsorptive amino acids were sufficient to stimulate myofibrillar and sarcoplasmic protein FSR and suppress whole-body and myofibrillar PB. This was associated with an acute downregulation of muscle E3 ligase gene expression of the ubiquitin-proteasome system and markers of autophagy. Dilution of plasma deuterated N τ -MH may be a reliable and sensitive alternative method to measure PB in various clinical conditions, precluding the need for muscle biopsies in frail subjects. (Supported by CIHR and MUHC-Research Institute).

1-83

Krill Oil supplementation modifies endocannabinoid congeners profile in cachectic advanced cancer patients

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Cancer Cachexia syndrome is characterized by anorexia, reduced food intake, muscle wasting and lipid profile disorder, mainly due to an hyperactivated immune system, chronic elevated levels of pro-inflammatory cytokines and insulin resistance, which induce a re-allocation of energy substrate store and use. Since the endocannabinoid system plays a crucial role in energy and lipid metabolism homeostasis, and krill oil has been shown to modulate endocannabinoid biosynthesis in previous studies, we aimed at verifying whether cachexia-induced lipid and energy disorders were associated with unbalanced endocannabinoid and congeners plasma levels, and whether krill oil supplementation was able to re-establish their physiological concentrations, and hence to improve lipid and energy metabolism.

Fortyfive IV stage cachectic patients (M/F 29/16; age range 55-85 y) with cancer at different sites were enrolled. Twenty-one healthy subjects were included as controls. Patients received 6 capsules (3 g/day) of krill oil, which contains omega-3 polyunsaturated fatty acids mainly in the phosphatidylcholine form (Superba[®] Krill Oil, Aker Biomarine, Norway). Treatment duration was 8 weeks. Cachectic patients had lower levels of the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2AG) and higher concentrations of the endocannabinoid congener oleoylethanolamide (OEA), which decreased significantly after Krill oil treatment. Beside, plasma lipohydroperoxide levels have been found lower than baseline.

Decrease of OEA was associated to reduced anorexia, improved lipid profile and lower levels of circulating pro-inflammatory cytokines. We conclude that krill oil treatment, probably by modulating OEA biosynthesis, may contribute to re-establish a physiological control of lipid and energy metabolism in cachectic patients.

1-84

Differences in phenotype and transcriptome distinguish IL-6-mediated versus myostatin-mediated cachexia

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Most cachexia research focuses on muscle wasting due to its contribution to decreasing function/quality of life. However, muscle loss often occurs in the context of profound phenotypic changes outside muscle. We suspect that cachexia phenotypes vary considerably across and within cancer types, due to different molecular drivers. We show the cytokine IL-6 and the TGF-beta family member myostatin cause wasting through dramatically different physiological and genomic effects. IL-6 is long known to induce muscle wasting, which we showed is largely through STAT3. Previously we also showed that administration of IL-6 induces hypertrophy of liver, spleen and intestines, through both mitosis and anti-apoptosis, in direct opposition to muscle and fat loss. Myostatin also induces muscle wasting, largely dependent upon SMAD2/3 activation. Here we show that mice treated with excess myostatin lose organ mass concomitantly and coordinately with muscle and fat. Thus IL-6-induced muscle wasting is effected through redistribution of protein from skeletal muscle to organs, while myostatin-induced wasting reflects global loss of protein stores. Mechanistically, severe cachexia effected by systemic IL-6 activated only STAT3 in skeletal muscle, while systemic myostatin activated SMAD2 and STAT3. Expression profiling revealed that IL-6 changed fewer genes in muscle than myostatin at equivalent levels of muscle loss. In severe cachexia, common genes were 66% of IL-6-altered genes but only 44% of myostatin-altered genes. Common top pathways included: generation

of precursor metabolites, contractile fiber, and mitochondria. IL-6 specific pathways included many related to mitochondria as well as sarcoplasm, sarcolemma, and muscle differentiation. Myostatin specific pathways included nucleolus, ribonuclear complex biogenesis, proteasome complex, ribosome biogenesis, and transcription factor binding. Taken together, these large differences in phenotype and

genomic response indicate that IL-6 and myostatin effect muscle wasting through distinct and only partially overlapping mechanisms. Furthermore, characterizing clinical cachexia phenotypes their drivers is necessary to develop targeted therapy.