Cholangiocarcinoma is an uncommon cancer that is associated with a dismal prognosis and significant weight loss and muscle wasting (cancer cachexia; Cooperman et al, 2000; Mosconi et al, 2009). A hallmark of this disease is elevated serum interleukin-6 (IL-6; Goydos et al, 1998) levels, a proinflammatory cytokine that also elicits protein catabolism in skeletal muscle (Bruce and Dyck, 2004). Muscle wasting is a defining feature of cancer cachexia and has major impacts on physical and respiratory function, immunity, chemotherapy response and overall survival (MacDonald et al, 2003; Prado et al, 2008, 2009; Saini et al, 2009; Dodson et al, 2011). Owing to the importance of muscle mass in physiological function and association between muscle loss and outcomes of cancer, alternations in muscle mass as a side effect of anticancer agents is of growing interest. Intracellular signals involved in skeletal muscle anabolism and catabolism have been elucidated. PI3K, AKT and mTOR are central to activating muscle protein synthesis by amino acids (Bodine et al, 2001; Edinger and Thompson, 2002; Saini et al, 2006; Durham et al, 2009). Induction of muscle anabolism by physical activity occurs by pathways involving RAF, MEK and MAPK/ERK kinases (Bodine et al, 2001; Fearon et al, 2011). Cancer therapies directed at these targets would be expected to provoke muscle wasting and this was shown for sorafenib (Antoun et al, 2010). By contrast, some mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK) inhibitors in the development for cancer therapy are anti-inflammatory. Selumetinib (AZD6244, ARRY-142886; AstraZeneca, Manchester, UK), an allosteric inhibitor of MEK1 and MEK2 phosphorylation of ERK (Bekaii-Saab et al, 2011), has tumour suppressive activity in preclinical models (Revill et al, 2006) and has been proven to inhibit IL-6 production (Tai et al, 2007). As pro-inflammatory cytokines promote muscle protein catabolism (Zaki et al, 2004; Argiles et al, 2009; Murphy and Lynch, 2009), and IL-6 is considered one of the principal catabolic actors in skeletal muscle (Bruce and Dyck, 2004), such agents may mitigate muscle wasting.

In our recent phase II trial of selumetinib (Bekaii-Saab et al, 2011), patients receiving selumetinib experienced an average of 3.9 kg confirmed nonfluid weight gain. Considering the observed weight gain of patients in our phase II study, we investigated muscle and/or fat tissue gain using computed tomography (CT) as described below. The comparator group included patients with advanced cholangiocarcinoma who received standard therapies.

**MATERIALS AND METHODS**

Studies were approved by Research Ethics Boards of Ohio State University and Alberta Cancer Board.
Selumetinib treatment group

Patients with advanced cholangiocarcinoma participated in a phase II study of selumetinib (100 mg PO b.i.d.; Bekaii-Saab et al, 2011). The formulation was selumetinib-free base in a liquid vehicle Captisol (sulpha-butyl-ethyl B-cyclodextrin). Study inclusion and exclusion criteria have previously been published (Bekaii-Saab et al, 2011).

Standard therapy group

The Cross Cancer Institute is the only cancer centre serving northern Alberta, Canada (population: 1 800 000). A database of all cases (Alberta Cancer Registry) codes primary cancers by site, morphology, clinical and demographic information. For this study, all invasive cholangiocarcinoma cases diagnosed between 1997 and 2007 and included in the Cancer Registry were identified (ICD-10 MO codes: 8140/3, 8141/3, 8160/3, 8162/3, 8180/3) and these were included if they had been evaluated by CT at diagnosis and at least once after starting treatment.

No patients in either group were prescribed anabolic interventions for anorexia-cachexia syndrome (e.g., megestrol acetate, oxandrolone or corticosteroids).

Body composition measurements

Digitally stored CT scans were analysed using Slice-O-Matic software V4.2 (Tomovision, Montreal, Canada). The directly determined measure was cm² of total skeletal muscle and total adipose tissue at the third lumbar vertebra (L3), a bony landmark previously validated (Mourtzakis et al, 2008) and utilised (Prado et al, 2007, 2008, 2009) in studies of cancer patients. The precision error of measurements is ~1.5% (Mourtzakis et al, 2008) with a minimum detectable change of approximately 3 cm². Changes in muscle or adipose tissue are reported as mean cm² (s.d.) lost or gained over time and also divided into three categories: (A) loss ≥6.0 cm², (B) stable ±5.9 cm² or (C) gain ≥6.0 cm² of muscle. These cutoffs are equivalent to loss/gain of ≥1 kg of skeletal muscle on a whole-body basis (Shen et al, 2004), which are of sufficient magnitude to associate with alterations in muscle strength (Frontera et al, 1988). For adipose tissue, categories were based on the equivalence of 14.7 cm² total fat at L3 and 1 kg tissue on a whole-body basis (Shen et al, 2004).

Statistics

Data are expressed as mean ± s.d. or median/s.e. for continuous variables. Comparisons for categorical variables were conducted using test of proportions, while Student’s t-test was used for continuous variables. Kaplan–Meier curves and log-rank tests were used to compare study groups in relation to survival. Analysis was conducted using SPSS software version 18.0 (SPSS, Chicago, IL, USA). All P-values were two-sided and levels of significance were P<0.05.

RESULTS

Demographics of study participants are described in Table 1. A total of 20 patients from the selumetinib phase II study had images that included the third lumbar vertebra. Patients with cholangiocarcinoma receiving standard treatment (n = 30) received the following treatments for either first- or second-line therapy: carboplatin, paclitaxel, etoposide (n = 4), gemcitabine with or without capcetabine (n = 6), epirubicin, carboplatin, capcetabine (n = 4), and radiation (n = 7). Nine patients received best supportive care.

The mean interval between scans was 91.5 days for selumetinib-treated patients and 85.5 days for cholangiocarcinoma patients. To account for variation in the exact duration of scan intervals, changes in tissue areas are expressed as: (cm² lost or gained/number of days between scans) × 100.

Overall, selumetinib-treated cholangiocarcinoma patients gained skeletal muscle, in contrast to those receiving standard therapy, who were markedly catabolic (Table 1, Figure 1); 84.2% of patients gained muscle after initiating selumetinib, compared with 16.7% of patients who were on standard treatment (P<0.001, Figure 1). Selumetinib-treated patients muscle cross-sectional area increased by +13.8 (11.9)cm²/100 days compared with a loss of −7.3 (14.3)cm²/100 days for non-selumetinib-treated patients (P<0.001; Table 1). This translates to approximately +2.3 vs −1.2 kg of skeletal muscle on a whole-body basis, respectively. Tissue gains noted for selumetinib-treated patients were restricted to skeletal muscle (Table 1). Adipose tissue was lost in both groups. There were no

| Table 1 | Patient characteristics and muscle and adipose tissue response to cholangiocarcinoma therapy |
|----------------|---------------------------------|
|               | Phase II study selumetinib therapy group | Cholangiocarcinoma standard therapy group |
| Patients, n   | 20                               | 30                              |
| Gender, male, % | 30                               | 56.7                            |
| Stage         | 100% Stage IV                     | 100% Stage IV                   |
| Scan interval, days median (s.e.) | 91.5 (6.7)                      | 85.5 (5.1)                     |
| Time to death, days median (s.e.) | 295 (4.5)a                      | 277 (56.1)a                    |
| Age, mean ± s.d. | 54.5 ± 14.4                     | 58.6 ± 12.2                    |
| Body mass index, kg m⁻², mean ± s.e. | 31.2 ± 9.4                      | 25.9 ± 5.0                     |
| Muscle change/100 days cm² | Mean (s.d.) | −11.7 (9.9)a | −7.3 (14.3)b |
| Estimated kg | 2.3                               | 1.2                             |
| Adipose tissue change/100 days cm² | Mean (s.d.) | −97.2 (143.2)a | −56.2 (85.4)a |
| Estimated kg | −6.6                              | −3.8                            |

Figure 1  Percentage of patients exhibiting loss >1 kg, no change and gain >1 kg of skeletal muscle after initiation of selumetinib therapy or standard therapy. P-value calculated using test of proportions.

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observed differences in muscle or adipose tissue changes (gain, stable or loss) between men and women in the standard therapy group vs the selumetinib group (P = 0.478 for muscle change and P = 0.557 for adipose tissue change).

Survival of the two groups is illustrated in Figure 2a. Median time to death was not different between selumetinib vs standard therapy (Table 1, Figure 2A). Because the likelihood of muscle loss increases as death approaches (Liefers et al, 2009), the selumetinib and standard therapy patients were further compared after stratification by time to death (Figure 2B). Regardless whether patients were started on selumetinib within 150 days of death or earlier, the selumetinib-treated patients showed significant gain of skeletal muscle compared with the standard care group.

DISCUSSION

Cholangiocarcinoma is one of the most lethal cancers and is typically associated with cachexia. We show that selumetinib, an agent that holds promising activity in cholangiocarcinoma (Bekaii-Saab et al, 2011), induces rapid and significant skeletal muscle gain. Muscle gain is unanticipated in advanced biliary cancer and was not observed in our comparator group of cholangiocarcinoma patients on standard therapy.

Selumetinib may have direct or indirect action on muscle. Muscle contains both MEK 1 and 2, which are involved in the promotion of myogenic differentiation (Jo et al, 2011). Selumetinib has also been shown to inhibit secretion of cytokines such as IL-6 (Tai et al, 2007), IL-1β and tumour necrosis factor-α, which are implicated in the promotion of cancer cachexia (Zhang et al, 2007, 2008). While the mechanism of action for this anabolic reaction for selumetinib remains unproven, it seems likely that the observed increase in muscle is related to inhibition of cytokine secretion, as inhibition of MEK1/2 would be expected to actually inhibit muscle growth. We previously showed that another tyrosine kinase inhibitor, sorafenib, for example, provokes muscle loss in a randomised, placebo-controlled study (Antoun et al, 2010). In contrast, our current results indicate that the weight gain associated with selumetinib treatment (Bekaii-Saab et al, 2011) is related to increased muscle mass.

Neither patients treated with selumetinib nor those treated standard care group gained adipose tissue. This is consistent with published data demonstrating that muscle and fat are not necessarily gained or lost in concert (Prado et al, 2008). Additionally, a recent international consensus definition of cancer cachexia has characterised cachexia by muscle loss occurring with or without the loss of adipose tissue (Fearon et al, 2011).

A limitation of this work is the lack of a placebo-controlled design. Nonetheless, our results are interesting and indicate a finding consistent across the study that has not been previously described with other biologic or chemotherapeutic agents in various cancers, including cholangiocarcinoma where cachexia is one of the major causes of morbidity and mortality. Our results add to the evidence suggesting that selumetinib is a particularly promising compound in patients with biliary cancer, as previously published (Bekaii-Saab et al, 2011).

These potential benefits for muscle function or other outcomes of selumetinib and potentially of other MEK inhibitors remain to be tested in randomised trials. Future randomised trials with this group of agents should include prospective assessment of inflammatory markers such as IL-6 and other cytokines implicated in cachexia, as well as outcomes that may reveal benefits of skeletal muscle gain. It would be of interest to continue evaluating new targeted cancer therapies for potential actions on muscle. A potential survival benefit of cachexia therapy was raised by the study of Zhou et al (2010), who showed that blocking muscle wasting by antagonism of the action of myostatin can have significant beneficial effects on survival in an animal model of cachexia. This result is currently being tested in a randomised phase II trial in pancreatic cancer (Eli Lilly and Company, 2012).

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