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Circulating Activin A predicts survival in cancer patients

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Abstract

Background Several experimental evidences pinpoint the possible role of Activin A (ActA) as a driver of cancer cachexia. Supporting this hypothesis, we showed recently that human cancer cachexia is associated with high ActA levels. Moreover, ActA levels were correlated with body weight loss and skeletal muscle density, two prognostic factors in cancer patients. Our goal was therefore to investigate the value of ActA to predict survival in cancer patients.

Methods Patients with colorectal or lung cancer were prospectively enrolled at the time of diagnosis or relapse between January 2012 and March 2014. At baseline, patients had clinical, nutritional, and functional assessment. Body composition and skeletal muscle density were measured by CT scan, and plasma ActA concentrations were determined. Overall survival (OS) was analysed since inclusion to 24 months later.

Results Survival data were available for 149 patients out of 152. Patients with high ActA (≥408 pg/mL) had lower OS than those with low levels, regardless the type of cancer (OS in colorectal cancer, 50% vs. 79%, $P < 0.05$; and in lung cancer, 27% vs. 67%, $P = 0.001$). The multivariable analysis confirmed the prognostic value of ActA independently of tumour stage or inflammatory markers, particularly in lung cancer. Low muscularity was also an independent prognostic factor.

Conclusions Our study demonstrates that high ActA level is an independent prognosis factor of survival in cancer patients. More than a basic marker of the severity of the neoplastic disease or of the inflammatory process, ActA seems to influence survival by contributing to the development of cachexia and loss of skeletal muscle mass.

Keywords Activin A; Cachexia; Lung cancer; Colorectal cancer; Biomarker

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Introduction

Cancer cachexia is a muscle-wasting syndrome, which affects up to 80% of advanced cancer patients and is considered to be responsible of 20% of all cancer deaths.[1] Despite intense research, mechanisms of cachexia are still poorly characterized, and therapeutic targets are desperately lacking. The muscle-wasting process in cachexia is demonstrated by a reduction of muscle size and also an increase in muscle fat infiltration,[2] as assessed by the muscle density measured by CT scan. Interestingly, low muscularity and muscle fat infiltration are recognized as independent risk factors of poor survival in cancer patients, regardless of body weight.[3–6] Therefore, it is expected that inhibition of muscle-wasting process may represent an effective strategy to treat cancer cachexia. However, the nature of the key players responsible of muscle atrophy in cancer cachexia is still elusive.

Activin A (ActA), a member of transforming growth factor-β (TGF-β) superfamily, is a homodimer of β-Activin chains and exerts most of its biological actions by binding to the transmembrane Activin type II receptor B (ActRIIB), a serine/threonine kinase receptor.[7] ActA is present in the circulation either in bioactive free form or bound to Follistatin (FS), the main regulator of its biological activity.

Firstly identified as gonadal-derived regulator of pituitary follicle-stimulating hormone (FSH), ActA is in fact expressed in a wide range of tissues, and many biological activities are assigned to it (skeletal muscle tissue regulation, gonadal function, inflammation, carcinogenesis, etc.).[7]

Recently, several evidences pinpoint the possible role of ActA as a critical driver of muscle atrophy in cancer cachexia. In one hand, increased local or circulating concentrations of ActA induce skeletal muscle atrophy.[8–10] Indeed, the binding of ActA to its muscle ActIIB receptor activates an atrophy gene programme via the phosphorylation of Smad2/3. This receptor is also shared with Myostatin, another TGF-β superfamily member and a negative growth factor of muscle mass.[11] In another hand, circulating levels of ActA have been reported to be increased in animal models of cancer cachexia.[12] Finally, inhibition of ActA reverses cachexia and increases survival in these animal models.[8] In humans, circulating levels of ActA are increased in various pathologies, such as cancer, acute respiratory distress syndrome, chronic obstructive pulmonary inflammation, and in many conditions associated with inflammation.[13,14] Recently, our team showed in a population of cancer patients that cachexia, characterized by decreased muscle mass and function, is associated with increased circulating concentrations of ActA,[15] suggesting this hormone might contribute to the development of human cancer cachexia. Moreover, circulating ActA was correlated positively with weight loss and negatively with skeletal muscle density (SMD) that both appear to be prognostic factors of poor survival in cancer patients. In contrast, plasma Myostatin was reduced in our population of cachectic patients, bringing no evidence that circulating Myostatin plays a role in human cancer cachexia development.

Taken together, these observations suggest that ActA may be a key driver of cancer cachexia and in particular of the muscle-wasting process, which is directly correlated with survival. Therefore, given this broad spectrum of evidences, we postulated that high levels of circulating ActA may be associated with poor prognosis in cancer patients. A link between high circulating ActA and low survival has been previously highlighted in myeloma,[16] pancreatic,[17] and lung cancers.[18] The aim of this study was therefore to assess the prognostic value of ActA as a marker of survival in a population of colorectal and lung cancer patients. In addition, we investigated whether the prognostic value of ActA is dependent of the type or the stage of cancer and related to the skeletal muscle parameters such as muscle mass or muscle density.

Patients and methods

Patients

Overall survival (OS) of patients, treated for colorectal or lung cancer at the Cliniques Universitaires Saint-Luc in Brussels, Belgium, between January 2012 and March 2014 and enrolled in our ACTICA study,[15] were analysed since the day of the inclusion visit to 24 months later. Patients were recruited at the diagnosis or at relapse, before any therapeutic intervention. Progression-free survival (PFS) was also assessed in first diagnosis colorectal cancer patients, given the good prognostic of this cancer and therefore the low mortality rate, during 24 months evenly. The protocol of the study was approved by the local research Ethics Committee of the Université Catholique de Louvain. Written consent was given prior to entry into the study. Subjects were at least 18 years old, had an expected survival of more than 3 months, and no previous history of other cancer in the last 5 years. Exclusion criteria were as follows: non-caucasian subjects, obvious malabsorption, major depression, artificial nutrition, high doses of steroids ($>$1 mg/kg hydrocortisone equivalent), hyperthyroidism, other causes of malnutrition, major walking handicap, Eastern Cooperative Oncology Group (ECOG) performance status $\geq$4, and psychological, familial, social, or geographic conditions that would preclude participation in the full protocol.

Study measurements

Patient and tumour characteristics

Body weight and height were measured at the inclusion of the study. Body weight changes during the previous 6 months were calculated and expressed as percentage of pre-illness body weight, which was recalled by the patient and verified when possible from medical notes. Cachexia was defined, according to the definition proposed by Fearon *et al*., as an involuntary weight loss $>$5% over the past 6 months or a weight loss $>$2% associated with body mass index $<$20 kg/m^2 or low muscularity.[19] Staging of cancer was based on the TNM Classification of Malignant Tumours score and on the 7th Edition of American Joint Committee on Cancer.[20]

Nutritional and functional assessment

Anorexia was evaluated by the Simplified Nutritional Appetite Questionnaire (SNAQ) score and was defined by an SNAQ score $<$14.[21] The functional status was assessed by two previously validate scales, namely, ECOG and EORTC QoL questionnaire (QLQ-C30).[22]

Skeletal and fat mass measurement

Skeletal muscle and fat mass were assessed by abdomen CT scan, performed for standard cancer care between 3 months before and 1 month after the inclusion date and before any therapeutic intervention. The median of the time interval between the CT scan and the assessment was 21 (0–104) days. A transverse CT-scan image from the third lumbar vertebrae (L3) was analysed for each patient and tissue area estimated, using previously described Hounsfield unit (HU) thresholds and quantified by the SLICE-O-MATIC software,

version 4.3 (Tomovision, Montreal, Canada). The most common and accepted HU range for skeletal muscle tissue is −29 to +150 HU and for adipose tissue is −190 to −30 HU. Cross-sectional area for muscle and adipose tissues was normalized for stature and expressed as muscle and fat indexes (cm^2/m^2).[2,23] All CT-scan images were analysed by a single trained observer, who was blinded to patient's status. The intra-observer coefficient of variation was 2.2% for muscle index measurements. Low muscularity is defined by a muscle index <55 cm^2/m^2 for men and <39 cm^2/m^2 for women as proposed by Fearon *et al.*[19]

Skeletal muscle density measurement was based on the mean muscle radiation attenuation for the entire muscle area in HU. The generally accepted lower limit of SMD is 30 HU, a value defined as two standard definitions below the mean attenuation value of muscles of young healthy persons.[2,24]

Biomarkers

Blood samples were collected from patients at the time of recruitment, in standardized conditions. Total plasma ActA, Myostatin, and Follistatin (FS288, FS300, and FS315) were measured by solid-phase two-site enzyme linked immunoassays (R&D Systems) according to the recommendations of the manufacturer. Nutritional and inflammatory markers [albumin, pre-albumin, and C-reactive protein (CRP)] were determined by clinical routine methods in our Clinical Chemistry Department.

Statistical analysis

Comparisons between groups were performed using non-parametric Mann Whitney U-test for continuous parameters and by χ^2 test for categorical variables. Data are expressed as median (min–max).

The OS was defined by the time from inclusion until patient death from any cause, while PFS was defined by the time from the initiation of anti-cancer therapies until the first observation of disease progression, both during the follow-up of 24 months. OS and PFS were analysed using the Kaplan–Meyer method, and survival curves were compared using the log-rank test. We firstly analysed OS according to ActA quartiles. Then, the outcome-oriented approach of Contal and O'Quigley was used to determine the best ActA and Mstn cut-offs to separate patients based on the outcome (death).[25] This method is based on the log-rank statistic and provides *P*-values corrected for examining multiple potential cut-off points. The cut-off point was determined using an SAS macro provided by Mandrekar *et al.*[26]

Univariable and multivariable analysis for OS were achieved using the Cox proportional hazards model. Hazard ratios (HR) and 95% confidence interval (CI) were obtained. Variables examined by univariable analysis were firstly standard prognostic parameters such as patient age ($\geq$65 years compared with younger), tumour nature (lung cancer compared with colorectal cancer) and stage (stages I, III, and IV compared with stage II), ECOG scale (scores 1, 2, and 3 compared with score 0), and high plasma lactate deshydrogenase (LDH) levels (LDH $\geq$ 250 UI/L); secondly, skeletal muscle parameters such as presence of cachexia, low muscularity, and low SMD ($<$30 HU); and thirdly biological parameters such as high ActA ($\geq$ 408 pg/mL), low Mstn ($<$1599 pg/mL), and conventional nutritional and inflammatory markers such as low albumin levels ($<$3.5 g/dL) and CRP levels. Higher bound for inclusion of candidate variables in the multivariate model was set to 10%. Backward stepwise selection was used to select optimal multivariate model.

Statistical significance was set at $P < 0.05$. Analysis was performed using SAS software (version 9.4; SAS Institute Inc, Cary, NC, USA).

Results

Patient and tumour characteristics

Survival data at 24 months were available for 149 patients while three patients were lost for the follow-up. At the inclusion, the patient median age was 67 years with a predominance of male subjects (57%) (*Table* 1). A majority of patients presented colorectal (63%) or advanced cancer (stages III or IV) (65%). OS at 24 months was 71%. As expected, in deceased patients compared with alive patients, lung cancer was more prevalent (70% vs. 24%, $P < 0.0001$) as well as advanced cancer, based on stages III (39% vs. 24%, $P = 0.0007$) and IV (55% vs. 30%, $P = 0.0007$). At baseline, deceased patients had a lower body mass index (24 kg/m^2 vs. 25 kg/m^2, $P = 0.026$) and had lost more body weight (5.4% vs. 2.4%, $P = 0.019$) than alive patients. Moreover, deceased patients had more severe anorexia (SNAQ score: deceased vs. alive patients: $14^{6–19}$ vs. $16^{8–20}$; $P = 0.005$), more symptoms (QLQ-C30: deceased vs. alive patients: $33^{2–66}$ vs. $15^{6–69}$; $P < 0.0001$), a poorer quality of life (QLQ-C30: deceased vs. alive patients: $58^{16–100}$ vs. $66^{0–100}$; $P = 0.002$) and a lower functional capacity (QLQ-C30: deceased vs. alive patients: $64^{18–97}$ vs. $82^{33–100}$; $P < 0.0001$ and ECOG; $P < 0.0001$) than alive patients.

Low muscularity and skeletal muscle density are associated with poor prognosis in cancer patients

In comparison with alive patients, deceased patients had a higher prevalence of low muscularity (54% vs. 35%, $P = 0.041$) and exhibited a lower SMD (26.6 HU vs. 32.4 HU, $P = 0.017$) and fat index (81 cm^2/m^2 vs. 111 cm^2/m^2,

Table 1 Patient and tumour baseline characteristics

	Total	Alive	Deceased	*P*-value (deceased vs. alive)
n	149	106	43	
Age (years)	67 (25–95)	66 (25–90)	68 (40–95)	0.278
Sex ratio M/F (%)	57/43	59/41	54/45	0.576
Baseline BMI (kg/m^2)	25 (16–47)	25 (16–47)	24 (16–45)	0.026
Weight loss (%)	3.6 (0.0–25.0)	2.4 (0.0–25.0)	5.4 (0.0–21.1)	0.019
Cachexia (%)	48	44	58	0.127
Tumour nature				<0.0001
Colorectal (%)	63	76	30	
Lung (%)	37	24	70	
Type				0.300
New diagnosis (%)	82	84	77	
Relapse (%)	18	16	23	
Stage (*n* = 121)				0.0007
I/II/III/IV (%)	17/18/28/37	23/23/24/30	0/6/39/55	

M, male; F, female; BMI, body mass index.

P = 0.040) (*Table* 2). Low muscularity was associated with shorter OS in total population (OS for patients with low muscularity vs. normal muscularity: 60% vs. 77%, HR: 1.98, CI: 1.07–3.93, $P < 0.05$), particularly in lung cancer patients (OS: 24% vs. 55%, HR:2.32, CI: 1.12–5.77, $P < 0.05$) and in stage IV of lung cancer (OS: 0% vs. 50%, HR:4.09, CI: 1.49–21.80, $P < 0.05$), but not significantly in colorectal cancer (81% vs. 90%, HR: 2.17, CI: 0.70–7.10, *P* = 0.175) (*Figure* 1). Low SMD (<30 HU) was associated with shorter OS in the total population (OS for patients with low SMD vs. normal SMD: 62% vs. 76%, HR: 2.01, CI: 1.08–3.86, $P < 0.05$) and in colorectal cancer (OS: 78% vs. 92%, HR: 3.18, CI: 1.02–10.47, $P < 0.5$), but not in lung cancer. These analyses were also performed excluding patients (*n* = 16) with CT scan performed farthest from study inclusion date (6 to 12 weeks before inclusion). Exclusion of these patients did not impact our results (data not shown).

High levels of Activin A are associated with poor prognosis in cancer patients

In our previous study, ActA levels were positively correlated with weight loss ($R = 0.323$, $P < 0.001$) and negatively with SMD ($R = -0.225$, $P < 0.01$) that both appear to be linked to survival in our population.[15] Consistently with this observation, baseline circulating ActA concentrations were higher in deceased patients compared with alive patients (561 pg/mL vs. 414 pg/mL, *P* = 0.001), whereas Follistatin remained unchanged between the two groups (*Table* 3). The Kaplan–Meyer analysis of survival by ActA quartiles showed that higher is the level of ActA, lower is the prognosis in total population and particularly in lung cancer (*Figure* 2). There was no significant difference in survival according to quartiles of ActA in colorectal cancer patient, probably because of the lower number of events. There was no difference concerning ActA levels between lung and colorectal cancer patients [respectively, 457 pg/mL (228–2540) and 447 pg/mL (165–17660), *P* = 0.475].

The most discriminating value of ActA to predict survival in our population was 408 pg/mL. We observed that patients with high levels of ActA (≥408 pg/mL) had lower OS than those with levels below this threshold, regardless the type of cancer (OS in colorectal cancer: 65% vs. 85%, HR 4.43, CI: 1.10–9.76, $P < 0.05$ and in lung cancer: 27% vs. 67%, HR: 4.31, CI: 1.63–6.96, *P* = 0.001) (*Figure* 2). Moreover, in first diagnosis colorectal cancer group, patients with high levels of ActA had lower PFS than those with low levels (HR 4.52, CI: 1.72–7.85, $P < 0.001$) (*Figure* 3).

Conversely and consistent with our previous observations, Myostatin was lower in deceased patients (1458 pg/mL vs. 1912 pg/mL, *P* = 0.030) compared with alive patients (*Table* 3). The threshold value for Myostatin to predict survival was 1599 pg/Ml. Low levels of Myostatin (<1599 pg/mL) were associated with lower OS in total population (OS: 58% vs. 79%, HR: 2.17, CI: 1.18–3.97, $P < 0.05$), but the significance of this observation was not sustained when we analysed it for each type of cancer.

Table 2 Skeletal muscle and fat mass baseline measurements

	Total	Alive	Deceased	*P*-value (deceased vs. alive)
n	136	97	39	
Muscle index (cm^2/m^2)	49 (31–84)	49 (32–80)	48 (31–84)	0.249
Low muscularity (%)	40	35	54	0.041
SMD (HU)	31.5 (0.2–62.2)	32.4 (0.2–62.2)	26.6 (10.9–54.8)	0.017
Fat index (cm^2/m^2)	102 (1–311)	111 (1–291)	81 (8–311)	0.040

SMD, skeletal muscle density in Hounsfield units (HU).

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Figure 1 Kaplan–Meier survival curves according to muscularity (above) and skeletal muscle density (SMD) (below) for total population (*n* = 138), colorectal cancer (*n* = 87), and lung cancer (*n* = 51).

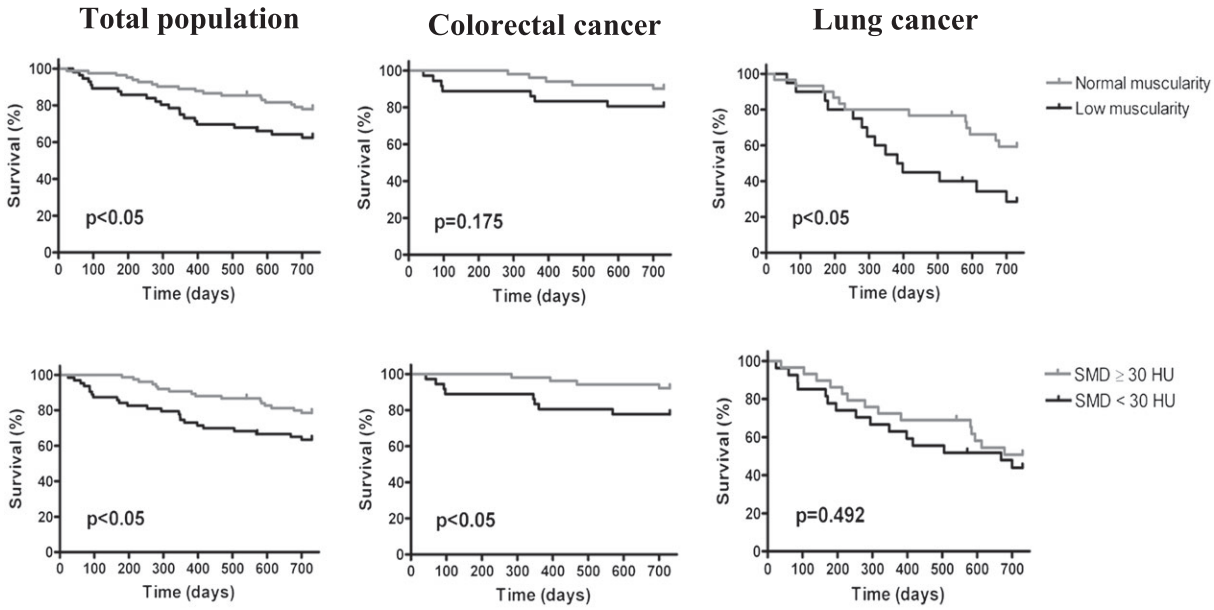


Table 3 Biomarkers

	Total	Alive	Deceased	*P*-value (deceased vs. alive)
n	149	106	43	
Activin A (pg/mL)	456 (165–17660)	414 (165–9402)	561 (282–17660)	0.001
Follistatin (pg/mL)	2167 (778–7534)	2100 (778–7534)	2399 (1175–7249)	0.064
Myostatin (pg/mL)	1802 (167–4989)	1912 (167–4989)	1458 (556–4458)	0.030
CRP (mg/dL)	0.38 (0.03–25.74)	0.23 (0.03–25.74)	1.60 (0.10–25.30)	<0.0001
Albumin (g/dL)	4.4 (2.8–5.1)	4.4 (2.8–5.1)	4.1 (3.0–5.0)	0.002
Pre-albumin (mg/dL)	20.8 (2.2–49.5)	21.7 (3.5–49.5)	18.8 (2.2–35.7)	0.034

CRP, C-reactive protein.

High levels of Activin A are associated with poor prognosis in cancer patients independently of tumour stage

High levels of Activin A were associated with advanced cancer. Indeed, levels of ActA were more elevated in patients with metastases compared with those without [622 pg/mL (235–17660) vs. 392 pg/mL (191–5024), $P < 0.0001$]. Moreover, patients with high ActA levels (≥408 pg/mL) had more frequently stage IV tumour than patients with ActA below this cut-off (49% vs. 17%, $P < 0.01$).

However, the multivariable analysis showed that high ActA was a prognostic factor of survival (HR: 4.07, CI: 1.44–11.52, $P = 0.008$) independently of tumour stage, and also independently of other prognostic variables, such SNAQ score, low SMD, and low Myostatin and CRP levels in total population (*Table* 4). Especially, high ActA was an independent prognostic factor in lung cancer (HR: 3.17, CI: 1.09–9.22, $P = 0.034$), but not in colorectal cancer (in univariable analysis HR: 4.44, CI: 0.98–20.01, $P = 0.053$). In lung cancer, high levels of ActA were associated with poor prognosis independently of the tumour stage, as illustrated in the sub-group of patients with advanced stage of disease (*Figure* 4).

Furthermore, as ActA levels, low muscularity appeared also as an independent poor prognostic factor (HR: 3.63, CI: 1.58–8.30, $P = 0.002$). Other expected prognostic factors were highlighted in our analyses, such as tumour nature (lung cancer), ECOG, and low albumin levels.

Discussion

Our study links high circulating ActA levels with poor prognosis in cancer patients independently of other

Figure 2 Kaplan–Meier survival curves according to plasma Activin A levels [quartiles and cut-off statistically determinated (408 pg/mL)] for total population (n = 152), colorectal cancer (n = 94), and lung cancer (n = 58).

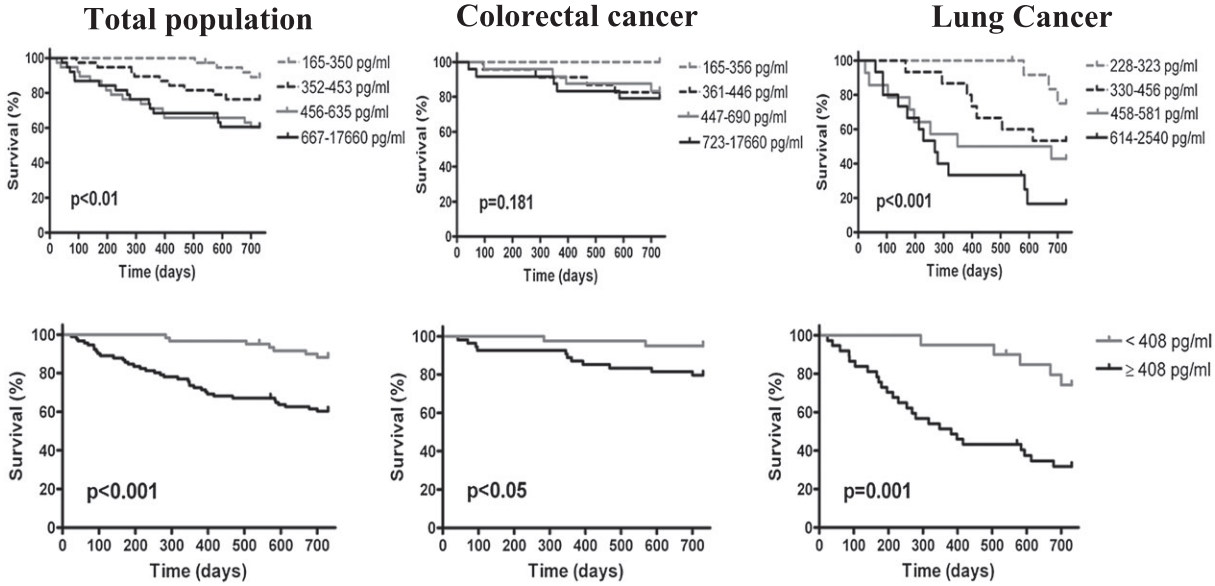
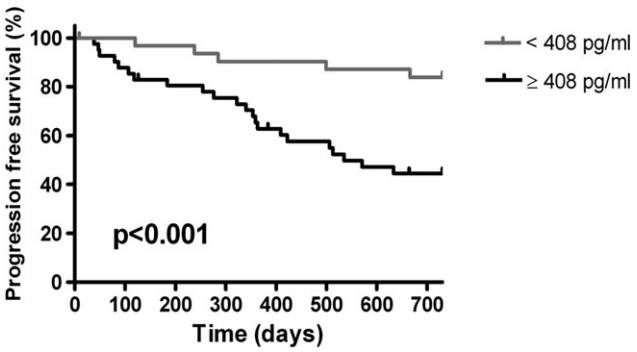


Figure 3 Kaplan–Meyer progression-free survival curves according to plasma Activin A levels in first diagnosis colorectal cancer (n = 73).



prognostic factors. Because we previously showed that circulating ActA levels are increased in cachectic patients, our current results support the view that ActA predicts poor survival by contributing to cancer cachexia, in particular muscle wasting, a well-established independent risk factor for poor survival in cancer patients.[3–5]

These results extend previous works that showed that high circulating ActA is associated with poor prognosis in myeloma,[16] in advanced pancreatic cancer,[17] and in lung adenocarcinoma.[18] However, we are the first to use statistical method to define the most discriminating cut-off value of ActA to predict survival. In the previous studies, the ActA cut-off value was defined respectively by the lowest quartile of ActA (422 pg/mL), the mean of ActA (800 pg/mL), and the median of ActA (value not given). Consistently with these data, our cut-off value for ActA (408 pg/mL) is close to the median of ActA levels in our total population and to the cut-off used by Terpos *et al.* in myeloma.[16] The higher ActA cut-off value, highlighted by Togashi *et al.*,[17] could be explained by the fact that their patients with unresecable pancreatic cancer were probably more cachectic than our patients. It would be therefore interesting to validate our cut-off value for ActA in other types of cancer, such as pancreatic cancer.

The prognostic value of ActA seems particularly robust in lung cancer compared with colorectal cancer, and it remains discriminating for prognosis even in the sub-group of patients with advanced stages of disease (stages III and IV). The prognostic value of ActA seems therefore variable depending on the nature of tumour, although the levels of ActA were not different between the two types of cancer. However, the absence of relevance of the ActA as a prognostic factor in multivariable analysis in colorectal tumour could be explain by the better prognosis of this type of cancer and the lower number of deaths during the 24-month follow-up. Nevertheless, high ActA levels were associated with shorter PFS in colorectal cancer.

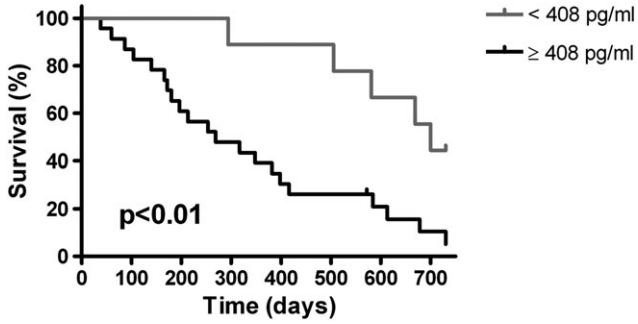
Several hypotheses may be proposed to explain why ActA levels are associated with poor prognosis in cancer patients. Firstly, the tumour expression of ActA, indirectly assessed by circulating levels, may reflect the severity or the extent of the cancer. The tumour expression of ActA and its role in carcinogenesis are variable depending of the tumour type.[27] In esophageal and head and neck carcinoma, high ActA expression seems to be linked to a more aggressive tumour. Indeed, high expression of ActA in these tumour tissues is

Table 4 Univariable and multivariable analysis for prognostic factors of overall survival

	Univariable		Multivariable	
Factors	Hazard ratio (95%CI)	*P*-value	Hazard ratio (95%CI)	*P*-value
Age ≥ 65 years	1.33 (0.72–2.45)	0.360		
Lung cancer[a]	5.03 (2.61–9.67)	<0.001	6.06 (2.45–15.04)	<0.001
Tumour stage[b]				
I	0.00 (0.00-NE)	0.255		
III	4.65 (1.05–20.62)	0.043		
IV	5.31 (1.23–22.9)	0.025		
ECOG[c]				
1	3.39 (1.68–6.81)	<0.001	1.48 (0.54–4.08)	0.450
2	8.30 (3.15–21.91)	<0.001	5.87 (1.78–19.41)	0.004
3	13.12 (4.21–40.84)	<0.001	3.37 (0.57–19.91)	0.180
SNAQ score	0.82 (0.74–0.91)	<0.001		
Cachexia	1.81 (0.88–3.74)	0.108		
Low muscularity	1.96 (1.04–3.68)	0.036	3.63 (1.58–8.30)	0.002
SMD < 30 HU	2.01 (1.06–3.81)	0.032		
Activin A ≥ 408 pg/mL	4.24 (1.89–9.53)	<0.001	4.07 (1.44–11.52)	0.008
Myostatin < 1599 pg/mL	2.16 (1.18–3.96)	0.013		
LDH ≥ 250 UI/L	1.15 (0.56–2.35)	0.699		
CRP (mg/dL)	1.06 (1.02–1.11)	0.008		
Albumin < 3.5 g/dL	4.50 (2.15–9.41)	<0.001	2.93 (1.17–7.34)	0.022

[a]Reference was colorectal cancer.
[b]Reference was tumour stage II.
[c]Reference was ECOG 0.
SMD, skeletal muscle density; LDH, lactate deshydrogenase; CRP, C-reactive protein; SNAQ, Simplified Nutritional Appetite Questionnaire.

Figure 4 Kaplan–Meyer survival curves according to plasma Activin A levels in stages III and IV lung cancer (*n* = 33).



correlated with tumour de-differentiation, metastasis, survival, and recurrence.[28,29] In lung adenocarcinoma, ActA is overexpressed in tumour tissue compared with normal lung tissue, and this overexpression of ActA is correlated with worse prognosis in stage I disease.[30] Moreover, *in vitro*, ActA induces lung cancer cell lines (H460 and SKLU1) proliferation.[30] In colorectal cancer, the ActA expression is increased in tumour tissue compared with normal colorectal tissue or benign polyp[31] and more markedly in stage IV tumour.[32] Additionally, the circulating levels of ActA are higher in patients with colorectal cancer compared with healthy controls and are correlated with disease stage.[31] These data pinpoint a link between ActA and the severity and/or the stage of the neoplastic disease in several types of cancer, suggesting a hypothesis to explain why ActA is a factor of poor prognosis in cancer patients. Nevertheless, we showed that in the total population, and especially in lung cancer, high ActA predicts poor prognosis independently of stage disease, suggesting that ActA influences the cancer prognosis by others mechanisms.

Secondly, circulating ActA level might be a marker of systemic inflammation associated with cancer. Effectively, ActA is a key player of the inflammatory process.[14] *In vitro*, human neutrophils release stored ActA in response to TNFα.[33] In animals, ActA increases quickly in circulation in response to acute inflammatory stimulus, such as an lipopolysaccharide (LPS) injection.[34] In humans, elevated ActA levels have been observed in several inflammatory diseases, such as septicemia[35] or acute distress syndrome,[36] and are associated with the severity of the disease and poor prognosis. Neoplastic process is associated with systemic inflammation, mediated by several cytokines such as IL-1β, IL-6, TNFα, and ActA.[37,38] High levels of inflammation, characterized by elevated CRP and low albumin levels are clearly associated with body weight loss and reduced survival in cancer patients.[37] Therefore, high levels of ActA could predict prognosis by reflecting the severity of tumour-induced inflammation. However, whereas rationally ActA levels are correlated with CRP,[15] the multivariable analysis does not highlight CRP as a prognostic factor unlike ActA. The value of ActA to predict survival seems accordingly more important that a basic inflammatory marker. Moreover, the prognostic value of ActA in the neoplastic disease seems more reproducible that others cytokines such as IL-6 or TNFα, for which the link between circulating levels and clinical outcome is not clear.[38–40] In addition, clinical trials failed to highlight

a clear benefit of anti-IL-6 or anti-TNFα treatments on lean muscle mass, suggesting that blunting these pro-inflammatory cytokines is not sufficient to enhance muscle mass in cancer patients.[38] Therefore, the prognostic value of ActA seems to go beyond its role in the inflammatory process.

Thirdly, ActA might play a direct role in the development of muscle atrophy, a crucial hallmark of cancer cachexia. Even in the absence of underlying disease, ActA exerts a direct atrophying effect on muscle in animal studies.[8,9] Therefore, high circulating ActA levels may contribute to the muscle-wasting process leading to low muscularity, a well-established prognostic factor in cancer.[3–5] In addition, in an animal model of cancer cachexia, ActA inhibition reverses the cachexia phenotype together with an increased survival, without any effect on tumour mass.[8] Moreover, we highlighted previously that cachexia is associated with high levels of ActA.[15] These data suggest that high levels of ActA may affect survival by decreasing skeletal muscle mass or quality associated with loss of functional capacity. Supporting this hypothesis, we observed that both low muscularity and ActA are significantly associated with poor prognosis in our patients, particularly those with lung cancer. In other hand, ActA levels were negatively correlated with SMD,[15] a marker of muscle quality, which was also associated with poor prognosis in our population. Interestingly, excepted nature tumour, all other parameters highlighted as prognostic factors, such as low albumin levels and decrease of functional capacity, are linked to skeletal muscle loss. Nonetheless, we could not find any correlation between muscle mass and ActA levels. This may be due to several factors. Foremost, in contrast to weight loss, skeletal muscle mass measurement is a static parameter, which does not allow to properly appreciate muscle mass changes over time. In addition, muscle mass is extremely variable across the general population. Hence, a normal muscularity at baseline does not exclude a previous loss of muscle mass. Finally, in addition to ActA, other processes like immobilization or anorexia could also contribute to the loss of muscle mass.

Given that future studies will be necessary to validate the prognostic value of ActA in cancer patients and its link with skeletal muscle mass loss and cachexia. This hypothesis is particularly attractive, because inhibitors of ActA are currently under clinical investigation.

In contrast to ActA, low circulating Myostatin levels were associated with poor prognosis in our total population. This observation is consistent with our previous study, showing an association between cancer cachexia and low circulating Myostatin.[15] Although Myostatin is doubtless an inhibitor of skeletal muscle mass development, its role in muscle atrophy remains unclear. Indeed, in contrast to early observations highlighting a link between elevated levels of Mstn and low muscularity situations,[41–43] recent studies showed a decrease of circulating Myostatin in conditions characterized by reduced skeletal muscle mass such as ageing, but without correlation between Myostatin levels and muscle mass.[44–46] In agreement with our results, Breitbart *et al*. observed in cancer patients with body weight loss a decrease of circulating Myostatin propeptide levels, reflecting a decrease of Myostatin production.[47] The lack of consistency between these studies might be explained by the use of various immunoassays detecting different forms of Myostatin and a possible cross-reactivity with other TGF-β members. We used for this study a recently developed commercial ELISA assays (R&D) without cross-reactivity with others TGF-β members, such as GDF-11 and GDF-15. Other hypotheses could nuance the interpretation of the value and the pathophysiological role of circulating Myostatin. We measured total Myostatin concentrations and not only the biologically active form. Moreover, Myostatin, which is mainly produced by skeletal muscle, has perhaps rather an autocrine and paracrine than an endocrine action on skeletal muscle. Therefore, the circulating concentrations of Mstn might perhaps not reflect its amount and activity at the muscle level.

The limitation of this study is the length of the follow-up, particularly for the colorectal cancer. Indeed, the prognosis of colorectal cancer (65% of surviving patients after 5 years) is much better compared with lung cancer (18% of surviving patients after 5 years).[48] Therefore, we list sparsely death events in the colorectal cancer group. A longer follow-up may therefore be necessary to decipher the prognostic value of ActA in colorectal cancer patients. But, in this population, the PFS analysis strengthens the prognostic value of ActA. Furthermore, although the OS analysis in the subgroups was limited by the small number of patients in each of them, the prognostic value of ActA remains significant, particularly in lung cancer, suggesting the robustness of this observation. The strength of the study is its prospective nature, which allowed a complete and strong characterization of each patient, with few missing data, especially concerning the skeletal muscle parameters and biomarkers measurements. The clinical evaluation, functional, and nutritional assessment scales were achieved by one single experienced investigator. Skeletal muscle parameters were analysed on CT scan by a single trained observer, who was blinded to patient's status and were interpreted according to well-established definitions. Finally, plasma samples were collected under standardized conditions, and ActA was measured with a robust ELISA kit (R&D Systems), for which no interference with Follistatin has been demonstrated.[18]

Our study demonstrated that high ActA levels are associated with poor prognosis in cancer patients independently of other prognostic factors. More than a basic marker of the severity of the neoplastic disease or of the inflammatory process, ActA seems to influence survival by contributing to the development of cachexia and especially loss of skeletal muscle mass.

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Conflict of interest

The authors have nothing to disclose.

References

1. Fearon KC, Glass DJ, Guttridge DC. Cancer cachexia: mediators, signaling, and metabolic pathways. *Cell Metab* 2012;**16**:153–166.
2. Aubrey J, Esfandiari N, Baracos VE, Buteau FA, Frenette J, Putman CT, et al. Measurement of skeletal muscle radiation attenuation and basis of its biological variation. *Acta Physiol (Oxf)* 2014;**210**:489–497.
3. Prado CM, Lieffers JR, McCargar LJ, Reiman T, Sawyer MB, Martin L, et al. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. *Lancet Oncol* 2008;**9**:629–635.
4. Martin L, Birdsell L, Macdonald N, Reiman T, Clandinin MT, McCargar LJ, et al. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. *J Clin Oncol* 2013;**31**:1539–1547.
5. Kazemi-Bajestani SM, Mazurak VC, Baracos V. Computed tomography-defined muscle and fat wasting are associated with cancer clinical outcomes. *Semin Cell Dev Biol* 2016;**54**:2–10.
6. Sjoblom B, Gronberg BH, Wentzel-Larsen T, Baracos VE, Hjermstad MJ, Aass N, et al. Skeletal muscle radiodensity is prognostic for survival in patients with advanced non-small cell lung cancer. *Clin Nutr* 2016;**35**:1386–1393.
7. Xia Y, Schneyer AL. The biology of activin: recent advances in structure, regulation and function. *J Endocrinol* 2009;**202**:1–12.
8. 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**:531–543.
9. Chen JL, Walton KL, Winbanks CE, Murphy KT, Thomson RE, Makanji Y, et al. Elevated expression of activins promotes muscle wasting and cachexia. *FASEB J* 2014;**28**:1711–1723.
10. Gilson H, Schakman O, Kalista S, Lause P, Tsuchida K, Thissen JP. Follistatin induces muscle hypertrophy through satellite cell proliferation and inhibition of both myostatin and activin. *Am J Physiol Endocrinol Metab* 2009;**297**:E157–E164.
11. McPherron AC. Metabolic Functions of Myostatin and Gdf11. *Immunol Endocr Metab Agents Med Chem* 2010;**10**:217–231.
12. Matsuyama T, Ishikawa T, Okayama T, Oka K, Adachi S, Mizushima K, et al. Tumor inoculation site affects the development of cancer cachexia and muscle wasting. *Int J Cancer* 2015;**137**:2558–2565.
13. Harada K, Shintani Y, Sakamoto Y, Wakatsuki M, Shitsukawa K, Saito S. Serum immunoreactive activin A levels in normal subjects and patients with various diseases. *J Clin Endocrinol Metab* 1996;**81**:2125–2130.
14. Hedger MP, de Kretser DM. The activins and their binding protein, follistatin-diagnostic and therapeutic targets in inflammatory disease and fibrosis. *Cytokine Growth Factor Rev* 2013;**24**:285–295.
15. Loumaye A, de Barsy M, Nachit M, Lause P, Frateur L, van Maanen A, et al. Role of Activin A and myostatin in human cancer cachexia. *J Clin Endocrinol Metab* 2015;**100**:2030–2038.
16. Terpos E, Kastritis E, Christoulas D, Gkotzamanidou M, Eleutherakis-Papaiakovou E, Kanellias N, et al. Circulating Activin-A is elevated in patients with advanced multiple myeloma and correlates with extensive bone involvement and inferior survival; no alterations post-lenalidomide and dexamethasone therapy. *Ann Oncol* 2012;**23**:2681–2686.
17. Togashi Y, Kogita A, Sakamoto H, Hayashi H, Terashima M, de Velasco MA, et al. Activin signal promotes cancer progression and is involved in cachexia in a subset of pancreatic cancer. *Cancer Lett* 2015;**356**:819–827.
18. Hoda MA, Rozsas A, Lang E, Klikovits T, Lohinai Z, Torok S, et al. High circulating Activin A level is associated with tumor progression and predicts poor prognosis in lung adenocarcinoma. *Oncotarget* 2016;**7**:13388–13399.
19. Fearon K, Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL. Definition and classification of cancer cachexia: an international consensus. *Lancet Oncol* 2011;**12**:489–495.
20. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010;**17**:1471–1474.
21. Wilson MM, Thomas DR, Rubenstein LZ, Chibnall JT, Anderson S, Baxi A, et al. Appetite assessment: simple appetite questionnaire predicts weight loss in community-dwelling adults and nursing home residents. *Am J Clin Nutr* 2005;**82**:1074–1081.
22. Minton O, Stone P. A systematic review of the scales used for the measurement of cancer-related fatigue (CRF). *Ann Oncol* 2009;**20**:17–25.
23. Mourtzakis M, Prado CM, Lieffers JR, Reiman T, McCargar LJ, Baracos VE, et al. A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. *Appl Physiol Nutr Metab* 2008;**33**:997–1006.
24. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *J Appl Physiol (1985)* 2000;**89**:104–110.
25. Contal C, O'Quigley J. An application of changepoint methods in studying the effect of age on survival in breast cancer. *Comput Statist Data Anal* 1999;253–270.
26. Mandrekar JN, Mandrekar SJ, Cha SS. Cutpoint determination methods in survival analysis using SAS®. SAS® users group international proceedings 28 2003.
27. Loomans HA, Andl CD. Intertwining of Activin A and TGFbeta signaling: dual roles in cancer progression and cancer cell invasion. *Cancers (Basel)* 2014;**7**:70–91.
28. Wang Z, Zhang N, Song R, Fan R, Yang L, Wu L, et al. Activin A expression in esophageal carcinoma and its association with tumor aggressiveness and differentiation. *Oncol Lett* 2015;**10**:143–148.
29. Kelner N, Rodrigues PC, Bufalino A, Fonseca FP, Santos-Silva AR, Miguel MC, et al. Activin A immunoexpression as predictor of occult lymph node metastasis and overall survival in oral tongue squamous cell carcinoma. *Head Neck* 2015;**37**:479–486.
30. Seder CW, Hartojo W, Lin L, Silvers AL, Wang Z, Thomas DG, et al. Upregulated

INHBA expression may promote cell proliferation and is associated with poor survival in lung adenocarcinoma. *Neoplasia* 2009;**11**:388–396.
31. Wu S, Qi Y, Niu LM, Xie DX, Cui XL, Liu ZH. Activin A as a novel biomarker for colorectal adenocarcinoma in humans. *Eur Rev Med Pharmacol Sci* 2015;**19**: 4371–4378.
32. Wildi S, Kleeff J, Maruyama H, Maurer CA, Buchler MW, Korc M. Overexpression of activin A in stage IV colorectal cancer. *Gut* 2001;**49**:409–417.
33. Chen Y, Wu H, Winnall WR, Loveland KL, Makanji Y, Phillips DJ, et al. Tumour necrosis factor-alpha stimulates human neutrophils to release preformed activin A. *Immunol Cell Biol* 2011;**89**:889–896.
34. Jones KL, Mansell A, Patella S, Scott BJ, Hedger MP, de Kretser DM, et al. Activin A is a critical component of the inflammatory response, and its binding protein, follistatin, reduces mortality in endotoxemia. *Proc Natl Acad Sci U S A* 2007;**104**:16239–16244.
35. Michel U, Ebert S, Phillips D, Nau R. Serum concentrations of activin and follistatin are elevated and run in parallel in patients with septicemia. *Eur J Endocrinol* 2003; **148**:559–564.
36. de Kretser DM, Bensley JG, Pettila V, Linko R, Hedger MP, Hayward S, et al. Serum activin A and B levels predict outcome in patients with acute respiratory failure: a prospective cohort study. *Crit Care* 2013;**17**:R263.
37. Tsoli M, Robertson G. Cancer cachexia: malignant inflammation, tumorkines, and metabolic mayhem. *Trends Endocrinol Metab* 2013;**24**:174–183.
38. Cohen S, Nathan JA, Goldberg AL. Muscle wasting in disease: molecular mechanisms and promising therapies. *Nat Rev Drug Discov* 2015;**14**:58–74.
39. Scheede-Bergdahl C, Watt HL, Trutschnigg B, Kilgour RD, Haggarty A, Lucar E, Vigano A. Is IL-6 the best pro-inflammatory biomarker of clinical outcomes of cancer cachexia? *Clin Nutr* 2012;**31**:85–88.
40. Suh SY, Choi YS, Yeom CH, Kwak SM, Yoon HM, Kim DG, et al. Interleukin-6 but not tumour necrosis factor-alpha predicts survival in patients with advanced cancer. *Support Care Cancer* 2013;**21**:3071–3077.
41. Yarasheski KE, Bhasin S, Sinha-Hikim I, Pak-Loduca J, Gonzalez-Cadavid NF. Serum myostatin-immunoreactive protein is increased in 60–92 year old women and men with muscle wasting. *J Nutr Health Aging* 2002;**6**:343–348.
42. Gonzalez-Cadavid NF, Taylor WE, Yarasheski K, Sinha-Hikim I, Ma K, Ezzat S, et al. Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. *Proc Natl Acad Sci U S A* 1998;**95**:14938–14943.
43. Breitbart A, Auger-Messier M, Molkentin JD, Heineke J. Myostatin from the heart: local and systemic actions in cardiac failure and muscle wasting. *Am J Physiol Heart Circ Physiol* 2011;**300**: H1973–H1982.
44. Lakshman KM, Bhasin S, Corcoran C, Collins-Racie LA, Tchistiakova L, Forlow SB, et al. Measurement of myostatin concentrations in human serum: circulating concentrations in young and older men and effects of testosterone administration. *Mol Cell Endocrinol* 2009;**302**:26–32.
45. Ratkevicius A, Joyson A, Selmer I, Dhanani T, Grierson C, Tommasi AM, et al. Serum concentrations of myostatin and myostatin-interacting proteins do not differ between young and sarcopenic elderly men. *J Gerontol A Biol Sci Med Sci* 2011;**66**:620–626.
46. Szulc P, Hofbauer LC, Rauner M, Goettsch C, Chapurlat R, Schoppet M. Serum myostatin levels are negatively associated with abdominal aortic calcification in older men: the STRAMBO study. *Eur J Endocrinol* 2012;**167**:873–880.
47. Breitbart A, Scharf GM, Duncker D, Widera C, Gottlieb J, Vogel A, et al. Highly specific detection of myostatin prodomain by an immunoradiometric sandwich assay in serum of healthy individuals and patients. *PLoS One* 2013;**8**:e80454.
48. Online document. National Cancer Institute at the National Institutes of Health. http://www.cancer.gov/types. Accessed 13 June 2016.
49. von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2015. *J Cachexia Sarcopenia Muscle* 2015;**6**:315–316.