ORIGINAL ARTICLE

Journal of Cachexia, Sarcopenia and Muscle (2015)
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12033

Plasma growth differentiation factor 15 is associated with weight loss and mortality in cancer patients

Lorena Lerner[1], Teresa G. Hayes[2,3], Nianjun Tao[1], Brian Krieger[1], Bin Feng[1], Zhenhua Wu[1], Richard Nicoletti[1], M. Isabel Chiu[1], Jeno Gyuris[1] & Jose M. Garcia[2,3,4]*

[1]*AVEO Pharmaceuticals, Cambridge, MA, USA;* [2]*Department of Medicine, Center for Translational Research on Inflammatory Diseases (CTRID), Michael E DeBakey Veterans Affairs Medical Center, Houston, TX, USA;* [3]*Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX, USA;* [4]*Department of Molecular and Cell Biology and Huffington Center on Aging, Baylor College of Medicine, Houston, TX, USA.*

Abstract

Background Cancer-related weight loss is associated with increased inflammation and decreased survival. The novel inflammatory mediator growth differentiation factor (GDF)15 is associated with poor prognosis in cancer but its role in cancer-related weight loss (C-WL) remains unclear. Our objective was to measure GDF15 in plasma samples of cancer subjects and controls and establish its association with other inflammatory markers and clinical outcomes.

Methods We measured body weight, appetite, plasma GDF15, and other inflammatory markers in men with cancer-related weight loss (C-WL, $n = 58$), weight stable patients with cancer (C-WS, $n = 72$), and non-cancer controls (Co, $n = 59$) matched by age and pre-illness body mass index. In a subset of patients we also measured handgrip strength, appendicular lean body mass (aLBM), Eastern Cooperative Oncology Group (ECOG), and Karnofsky performance scores.

Results GDF15, interleukin (IL)-6 and IL-8 were increased in C-WL versus other groups. IL-1 receptor antagonist, IL-4, interferon–gamma, tumour necrosis factor alpha, and vascular endothelial growth factor A were increased in C-WL versus C-WS, and Activin A was significantly downregulated in Co versus other groups. C-WL patients had lower handgrip strength, aLBM, and fat mass, and Eastern Cooperative Oncology Group and Karnofsky performance scores were lower in both cancer groups. GDF15, IL-6, and IL-8 significantly correlated with weight loss; GDF15 negatively correlated with aLBM, handgrip strength, and fat mass. IL-8 and Activin A negatively correlated with aLBM and fat mass. GDF15 and IL-8 predicted survival adjusting for stage and weight change (Cox regression $P < 0.001$ for both).

Conclusion GDF15 and other inflammatory markers are associated with weight loss, decreased aLBM and strength, and poor survival in patients with cancer. GDF15 may serve as a prognostic indicator in cancer patients and is being evaluated as a potential therapeutic target for cancer-related weight loss.

Keywords GDF15; Inflammation; Cachexia; MIC-1; Cytokines; IL-6; IL-8; Activin A

Received: 7 October 2014; Revised: 24 February 2015; Accepted: 16 March 2015
**Correspondence to: Jose M. Garcia, MD, PhD, Div. of Endocrinology, Diabetes and Metabolism, Michael E. DeBakey Veterans Affairs Medical Center, 2002 Holcombe Blvd, Bldg 109, Rm 210, Houston, TX 77030, USA.: Tel: (713)794-7989, Fax: (713)794-7771, Email: jgarcia1@bcm.edu*

Introduction

Over 1.5 million individuals in the USA are diagnosed with cancer each year. Energy metabolism is usually affected in this population, leading to an involuntary decrease in body weight because of fat and muscle loss known as cachexia. This condition eventually is present in up to 80% of patients with advanced cancer and is associated with decreased response to therapy, quality of life, and survival.[1–3] Weight loss related to cancer has been associated with increased inflammation. Moreover, inflammation has been directly implicated in all components of cachexia including weight loss, decreased food intake, increased muscle proteolysis, increased adipose tissue lipolysis, and energy expenditure.[4–6]

The novel inflammatory marker growth differentiation factor 15 (GDF15), also known as macrophage inhibitory cytokine-1, is a cytokine member of the transforming growth factor beta family. This factor circulates in plasma at

detectable concentrations in healthy individuals but is significantly elevated in cancer.[7] More recently, it has been postulated to play a role in the development of cancer-related weight loss (C-WL). In animal models, GDF15 was shown to induce anorexia, fat, and lean body mass loss.[8] While its role in human cancer is not yet understood, some but not all studies report an association between GDF15 levels and weight loss and poor prognosis and decreased survival.[9,10]

There is an ongoing search for anabolic, catabolic, and anti-catabolic circulating biomarkers that would aid in establishing the diagnosis and prognosis in patients at risk or with ongoing C-WL. In this study, we aim at determining the association, if any, between circulating levels of a panel of cytokines, including GDF15, with body weight, lean and fat mass (FM), muscle strength, performance status, and survival in a cross-sectional study of patients with cancer with and without weight loss and a non-cancer control group.

Materials and methods

Study design

This study was performed at the Michael E. DeBakey VA Medical Center (MEDVAMC) in Houston, TX. The protocol was approved by the Institutional Review Boards of Baylor College of Medicine and MEDVAMC. Patients were recruited from November 2006 to August 2010 from the MEDVAMC. All clinical investigations were conducted in accordance with the guidelines in The Declaration of Helsinki. This was a cross-sectional study of male patients with cancer with and without significant weight loss (greater than 5% over the previous 6 months), and male non-cancer controls matched by age and body mass index to the two cancer groups. The study also had a retrospective follow-up component when information was gathered from the electronic medical records to collect survival data. Subjects were followed until November 2011 or until death, whichever occurred first (median: 1485 days). The Social Security Death Index[11] and medical record were used to enter date of death.

Eligibility criteria

Subjects were ≥18 years old and provided written informed consent. The study included (1) individuals with cancer, excluding non-melanoma skin cancer and cancer-related weight loss (C-WL, $n = 58$); (2) individuals with cancer, excluding non-melanoma skin cancer, without weight loss [cancer-weight stable (C-WS), $n = 72$]; and (3) weight-stable subjects without cancer (controls, Co, $n = 59$). Exclusion criteria included Eastern Cooperative Oncology Group (ECOG) score >2, body mass index >35 (because of a weight limitation of the dual-energy x-ray absorptiometry scanner), history of congestive heart failure, active infections, or other inflammatory conditions associated with weight loss, evidence of ascites or clinically significant oedema, use of anabolic agents, appetite stimulants (including corticosteroids other than dexamethasone at the time of IV chemotherapy administrations), alcohol abuse, tube feeding or parenteral nutrition in the prior three months, participation in a clinical trial with investigational agents within one month prior to recruitment, and use of androgens or anti-androgens. None of the cancer subjects were receiving curative chemotherapy.

Study measures

Body weight changes were assessed using weights recorded in the electronic medical records 12 months before enrolment (baseline), 6 months before enrolment, or at the time of enrolment in the study. Subjects were weighed on a calibrated scale, and appetite was assessed by the previously validated Anderson Symptom Assessment Scale visual analogue scale.[12,13]

In a subset of subjects (34 C-WL, 36 C-WS and 40 Co), the following outcomes were also measured: Functional assessment was performed by previously validated ECOG, Karnofsky performance scale (KPS), and Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) questionnaires.[14] All questionnaires except ECOG were adjusted during data analysis so that the lowest number meant worst symptoms and the highest number meant no symptoms at all. Lean body mass (LBM) and FM were measured by dual-energy x-ray absorptiometry (Lunar Prodigy DF + 14333, Software 6.7v, GE). Grip strength was assessed with a hand-held dynamometer (Jamar Hydraulic Dynamometer, model #5030J1, J.A. Preston Corp., Clifton, NJ, USA). Some of these results have been reported previously.[15]

The following markers were measured in an early morning fasting plasma samples: Activin A, eotaxin, fibroblast growth factor basic (FGFb), granulocyte-colony stimulating factor (G-CSF), GDF15, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma, interleukin (IL)-10, IL-12p70, IL-13, IL-15, IL-17, IL-1β, IL-1 receptor a (IL-1Ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, interferon gamma-induced protein 10 (IP-10 or CXCL10), monocyte chemotactic protein 1 (MCP-1 or CCL2), macrophage inflammatory protein-1 alpha (MIP-1α or CCL3), macrophage inflammatory protein-1 beta (MIP-1β or CCL4), platelet-derived growth factor B (PDGF-bb), regulated on activation, normal T cell expressed and secreted (RANTES or CCL5), tumour necrosis factor alpha (TNF-α), and vascular endothelial growth factor A (VEGFA). Individual enzyme-linked immunosorbent assay kits were used for measurements of GDF15 and Activin A (R&D Systems, Minneapolis, MN, USA). The rest of the cytokines were measured using Bio-Plex Pro™ Human Cytokine 27-Plex Assay from Bio-Rad® (Luminex®, magnetic beads, Bio-Rad, Hercules, CA, USA) and analysed by using the Bio-Plex Manager 2.0 software (all from Bio-Rad Laboratories). Albumin and highly

sensitive C-reactive protein (hs-CRP) were also measured as we previously published.[15] All procedures were carried out according to the manufacturer's instructions. Because no normal values have been established for these analytes and our sample is too small to establish such values, information is presented using mean ± SD or SEM or quartiles.

Statistical analysis

SPSS v17.00 (SPSS Inc., Chicago, IL, USA) and R v2.14.1 (http://www.r-project.org/) were used for statistical analysis. Variables were summarized descriptively by group using *N*, mean and standard error for normally distributed continuous variables, by median and interquartile range for non-normally distributed variables, and by frequencies (%) for nominal or ordinal variables. A three-group (controls vs C-WL vs C-WS) analysis of variance was used to analyse normally distributed continuous variables, and non-parametric tests were used for variables non-normally distributed. Data from analytes were converted to categorical variables (detectable vs non-detectable) when more than 25% of samples were below the level of detection for the assay (eotaxin, G-CSF, GM-CSF, IL-10, IL-12p70, IL-13, IL-15, IL-17, IL-1β, IL-2, IL-5, IL-7, and MIP-1α). Statistical comparisons for these categorical variables were performed using the chi-squared test. For multiple comparisons, the Dunn's test was used. *P* values ≤0.05 were considered statistically significant. It was anticipated that cancer stage may be highly associated with C-WL and survival. The association between GDF15 and other cytokines and C-WL and survival was measured by a logistic regression model or Cox proportional hazards model to determine which variables, in addition to cancer stage, were significant predictors of C-WL and mortality. The measure of association was given by the *P* value. Pearson's correlations, or non-parametric Spearman's correlation when the data were not normally distributed, were obtained between continuous variables. See supplemental data for more information.

Results

Subjects were all male with similar body weights 12 months before entering the study. Subjects in the C-WS group were slightly older than in the other two groups, although this was probably not clinically relevant. As expected, C-WL subjects had significantly lower body weight and had experienced a significant degree of weight loss over the previous 6 and 12 months compared with non-cancer controls and to subjects with cancer and without weight loss (*Table* 1). There were no significant differences in ethnicity or rate of comorbidities between groups but controls had a lower rate of exposure to opioids compared with the other groups, as expected. There were no significant differences regarding diagnosis, stage, opioid, or chemotherapy use between the

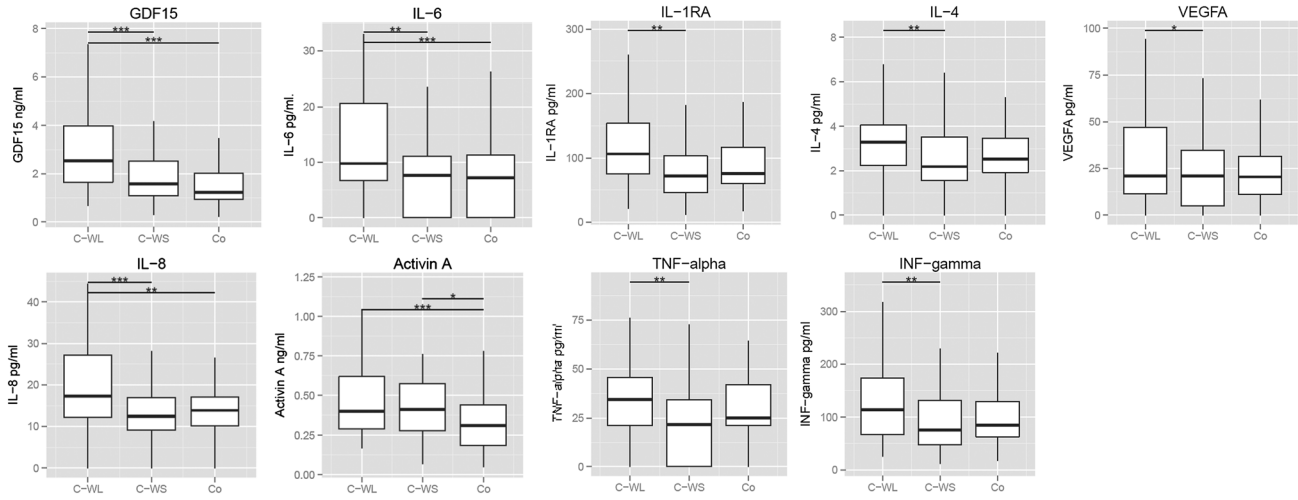
Table 1 Baseline characteristics

Characteristics	Controls	Cancer weight stable	Cancer weight loss	*P* value
No. of subjects	59	72	58	
Age in years	63.2			0.025
Mean	9.5	66.8 £	63.1	
SD		9.6	7.6	
Race (n (%))				0.21
Caucasian	38 (64)	50 (69)	45 (78)	
African American	14 (24)	14 (19)	12 (21)	
Hispanic	7 (12)	6 (8)	1 (2)	
Asian		2 (3)		
Body weight 12 months before enrolment (kg)				0.37
Mean	91.4	87.8	89.7	
SD	13	14.4	15.4	
Enrolment body weight (kg)				<0.001
Mean	90.9**	87.1 **	78.2	
SD	12.6	15.3	13.6	
Weight change 6 months prior to enrolment (%)				<0.001
Mean	−0.38 **	0.31 **	−11.7	
SD	3.1	4.2	7.3	
Weight change 12 months prior to enrolment (%)				<0.001
Mean	−0.41 **	−0.99 **	−12.5	
SD	4.1	4.9	7.1	
Comorbidities (*n* (%))				
Diabetes	13 (22)	24 (33)	11 (19)	0.19
COPD	3 (5)	4 (6)	8 (14)	0.19
Opioid use (*n* (%))	2 (3) £	12 (18)	18 (31)	0.002
Cancer type (*n* (%))				0.2
Lung		20 (28)	21 (36)	
GI		13 (18)	14 (24)	
GU		16 (22)	6 (10)	
Head and neck		1 (1)	3 (5)	
Other		22 (31)	14 (25)	
Stage † (*n* (%))				0.06
I		12 (17)	2 (3)	
II		10 (14)	13 (22)	
III		19 (26)	18 (31)	
IV		24 (33)	23 (40)	
Chemotherapy regimen (*n* (%))				0.57
Platinum-based		16 (22)	17 (29)	
Taxanes		7 (10)	6 (10)	
5-Fluorouracil		9 (13)	11 (19)	
Doxorubicin		2 (3)	2 (3)	
Tyrosine kinase inhibitor		4 (6)	3 (5)	
aLBM (kg)				0.01
Mean	18.5*	17.6	16.2	
SD	2.89	3.3	3.2	
FM (kg)				<0.001
Mean	29.9**	25.9	21.6	
SD	7.22	7.7	7.45	
HGS (kg)				0.04
Mean	140	151*	115	
SD	68	45	65	
ASAS appetite (1–10)				0.32
Mean	5.72	5.7	5.23	
SD	1.45	1.72	2.51	

*$P < 0.05$ compared with C-WL; £ $P < 0.05$ compared with other groups. **$P < 0.001$ compared with C-WL; § $P < 0.001$ compared with controls. †Staging performed only for solid tumours. COPD, chronic obstructive pulmonary disease; GI, gastrointestinal tumours; GU, genito-urinary tumours; aLBM, appendicular lean body mass; FM, fat mass; HGS, handgrip strength; and ASAS, Anderson symptoms assessment scale.

DOI: 10.1002/jcsm.12033

Figure 1 Upregulated cytokine levels in weight losing patients with cancer. Data are represented as box plots (median and quartiles). Circulating levels of cytokines in control patients (Co), weight stable cancer patients (C-WS), and weight losing cancer patients (C-WL). Non-parametric one-way ANOVA Kruskal–Wallis test and multiple comparisons test Dunn's *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.



two cancer groups, although C-WL subjects trended to have more advanced stage disease. Analysis in a subset of patients showed that C-WL subjects had lower grip strength, aLBM, FM, and appetite scores compared with the other groups, although differences were not significant for the latter variable, as we have described previously.[15] Also, and as previously described, weight losing when compared with weight stable cancer patients and non-cancer controls had lower albumin (3.4 ± 0.1, 3.8 ± 0.07 and 3.9 ± 0.04 g/dL, respectively, $P < 0.001$) and higher hs-CRP levels (3.5 ± 1.1, 1.76 ± 0.8 and 0.49 ± 0.1 mg/dL, respectively $P < 0.03$).[15]

GDF15 and other inflammatory markers are increased in cancer-related weight loss

Levels of GDF15, IL-6, and IL-8 were significantly increased in C-WL versus other groups (*Figure* 1). Activin A was significantly elevated in both cancer groups compared with controls. IL-1Ra, IL-4, INF γ, TNFα, and VEGFA were also elevated in C-WL, but the difference only reached significance when compared with C-WS. Among the inflammatory cytokines whose presence was not detectable in more than a quarter of the samples tested, IL-10, IL-12p70, and IL-17 were significantly elevated in C-WL compared with the two other groups (*Table* 2). Other cytokine pairs with significant differences were also noted: increased G-CSF in C-WS versus other groups, increased IL-7 in C-WL versus C-WS, and increased MIP-1α in C-WL versus Co. There were no significant differences between groups in eotaxin, fibroblast growth factor basic, GM-CSF, IL-13, IL-15, IL-1β, IL-2, IL-5, IL-9, IP-10, MCP-1, MIP-1β, PDGFbb, and RANTES levels (data not shown). Exposure to radiation therapy has been associated with acute (4 h after exposure) elevations in GDF15 mRNA levels.[16] In our cohort, only one subject in the cancer-weight stable group was receiving radiation therapy at the time of enrolment, although it had not been exposed to radiation 24 h before enrolment; 48 other subjects had a history of past exposure (more than 1 month before enrolment) to radiation therapy. Moreover, there was no difference in GDF15 levels or in other cytokine levels between subjects exposed and those unexposed to radiation therapy (data not shown).

Table 2 Increased presence of inflammatory cytokines in weight losing cancer patients

Cytokine	Co (% of samples detected)	C-WS (% of samples detected)	C-WL (% of samples detected)
IL-10	25	26	45**,***
IL-12p70	56	53	76**,***
IL-17	47	46	67**,***
MIP-1α	12	17	29**
IL-7	39	31	57****
G-CSF	49	75*	69***

Chi-square test.
*$P < 0.01$ compared Co to C-WS.
**$P < 0.05$ compared Co to C-WL.
***$P < 0.05$ compared C-WS to C-WL.
****$P < 0.01$ compared C-WS to C-WL.

Table 3 Correlations between inflammatory markers and clinical measures

	aLBM	Fat Mass	Grip
GDF15	−0.34 ($p < 0.001$)	−0.25 ($p = 0.012$)	−0.29 ($p = 0.003$)
IL-8	−0.27 ($p = 0.006$)	−0.30 ($p = 0.002$)	−0.17 ($p = 0.075$)
Activin A	−0.30 ($p = 0.002$)	−0.22 ($p = 0.028$)	−0.16 ($p = 0.105$)

Correlation analyses performed by Pearson's correlation or Spearman's correlation when data were not normally distributed. aLBM, appendicular lean body mass.

Table 4 Association between weight loss and cytokines in the lung cancer patient subset (21 C-WL, 20 C-WS)

Analyte	Beta	*P* value
GDF15	−1.0685	0.0088
VEGF	−0.5251	0.0105
MIP-1b	−1.3202	0.0155
IL-12	−0.3602	0.0157
PDGFb	−0.4887	0.0209
RANTES	−0.6513	0.0240
IL-6	−0.4336	0.0319
IL-8	−0.6927	0.0600
FGFb	−0.5923	0.0648
TNFα	−0.2418	0.0800
Activin A	−0.6078	0.1170
IFNγ	−0.3372	0.1511
IL-1Ra	−0.3811	0.1658
IL-10	−0.2030	0.1917
IL-4	−0.4575	0.1980
MCP-1	−0.4698	0.2250
IL-17	−0.1479	0.2475
IL-7	−0.1550	0.2812
IL-13	−0.2052	0.3183
IL-9	−0.1237	0.3198
GM-CSF	−0.1127	0.3567
IL-5	−0.1584	0.5462
Eotaxin	−0.0547	0.5926
IL-15	0.1127	0.5998
MIP-1α	−0.1140	0.6071
G-CSF	−0.0563	0.6540
IL-2	−0.0651	0.7981
IP-10	0.0422	0.9068
IL-1b	−0.0177	0.9529

Multivariate logistic regression analysis adjusting by tumour stage was used. Beta represents the association coefficient estimate.

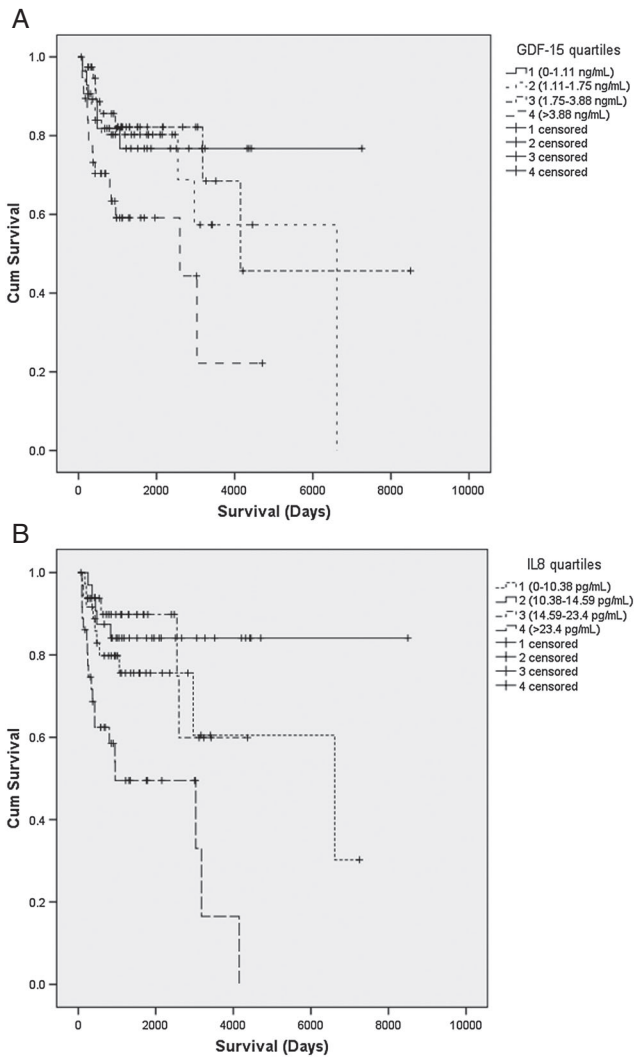
Association between GDF15 and other inflammatory markers and clinical parameters in cancer subjects

Levels of GDF15 correlated positively with those of IL-6, IL-1Ra, IL-2, IL-4, IL-9, IL-10, IP-10, INFγ, IP-10, MCP-1, MIP-1α, MIP-1β, TNFα, VEGFA, and Activin A in patients with cancer. Also, GDF15, IL-6, IL-8, and IL-1ra levels significantly correlated with 6 month weight loss (see Supporting Information, *Table* S1). GDF15 negatively correlated with aLBM, handgrip strength, and FM. IL-8 and Activin A negatively correlated with aLBM and FM (*Table* 3). There was no association between GDF15 and appetite scores as measured by the Anderson Symptom Assessment Scale (not shown). Performance status as measured by ECOG was positively correlated with Activin A and negatively correlated with IL-2; KPS was negatively correlated with Activin A and positively correlated with IL-2; regarding FACIT-F scores, physical domain scores were negatively correlated with GDF15 (*Table* S2). The association between hs-CRP and albumin and other clinical parameters has been reported previously.[15]

Subset analyses in lung cancer subjects

Subset analyses were performed with data from patients with lung cancer, the largest group of patients available by cancer diagnosis. Sample size was too small in other tumour types to conduct these statistical analyses. In this subset, multivariate logistic regression was used to evaluate the association between the cytokine levels and C-WL after adjusting for tumour stage. GDF15, VEGFA, MIP-1β, IL-12, PDGFbb, RANTES, and IL-6 were significantly associated with weight loss in patients with lung cancer (*Table* 4). In order to perform a multivariate logistic regression analysis, cytokines were removed from further analyses when the Pearson correlation coefficients were greater than 0.5. Activin A, IL-6, and IL-8 were strongly correlated among themselves; RANTES, IL-8, VEGFA, and PDGFbb were also strongly correlated with each other (*Table* S3). Therefore, only GDF15, IL-6, PDGFbb, MIP-1β, and IL-12 were included for further analysis. Of the five cytokines subjected to multivariate logistic

Figure 2 Kaplan–Meier survival curves according to GDF15 quartiles (ng/mL, *A*) and to IL-8 quartiles (pg/mL, *B*).



DOI: 10.1002/jcsm.12033

regression analysis adjusted by tumour stage, GDF15 was identified as the only predictor of weight loss (*Table* S4).

Survival analysis

Survival analysis showed that GDF15 and IL-8 predicted survival after adjusting for stage and weight change (Cox regression $P < 0.001$ for both). Cancer diagnosis per se was not a significant predictor of survival in this model (not shown). Kaplan–Meier analysis by GDF15 and IL-8 quartiles showed that lower GDF15 and IL-8 levels were associated with better survival ($P < 0.05$ for both; *Figure* 2), while conversely, the highest quartile was associated with the poorest survival.

Discussion

Several lines of evidence suggest a prognostic link between weight loss and cancer. For example, weight loss and anorexia were reported to be associated with poor prognosis and worse survival in cancer and other chronic conditions in several studies.[17,18] Others have shown that both weight loss and anorexia are associated with greater mortality in patients with terminal cancer.[19] Despite the enormous burden on patients, cancer-related anorexia and weight loss are often undiagnosed and remain untreated. Also, inflammatory markers are thought to play a role in the development of these symptoms, and the prognostic value of some of these markers has been examined before.[20–23]

The transforming growth factor beta family of cytokines has recently been identified as a potential therapeutic target for C-WL, given that it is one of the key regulators of lean body mass and body weight. A novel member of this family known as macrophage inhibitory cytokine-1/GDF15, originally identified as a key regulator of macrophage activation, was shown to induce anorexia and weight loss in mice bearing tumours over-expressing GDF15. Moreover, these effects were neutralized by administration of monoclonal antibodies targeting GDF15.[8]

In humans, the role of GDF15 is still controversial, with some studies reporting an association with weight loss and survival in patients with prostate cancer while other studies reporting an association between GDF15 and other inflammatory markers but not with nutritional status or survival in patients with gastrointestinal tumours.[9,10] The purpose of this study was to further characterize the role of GDF15 and other inflammatory markers in humans with cancer with and without weight loss compared with non-cancer controls and to establish the association between these markers and clinically relevant outcomes including body composition, body weight, muscle strength, and overall survival.

In this study, circulating levels of GDF15 were significantly increased in patients with cancer with weight loss compared with patients with cancer without weight loss and non-cancer controls. Moreover, GDF15 levels were associated with weight loss, decreased FM, muscle mass, strength, physical performance scores, and survival in patients with cancer. This is very relevant because LBM loss is a negative and independent prognostic factor in patients with cancer[18] and decreased grip strength is associated with poor survival in this population.[24] Lean body mass in the extremities (also known as appendicular LBM or aLBM) is a good surrogate for muscle mass, given that most of the lean tissue in this area is skeletal muscle,[25] unlike the lean body mass in the trunk that includes a large amount of non-skeletal lean tissue such as liver, gastrointestinal tract, and heart. Although causality cannot be inferred from the current study, these associations between GDF15 levels, muscle mass, and strength and survival suggest that it could be used as a prognostic biomarker in this setting as others have previously proposed.[26]

GDF15 has been postulated to play a role in cancer-induced weight loss by inducing anorexia in animal models; hence, it is intriguing that appetite scores were not associated with GDF15 levels in our study in spite of the clear association with other parameters that are clinically relevant such as LBM, body weight, and survival. Our study was not set up to answer this question but it is possible that the acute effect of GDF15 on appetite demonstrated in rodents is not maintained chronically or that other factors inherent to patients with cancer (i.e. administration of chemotherapy and opioids) interfere with this association. More studies will be needed to explore this issue in more depth.

The association between GDF15 and decreased aLBM, FM, and grip strength in patients with cancer suggests that this molecule may be involved in the regulation of body composition and muscle function in this setting. Although the mechanism cannot be elucidated from this study, several hypotheses could explain these findings. GDF15 has been proposed to cause anorexia through a direct effect on the hypothalamus, at least in rodents.[8] Also, other members of the transforming growth factor beta receptor superfamily such as myostatin or Activin A directly regulate muscle mass by promoting proteolysis and decreasing protein synthesis.[27–29] Whether GDF15 shares the same pathways in muscle remains to be determined and should be the focus of future studies.

In rodent models, Activin A induces weight loss and inhibition of the Activin receptor type IIB in skeletal muscle reverses the phenotype and increases survival.[28] However, data in humans of the potential role of Activin A in this setting are lacking. Here, we report that circulating levels of Activin A were increased in patients with cancer with weight loss and that it correlated with LBM, FM, and performance status scores. This is consistent with the notion that in

addition to GDF15, plasma Activin A levels may also be used as a biomarker of cachexia in humans and may serve to guide future therapeutic interventions. The association between Activin A and LBM also supports the concept that the cellular target of this pathway is skeletal muscle.

Several other pro-inflammatory cytokines including IL-6, TNF-α, and IL-1β have been associated with cachexia and anorexia in both humans and rodents[3,20,30] but the role of IL-8 in C-WL is less well characterized. In our cohort, IL-8 was associated not only with aLBM and FM but also with survival. To our knowledge, this is the most comprehensive characterization of the associations between IL-8 and other clinical parameters in this setting. Given that genetic polymorphisms of IL-8 have been associated with an increased risk for C-WL[31] and that in-vitro studies have shown that IL-8 induces the synthesis of hepatic inflammatory mediators,[32] it is tempting to think that there is a causal relationship between IL-8 induction and the aforementioned outcomes. More studies will be needed to test this hypothesis. IL-2 was not different between groups, but it correlated well with better ECOG and KPS. The role of IL-2 in cancer cachexia is not well established, but it is reportedly an anti-inflammatory cytokine.[33] Larger and more mechanistic studies will be needed to determine the relevance of this association.

There are several strengths to this study. The patients were all males and fairly homogeneous with regards to comorbidities. This is, to our knowledge, the most comprehensive report of clinically relevant outcomes in C-WL such as aLBM, grip strength, survival, and GDF15 levels. The inclusion of non-terminally ill patients in this study allowed us to explore the role of these variables in a wider variety of cancer patients. The extended length of time given to follow-up for this cohort of patients provided additional strength to the resulting survival analysis.

This study also presented certain limitations. Its relatively small sample size and the heterogeneity of the samples with regards to tumour type or stage may have limited the power of the study to find additional, potentially significant differences among its various outcomes. Nevertheless, a post hoc analysis limited to the most common tumour type-lung cancer also showed an association between GDF15 and weight loss even after adjusting for tumour stage. Unfortunately, the sample size for other cancer diagnoses was too small to perform statistical analyses and to establish if these biomarkers predict weight loss in other settings.

The study was performed at the Veterans Affairs Medical Center in Houston, and the population at this institution is >95% male. Hence, only men were included in the study, and these results may not be immediately applicable to a female population. Moreover, levels in circulation may not reflect tissue action in the target organs (i.e. hypothalamus, adipose tissue, or muscle), and the potential effects of GDF15 on muscle proteolysis were not evaluated. Nevertheless, these limitations do not diminish the significant conclusions derived from the present study.

In summary, we show here that GDF15 and other inflammatory markers are associated with weight loss, decreased muscle mass, and strength and poor survival in patients with cancer. If larger human studies confirm these findings, GDF15 may potentially serve as a prognostic indicator in patients with cancer. GDF-15 is also being evaluated as a potential therapeutic target for the treatment of C-WL.[34,35]

Acknowledgements

The authors of this manuscript certify that they comply with the ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia, and Muscle 2010;1:7–8 (von Haehling S., Morley J. E., Coats A. J. S., Anker S. D.).

Funding

This study was funded by AVEO Pharmaceuticals, by the Department of Veterans Affairs (MERIT grants: I01-BX000507 and I01 CX000174) and the National Institutes of Health (AG040583). We thank the Baylor Diabetes and Endocrinology Research Center (P30 DK079638) for their help.

Supporting information

Supporting information may be found in the online version of this article.

Table S1. Correlations between inflammatory markers and 6 month body weight loss.
Table S2. Association between inflammatory cytokines and performance status and FACIT-F scores.
Table S3. Correlation coefficients among the top nine cytokines that are associated with cancer-related weight loss in lung cancer.
Table S4. Association between weight loss and cytokine levels using multivariate analysis, adjusting by tumor stage using logistic regression in lung cancer patients subset.
Weight loss ~ Tumor stage + GDF15+Activin A+IL-6+IL-8+VEGFA.

Conflict of interest

L .L., N.T., B.K., B.F., R.N., and J.G. are employees of AVEO Pharmaceuticals. M.I.C. and J.W. are former employees of AVEO Pharmaceuticals. J.M.G. receives research support and is a consultant for AVEO Pharmaceuticals.

References

1. Deans C, Wigmore SJ. Systemic inflammation, cachexia and prognosis in patients with cancer. *Curr Opin Clin Nutr Metab Care* 2005; **8**: 265–9.
2. Maltoni M, Nanni O, Pirovano M, Scarpi E, Indelli M, Martini C, *et al*. Successful validation of the palliative prognostic score in terminally ill cancer patients. *Italian Multicenter Study Group on Palliative Care Journal Pain Symptom Manage* 1999; **17**: 240–7.
3. Utech AE, Tadros EM, Hayes TG, Garcia JM. Predicting survival in cancer patients: the role of cachexia and hormonal, nutritional and inflammatory markers. *Journal Cachexia Sarcopenia Muscle* 2012; **3**: 245–51.
4. Batista ML Jr., Peres SB, McDonald ME, Alcantara PS, Olivan M, Otoch JP, *et al*. Adipose tissue inflammation and cancer cachexia: possible role of nuclear transcription factors. *Cytokine* 2012; **57**: 9–16.
5. Wallenius K, Wallenius V, Sunter D, Dickson SL, Jansson JO. Intracerebroventricular interleukin-6 treatment decreases body fat in rats. *Biochem Biophys Res Commun* 2002; **293**: 560–5.
6. Lyngso D, Simonsen L, Bulow J. Metabolic effects of interleukin-6 in human splanchnic and adipose tissue. *J Physiol* 2002; **543**: 379–86.
7. Valenzuela SM, Martin DK, Por SB, Robbins JM, Warton K, Bootcov MR, *et al*. Molecular cloning and expression of a chloride ion channel of cell nuclei. *J Biol Chem* 1997; **272**: 12575–82.
8. Johnen H, Lin S, Kuffner T, Brown DA, Tsai VW, Bauskin AR, *et al*. Tumor-induced anorexia and weight loss are mediated by the TGF-beta superfamily cytokine MIC-1. *Nat Med* 2007; **13**: 1333–40.
9. Brown DA, Lindmark F, Stattin P, Balter K, Adami HO, Zheng SL, *et al*. Macrophage inhibitory cytokine 1: a new prognostic marker in prostate cancer. *Clin Cancer Res* 2009; **15**: 6658–64.
10. Skipworth RJ, Deans DA, Tan BH, Sangster K, Paterson-Brown S, Brown DA, *et al*. Plasma MIC-1 correlates with systemic inflammation but is not an independent determinant of nutritional status or survival in oesophago-gastric cancer. *Br J Cancer* 2010; **102**: 665–72.
11. Death Master File [database on the Internet]. 2005 [cited 7 August 2010]. Available from: http://www.segenealogy.com/_search/vital_ssdi.htm.
12. Bruera E, Kuehn N, Miller MJ, Selmser P, Macmillan K. The Edmonton Symptom Assessment System (ESAS): a simple method for the assessment of palliative care patients. *J Palliat Care* 1991; **7**: 6–9.
13. Heedman PA, Strang P. Symptom assessment in advanced palliative home care for cancer patients using the ESAS: clinical aspects. *Anticancer Res* 2001; **21**: 4077–82.
14. Cella DF, VonRoenn J, Lloyd S, Browder HP. The Bristol-Myers Anorexia/Cachexia Recovery Instrument (BACRI): a brief assessment of patients' subjective response to treatment for anorexia/cachexia. *Qual Life Res* 1995; **4**: 221–31.
15. Burney BO, Hayes TG, Smiechowska J, Cardwell G, Papusha V, Bhargava P, *et al*. Low testosterone levels and increased inflammatory markers in patients with cancer and relationship with cachexia. *J Clin Endocrinol Metab* 2012; **97**: E700–9.
16. Joiner MC, Thomas RA, Grever WE, Smolinski JM, Divine GW, Konski AA, *et al*. Developing point of care and high-throughput biological assays for determining absorbed radiation dose. *Radiother Oncol* 2011; **101**: 233–6.
17. Dewys WD, Begg C, Lavin PT, Band PR, Bennett JM, Bertino JR, *et al*. Prognostic effect of weight loss prior to chemotherapy in cancer patients. *Eastern Cooperative Oncology Group American Journal Medicine* 1980; **69**: 491–7.
18. Reuben DB, Mor V, Hiris J. Clinical symptoms and length of survival in patients with terminal cancer. *Arch Intern Med* 1988; **148**: 1586–91.
19. Vigano A, Donaldson N, Higginson IJ, Bruera E, Mahmud S, Suarez-Almazor M. Quality of life and survival prediction in terminal cancer patients: a multicenter study. *Cancer* 2004; **101**: 1090–8.
20. Garcia JM, Garcia-Touza M, Hijazi RA, Taffet G, Epner D, Mann D, *et al*. Active ghrelin levels and active to total ghrelin ratio in cancer-induced cachexia. *J Clin Endocrinol Metab* 2005; **90**: 2920–6.
21. Laviano A, Gleason JR, Meguid MM, Yang ZJ, Cangiano C, Rossi FF. Effects of intra-VMN mianserin and IL-1ra on meal number in anorectic tumor-bearing rats. *J Investig Med* 2000; **48**: 40–8.
22. Tas F, Duranyildiz D, Argon A, Oguz H, Camlica H, Yasasever V, *et al*. Serum levels of leptin and proinflammatory cytokines in advanced-stage non-small cell lung cancer. *Med Oncol* 2005; **22**: 353–8.
23. Simons JP, Schols AM, Buurman WA, Wouters EF. Weight loss and low body cell mass in males with lung cancer: relationship with systemic inflammation, acute-phase response, resting energy expenditure, and catabolic and anabolic hormones. *Clin Sci (Lond)* 1999; **97**: 215–23.
24. Gale CR, Martyn CN, Cooper C, Sayer AA. Grip strength, body composition, and mortality. *Int J Epidemiol* 2007; **36**: 228–35.
25. Sattler F, Bhasin S, He J, Chou CP, Castaneda-Sceppa C, Yarasheski K, *et al*. Testosterone threshold levels and lean tissue mass targets needed to enhance skeletal muscle strength and function: the HORMA trial. *J Gerontol A Biol Sci Med Sci* 2011; **66**: 122–9.
26. Tsai VW, Husaini Y, Manandhar R, Lee-Ng KK, Zhang HP, Harriott K, *et al*. Anorexia/cachexia of chronic diseases: a role for the TGF-beta family cytokine MIC-1/GDF15. *Journal Cachexia Sarcopenia Muscle* 2012; **3**: 239–43.
27. Lin J, Arnold HB, Della-Fera MA, Azain MJ, Hartzell DL, Baile CA. Myostatin knockout in mice increases myogenesis and decreases adipogenesis. *Biochem Biophys Res Commun* 2002; **291**: 701–6.
28. Zimmers TA, Davies MV, Koniaris LG, Haynes P, Esquela AF, Tomkinson KN, *et al*. Induction of cachexia in mice by systemically administered myostatin. *Science* 2002; **296**: 1486–8.
29. Zhou X, Wang JL, Lu J, Song Y, Kwak KS, Jiao Q, *et al*. Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival. *Cell* 2010; **142**: 531–43.
30. Fong Y, Moldawer LL, Marano M, Wei H, Barber A, Manogue K, *et al*. Cachectin/TNF or IL-1 alpha induces cachexia with redistribution of body proteins. *Am J Physiol* 1989; **256**: R659–65.
31. Bo S, Dianliang Z, Hongmei Z, Xinxiang W, Yanbing Z, Xiaobo L. Association of interleukin-8 gene polymorphism with cachexia from patients with gastric cancer. *J Interferon Cytokine Res* 2010; **30**: 9–14.
32. Wigmore SJ, Fearon KC, Maingay JP, Lai PB, Ross JA. Interleukin-8 can mediate acute-phase protein production by isolated human hepatocytes. *Am J Physiol* 1997; **273**: E720–6.
33. Banchereau J, Pascual V, O'Garra A. From IL-2 to IL-37: the expanding spectrum of anti-inflammatory cytokines. *Nat Immunol* 2012; **13**: 925–31.
34. Lerner L, Hayes T, Tao N, Krieger B, Feng B, Nicoletti R, *et al*. Plasma growth differentiating factor-15 (GDF-15) and other inflammatory markers are associated with weight loss and poor prognosis in cancer patients. *Cancer Res* 2013; **73**: 3504.
35. Lerner L, Abbott S, Bai A, Chen T, Chiu MI, Liu Q, *et al*. Anti-gdf15 antibodies. Google Patents; 2013.