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News & Views

Cysteine-Rich Protein Reverses Weight Loss in Lung Cancer Patients Receiving Chemotherapy or Radiotherapy

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ABSTRACT

Oxidative stress plays a role in the tumor-cytotoxic effect of cancer chemotherapy and radiotherapy and also in certain adverse events. In view of these conflicting aspects, a double-blind trial over a 6-month period was performed to determine whether a cysteine-rich protein (IMN1207) may have a positive or negative effect on the clinical outcome if compared with casein, a widely used protein supplement low in cysteine. Sixty-six patients with stage IIIB-IV non–small cell lung cancer were randomly assigned to IMN1207 or casein. Included were patients with a previous involuntary weight loss of ≥3%, Karnofsky status ≥70, and an estimated survival of >3 months. Thirty-five lung cancer patients remained on study at 6 weeks. Overall compliance was not different between treatment arms (42–44% or 13 g/day). The patients treated with the cysteine-rich protein had a mean increase of 2.5% body weight, whereas casein-treated patients lost 2.6% ($p = 0.049$). Differences in secondary endpoints included an increase in survival, hand-grip force, and quality of life. Adverse events were mild or moderate. Further studies will have to show whether the positive clinical effects can be confirmed and related to specific parameters of oxidative stress in the host. *Antioxid. Redox Signal.* 10, 395–402.

INTRODUCTION

In cancer patients, oxidative stress is both a curse and a blessing. Oxidative stress plays a major role in the tumor-toxic effects of chemotherapy and radiotherapy and, incidentally, also has substantial adverse effects on the host tissue of the patients. Evidence indicates that loss of body cell mass (cancer cachexia) results to some extent from aberrant inflammation (13, 15) and is significantly correlated with an oxidative shift in plasma redox status, as indicated by a decreased ratio of reduced to oxidized cysteine (16). Substantial weight loss usually compromises the quality of life of cancer patients and is a contributor to morbidity and mortality (5, 10, 18, 25). Attempts to prevent the loss of body cell mass by nutritional intervention have, by and large, not been satisfactory (12, 13, 15, 22, 25, 27). A preliminary study of patients with different types of cancer revealed, however, that treatment with the glutathione precursor *N*-acetylcysteine reversed the loss of body cell mass and the oxidative shift in plasma redox status (16). Several redox-regulated signaling pathways are known to be involved in catabolic processes (reviewed in 11).

However, as the tumor-cytotoxic effects of cancer chemotherapy and radiotherapy typically involve oxidative stress, concern has been expressed that antioxidative treatment may interfere with these therapies and thus exacerbate mortality (see 9, 4, 24). Casein (*i.e.*, the protein base of the majority of clinically used enteral nutritional supplements) contains only minute quantities of cysteine. As cysteine is a limiting biosynthetic precursor of glutathione, it is expected to ameliorate the oxidative stress.

In view of these conflicting aspects, we now determined in a placebo-controlled double-blind clinical trial whether replac-

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ing casein with a high-cysteine whey-derived protein formulation (Table 1) may have a negative effect on the clinical outcome, as feared by some, or a positive effect, as suggested by the preliminary study on *N*-acetylcysteine (16). Specifically, the present study tested the hypothesis that loss of body weight and body cell mass in advanced frail cancer patients with relatively poor prognosis may be ameliorated, and the quality of life and functional performance may be improved by the high-cysteine whey-derived protein formulation compared with casein.

Both proteins are approximately isocaloric and isonitrogenous. A comparison of these two protein supplements has to be seen in the context of the general protein intake. A few results point to the generally low protein intake of patients with advanced cancer, even in patients who consumed commercially available nutritional supplements (14, 17). According to a recent review on protein requirements (20), this level of protein intake would likely be insufficient to support nitrogen balance, even in healthy individuals of a similar age. In several recent studies of pancreatic cancer patients, complex protein–containing supplements have mediated modest improvements, but the specific contribution of the protein remained unclear (1, 2, 14). In view of the notoriously low protein intake of cancer patients, it is believed that the quality of the protein is particularly important because any deficiency in the composition of a given protein may not be overcome by increasing the protein intake.

PATIENT ENROLLMENT, TRIAL PROFILE, BASELINE CHARACTERISTICS, AND COMPLIANCE

Patients were enrolled between October 2003 and February 2006 (Appendices 1–5). Sixty-six patients with lung cancer and 22 with colorectal cancer were recruited and randomly assigned to the treatment groups in two separate strata. Only seven patients consumed a minimum of 75% of their study medication (see Appendix 2) as required for the "per protocol" (PP) analysis according to the trial protocol. We therefore included in our analysis all patients who returned their canisters and completed at least the second visit (the "evaluable patients"). The trial profile (Fig. 1) and the baseline data of the evaluable colorectal cancer patients revealed that they were not well matched, and their numbers too small to be statistically meaningful. [Specifically, the mean age (63.6 ± 10.1 *vs*. 41.7 ± 7.0 years), baseline TNF-α levels (1.8 ± 0.7 *vs*. 3.1 ± 1.9 pg/ml), plasma glutamine levels (592 ± 129 *vs*. 461 ± 100 μM), and ESAS 8 (1.25 ± 2.82 *vs*. 7.00 ± 1.73) were significantly mismatched baseline parameters). We, therefore, report only the results from the stratum of lung cancer patients (Table 2). The mean compliance of the 35 evaluable lung cancer patients with casein or the cysteine-rich protein was $44 \pm 34\%$ and $42 \pm 29\%$, respectively, implying that the patients consumed, on the average, ~13 g/day of either protein.

Table 1. Amino Acid Composition of the Cysteine-rich Protein (CysP) and the Control Protein (casein)

	CysP	*Casein*
cys	0.50	0.05
met	0.14	0.18
glu	1.15	1.41
asp	1.04	0.56
leu	1.02	0.66
ile	0.46	0.37
val	0.46	0.49
ala	0.55	0.32
ser	0.39	0.50
gly	0.22	0.20
lys	0.74	0.49
arg	0.18	0.26
his	0.11	0.15
pro	0.35	0.88
phe	0.23	0.28
tyr	0.24	0.25
thr	0.40	0.32
trp	0.08	0.03

Amino acids are indicated as moles/kg.

EFFECTS ON PRIMARY END POINTS, MUSCLE FUNCTION, AND KARNOFSKY PERFORMANCE STATUS (SEE APPENDICES 6–8)

Patients completing the 6-month visit on the cysteine-rich protein showed, in contrast to the casein-treated group, a significant increase in body cell mass and hand-grip force (see Appendix 7) and a trend ($p = 0.09$) in the Karnofsky status compared with baseline values (Table 3). The treatment arms, however, were not significantly different, perhaps because of the small number of surviving patients.

In view of the poor condition of the patients and the resulting loss due to death or discontinuation, it seemed appropriate to analyze the data of all 35 "evaluable patients." This analysis showed that the mean changes in body weight of the casein-treated and cysteine-rich protein–treated groups were significantly different already after 6 weeks of treatment ($-1.21 \pm 3.90\%$ ($n = 17$) and $+1.36 \pm 2.94\%$ ($n = 18$), respectively; $p = 0.038$). To illustrate the changes throughout the study period, the changes between baseline and last observation according to the "last-observation-carried forward" (LOCF) method (see Appendix 8) and the means of the changes that were seen at 6 weeks, 3 months, and 6 months (or last observation) are shown in the middle and right panels of Table 3, respectively. The changes in body weight between treatment groups remained significantly different during the entire observation period (Table 3). The change in body cell mass in the cysteine-rich protein group was also different if compared either with the control group ($p = 0.01$) or with the baseline value ($p = 0.02$). However, the body cell mass data are less precise, as indicated by the large standard deviation, and may therefore be viewed only as supportive evidence.

EFFECTS ON LABORATORY PARAMETERS (SEE APPENDIX 7)

The two treatment arms showed no significant differences in the changes in laboratory end points, including C-reactive pro-

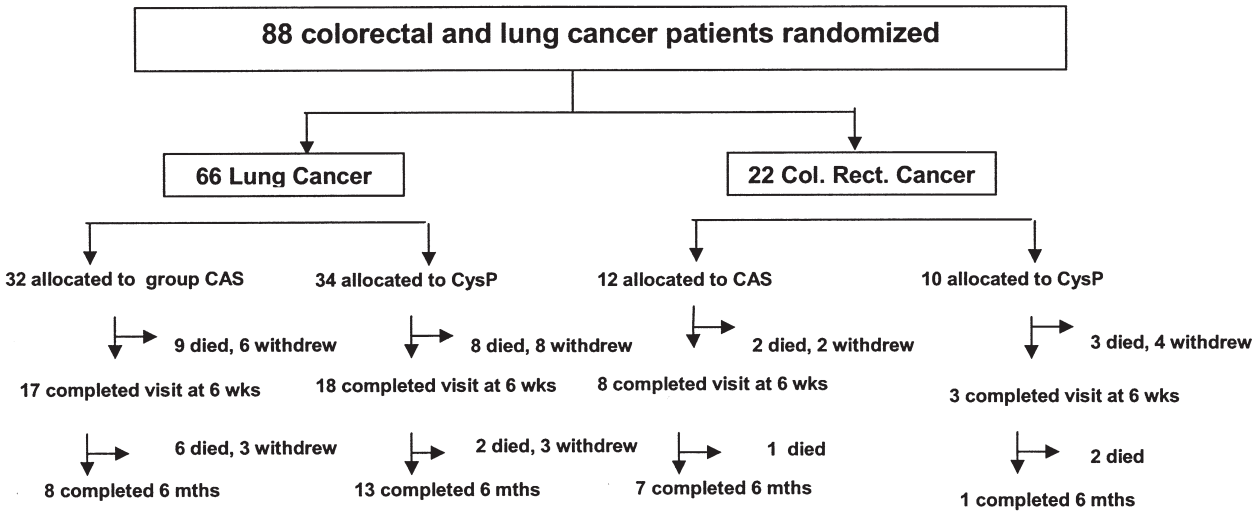

FIG. 1. Trial profile. CAS, casein group; CysP, cysteine-rich protein group (IMN1207).

tein, TNF-α, IL-6, and albumin concentrations (data not shown). The changes in glutathione (-58.9 ± 242.7 *vs.* $+ 110.7 \pm 300.7$ μM) (see Appendix 7) and plasma cysteine ($+9.8 \pm 23.5$ *vs.* $+34.8 \pm 150.4$ μM) showed higher values for patients treated with the cysteine-rich protein but were not significantly different. The high standard deviations indicate that these data are not very precise.

MORTALITY: EFFECT OF THE CYSTEINE-RICH PROTEIN ON THE SURVIVAL OF PATIENTS WITH CHEMOTHERAPY AND/OR RADIOTHERAPY

Of the 66 randomized lung cancer patients and 22 colorectal cancer patients, 25 and 36 patients with lung cancer and eight and 15 patients with colorectal cancer died during the trial of disease-related causes within 6 months and 12 months, respectively (see Fig. 1). The Kaplan–Meier survival curves (see Appendix 8) of the two treatment arms of the total lung cancer group were not different from one another (Fig. 2a), whereas the two treatment arms of the 35 evaluable patients (Fig. 2b) were different ($p = 0.024$; 95% hazard ratio, 0.067 to 0.916). Approximately 80% of the patients treated with cysteine-rich protein and <50% of the patients in the control group survived 12 months. The survival of lung cancer patients with chemotherapy and/or radiotherapy ($n = 28$) (Fig. 2c) also showed a positive trend in the group with the cysteine-rich protein ($p = 0.058$).

EFFECTS ON CHANGES IN QUALITY OF LIFE (SEE APPENDIX 6)

Several quality-of-life parameters, including hand-grip force, Karnofsky status, the feeling of being nervous and worried (McGill QOLC2), and appetite and depression (ESAS) (see Appendix 6) showed a marked deterioration shortly before death, as indicated by the data from a subgroup of six patients in whom these measurements had been performed within the last 17 days before death. These six patients showed a significantly ($p <$

TABLE 2. BASELINE CHARACTERISTICS

Assignment	*Casein*	*CysP*
No.	17 (32)	18 (34)
Female/male	6/11 (11/12)	4/14 (6/28)
Age (yr)	63.5 ± 10.9 (63.6 ± 11.4)	64.3 ± 10.7 (63.8 ± 10.1)
Height (cm)	167.2 ± 8.4	169.5 ± 11.7
Body weight (kg)	64.5 ± 16.7	66.1 ± 14.3
Body cell mass (kg)	22.5 ± 6.8	22.4 ± 4.6
CRP (mg/L)	47.4 ± 45.6[b] (73.3 ± 82.7)	35.3 ± 54.1[b] (60.1 ± 81.3)
TNF-α (pg/ml)	15.5 ± 52.3[b] (10.4 ± 38.5)	2.6 ± 1.7[b] (2.6 ± 1.5)
IL-6 (pg/ml)	6.2 ± 3.9	4.6 ± 3.3
Glutamine (μM)	551 ± 160[a] (513 ± 182)	564 ± 147 (566 ± 144)
Cysteine (μM)	29.0 ± 15.5[a]	30.7 ± 14.9
RBC-GSH (μM)	476 ± 219[a]	443 ± 292
Hand-grip force (kg)	28.6 ± 10.7	29.0 ± 8.6
Karnofsky index (units)	78.2 ± 7.3	78.9 ± 8.3
ESAS 8 (units) Constipation	2.94 ± 3.55[a] (2.59 ± 3.23)	1.67 ± 2.59 (2.09 ± 2.84)

Data ± SD refer to the patients who completed at least two visits. Data in brackets refer to the total group of randomized patients. All groups consisted mainly of whites (>85%).

[a]Data were available from 16 casein-treated patients.

[b]TNF-α data were available from 15 casein-treated and 17 IMN1207 (CysP)-treated patients. C-reactive protein (CRP) data were available from only 15 casein-treated and 16 IMN1207 (CysP)-treated patients.

TABLE 3. EFFECT OF PROTEIN SUPPLEMENTS ON CHANGES IN OBJECTIVE PARAMETERS

	6-mo Values[a]		*Last observation (LOCF)*[b]		*Mean of 6-wk, 3-mo, and 6-mo values*[c]	
Rel. or Abs. changes	*Casein* n = *8*	*CysP* n = *13*	*Casein* n = *17*	*CysP* n = *18*	*Casein* n = *17*	*CysP* n = *18*
Body weight (%)	−1.01 ± 8.52 Cl, (−8.14/+6.11)	2.38 ± 7.21 (−1.98/+6.73)	**−2.63 ± 8.07**[d] **(−6.78/+1.52)**	**2.50 ± 6.74**[d] **(−0.85/+5.85)**	**−1.88 ± 5.46**[e] **(−4.69/+0.93)**	**1.79 ± 4.45**[e] **(−0.43/+4.00)**
Body cell mass (%)	+10.34 ± 14.10 Cl, (−23.00/+43.69)	**+14.09 ± 15.84**[f] **(+3.46/+24.73)**	**−5.47 ± 34.63**[g] **(−23.27/+12.33)**	**11.55 ± 18.05**[g,h] **(+1.93/+21.16)**	**−2.85 ± 30.93**[i] **(−18.76/13.05)**	**+8.34 ± 17.89**[e] **(−1.20/+17.87)**
Hand-grip force (%)	+8.49 ± 16.21 Cl, (−6.49/+23.48)	**+12.41 ± 16.52**[j] **(+2.43/+22.39)**	−2.22 ± 22.57 (−13.83/+9.38)	2.57 ± 24.75 (−9.73/+14.88)	0.17 ± 18.38 (−9.28/+9.62)	2.50 ± 18.87 (−6.88/+11.89)
Karnofsky index (units)	+3.75 ± 11.8 Cl, (−6.18/+13.68)	5.38 ± 10.50[k] (−0.96/+11.73)	−2.94 ± 17.23 (−11.80/+5.92)	0.56 ± 16.62 (−7.71/+8.82)	−2.16 ± 11.28 (−7.96/+3.65)	2.50 ± 11.98 (−3.46/+8.46)

Bold data indicate a statistical significance.
[a]Data show the means ± SD and 95% confidence intervals (CIs) of the measurements at the 6-mo visit.
[b]Measurements at the end of the observation period from patients completing at least two visits.
[c]Means of three values for each subject (*i.e.,* the measurements at 6 wk, 3 mo, and 6 mo).
[d]$p = 0.049$ for difference between groups.
[e]$p = 0.036$ for difference between groups.
[f]$p = 0.015$ for difference to baseline.
[g]$p = 0.010$ for difference between groups, by Kruska-Wallis test.
[h]$p = 0.022$ for difference to baseline.
[i]$p = 0.005$ for difference between groups, by Kruska-Wallis test.
[j]$p = 0.019$ for difference to baseline.
[k]$p = 0.089$ for difference to baseline.

0.05) greater decrease in Karnofsky index and three other quality-of-life parameters (feeling nervous and worried, not feeling well, and depression) than the other 29 patients, irrespective of the treatment group. In the remaining 29 patients (who composed the majority of the population and included two patients whose last data had been determined 41 and 56 days before death, respectively), the group with the cysteine-rich protein showed a significant improvement in all of these parameters if compared with baseline values, whereas the casein-treated group showed no significant improvement (Table 4). Also, no significant changes were seen in other McGill or ESAS parameters (data not shown). The combined treatment arms of the imminently dying patients and the combined arms of the remaining patients were significantly different in the changes in Karnofsky status, feeling nervous and worried, not feeling well, and degree of depression (not shown). The cut-off point between day 17 and day 41 has been arbitrarily chosen.

Clinical assessment of disease activity

The reported changes in disease activity (regression, stable disease, or progression) in the two treatment groups were not significantly different.

ADVERSE REACTIONS

Of the 33 lung cancer patients allocated to the cysteine-rich protein, four complained about events *possibly* related to the protein (*i.e.*, one person with moderate nausea, one with moderate nausea plus abdominal discomfort, one with mild emesis, and one with mild dryness of oral mucous membrane. One patient complained of mild increased nausea that was considered *probably* related to the protein. Among the colorectal cancer patients allocated to the cysteine-rich protein, one patient reported serious vomiting, moderate diarrhea, and moderate nausea, which were altogether considered probably related to the protein. Among the 33 lung cancer patients allocated to casein, one patient reported a mild case of nausea probably related to the protein, and three patients reported events possibly related to the protein (*i.e.*, a case of mild transient vomiting, and one case each of mild and moderate constipation). Of the colorectal cancer patients allocated to casein, one reported mild nausea, which was considered definitely related to the protein, and one reported mild constipation that was possibly related to the protein.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

The results show that the survival of non–small cell lung cancer patients with chemotherapy or radiotherapy or both was not decreased by supplementation with the cysteine-rich protein. The results thus alleviate the concern that treatment with (certain) antioxidants may interfere with the tumor-cytotoxic effects of chemotherapy and radiotherapy and thus increase mortality (see 4, 9, 24). They also alleviate the concern that (certain) nutritional programs may enhance tumor growth.

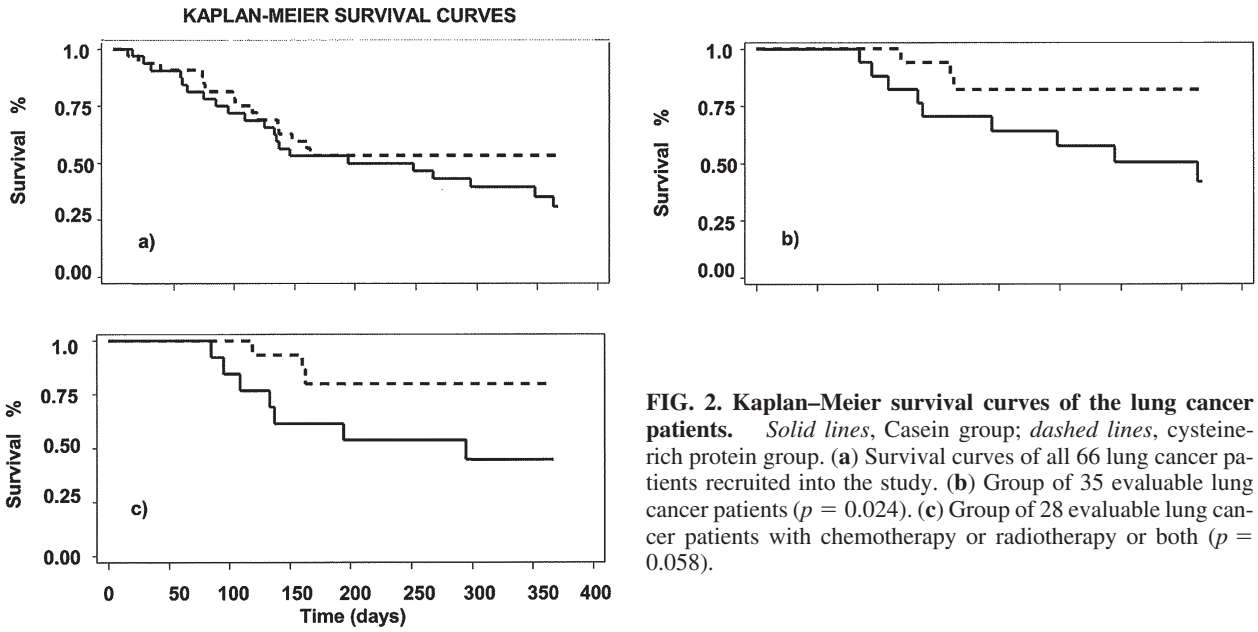

FIG. 2. Kaplan–Meier survival curves of the lung cancer patients. *Solid lines*, Casein group; *dashed lines*, cysteine-rich protein group. (**a**) Survival curves of all 66 lung cancer patients recruited into the study. (**b**) Group of 35 evaluable lung cancer patients ($p = 0.024$). (**c**) Group of 28 evaluable lung cancer patients with chemotherapy or radiotherapy or both ($p = 0.058$).

The data show that treatment with the cysteine-rich protein was sufficient to reverse cancer-related weight loss and the loss of body cell mass in our non–small cell lung cancer patients. This was associated with an improvement of muscle force and certain quality-of-life parameters, provided that measurements shortly before death were excluded. As glutathione and cysteine measurements did not yield significant differences, the trial produced no data proving the trial hypothesis that the reversal of

TABLE 4. CHANGES BASED ON LAST MEASUREMENTS DETERMINED >17 DAYS BEFORE DEATH

Rel. or Abs. changes	*Casein*	*CysP*
n	13	16
Body weight (%)	−3.09 ± 8.60[c]	+2.46 ± 6.90[c]
Hand-grip force (%)	1.71 ± 21.45	**+9.10 ± 16.62**[d] **CI, +0.24/+17.96**
Karnofsky index (units)	+2.31 ± 13.01	+5.00 ± 9.66[e]
McGill QOLC2 (units)[e] Feeling nervous and worried	0.23 ± 3.24	**−1.53 ± 2.50**[b f] **CI, −2.92/−0.15**
ESAS 2 (units)[e] Not feeling well[a]	−0.85 ± 3.83	−1.20 ± 2.68[b g]
ESAS 5 (units) Lack of Appetite	−1.23 ± 5.40	**−2.07 ± 2.60**[b h] **CI, (−3.51/−0.62)**
ESAS 11 (units)[e] Depression	−0.54 ± 4.22	**−1.60 ± 2.77**[b i] **CI, −3.14/−0.06**

CysP, cysteine-rich protein.

[a]Subjective feeling of physical, emotional, social, spiritual, and financial well-being.

[b]McGill QOL and ESAS data available from 15 patients only.

For detailed information about McGill QOL and ESAS parameters, see refs. 23–25.

Bold data indicate a statistical significance.

[c]$p = 0.064$ for difference between groups.

[d]$p = 0.044$ for difference to baseline.

[e]$p = 0.056$ for difference to baseline.

[f]$p = 0.033$ for difference to baseline.

[g]$p = 0.105$ for difference to baseline.

[h]$p = 0.008$ for difference to baseline.

[i]$p = 0.042$ for difference to baseline.

weight loss may be largely mediated by cysteine and glutathione. The available data do not exclude that amino acids other than cysteine or other properties of the cysteine-rich protein such as digestibility may (also) contribute to the observed efficacy. However, the important role of cysteine was supported by the facts that increases in body cell mass have previously been observed in studies with other cysteine derivatives (16, 20) and that the two proteins in the present study differed most strongly in cysteine content. The whey-derived protein was especially designed to have an even higher cysteine content than normal whey protein, and cysteine is a biosynthetic precursor not only of proteins but also of the cellular antioxidant, glutathione. The facts that the decrease in body cell mass in old age and cancer patients was found to be correlated with an oxidative shift in the plasma redox status (16), and certain redox-sensitive signaling pathways are involved in catabolic processes (reviewed in 11), also support a role of glutathione in wasting. This paradigm links the differential effects of the two proteins in our trial to established molecular mechanisms. The trial confirmed an important prediction of the hypothesis. Oxidative stress has also been implicated in the adverse effects of chemotherapy and radiotherapy (4, 9, 19, 23). Postradiotherapy plasma glutathione was associated with outcome in patients with head and neck squamous cell carcinoma (3).

This study notably demonstrates that a certain protein formulation can be effective by itself as an anticancer cachexia therapy. The superior efficacy of the cysteine-rich protein in comparison to casein is important for these patients, in view of their early satiety and the resulting inability to eat more protein. Both treatment groups showed a mean compliance of $<50\%$ corresponding to $\sim$13 g protein/day. This was reminiscent of the generally low dietary protein intake in cancer patients (14, 21).

The study demonstrates the efficacy of the cysteine-rich protein to support weight gain and increase in body cell mass in patients with advanced non–small cell lung cancer. The effect on survival merits further study. The weight of the evidence is limited by the small number of evaluable patients. As the study focused on the relatively narrow selection of advanced cancer patients who had already shown substantial weight loss but were nevertheless anticipated to survive for at least another 6 months, the recruitment rate was inevitably slow. Nevertheless, the sample size was similar to patient populations in a number of related publications.

The statistical significance in the most important end points under test provides direction for a larger follow-up trial. Muscle function and several quality-of-life parameters were also improved, except in imminently dying patients. The data suggest that the potential efficacy of a therapeutic intervention may be missed if data from imminently dying patients are mixed with data from patients surviving all or a large part of the study period. This point should be prospectively taken into account in the design of trials in the future.

Despite the positive results, it is believed that this treatment addresses only one aspect of cancer cachexia. Even better effects may possibly be achieved if the cysteine-rich protein is combined with other anticachexia treatments.

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ABBREVIATIONS

CRP, C-reactive protein; EPO, erythropoietin; ESAS, Edmonton symptom Assessment Scale; HR, hazard ratios; LOCF, last observation carried forward; PP, per protocol; QOL, quality of life; TNF-α: tumor necrosis factor α.

APPENDIX: PATIENTS AND METHODS

1. Study design

This multicenter, randomized, double-blind phase II study (Health Canada Number 085608) was designed to evaluate the safety and efficacy of the cysteine-rich protein isolate, IMN1207 (Immunotec Research Ltd., Vaudreuil, QC, Canada) in the prevention of wasting in two strata of patients (*i.e.*, patients with metastatic colorectal and stage IIIB-IV non–small cell lung cancer, respectively, over 6 months). The study treatment consisted of oral administration of the cysteine-rich protein or another protein, casein, instead of a placebo. Participants received seven 400-g canisters of the powdered medications twice (*i.e.*, at the start and at the 3-month visit. They also received a 10-g scoop, shaker, blender, and instructions to ingest three scoops (3×10 g) daily. The study medication was administered in conjunction with standard of care for cancer type and stage.

The study was conducted according to the standards of good clinical practice and national regulations. The protocol was approved by the local ethics committees. All patients were informed of all procedures and gave written informed consent. Regular monitoring and sample audits were performed at the trial centers. The sponsor held the data but placed no limitations on data analysis. Investigators participated in the design of the protocol and had full access to the raw data and analysis.

2. Study medication

The amino acid composition of the cysteine-rich protein and the control protein (Table 1) was determined by using an amino acid analyzer. Preparation of cysteine-rich protein and casein, labeling, packaging, stability testing, and shipment were conducted by Immunotec Research, Ltd., in collaboration with WellSpring Pharmaceutical Corporation, Canada. The products met all stability testing specifications of Health Canada.

3. Inclusion and exclusion criteria

Included were metastatic colorectal or non–small-cell lung cancer patients in two separate strata. Here we report the results from the lung cancer patients only. Included were patients of 21 years or older with an involuntary decrease in body weight of $>3\%$ during 3 months immediately preceding study entry, Karnofsky performance status $\geq 70\%$, life expectancy >3 months, serum creatinine <3.0 mg/dl or 265 μM, bilirubin in the normal range, and SGPT/ALT <6 times the upper limit of normal and reliable contraception in the case of women of childbearing potential. Excluded were patients with a history of angioedema, allergic reactions to any agent used in this study, uncontrolled meta-

static brain tumors, milk protein intolerance, ascites, edema, significant anemia, or subjects currently using *N*-acetylcysteine, α-lipoic acid, or dry whey protein supplements.

4. Determination of sample size

Based on historic data, a mean decrease of 4% body weight over a 3-month period with a standard deviation of 4% was expected in the control group. With $\alpha = 0.05$, a sample size of 2×30 patients was expected to give an 80% chance of detecting a hypothetical 3% difference in weight loss between treatment groups.

5. Recruitment, randomization, and evaluation

Patient randomization and data management were performed by GEREQ, Montreal, Quebec. Data monitoring and site visits were performed by Canreg, Inc., Dundas, Ontario. Patients were recruited and screened for eligibility at the clinical trial centers (*i.e.*, Department of Oncology at McGill University, Montreal; Cross Cancer Institute, Edmonton; Juravinski Cancer Center, Hamilton; and Allan Blair Cancer Center, Regina). At a central independent biostatistical company (GEREQ), patients were subsequently stratified into two strata with lung cancer and colorectal cancer, respectively, and randomized into the two treatment arms. The blinding code was kept at the statistician's office. At the trial sites, randomized patients underwent clinical examinations before the start of treatment (week 0), and at week 6, month 3, and month 6. At these time points, additional blood and urine samples were taken for laboratory tests, the Karnofsky performance status, quality of life (QOL), symptoms, and appetite (8), as well as ESAS (6, 7), and hand-grip strength were determined, and a CT scan or MRI was performed as part of patient's cancer therapy. Laboratory tests included hematologic profile, clinical biochemistry, biologic markers, urine analysis, and pregnancy tests (women only). The data were recorded at the trial sites. Compliance, as defined by the intake of study medication, was determined by weighing the returned canisters. Safety was continuously monitored by central collection of records of all serious adverse events including mortality.

6. Primary and secondary endpoints

Primary end points were the relative (%) change in body weight and percentage change in absolute body cell mass over a 6-month period. Secondary end points included the assessment of strength by hand-grip dynamometry, Karnofsky performance status, assessment of the quality of life based on the McGill QOL (8), and "symptom burden" based on the Edmonton Symptom Assessment Scale (ESAS) (6, 7), mortality, changes in glutathione in red blood cells, plasma concentrations of cysteine, erythropoietin (EPO), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and C-reactive protein (CRP), and disease activity (regression, stable disease, or progression).

7. Determination of body cell mass and blood parameters

Body cell mass (16) was determined by bioelectrical impedance analysis [Biodynamics (Model 450), Seattle, WA]. Patients were advised to drink only 400 ml and not to eat during the last 8 hours before analysis. Clinical assessments and some laboratory tests including C-reactive protein (CRP) were performed directly at the trial sites. Plasma amino acids, EPO, IL-6, and TNF-α were analyzed centrally by the Clinical Research and Clinical Trials Laboratory, Hamilton, Ontario, Canada. Free plasma amino acids including cysteine were determined by using an amino acid analyzer. Glutathione was assayed by Immunosciences Lab., Inc., Beverly Hills, CA, U.S.A., by using the commercial test kit BIOXYTECH R GSH-420 TM (catalog no. 21023). Red blood cells were obtained from 0.5 ml blood by centrifugation for 5 min at 2,500 *g* and 4°C. After removal of the plasma, the cells were washed 3 times in cold saline, resuspended in 4 volumes of cold water, and vortexed thoroughly. A volume of 0.1 ml lysate was then mixed with 0.3 ml of "precipitation reagent" (aqueous solution of trichloroacetic acid) in a microcentrifuge tube, vortexed for at least 15 sec, and then centrifuged at 10,000 *g* for 5 min at room temperature. A volume of 0.2 ml of the resulting supernatant was thoroughly mixed with 0.2 ml of a pH 7.8 "buffer" (potassium phosphate/diethylene triaminepentaacetic acid/lubrol) and 0.2 ml "reducing agent" [tris (2-carboxyethyl) phosphine in HCl]. After addition of 0.2 ml "chromogen" (1-methyl-4-chloro-7-trifluoromethylquinolinium methylsulfate in HCl), the solution was again thoroughly mixed and subsequently mixed with 0.2 ml "color developer" (aqueous NaOH solution). After incubation for 30 min at room temperature in the dark, absorbance was measured at 420 nm.

8. Statistical analysis

The statistical analysis was performed by an independent statistical company (Boreal Primum, Montreal, Quebec). The data were analyzed separately for the two strata (disease sites) and expressed as means $\pm$ SD. Treatment effects were statistically evaluated by comparison with the control group (two-sided *t* test for independent variables, if not indicated otherwise) and by comparison with the corresponding baseline values (*t* test for dependent variables). Comparison of the treatment groups was based on the data obtained at 6 months or on the last-observation-carried-forward (LOCF) method for patients who completed at least two visits. Survival curves were generated with the use of Kaplan–Meier estimates for treatment and compared with the log-rank test. Hazard ratios (HRs) were estimated by using a Cox proportional-hazard model. The results were judged by the *p* value. A *p* value <0.05 was regarded as statistically significant.

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