Effect of adenosine 5′-triphosphate infusions on the nutritional status and survival of preterminal cancer patients

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The aim of the study was to investigate the effect of intravenous infusions of adenosine 5′-triphosphate (ATP) on nutritional status and survival in preterminal cancer patients. Ninety-nine preterminal cancer patients (estimated life expectancy 1–6 months) with mixed tumor types were randomly allocated to receive either intravenous ATP weekly (8–10 h/week, maximum 50 μg/kg/min) for 8 weeks, or no ATP (control group). Nutritional status parameters were assessed until 8 weeks, and analyzed by repeated-measures analysis of covariance. Cox proportional hazards models were fitted to assess the effect of ATP on short-term (0–8 weeks) and long-term (0–6 months) survival. Fifty-one patients were randomized to ATP and 48 to the control group. Results showed a significant favorable effect of ATP on triceps skin fold thickness [between-group difference per 8 weeks 1.76 mm, 95% confidence interval (CI): 0.48–3.12 mm; *P*=0.009] and on short-term survival [0–8 weeks hazard ratio (HR): 0.40, 95% CI: 0.17–0.95; *P*=0.037]. In weight-stable patients and in lung cancer patients, long-term survival (0–6 months) was also significantly better in ATP-treated patients (weight-stable patients HR: 0.40, 95% CI: 0.19–0.83; *P*=0.014; patients with lung cancer: HR: 0.35, 95% CI: 0.14–0.88; *P*=0.025). In conclusion, in this population of preterminal cancer patients, ATP infusions, at the dose and schedule studied, had a favorable effect on triceps skin fold thickness and survival, especially in weight-stable patients and patients with lung cancer. Larger studies are warranted to confirm these findings and to further define the effect of ATP on tumor growth and survival. *Anti-Cancer Drugs* 20:625–633 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Cachexia is one of the most common and devastating symptoms in patients with advanced cancer ranging from 24% early at the diagnosis of advanced cancer to more than 80% at the terminal stages [1]. The presence of weight loss is associated with decreased survival [2,3], and in some cases, excessive weight loss may itself be the cause of death [4]. Cytokines are thought to play a pivotal role in the development and progression of cancer cachexia [5–7]. So far, neither nutritional supplementation [1,8] nor pharmacological interventions [6,7,9] have been able to reverse cachexia. Research on novel approaches to counteract cancer cachexia, therefore, remains highly relevant.

A potential agent in the treatment of cancer cachexia is adenosine 5′-triphosphate (ATP). A randomized clinical trial (RCT) in patients with non-small-cell lung cancer (NSCLC) [10,11] showed that ATP infusions, given over 30 h at 2- to 4-week intervals (infusion rate maximum 75 μg/kg/min), had marked beneficial effects on nutritional intake, body weight, fat, and muscle mass. ATP treatment was also associated with a highly significant survival benefit in a subgroup of patients with stage IIIB NSCLC [12]. Moreover, ATP prevented the progressive increase in plasma concentrations of C-reactive protein and the decrease in albumin which were seen in the control group [10], providing a possible mechanism underlying the favorable effects of ATP on the nutritional status in these patients. The anti-inflammatory properties of ATP were recently confirmed by Swennen *et al.* [13,14] who, using an ex-vivo model of stimulated whole blood, demonstrated that ATP inhibited TNF-α and enhanced IL-10 release [13] through stimulation of the $P2Y_{11}$ and $P2Y_{12}$ receptor, respectively [14].

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Based on these promising results, we initiated a RCT to investigate the effects of ATP on the nutritional status and survival in patients with different types of cancer in the preterminal stage, defined as a life expectancy less than 6 months.

Patients and methods

Patients

From March 2002 to October 2006, 100 patients were included in the study. The Departments of Medical Oncology and Pulmonology in five centers and 50 general practitioners in the southern half of The Netherlands participated in patient recruitment.

Patients were eligible if they had histologically or cytologically proven cancer, without curative treatment options, with an estimated life expectancy of 1–6 months, a World Health Organization (WHO) performance status 1 or 2, suffering from at least one of the following complaints: fatigue, weight loss more than 5% over the previous 6 months, or anorexia. Patients with symptomatic angina pectoris, symptomatic heart failure or any form of atrioventricular block (assessed by electrocardiography), as well as patients with cognitive dysfunction, or concurrent palliative chemotherapy were excluded. The study was approved by the ethics committees of all hospitals involved in the study, and all patients signed written informed consent before the study.

Study design

After baseline measurements, patients were stratified for region, tumor type, and weight loss (≤ 5 vs. $> 5\%$ over the last 6 months), and then randomly assigned to receive either usual care, standard nutritional advice and ATP (ATP group), or usual care and standard nutritional advice alone (control group), using computer-generated random numbers with permutation blocks of 4. Outcome parameters were assessed until 8 weeks.

Intervention

Patients allocated to the ATP group received weekly 8–10 h ATP infusions over a period of 8 weeks [15]. The first ATP infusion was offered under medical supervision at the day care center of the participating hospitals. ATP infusions were started at a rate of 20 μg/kg/min and increased in steps of 10 μg/kg/min per 10 min, until reaching a maximum rate of 50 μg/kg/min, or the maximally tolerated dose if this was lower, in case of side effects. Thereafter, ATP was infused at a continuous rate. Subsequent infusions were given at the maximally tolerated dose in the patient's home.

Outcome assessment

Body weight was measured without shoes, using an electronic scale (Soehnle 7407 Translucia, Soehnle Professional GmbH & Co. KG, Backnang, Germany). Triceps skin fold thickness was measured in triplicate at the dominant arm, with a Holtain skin fold caliper (CMS weighing equipment LTD, London UK), and the median used for further calculations. Mid-upper arm circumference was measured in duplicate at the dominant side with a flexible measuring tape, and the mean value was used for further calculations. To exclude inter-observer variability, longitudinal measurements in one patient were performed by one observer. Nutritional intake was assessed by a 3-day food diary, including meal preparation details, and checked by an experienced dietician. Intake of energy and protein was calculated using the Dutch Food Composition Table 2001 (NEVO, The Hague, The Netherlands), by the Software Program Komeet (version 4.0.58; BaS Nutrition Software, Arnhem, The Netherlands).

Statistical analysis

Data were doubly entered and analyzed according to the intention-to-treat principle. Curves were fitted to describe the time course of nutritional status parameters, and differences over time between the two groups appraised by repeated-measures analysis of covariance, using SAS Proc Mixed, version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). Independent variables were the treatment indicator variable, the baseline measurement, measurement time, and the interaction between time and treatment. Statistical significance of treatment effects was assessed by testing the interaction between time and treatment. For weight analyses, patients with edema and/or ascites were excluded.

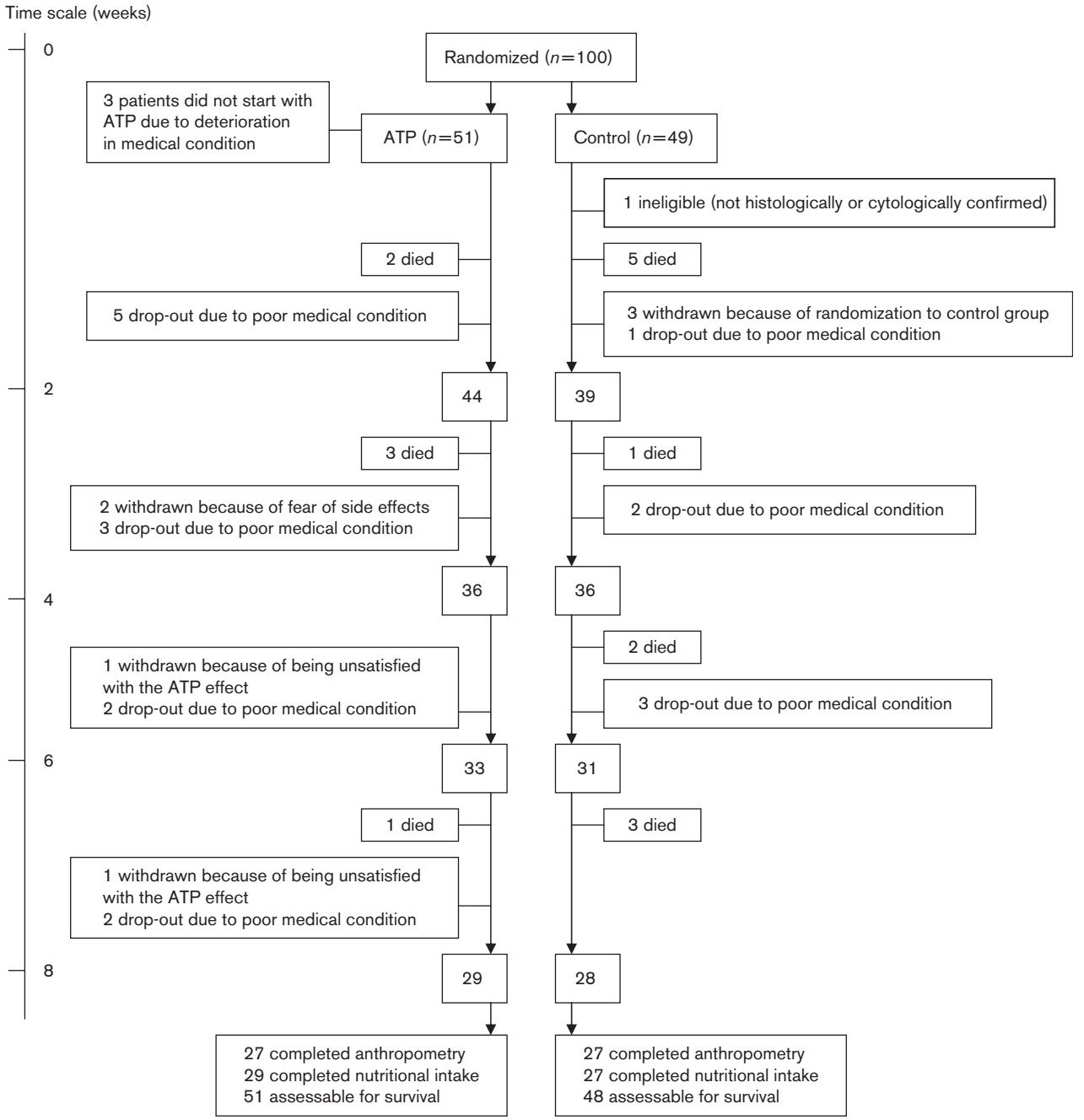
Cox regression models were fitted including interaction terms between treatment and dummy variables for short-term (i.e. during the 8-week ATP intervention) and long-term survival (i.e. 0–6 months, based on the maximum life expectancy of 6 months in the study population). For the latter analysis (0–6 months), dummy variables for 0–8 weeks and 8 weeks to 6 months were combined into a single dummy variable (0–6 months) as Cox proportional hazards assumption was tested and not rejected. Median survival time was determined by extrapolation from confounder-adjusted Kaplan–Meier plots. Because of previously shown survival benefit of ATP treatment in weight-losing stage IIIB NSCLC patients [12], we repeated the same analyses in (i) patients stratified by weight loss, defined as ≤ 5 or greater than 5% weight loss over the past 6 months [16], and (ii) lung cancer patients. All models were adjusted for confounders. Two-tailed P values less than 0.05 were considered statistically significant.

Results

Study population

One hundred patients were randomly assigned to the ATP ($n = 51$) or control ($n = 49$) group (Fig. 1). One patient in the control group was excluded because cancer was not confirmed histologically or cytologically. Sixteen

Fig. 1



Flow diagram of randomized patients. In two patients (both control group) one measurement is missing at T2 and in one patient (control group) one measurement is missing at T6. At T8 anthropometry was not assessed because of poor medical condition in three patients (two in ATP group; one in control group). Nutritional intake was not assessed in one patient in the control group. ATP, adenosine 5′-triphosphate.

patients (seven ATP, nine controls) died or dropped out before the first follow-up measurement, leaving 83 assessable patients (44 ATP, 39 controls). Fifty-seven patients (29 ATP, 28 controls) completed the full 8-week study period. Eleven patients died in the control group and six patients in the ATP group due to disease progression, unrelated to the study product. Total drop-out rates were equally distributed over ATP ($n = 22$) and control patients ($n = 20$).

Baseline characteristics

Mean age of the total study population was 66.4 years, and 66% of the patients were male. Lung cancer was the most frequent tumor type (45%), followed by colon

cancer (13%). Seventy percent of the patients had WHO performance index 1, 30% had WHO 2.

As shown in Table 1, there was some unbalance between the randomization groups in favor of the control group: randomized and assessable patients in the ATP group had a worse performance status, a lower energy intake, a higher frequency of edema and ascites; also weight loss $\geq 10\%$ at baseline was found more frequently in the ATP group.

Table 1 Baseline patient characteristics of randomly assigned and assessable patients

	ATP		Control	
	Randomly assigned[b] ($N=51$), N (%) or mean ± SD	Assessable[a,c] ($N=44$), N (%) or mean ± SD	Randomly assigned[b] ($N=49$), N (%) or mean ± SD	Assessable[a,c] ($N=39$), N (%) or mean ± SD
Sex				
Male	35 (69)	34 (77)	31 (63)	26 (67)
Female	16 (31)	10 (23)	18 (37)	13 (33)
Age (years)	68 ± 9.8	68 ± 9.6	65 ± 10.5	65 ± 11.0
Years of cancer at inclusion	2.3 ± 2.7	2.5 ± 2.9	2.7 ± 3.3	2.7 ± 3.3
Tumor type				
Head and neck	–	–	5 (10)	5 (13)
Esophagus	1 (2)	1 (2)	3 (6)	1 (3)
Stomach	2 (4)	–	3 (6)	3 (8)
Pancreas	3 (6)	2 (5)	–	–
Colon	8 (16)	8 (18)	5 (10)	3 (8)
Lung	21 (41)	19 (43)	24 (49)	21 (54)
(Non) Hodgkin	1 (2)	–	1 (2)	–
Melanoma	2 (4)	2 (4)	2 (4)	1 (3)
Sarcoma	1 (2)	1 (2)	–	–
Bladder	–	–	1 (2)	1 (3)
Kidney	3 (6)	3 (7)	–	–
Ovary	1 (2)	1 (2)	–	–
Prostate	5 (10)	5 (11)	4 (8)	3 (8)
Liver	2 (4)	2 (5)	–	–
Cervix	–	–	1 (2)	1 (3)
Mammary	1 (2)	–	–	–
Smoking				
Yes	23 (45)	20 (45)	15 (31)	13 (33)
No	28 (55)	24 (55)	33 (67)	25 (64)
Missing	–	–	1 (2)	1 (3)
WHO performance index				
1	33 (65)	30 (68)	37 (76)	33 (85)
2	18 (35)	14 (32)	12 (24)	6 (15)
Fatigue				
Yes	48 (94)	41 (93)	48 (98)	39 (100)
No	3 (6)	3 (7)	1 (2)	0 (0)
Loss of appetite				
Yes	36 (71)	31 (70)	31 (63)	23 (59)
No	15 (29)	13 (30)	18 (37)	16 (41)
EORTC QLQ-C30				
Physical functioning	44.0 ± 25.9	49.1 ± 23.9	45.3 ± 26.2	50.1 ± 24.7
Fatigue	65.6 ± 27.0	62.9 ± 27.2	58.3 ± 26.6	54.1 ± 24.3
Appetite loss	49.7 ± 37.9	48.5 ± 37.7	43.1 ± 34.4	41.0 ± 34.6
Energy intake (MJ/day)	6.6 ± 2.1	6.8 ± 2.1	7.4 ± 2.3	7.7 ± 2.1
Weight[d]	69.9 ± 15.2	71.4 ± 15.3	68.4 ± 17.2	72.0 ± 16.9
Body mass index (kg/m^2)[d]	23.3 ± 4.5	23.7 ± 4.8	23.1 ± 4.6	24.1 ± 4.4
Weight change in previous 6 months (%)	− 3.5 ± 7.9	− 2.8 ± 7.6	− 2.6 ± 9.0	− 1.0 ± 9.0
$\geq 5\%$ weight loss in previous 6 months	18 (35)	13 (30)	17 (35)	10 (26)
Overall weight change (%)[e]	− 10.9 ± 10.0	− 9.9 ± 10.0	− 9.1 ± 13.5	− 6.1 ± 12.4
$\geq 10\%$ overall weight loss	27 (53)	22 (50)	20 (41)	12 (31)
Edema and/or ascites	14 (28)	12 (27)	8 (17)	6 (15)
Edema	12 (24)	11 (25)	8 (17)	6 (15)
Ascites	6 (12)	4 (9)	2 (4)	0 (0)
Upper arm circumference (cm)	29.4 ± 4.4	29.5 ± 4.4	28.6 ± 4.5	29.6 ± 4.0
Triceps skin fold thickness (mm)	15.3 ± 6.9	15.1 ± 6.8	16.4 ± 8.2	17.6 ± 7.9

The EORTC QLQ-C30 (European Organization for Research and Treatment of Cancer Core Questionnaire) is a questionnaire developed to assess various domains of the quality of life of cancer patients.

ATP, adenosine 5′-triphosphate; WHO, World Health Organization.

[a]At least one follow-up analysis was performed.

[b]Data available for energy intake, $n=49$ (ATP) and $n=48$ (control); for upper arm circumference, $n=51$ (ATP) and $n=47$ (control); for triceps skin fold thickness, $n=50$ (ATP) and $n=46$ (control).

[c]Data available for energy intake, $n=42$ (ATP) and $n=39$ (control).

[d]Patients with ascites or edema were excluded from analysis. Randomly assigned: for weight, $n=27$ (ATP) and $n=31$ (control); for body mass index, $n=25$ (ATP) and $n=31$ (control). Assessable: for weight, $n=22$ (ATP) and $n=24$ (control); for body mass index, $n=21$ (ATP) and $n=24$ (control).

[e]Overall weight change = current weight minus stable preillness weight.

Intervention

Of the 51 patients assigned to the ATP group, three patients (6%) did not start the infusion because of rapid deterioration of their condition. The remaining 48 patients received a mean of 5.5 ATP infusions per patient over the 8-week period. Twenty-four patients (47%) completed all eight ATP infusions; of these, 12 patients pledged to continue ATP administration after completion of the regular 8-week study period. Over the subsequent 4 months, these patients received a mean of seven ATP infusions per patient [15].

The mean dose of ATP administered was 45 μg/kg/min in the first ATP infusion to 40 μg/kg/min in the eighth infusion, and 36 out of 48 patients received a mean ATP dose of 40–50 μg/kg/min over all infusions. The total administered dose per patient per day was 1.42 ± 0.43 g ATP per day (mean ± SD).

The majority of ATP infusions (63%) were without side effects. Dyspnea was the most common side effect (14% of infusions), followed by chest discomfort (12%) and the urge to take a deep breath (11%). No symptoms of cardiac ischemia occurred in any of the infusions. All side effects were transient and resolved within minutes after lowering the ATP infusion rate [15].

Cointerventions

At baseline, 10 patients (five ATP, five control group) were treated with hormones and four patients with gefitinib (two ATP, two controls); no patients were treated with palliative chemotherapy or radiotherapy. During the study, one patient (control) received palliative radiotherapy for skeletal metastases, and two patients (control) started with chemotherapy. Hormone therapy was continued in nine patients (five ATP, four controls) and reduced in one patient (control). Gefitinib was started in one patient (control), raised in one patient (ATP), and continued in three patients (one ATP, two controls). Use of nonopioid or opioid analgesics, corticosteroids, antihypertensives, antiemetics, antidiarrhoics, laxatives, diuretics, antibiotics, antidiabetics, or drugs for chronic obstructive pulmonary disease was similar in the ATP and control group (data not shown).

Effect of adenosine 5′-triphosphate on nutritional status

Changes per week in anthropometric measurements during the 8-week intervention period in the ATP and control group are shown in Table 2 and changes in nutritional intake in Table 3. Both in ATP-treated and control patients, weight, mid-upper arm circumference, and triceps skin fold thickness decreased. However, triceps skin fold thickness decreased significantly less in the ATP group [change per 8 weeks −1.84 mm, 95% confidence interval (CI): −3.92 to 0.24 mm] than in the control group (−3.60, 95% CI: −5.84 to −1.36 mm; between-group difference 1.76, 95% CI: 0.48–3.12 mm, $P = 0.009$). No statistically significant difference between the two groups was observed for weight, mid-upper arm circumference, or intake of energy and

Table 2 Changes per 8 weeks in anthropometric measurements in patients treated with ATP and control patients, and between-group difference

	ATP		Control		Between-group difference	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Weight (kg)	−2.62	−6.16 to 0.96	−2.20	−6.24 to 1.92	−0.42	−2.24 to 1.44
Triceps skin fold thickness (mm)	−1.84	−3.92 to 0.24	−3.60	−5.84 to −1.36	1.76	0.48 to 3.12
Mid-upper arm circumference (cm)	−2.24	−3.36 to −1.20	−1.52	−2.80 to −0.24	−0.72	−1.52 to 0.08

Number of patients weight: ATP, $n=26$; control, $n=27$; triceps skin fold thickness: ATP, $n=48$; control, $n=45$; mid-upper arm circumference: ATP, $n=49$; control, $n=46$.
ATP, adenosine 5′-triphosphate; CI, confidence interval.

Table 3 Nutritional intake at 0, 4, and 8 weeks in the ATP and control group

	ATP			Control			
	0 ($n=49$)	4 ($n=36$)	8 ($n=29$)	0 ($n=47$)	4 ($n=34$)	8 ($n=26$)	*P* value (ATP vs. control)
Energy intake (MJ/day)	6.6 ± 0.3	6.6 ± 0.4	7.1 ± 0.4	7.4 ± 0.3	7.2 ± 0.3	7.7 ± 0.4	0.74
Protein intake (g/day)	60 ± 3	58 ± 4	65 ± 5	65 ± 4	61 ± 4	69 ± 5	0.52
Protein intake (%)	15 ± 0.5	15 ± 0.7	16 ± 0.8	15 ± 0.4	14 ± 0.6	15 ± 0.5	0.47
Carbohydrate intake (g/day)	187 ± 9	187 ± 11	198 ± 13	203 ± 9	206 ± 10	196 ± 11	0.59
Carbohydrate intake (%)	49 ± 1.2	50 ± 1.7	48 ± 1.8	47 ± 1.1	50 ± 1.9	43 ± 1.6	0.93
Fat intake (g/day)	60 ± 3	59 ± 4	63 ± 5	72 ± 4	65 ± 4	79 ± 6	0.98
Fat intake (%)	33 ± 1.2	34 ± 1.2	34 ± 1.4	36 ± 0.8	34 ± 1.4	39 ± 1.5	0.94
Water (l/day)	1.9 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	

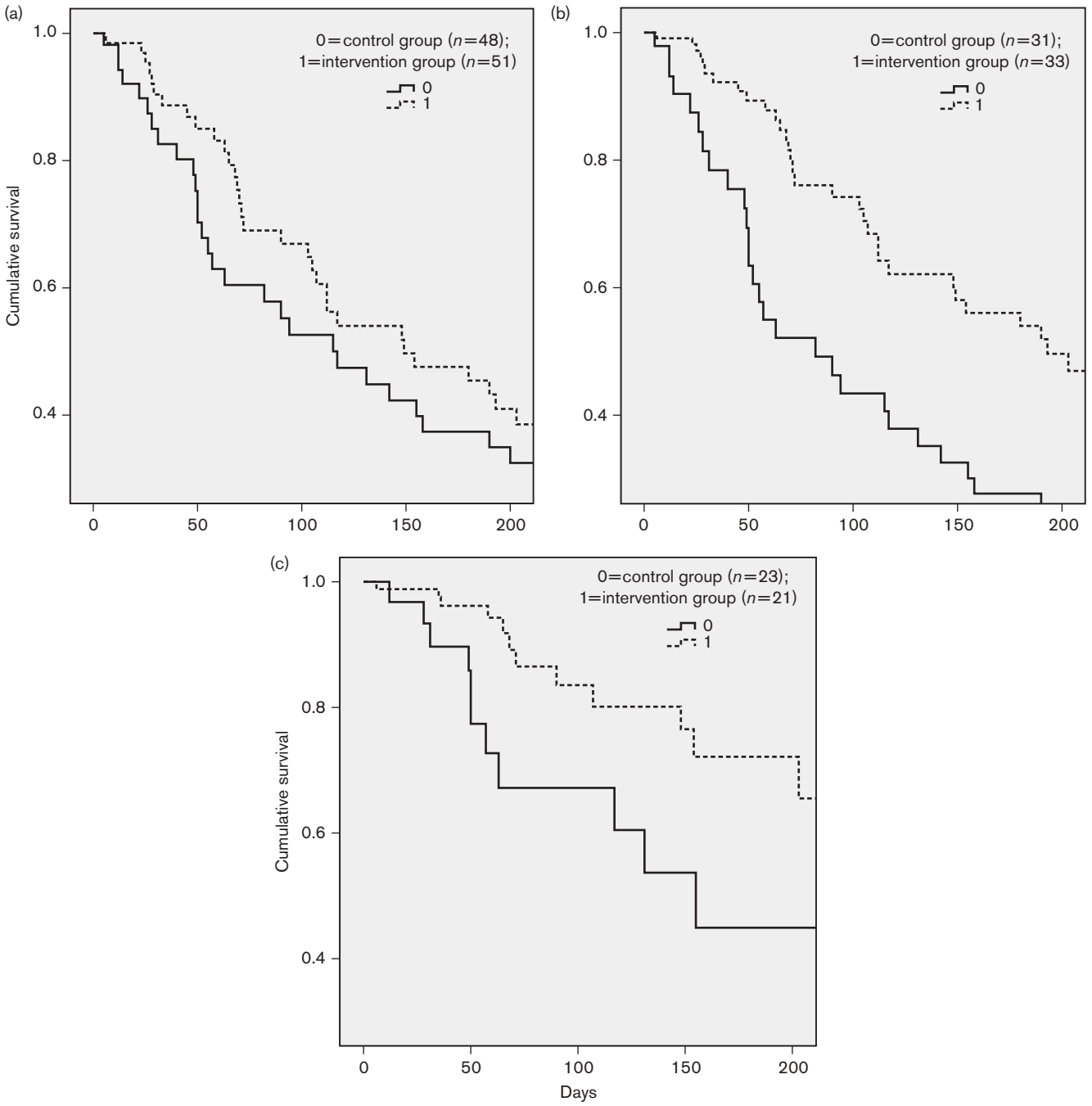
Values are expressed as mean ± SE.
P value based on repeated-measures analysis of covariance.
ATP, adenosine 5′-triphosphate.

protein. In the subgroup of patients with lung cancer, results were similar (data not shown).

Effect of adenosine 5′-triphosphate on survival

As shown in Fig. 2a, Cox regression revealed a statistically significant difference in short-term (0–8 weeks) survival in favor of ATP-treated patients [hazard ratio (HR) of ATP vs. control: 0.40, 95% CI: 0.17–0.95; $P = 0.037$]. Long-term survival (0–6 months) was not significantly different between the ATP and control group (HR: 0.71, 95% CI: 0.40–1.28; $P = 0.26$). Median survival was 150 days in the ATP group and 115 days in the control group. On the census date of 10 September 2007, nine patients (six ATP, three controls) were still alive.

Fig. 2



Kaplan–Meier plots of survival in patients treated with adenosine 5′-triphosphate (ATP) versus no ATP (control): (a) total group (ATP: $n=51$; control: $n=48$), (b) patients with less than 5% weight loss over the previous 6 months (ATP: $n=33$; control: $n=31$), (c) patients with lung cancer (ATP: $n=21$; control: $n=23$).

Stratified analysis in weight-stable and weight-losing patients showed a significant survival benefit of ATP treatment in weight-stable patients (Fig. 2b; ATP: $n = 33$; control: $n = 31$), both during the 8-week intervention (HR: 0.20, 95% CI: 0.08–0.54; $P = 0.001$) and for long-term survival (0–6 months HR: 0.40, 95% CI: 0.19–0.83; $P = 0.014$). No significant effect of ATP on survival was found in weight-losing patients (data not shown).

In patients with lung cancer ($n = 44$) (Fig. 2c), results also indicated a significant short-term and long-term survival benefit of ATP treatment (0–8 weeks HR: 0.17, 95% CI: 0.04–0.78; $P = 0.023$; 0–6 months HR: 0.35, 95% CI: 0.14–0.88; $P = 0.025$).

Discussion

In the present RCT, we investigated the effects of ATP infusions on the nutritional status and survival in patients with preterminal cancer of different tumor types. In view of the short life expectancy of this patient population (< 6 months), we chose to apply a frequent ATP treatment schedule over a short period of time (i.e. weekly 8–10 h infusions of ATP over 8 weeks).

Results showed a significant advantage in short-term survival (0–8 weeks) for ATP-treated patients relative to control patients (HR: 0.40), which was more pronounced in weight-stable patients (HR: 0.20) and patients with lung cancer (HR: 0.17). Moreover, ATP treatment even showed significant benefit for long-term survival (0–6 months) in both weight-stable patients (HR: 0.40) and patients with lung cancer (HR: 0.35). These results are intriguing especially as our study population was composed of refractory cancer patients with preterminal illness with an estimated life expectancy less than 6 months. Clearly, our findings will need confirmation in future rigorous studies.

The question could be raised why the observed survival benefit of ATP in our study population was especially present and lasted longer in weight-stable patients, as opposed to patients with recent weight loss. Recent weight loss is well known to be a predictor of survival [2,3], presumably because of a higher tumor stage [17,18]. Many malignant tumors produce humoral factors, such as cytokines or specific cachexia-inducing factors, thereby inducing weight loss [5–7,19]. Thus, the notion of better effects of ATP in weight-stable patients could indicate that ATP may be more effective in patients with less advanced disease. This hypothesis is supported by Agteresch *et al.* [12], who reported favorable effects of ATP on survival only in NSCLC patients with locally extended disease (stage IIIB), but not in patients with metastatic disease (stage IV). However, as we did not collect blood samples for ethical reasons, especially to minimize burden in the control group, our data do not provide further insight in the precise mechanism involved.

It might be argued that the additional fluid administration in the ATP infusion group could have been responsible for the survival benefit in the ATP group. However, results showed that patients in the control group had a higher total fluid intake/load than patients in the intervention group (2.1 ± 0.6 l in the control group, vs. 1.8 ± 0.7 l in the ATP group excluding the ATP infusion; Table 3). In addition, patients in the ATP group received just one infusion on an average of 284 ml (range: 27–556 ml) per day, so that total fluid intake including the infusion volume over the week in the ATP group still remained below fluid intake in the control group. We therefore conclude that the extra fluid administration by the infusions does not provide an explanation for the observed survival benefit in the ATP group.

The mechanism underlying the effect of ATP on survival remains to be elucidated. Agteresch *et al.* [12] speculated that the effect of ATP on survival could have been mediated by the anticachectic effect of ATP in their study population. However, based on our finding of stronger and longer lasting effects of ATP on survival in weight-stable patients, it would seem more likely that other mechanisms underlie the favorable effect of ATP on survival, such as a direct effect of ATP on malignant cell growth. Extracellular ATP has already been shown to effectively inhibit the growth of various types of malignant tumor cells [20–23] and to reduce tumor growth of various tumors *in vivo* [24–26]. In view of the preterminal disease stage of the patients, we did not monitor tumor growth; as a consequence, the above-mentioned interpretation remains speculative.

Our findings with regard to the effects of ATP on the nutritional status partly confirm results of the earlier RCT in patients with advanced NSCLC [10,11]. In this earlier trial, the favorable effects of ATP on triceps skin fold thickness came into effect within 8 weeks, whereas ATP effects on body weight and mid-upper arm circumference only emerged after a delay of 8–16 weeks. The absence of significant effects of ATP on body weight and mid-upper arm circumference in this study would suggest that our 8-week intervention in preterminal patients may have been too short to detect significant effects of ATP on the nutritional status, except for fat mass.

Another possible explanation for the lack of effect might be that the administered ATP dose was not high enough to cause an effect on nutritional status. Although knowledge about a possible threshold for effect of ATP infusion is not available, pharmacokinetic data from Agteresch *et al.* [27] show that compared with baseline, ATP infusions with a dose between 25 and 60 μg/kg/min

induce a 53–56% increase in erythrocyte ATP concentrations. Erythrocyte ATP concentrations are the presumed direct mediator of the physiological effects of ATP through P1 and P2 purinergic receptors [28]. As in this study, 46 out of 48 patients received a mean dose ≥ 25 μg/kg/min, it seems unlikely that the lack of effect of ATP on the nutritional status was due to a too low infusion rate.

The question could be raised whether the presence of edema, which was more frequently observed in the ATP group, could have biased our results in favor of the ATP group. Of note, as edema most frequently occurs in the lower extremities, this would be expected to have little influence on the size of triceps skin fold thickness in the mid-upper arm. Nevertheless, we verified our data by performing a stratified analysis for patients with and without edema. Both control patients with and without edema showed a clear decrease in triceps skin fold thickness. By contrast, in ATP treated patients, triceps skin fold thickness was completely stable regardless of whether edema was present or not, indicating that the effect of ATP on triceps skin fold thickness was independent of the presence of edema.

Some methodological issues need to be considered. First, because of the preterminal illness of our patient population, baseline data and time changes of outcome parameters showed high between-subject variability, limiting the power of the study to detect effects of ATP on nutritional status. The high drop-out rate, which was unavoidable in this preterminal study population, further reduced the power of the study.

Second, despite adequate stratification and randomization there was an imbalance in baseline characteristics between the randomization groups in favor of the control group. Randomized and assessable patients in the ATP group had a worse performance status, a lower energy intake, a higher frequency of edema and ascites; also weight loss $\geq 10\%$ at baseline was found more frequently in the ATP group. We eliminated the potential bias by this imbalance by adequately adjusting our data for potential confounding in multivariate analyses.

Third, as 40% of our study population were patients with lung cancer, the positive effect of ATP on survival reported for the whole population could just have been the result of the specific contribution of lung cancer patients. However, even though stratified analysis showed quantitatively less effect of ATP on survival in patients without lung cancer compared with lung cancer patients, the trend in both groups was identical.

Fourth, cointerventions (chemotherapy, antibiotics, corticosteroids) might also have influenced patients' nutritional status or survival. However, the only difference between the control and ATP group was the start of palliative radiotherapy in one control patient, palliative chemotherapy in two control patients and gefitinib in one control patient, all of which would therefore induce bias towards longer survival in the control group, and thus cannot explain our finding of a longer survival in ATP-treated patients.

Finally, for ethical reasons, our trial was not placebo controlled. As the belief that emotional state could affect survival exists among patients and professionals [29], the question might be raised whether the observed effect of ATP on survival in our study could have been because of a placebo effect (possibly inducing a better 'coping strategy') [29,30]. However, the majority of literature shows little evidence which would suggest that a 'fighting spirit' or psychological coping could play an important part in survival [30,31]. A review of observational studies showed no relationship between coping style and survival for the large majority of studies [30]. A meta-analysis [32] of RCTs studying the effect of psychological therapy on survival in women with metastatic breast cancer showed negative results in four out of five RCTs. More recently, a study by Andersen *et al.* [33] showed a reduced risk of breast cancer recurrence and death from breast cancer in patients receiving a psychologic intervention compared with controls receiving no intervention. However, this study was performed in patients without macroscopic tumor mass awaiting adjuvant chemotherapy, with long-term disease recurrence as primary end point. Furthermore, the intervention concerned not only included psychologic intervention but also strategies to increase daily activities, to improve dietary habits, and to stop smoking, all of which may have contributed to the observed reduced cancer recurrence in the intervention group. Although the literature on the effects of psychological interventions on survival is not conclusive, it would appear less likely that the observed survival benefit of ATP in this study was caused by a placebo effect, especially as the survival benefit of ATP was stronger in specific subgroups (i.e. weight-stable patients and patients with lung cancer) and, in these subgroups, had a long-term effect (i.e. 6 months), when any placebo effect would long have faded.

A number of novel aspects of this study should be highlighted. First, this is the first RCT in humans with intravenous ATP infusions at weekly intervals (as opposed to 2- to 4-week intervals in previous studies), with infusions lasting only 8–10 h (instead of 30–96 h) and at a maximal dose of 50 μg/kg/min (instead of 75–100 μg/kg/min) [10,34]. Furthermore, the intervention period lasted for only 8 weeks (instead of 24 weeks) [10]. The above-mentioned novel schedule of ATP administration showed significant favorable effects on survival, both at 8 weeks and, in lung cancer patients as well as in

weight-stable patients, even at 6 months, that is, 4 months after completion of the last ATP infusion.

Second, this is the first RCT with intravenous ATP administration in patients of mixed tumor types in a very late stage of the disease. Results corroborate the previously reported favorable effects of ATP on survival in NSCLC [12], but go beyond this by showing the same trend in patients of other tumor types, notwithstanding the considerably later disease stage than in the previous study [12]. Third, this is the first study in which ATP was administered at home, showing that ATP can be safely administered in the home setting [15].

In conclusion, our data indicate that ATP infusions at the dose and schedule studied may increase survival in preterminal cancer, especially in weight-stable patients and in patients with lung cancer. These findings will need confirmation in larger studies aimed at further defining the effect of ATP on tumor growth and survival in specific types of cancer and at different tumor stages.

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