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Safety, Tolerability, and Effect on Quality of Life of a Mixture of Amino Acids and Other Small Molecules in Cancer Patients

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Abstract

We performed two clinical studies to evaluate the safety, tolerability, and effect on quality of life of a product containing a mixture of amino acids, vitamins, and other small molecules. In the first one period, open-label, multiple-dose study, the safety and tolerability of a 1-week administration was evaluated in 24 healthy volunteers. In the second one period, open-label, multiple-dose, single-arm study, we investigated the safety, tolerability, and effect on quality of life of a 4-week administration in 50 cancer patients. The safety assessment included the monitoring of adverse events, changes in physical status, and clinical laboratory tests. Changes in quality of life were measured with the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 version 3.0 (EORTC QLQ-C30). We have found that administration of the investigated product is safe and well tolerated in healthy individuals and in cancer patients. Administration of the product to cancer patients significantly improved their quality of life (EORTC QLQ-C30 global health status score: baseline: 24.17 ± 9.2; end of treatment: 47.08 ± 14.56, $p < 0.001$). To evaluate the anticancer activity of the investigated product in humans, a randomized, blinded, combination clinical trial should be conducted.

Key words: amino acids, cancer, chemotherapy, quality of life, vitamins

Introduction

Cancer is the leading cause of death in the developed countries, with a worldwide death toll of almost 8 million people per year, and the worldwide occurrence is nearly 13 million new cases per year.[1] Despite the significant improvements in conventional therapies (surgery, radiotherapy, and/or chemotherapy) and the application of targeted and/or biological therapies, the high mortality rate of this disease group often motivates patients to seek alternative treatments in order to complement the conventionally applied therapies. In fact ~40% of cancer patients use some kind of complementary and alternative medicine, though there are considerable variations in this ratio determined by the geographic location and socioeconomic factors.[2]

Earlier we have hypothesized that in addition to the immunological and nonimmunological surveillance, a further defense mechanism might operate to prevent the development of tumors.[3] We thought that the small molecules that are selectively accumulated in cancer cells might participate in a nonimmunological antitumor surveillance mechanism, and started to investigate those small molecules (vitamins, amino acids, monosaccharides, nucleobases, etc.) that are differentially taken up by tumor and normal cells.[3] Since the elevated glucose uptake of cancer cells has been first reported by Otto Warburg,[4] it has been shown that many other molecules (amino acids and vitamins) are accumulated in cancer cells.[5,6] This accumulating feature of cancer cells is utilized in positron emission tomography,[7] and targeting strategies have been started to emerge on the basis of amino acid and vitamin accumulation.[8,9]

During our earlier work we have experimentally selected small molecules [L-arginine, L-histidine, L-methionine, L-phenylalanine, L-tyrosine, L-tryptophan, L-ascorbate, D-biotin, pyridoxine, riboflavin, adenine, and L(−)malate] whose mixture (''active mixture'') showed a selective toxic effect *in vitro*

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on a variety of tumor cell lines.[10,11] We have also shown by a series of *in vitro* experiments that the active mixture selectively induces apoptosis in cancer cells, and increases the anticancer effect of cytostatic agents and irradiation.[12–14] Recently we have demonstrated that the active mixture significantly inhibits the growth of experimental murine and human tumors *in vivo*, and treatment with active mixture increases the *in vivo* antitumor activity of cytostatic agents. In a preclinical model of colon cancer (Colon 26 adenocarcinoma) combined treatment with the active mixture and 5-fluorouracil or cisplatin resulted in a significantly enhanced tumor growth inhibition compared with the single treatments. Further, we have provided evidence that the active mixture induces apoptosis of cancer cells via the mitochondrial pathway, induces G1 arrest, and increases the number of apoptotic cells in tumor xenografts.[15]

In this article we describe the results of two clinical trials of a drink powder investigational product that was developed on the basis of the earlier detailed theoretical background and contains the components of the active mixture. In the first study we evaluated the safety and tolerability in healthy volunteers. In the second study we investigated the safety, tolerability, and effect on quality of life in cancer patients.

Materials and Methods

Investigational product

The investigational product was evaluated as a drink powder (manufactured by Meditop Pharmaceutical Ltd., Pilisborosjenő, Hungary for Culevit Ltd., Budapest, Hungary), and has the following composition (in 100 g of the product): L-arginine, 30.7 g; L-histidine, 12.2 g; L-methionine, 11.7 g; L-phenylalanine, 12.9 g; L-tyrosine, 498 mg; L-tryptophan, 6 g; L-ascorbic acid, 13.7 g; D-biotin, 11.8 mg; pyridoxine-hydrochloride, 392 mg; riboflavin-5-phosphate·$2H_2O$, 313 mg; adenine-hydrochloride·$1/2H_2O$, 885 mg; and L-(-)-malic acid, 9.19 mg.

Ethics and conduct of the studies

Both the safety and the quality-of-life studies were performed at the facilities of Drug Research Center Ltd., Balatonfüred, Hungary. The study was conducted in accordance with the European guidelines on Good Clinical Practice (ICH/CPMP/135/95) and Standard Operating Procedures of the clinical investigational site. The study started only after getting the Ethics Committee's written approval. Eligible volunteers signed an informed consent form.

Safety study in healthy volunteers

This study was a one period, open-label, multiple-dose study with the participation of 24 healthy male and female volunteers. The eligibility criteria were the following: age, 18–45 years; body–mass index (BMI = weight/height^2), 18.0–35.0 kg/m^2; ability and willingness to abstain from alcohol, tobacco products, caffeine, methylxanthine-containing beverages or foods (coffee, tea, Coke, chocolate, and ''power drinks''), and grapefruit or grapefruit juice from 48 hours prior to entrance into the clinic until the end of hospitalization.

The study included a screening visit (SC visit, within 14 days prior to treatment initiation), a day-1 visit (D1 visit, on the first day of treatment), a day-8 visit (D8 visit, on the day after treatment termination), and a follow-up visit (FUP visit, within 5 days after the last dosing).

Each volunteer was hospitalized 14 hours before the first dose and stayed in hospital for 8 days. Volunteers received 25.5 g/day of the investigational product dissolved each morning in 600 mL of water administered in 10 equal doses per day for 7 consecutive days. The safety assessment included monitoring for occurrence of adverse events (AEs), changes in physical status (vital signs, blood pressure, and heart rate), ECG, and clinical laboratory tests. The AEs were recorded every day during the hospitalization period. Just before product administration and twice a day during the treatment period, subjects were asked nonleading questions to determine the occurrence of AEs. In addition, all AEs reported spontaneously during the course of the study were recorded. The causality relationship of AEs to the administration of the investigational product was determined by the principal investigator according to the European guidelines on Good Clinical Practice (ICH/CPMP/135/95), and on the basis of the 2011/C 172/01 European guideline. ''Detailed guidance on the collection, verification, and presentation of AE/reaction reports arising from clinical trials on medicinal products for human use'' using the following categories: definitely related, probably related, possibly related, probably not related, and not related. The following safety parameters were measured on the SC, D1, D8, and FUP visits: clinical chemistry: sodium, potassium, chloride, glucose, total protein, cholesterol, triglyceride, uric acid, urea, creatinine, total bilirubin, alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), aspartate aminotransferase (AST, SGOT), alanine transaminase (ALT, SGPT), and lactate dehydrogenase (LDH); hematology: leucocytes (WBC), erythrocytes (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, lymphocytes, monocytes, eosinophils, basophils, and neutrophils; urinalysis: hemoglobin, urobilinogen, ketones, glucose, protein, specific gravity, blood, pH, and nitrites; vital signs: blood pressure, pulse rate (after 5 minutes of rest in the supine position), and standard 12-lead ECG (PR-interval, QRS-duration, QT-interval, RR-interval, and QTc-interval).

Quality-of-life study in cancer patients

This study was a one period, open-label, multiple-dose, single-arm study with the participation of 50 male and female cancer patients with different types of stage I–IV-malignant tumors. The eligibility criteria were the following: age, 18–65 years; BMI = weight/height^2, 16.0–40.0 kg/m^2; I–IV-stage cancer patients who are either receiving ongoing chemo- or radiotherapy (at any phase of the treatment), or not receiving ongoing chemo- or radiotherapy, but whose cancer is verified by the latest (not older than 3 months) medical documentation (hospital evaluation report or diagnostic imaging examination report [CT, MRI, or PET scan]); life expectancy, more than 6 months; and European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 version 3.0 (EORTC QLQ-C30) global health status score, under 30.

The study contained an SC visit (within 14 days prior to treatment initiation), three visits during the treatment period (D1, D7, and D28 visits, on the 1st, 7th, and 28th days of the treatment period), and an FUP visit (within 7 days after the last dosing). The patients received the first dose of the study

product at the investigational site. After this the patients continued to participate in the study as outpatients. The study participants received 25.5 g/day of the investigational product dissolved each morning in 600 mL of water administered in 10 equal doses per day for 28 consecutive days.

Safety assessments were performed at the SC, D1, D7, D28, and FUP visits and included the monitoring of occurrence of AEs, examination of the causality relationship of AEs to the administration of the investigational product, and the measurement of the same safety parameters as described for the safety study in healthy volunteers.

For efficacy assessment the EORTC QLQ-C30 was filled out at the SC, D1, and D28 visits.

Statistical analysis

Continuous laboratory variables of the safety study were analyzed by descriptive statistics performed for each visit, comprising the mean, median, minimum, maximum, standard deviation, and number of cases. Proportion of normal/abnormal laboratory values was also calculated. Categorical laboratory values were analyzed by frequencies and the number of cases for each visit.

In case of the quality-of-life study, the evaluation included descriptive statistics and hypothesis tests. Both safety and efficacy analyses were performed. In the safety analysis, descriptive statistics and individual data listings were used. Descriptive statistics included case number, mean, standard deviation, confidence interval, minimum, and maximum for continuous data; and frequency and proportion for categorical data. Efficacy analysis concentrated on the results of the EORTC QLQ-C30; descriptive statistics (case number, mean, standard deviation, confidence interval, minimum, and maximum) were applied for the quality-of-life value, and for the functional and symptom scales. Hypothesis testing was performed to examine the mean change of EORTC QLQ-C30 score values from the baseline to the last measurement. Kolmogorov–Smirnov test was used to test normality. Paired-sample *t*-test was used for normally distributed variables. For not normally distributed variables, Wilcoxon signed rank test was used. Statistical analyses were performed with the use of SPSS 19.0.0 software.

Results and Discussion

Administration of the investigational product is safe in healthy volunteers

The safety study included 12 male and 12 female healthy volunteers. All volunteers were Caucasians and Hungarian citizens. The mean age was 31.11 ± 7.374 years. Safety clinical chemistry and hematology laboratory parameters measured on the SC, D1, D8, and FUP visits showed no significant alterations compared with the screening values (Table 1). Urinalysis (hemoglobin, urobilinogen, ketones, glucose, protein, specific gravity, blood, pH, and nitrites), measurement of vital signs (blood pressure and pulse rate), standard 12-lead ECG (PR-interval, QRS-duration, QT-interval, RR-interval, and QTc-interval), and physical examination also failed to reveal any significant change compared with the screening values (data not shown).

There were no serious adverse events (SAEs) during the study. Seven AEs with mild or moderate intensities occurred in three volunteers (Table 2). One volunteer (R016) was dropped out from the study on the fourth day of the treatment period due to infectious mononucleosis; however, data of this volunteer were also included in the safety analysis. The relationship between the AEs and the investigational product was evaluated. Six AEs were judged not to be related to the study product and one AE (diarrhea) was considered probably not related to the investigational product.

The objective of this study was to evaluate the safety and tolerability of multiple doses of the investigational product in healthy volunteers. On the basis of our results the administered dose of the study product was safe and well-tolerated by healthy volunteers. The observed good safety profile of the investigational product is in agreement with previously reported data on the safety of the individual components. In fact the amount of L-arginine, L-histidine, L-phenylalanine, L-tyrosine, L-tryptophan, L-ascorbic acid, pyridoxine, and adenine present in the administered daily amount of the product (25.5 g) is below the amount specified in the maximum recommended therapeutic dose (MRTD) database.[16] The biotin content (3 mg) present in the administered daily amount of the product was 900-times higher than the amount specified in the MRTD database; however, there are reports of the safe use of pharmacological doses of biotin in the range of 0.6–20 mg/single dose.[17,18] The administered daily amount of riboflavin (80 mg) was 160-times higher than the amount specified in the MRTD database; however, there are reports of the safe use of 200–400 mg riboflavin per day.[19,20] Malic acid and methionine are not listed in the MRTD database; however, there are reports of the safe use of these substances (2.4 and 6 g, respectively) in the amount that is comparable with the daily administered amount (2.5 and 3 g, respectively) in our safety study.[21,22] Due to possible matrix effects, the safety profile of a mixture of 12 components could not be perfectly estimated on the basis of the safety of the individual components. Therefore, our results provide additional evidence on the safety of these substances when they applied in combination.

Administration of the investigational product is safe and improves quality of life in cancer patients

The quality-of-life study successfully recruited 50 patients. The intention to treat (ITT) population was defined as all subjects who received at least one dose of study medication. The ITT population contained 50 patients. All of these patients had D1 visit, but only 45 had D7 visit, 42 had D28 visit, and 40 had FUP visit. The per protocol (PP) population was defined as all subjects who received all doses of study medication and had no major protocol violations. The PP population included 40 patients. Three out of the 10 drop-outs occurred due to AEs. Two of these AEs (pneumonia and anemia) were serious and judged by the principal investigator not to be related to the administration of the study drug, while one of these AEs (gastric pain) had moderate intensity and was definitely related to the study drug. The remaining seven drop-outs occurred either because the patients failed to attend the scheduled study visit(s), or the patients changed their mind about their willingness to participate in the study. All patients were Caucasians with a mean age of 54.76 ± 12.11 and 54.50 ± 12.81 for the ITT and PP populations, respectively. The gender distribution was the following: women, 54% (ITT) and 55% (PP); men, 46% (ITT) and 45% (PP). The ITT

Table 1. Clinical Chemistry and Hematology Laboratory Parameters Measured During the Safety Study

Parameter	*Visit*	*Mean*	*SD*	*Parameter*	*Visit*	*Mean*	*SD*
Na (mM)	Screening	142.6	1.761	LDH (U/L)	Screening	284.9	41.20
	Day 1	142.6	1.596		Day 1	283.8	73.91
	Day 8	143.0	1.182		Day 8	266.8	65.31
	Follow-up	141.5	1.492		Follow-up	306.3	79.78
K (mM)	Screening	4.602	0.352	WBC (g/L)	Screening	6.858	1.538
	Day 1	4.441	0.397		Day 1	7.021	1.737
	Day 8	4.305	0.335		Day 8	7.333	1.685
	Follow-up	4.330	0.374		Follow-up	6.929	1.788
Cl (mM)	Screening	106.1	1.895	RBC (T/L)	Screening	4.768	0.402
	Day 1	106.1	1.700		Day 1	4.601	0.441
	Day 8	105.4	1.884		Day 8	4.785	0.458
	Follow-up	104.3	1.397		Follow-up	4.729	0.404
Glucose (mM)	Screening	5.124	0.356	Hemoglobin (mM)	Screening	8.879	0.764
	Day 1	4.403	0.478		Day 1	8.488	0.723
	Day 8	4.672	0.478		Day 8	8.808	0.783
	Follow-up	4.705	0.361		Follow-up	8.625	0.628
Total protein (g/L)	Screening	75.75	3.548	Hematocrit (L/L)	Screening	0.436	0.036
	Day 1	67.62	4.750		Day 1	0.413	0.041
	Day 8	72.16	3.151		Day 8	0.432	0.036
	Follow-up	72.15	3.153		Follow-up	0.423	0.031
Cholesterol (mM)	Screening	5.092	0.860	MCV (fl)	Screening	91.38	3.386
	Day 1	4.500	0.796		Day 1	90.08	4.529
	Day 8	4.288	0.775		Day 8	90.54	6.171
	Follow-up	4.338	0.746		Follow-up	89.67	3.535
Triglyceride (mM)	Screening	0.979	0.372	MCH (fmol)	Screening	1.864	0.079
	Day 1	1.088	0.396		Day 1	1.848	0.085
	Day 8	1.516	0.654		Day 8	1.842	0.098
	Follow-up	0.898	0.416		Follow-up	1.826	0.080
Uric acid (μM)	Screening	295.9	86.53	MCHC (mM)	Screening	20.40	0.301
	Day 1	276.4	71.35		Day 1	20.56	0.456
	Day 8	266.7	64.38		Day 8	20.36	0.581
	Follow-up	288.7	70.91		Follow-up	20.38	0.261
Urea (mM)	Screening	5.183	1.075	Platelets (g/L)	Screening	259.6	45.14
	Day 1	5.017	0.969		Day 1	239.3	44.34
	Day 8	4.571	0.894		Day 8	255.3	43.23
	Follow-up	4.542	1.055		Follow-up	265.7	39.22
Creatinine (μM)	Screening	70.92	14.12	Lymphocytes (%)	Screening	32.41	6.320
	Day 1	70.92	14.03		Day 1	39.90	6.021
	Day 8	70.50	13.45		Day 8	34.27	6.095
	Follow-up	68.25	12.00		Follow-up	36.03	7.169
Total bilirubin (μM)	Screening	8.804	5.896	Monocytes (%)	Screening	6.733	1.613
	Day 1	9.938	5.384		Day 1	7.917	2.601
	Day 8	9.142	6.072		Day 8	7.304	1.727
	Follow-up	8.704	4.089		Follow-up	7.350	1.839
ALP (U/L)	Screening	164.2	43.12	Eosinophils (%)	Screening	2.717	1.328
	Day 1	150.5	39.95		Day 1	3.388	1.518
	Day 8	162.3	41.47		Day 8	3.042	1.658
	Follow-up	167.0	41.71		Follow-up	3.129	2.220
GGT (U/L)	Screening	22.29	12.55	Basophils (%)	Screening	0.171	0.046
	Day 1	18.21	10.33		Day 1	0.279	0.198
	Day 8	20.00	11.55		Day 8	0.304	0.268
	Follow-up	20.42	9.753		Follow-up	0.263	0.097
AST (SGOT) (U/L)	Screening	21.63	4.362	Neutrophils (%)	Screening	57.93	7.123
	Day 1	18.96	8.795		Day 1	48.51	6.118
	Day 8	20.33	7.051		Day 8	54.89	6.660
	Follow-up	22.92	11.91		Follow-up	53.23	8.220
ALT (SGPT) (U/L)	Screening	21.33	6.882				
	Day 1	17.25	10.65				
	Day 8	24.21	12.33				
	Follow-up	25.88	16.74				

$n = 24$.

ALP, alkaline phosphatase; GGT, γ-glutamyl transferase; AST (SGOT), aspartate aminotransferase; ALT (SGPT), alanine transaminase; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; SD, standard deviation.

Table 2. Adverse Events Occurred During the Safety Study

Number of AEs	*Study day of occurrence*	*Randomization number*	*Description of AE*	*MedDRA PT*	*MedDRA code*	*Intensity*	*Relation to study product*
1	Day 1	R014	Headache	Headache	10019211	Mild	Not related
2	Day 2	R014	Headache	Headache	10019211	Mild	Not related
3	Day 3	R014	Headache	Headache	10019211	Mild	Not related
4	Day 6	R015	Diarrhea	Diarrhea	10012735	Mild	Probably not related
5	Day 7	R015	Nausea	Nausea	10028813	Moderate	Not related
6	Day 7	R015	Vomiting	Vomiting	10047700	Moderate	Not related
7	Day 1	R016	Frequent defecation, expansion, increased AST (56 U/L), ALT (59 U/L), and LDH (529 U/L) values, Epstein–Barr virus serology (VCA-IgG): 481 E/mL	Infectious mononucleosis	10021914	Moderate	Not related

AE, adverse event; MedDRA PT, Medical Dictionary for Regulatory Activities Preferred Terms.

population contained 14 patients with breast cancer, 10 patients with lung cancer, 4 patients with colon cancer, 4 patients with rectal cancer, 3 patients with head and neck cancer, 2 patients with gastric cancer, 2 patients with Hodgkin lymphoma, 2 patients with non-Hodgkin lymphoma, and 1 patient with each of the following cancers: thyroid cancer, liver cancer, liposarcoma, melanoma, pancreatic cancer, prostate cancer, kidney cancer, bladder cancer, and vaginal cancer. The diagnoses [with wording taken from the medical report(s) of the patient], the date of diagnosis, and the staging data of the disease for the ITT population are summarized in Table 3.

Safety analysis was performed on the ITT population. Clinical chemistry and hematology laboratory parameters measured on the SC, D1, D7, D28, and FUP visits showed no significant alterations compared with the screening values (Table 4). The additional safety examinations (urinalysis, measurement of vital signs, standard 12-lead ECG, and physical examination) also verified that the administration of the investigational product causes no significant change in the safety parameters compared with the screening values (data not shown). Two SAEs in 2 patients, and 14 AEs in 9 patients occurred during the study. The two SAEs were judged not to be related to the administration of the investigational product. Among the 14 AEs, 10 were not related, 1 was possibly related, and 3 were definitely related to the administration of the investigational product. The administration of the investigational product was stopped in case of the two SAEs and in the case of one AE. The SAEs and AEs occurred during the study are summarized in Table 5. It is important to note that the all of the AEs that were definitely

Table 3. Description of the Diagnoses, Date of Diagnosis, and Staging of Patients in the Quality-of-Life Study

Case number	*Randomization number*	*Diagnosis*	*Date of diagnosis*	*Stage*
1	1	Invasive ductal cancer of the breast	01/2011	T2N1M0
2	4	Non-Hodgkin lymphoma	10/2004	4b
3	6	Planocellular carcinoma of the hypopharynx	06/2010	III
4	9	Lower gum carcinoma	09/2011	T2N0M0
5	15	Breast cancer with pulmonary metastasis	02/2006	T1N2M1
6	16	Adenocarcinoma of the lung	01/2011	T2N0M0
7	29	Pulmonary carcinoma	01/2011	T4N2M0
8	37	Pulmonary carcinoma	09/2010	III
9	38	Non-Hodgkin lymphoma	11/2011	IV
10	39	Adenocarcinoma of the breast with vertebral metastasis	01/2012	T3N1M1
11	41	Follicular thyroid cancer with pulmonary, mediastinal, and bone metastasis	01/2004	T0N3M1
12	42	Cecal tumor	10/2011	T4N2M1
13	43	Sigmoid colon adenocarcinoma with liver metastasis	11/2011	T3N1M1
14	44	Pulmonary adenocarcinoma with brain, bone, and pulmonary metastasis	01/2010	T4N2M1
15	45	Planocellular carcinoma of the lungs	10/2011	T2N1M1
16	46	Pulmonary adenocarcinoma	01/2012	T2N2M0

(*continued*)

TABLE 3. (CONTINUED)

Case number	*Randomization number*	*Diagnosis*	*Date of diagnosis*	*Stage*
17	47	Malignant melanoma	12/2009	T3N3M1
18	48	Breast carcinoma, invasive ductal form	08/2011	T2N2M1
19	49	Prostate adenocarcinoma	02/2012	T2N0M0
20	50	Cancer of the rectum with pulmonary metastasis	01/2009	IV
21	51	Rectal carcinoma with liver metastasis	08/2011	T3N2M1
22	52	Colon tumor	11/2011	T3N1M0
23	53	Pulmonary adenocarcinoma	01/2011	T3N1M1
24	54	Right renal tumor	02/2012	T1N0M0
25	55	Pulmonary adenocarcinoma on the right side with pulmonary and mediastinal metastasis	08/2011	T4N2M1
26	56	Left breast carcinoma	11/2011	T1N0M0
27	57	Hodgkin lymphoma	12/2011	IV.B
28	58	Left inguinal liposarcoma with pulmonary metastasis	08/2011	T2N2M1
29	59	Right breast carcinoma with liver metastasis	04/2004	T2N1M1
30	60	Breast cancer	11/2011	T1N0M0
31	61	Rectal adenocarcinoma	09/2011	T3N1M0
32	62	Hepatocellular carcinoma	02/2012	T2N0M0
33	63	Pancreas adenocarcinoma	01/2012	T3N1M0
34	64	Vaginal carcinoma	02/2012	T1N0M0
35	65	Adenocarcinoma of the colon with pulmonary metastasis	01/2009	IV
36	66	Right breast carcinoma with bone and liver metastasis	02/2012	T1N1M1
37	67	Left breast carcinoma	02/2012	T2N1M0
38	68	Gastric tumor	02/2012	T1N0M0
39	69	Hodgkin lymphoma	11/2011	T2M1N1
40	70	Planocellular carcinoma of the hypopharynx	04/2012	T1N1M0
41	71	Right breast carcinoma	04/2012	T1N1M0
42	72	Gastric tumor	04/2012	T2N1M0
43	101	Breast cancer	04/2011	II
44	102	Breast cancer	12/2011	II
45	103	Bladder tumor	01/1996	I
46	104	Invasive ductal breast carcinoma	10/2011	II
47	105	Adenocarcinoma of the lung	09/2010	IV
48	106	Adenocarcinoma of the rectum	08/2011	IV
49	107	Planocellular carcinoma of the lung	04/2012	I
50	237	Right breast carcinoma with pulmonary and bone metastasis	02/2006	T1N1M1

TABLE 4. CLINICAL CHEMISTRY AND HEMATOLOGY LABORATORY PARAMETERS MEASURED IN THE INTENTION-TO-TREAT POPULATION DURING THE QUALITY-OF-LIFE STUDY

Parameter	*Visit*	*Mean*	*SD*	*Parameter*	*Visit*	*Mean*	*SD*
Na (mM)	Screening	139.59	2.721	LDH (U/L)	Screening	343.70	121.029
	Day 1	140.18	2.554		Day 1	348.70	119.545
	Day 7	139.97	2.230		Day 7	337.64	103.451
	Day 28	139.27	2.808		Day 28	371.57	205.091
	Follow-up	139.35	3.256		Follow-up	355.73	157.273
K (mM)	Screening	4.36	0.387	WBC (g/L)	Screening	7.51	4.581
	Day 1	4.40	0.401		Day 1	8.09	5.985
	Day 7	4.35	0.423		Day 7	8.11	3.560
	Day 28	4.35	0.419		Day 28	7.92	3.575
	Follow-up	4.49	0.552		Follow-up	7.84	4.702
Cl (mM)	Screening	102.46	3.255	RBC (T/L)	Screening	4.17	0.543
	Day 1	102.30	3.710		Day 1	4.13	0.561
	Day 7	102.69	3.525		Day 7	4.16	0.518
	Day 28	102.03	3.133		Day 28	4.18	0.484
	Follow-up	101.27	3.812		Follow-up	4.14	0.473
Glucose (mM)	Screening	5.81	1.519	Hemoglobin (mM)	Screening	7.84	1.069
	Day 1	5.87	2.053		Day 1	7.79	1.150
	Day 7	6.10	2.110		Day 7	7.87	0.927

(*continued*)

Table 4. (Continued)

Parameter	*Visit*	*Mean*	*SD*	*Parameter*	*Visit*	*Mean*	*SD*
	Day 28	6.47	2.333		Day 28	7.88	0.883
	Follow-up	6.07	2.433		Follow-up	7.83	0.837
Total Protein (g/L)	Screening	71.70	5.354	Hematocrit (L/L)	Screening	0.37	0.051
	Day 1	70.43	5.517		Day 1	0.37	0.054
	Day 7	71.27	5.413		Day 7	0.38	0.048
	Day 28	70.08	5.594		Day 28	0.38	0.042
	Follow-up	71.62	4.424		Follow-up	0.37	0.040
Cholesterol (mM)	Screening	4.96	1.139	MCV (fl)	Screening	88.42	7.508
	Day 1	4.78	1.121		Day 1	89.68	8.314
	Day 7	4.80	1.174		Day 7	90.44	6.989
	Day 28	4.85	1.293		Day 28	90.24	6.555
	Follow-up	4.95	1.587		Follow-up	90.05	7.005
Triglyceride (mM)	Screening	1.79	1.351	MCH (fmol)	Screening	1.88	0.167
	Day 1	1.95	1.385		Day 1	1.88	0.178
	Day 7	2.03	1.638		Day 7	1.89	0.128
	Day 28	2.06	1.702		Day 28	1.89	0.128
	Follow-up	2.11	1.565		Follow-up	1.89	0.124
Uric acid (μM)	Screening	287.82	95.404	MCHC (mM)	Screening	21.28	0.774
	Day 1	285.12	88.025		Day 1	20.98	0.731
	Day 7	286.47	72.880		Day 7	20.97	0.705
	Day 28	298.79	81.667		Day 28	20.96	0.681
	Follow-up	279.39	73.419		Follow-up	21.05	0.782
Urea (mM)	Screening	5.58	1.732	Platelets (g/L)	Screening	294.02	98.278
	Day 1	5.43	1.578		Day 1	296.90	114.066
	Day 7	6.06	1.727		Day 7	299.24	122.350
	Day 28	6.28	2.182		Day 28	288.38	98.428
	Follow-up	5.89	1.607		Follow-up	277.35	117.282
Creatinine (μM)	Screening	70.68	17.978	Lymphocytes (%)	Screening	26.02	13.424
	Day 1	70.56	18.211		Day 1	27.90	12.872
	Day 7	69.27	15.241		Day 7	24.91	11.218
	Day 28	69.83	15.765		Day 28	24.12	8.892
	Follow-up	71.18	15.312		Follow-up	26.47	10.137
Total bilirubin (μM)	Screening	8.10	4.083	Monocytes (%)	Screening	7.58	2.988
	Day 1	7.42	4.636		Day 1	8.58	4.691
	Day 7	6.70	3.221		Day 7	7.19	2.546
	Day 28	7.28	4.888		Day 28	7.06	2.707
	Follow-up	6.39	3.131		Follow-up	7.71	2.696
ALP (U/L)	Screening	227.40	101.626	Eosinophils (%)	Screening	2.51	2.157
	Day 1	237.06	122.189		Day 1	2.42	1.452
	Day 7	213.00	71.131		Day 7	2.27	1.214
	Day 28	210.60	64.897		Day 28	2.10	1.102
	Follow-up	216.10	76.016		Follow-up	2.28	1.099
GGT (U/L)	Screening	50.46	45.174	Basophils (%)	Screening	1.29	6.800
	Day 1	48.02	54.818		Day 1	0.55	0.830
	Day 7	44.71	38.147		Day 7	0.31	0.147
	Day 28	49.88	51.315		Day 28	0.30	0.131
	Follow-up	45.85	39.988		Follow-up	0.35	0.204
AST (SGOT) (U/L)	Screening	25.00	19.784	Neutrophils (%)	Screening	62.79	15.098
	Day 1	26.08	24.299		Day 1	60.27	15.258
	Day 7	26.11	12.274		Day 7	64.89	11.698
	Day 28	27.40	14.919		Day 28	66.42	9.788
	Follow-up	25.35	8.763		Follow-up	62.76	10.885
ALT (SGPT) (U/L)	Screening	23.32	15.421				
	Day 1	22.30	18.219				
	Day 7	24.71	16.682				
	Day 28	27.50	22.524				
	Follow-up	27.30	17.960				

$n = 50$ at screening; $n = 50$ at day 1; $n = 45$ at day 7; $n = 42$ at day 28; $n = 40$ at follow-up.

Table 5. Adverse Events Occurred During the Quality-of-Life Study

Randomization number	*Visit when the (S)AE was reported*	*Description of (S)AE*	*MedDRA SOC*	*MedDRA PT*	*Intensity*	*Administration of the study product*	*Relation to the study product*
SAEs							
38	Day 28	Hospitalization due to fever (40°C) and cough. Pneumonia and viral infection were diagnosed.	Surgical and medical procedures (10042613)	Hospitalization (10054112)	Severe	Stopped	Not related
			General disorders and administration site conditions (10018065)	Pyrexia (10037660)			
			Respiratory, thoracic, and mediastinal disorders (10038738)	Cough (10011224)			
			Infections and infestations (10021881)	Pneumonia (10035664)			
			Infections and infestations (10021881)	Louping ill (10024887)			
68	Day 7	Hospitalization due to serious anemia	Surgical and medical procedures (10042613)	Hospitalization (10054112)	Severe	Stopped	Not related
			Blood and lymphatic system disorders (10005329)	Anemia (10002034)			
AEs							
15	Day 7	Vomiting	Gastrointestinal disorders (10017947)	Vomiting (10047700)	Mild	Continued	Not related
15	Day 28	Nausea, vomiting	Gastrointestinal disorders (10017947)	Vomiting (10047700)	Mild	Continued	Definitely related
15	Follow-up	Acute bronchitis	Respiratory, thoracic, and mediastinal disorders (10038738)	Acute bronchitis (10000687)	Moderate	Not applicable	Not related
47	Day 28	Intense weakness, indisposition	General disorders and administration site conditions (10018065)	Asthenia (10003549) Malaise (10025482)	Moderate	Continued	Not related
60	Day 7	Vomiting	Gastrointestinal disorders (10017947)	Vomiting (10047700)	Severe	Continued	Not related
60	Day 28	Nausea, vomiting	Gastrointestinal disorders (10017947)	Vomiting (10047700)	Moderate	Continued	Not related
62	Day 28	Gastric pain	Gastrointestinal disorders (10017947)	Abdominal pain upper (10000087)	Moderate	Stopped	Definitely related
66	Day 28	Bone pain	Musculoskeletal and connective tissue disorders (10028395)	Bone pain (10006002)	Severe	Continued	Not related
66	Follow-up	Bone pain	Musculoskeletal and connective tissue disorders (10028395)	Bone pain (10006002)	Severe	Not applicable	Not related
69	Follow-up	Gastric pain	Gastrointestinal disorders (10017947)	Abdominal pain upper (10000087)	Moderate	Not applicable	Definitely related
71	Day 28	Gastric pain	Gastrointestinal disorders (10017947)	Abdominal pain upper (10000087)	Mild	Continued	Possibly related
71	Follow-up	Gastric pain	Gastrointestinal disorders (10017947)	Abdominal pain upper (10000087)	Mild	Not applicable	Not related
103	Day 1	Risen pain on the left side of the pubic bone, pain around sacrum	Musculoskeletal and connective tissue disorders (10028395)	Bone pain (10006002)	Moderate	Continued	Not related
105	Day 7	Risen pain	General disorders and administration site conditions (10018065)	Pain (10033371)	Moderate	Continued	Not related

SAE, serious adverse event; MedDRA SOC, Medical Dictionary for Regulatory Activities System Organ Class.

Table 6. Change of the EORTC QLQ-C30 Global Health Status/Quality-of-Life Value in the Quality-of-Life Study

Visit	*Mean*	*SD*	*95% CI*	p
Screening	23.9583	9.47096	[20.93; 26.99]	–
Day 1	24.1667	9.20640	[21.22; 27.11]	–
Day 28	47.0833	14.56271	[42.43; 51.74]	<0.001

$n=40$; p-value (day 28 vs. day 1, Wilcoxon signed rank test).
EORTC QLQ-C30, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 version 3.0; 95% CI, 95% confidence interval.

or possibly related to the administration of the study product affected the gastrointestinal system, and manifested as nausea, vomiting, or gastric pain with mild to moderate intensity.

On the basis of our results the administered dose of the investigational product was safe and well-tolerated by cancer patients. The similarly good safety profile of the study product observed in healthy volunteers and cancer patients indicates that there are no major differences between these populations in factors that determine the safety of the investigated product. However, due to the study-product-related gastrointestinal AEs observed in cancer patients, the occurrence of nausea, vomiting, and gastric pain should be closely monitored during the administration of the investigational product, especially if the patient has a gastric disorder in his/her medical history.

Efficacy analysis was performed on both the ITT and PP populations on the basis of the answers given to the questions of the EORTC QLQ-C30 at the SC, D1, and D28 visits. Differences between the values obtained at the D1 and D28 visits were compared. Essentially the same results were obtained for both populations; therefore, only the results of the PP population are presented. The average value of the global health status/quality of life has increased significantly with a change of 22.92 ($p<0.001$, Table 6). Evaluation of the symptom scales of the EORTC QLQ-C30 revealed that administration of the study product significantly decreased the scores of fatigue (change: -19.17, $p<0.001$), pain (change: -18.38, $p<0.001$), dyspnea (change: -11.67, $p=0.019$), and diarrhea (change: -16.67, $p=0.003$), while the changes in the scores of nausea and vomiting, insomnia, appetite lost, and constipation were not found to be statistically significant (Table 7). Analysis of the functional scales of the EORTC QLQ-C30 showed that administration of the study product significantly increased the scores of physical functioning (change: 9.5, $p=0.001$), role functioning (change: 18.75, $p<0.001$), emotional functioning (change: 20.32, $p<0.001$), cognitive functioning (change: 13.33, $p=0.007$), and social functioning (change: 14.59, $p=0.009$, Table 8). On the basis of these results, the investigational product improved the quality of life of cancer patients through at least two ways. On one hand, it alleviated some of the negative symptoms associated with the disease or the applied conventional therapy; on the other hand, it improved physical, behavioral, and effective functions.

The observed improvements in the quality of life of cancer patients may or may not be related to the experimentally demonstrated anticancer effects of the active mixture mentioned in the ''Introduction.'' Therefore, a

Table 7. Change of the EORTC QLQ-C30 Symptom Scales in the Quality-of-Life Study

Symptom scale	*Visit*	*Mean*	*SD*	*95% CI*	p
Fatigue	Screening	66.9444	22.71358	[59.68; 74.21]	–
	Day 1	63.0556	22.97686	[55.71; 70.40]	–
	Day 28	43.8889	24.51815	[36.05; 51.73]	<0.001[a]
Pain	Screening	60.8333	28.87985	[51.60; 70.07]	–
	Day 1	55.5556	30.66832	[45.61; 65.50]	–
	Day 28	37.1795	26.61770	[28.55; 45.81]	<0.001[a]
Dyspnea	Screening	28.3333	31.62278	[18.22; 38.45]	–
	Day 1	34.1667	36.58304	[22.47; 45.87]	–
	Day 28	22.5000	29.61048	[13.03; 31.97]	0.019[b]
Diarrhea	Screening	20.8333	27.92695	[11.90; 29.76]	–
	Day 1	27.5000	31.92539	[17.29; 37.71]	–
	Day 28	10.8333	19.07774	[4.73; 16.93]	0.003[b]
Nausea and vomiting	Screening	15.8333	25.30441	[7.74; 23.93]	–
	Day 1	18.7500	27.26558	[10.03; 27.47]	–
	Day 28	10.4167	22.22890	[3.31; 17.53]	0.122[b]
Insomnia	Screening	58.3333	34.38512	[47.34; 69.33]	–
	Day 1	47.5000	35.31503	[36.21; 58.79]	–
	Day 28	37.5000	30.37046	[27.79; 47.21]	0.116[a]
Appetite lost	Screening	32.5000	31.56642	[22.40; 42.60]	–
	Day 1	35.0000	31.07752	[25.06; 44.94]	–
	Day 28	25.0000	30.89362	[15.12; 34.88]	0.063[b]
Constipation	Screening	25.0000	34.38512	[14.00; 36.00]	–
	Day 1	20.8333	32.63150	[10.40; 31.27]	–
	Day 28	17.5000	26.13520	[9.14; 25.86]	0.515[b]

$n=40$; p-value (day 28 vs. day 1).
[a]For normally distributed values paired-sample t-test was used.
[b]For not normally distributed values Wilcoxon signed rank test was used.

Table 8. Change of the EORTC QLQ-C30 Functional Scales in the Quality-of-Life Study

Functional scale	*Visit*	*Mean*	*SD*	*95% CI*	p
Physical functioning	Screening	59.6667	20.19562	[53.21; 66.13]	–
	Day 1	56.6667	23.09401	[49.28; 64.05]	–
	Day 28	66.1667	18.07786	[60.39; 71.95]	0.001[a]
Role functioning	Screening	40.0000	28.44498	[30.90; 49.10]	–
	Day 1	42.5000	31.56642	[32.40; 52.60]	–
	Day 28	61.2500	22.76709	[53.97; 68.53]	< 0.001[a]
Emotional functioning	Screening	42.5000	25.58434	[34.32; 50.68]	–
	Day 1	43.6404	24.46378	[35.60; 51.68]	–
	Day 28	63.9583	25.83282	[55.70; 72.22]	< 0.001[a]
Cognitive functioning	Screening	64.5833	24.22223	[56.84; 72.33]	–
	Day 1	61.6667	25.09242	[53.64; 69.69]	–
	Day 28	75.0000	26.68803	[66.46; 83.54]	0.007[b]
Social functioning	Screening	49.5833	34.07038	[38.69; 60.48]	–
	Day 1	53.3333	32.51123	[42.94; 63.73]	–
	Day 28	67.9167	27.05579	[59.26; 76.57]	0.122[a]

$n = 40$; p-value (day 28 vs. day 1).
[a]For normally distributed values paired-sample t-test was used.
[b]For not normally distributed values Wilcoxon signed rank test was used.

randomized, blinded, combination clinical trial with appropriately selected primary endpoint (response based or progression-free survival based) is needed to evaluate the anticancer activity in humans.

Conclusions

We have found that administration of a mixture of amino acids, vitamins, and other small molecules is safe and well tolerated both in healthy individuals and in cancer patients. Administration of the investigational product to cancer patients significantly improved the quality of life with a decrease in the scores of certain symptom scales and an increase in the scores of certain functional scales of the EORTC QLQ-C30. On the basis of the results of the clinical trials, the investigational product has been registered in the category of Food for Special Medical Purposes (FSMP) and is on the market under the trade name of Culevit® Drink Powder in Hungary. The application of Culevit Drink Powder is recommended to cancer patients as a complementary treatment along the conventional therapies (surgery, radio-, chemo-, and biological therapies). To evaluate the anticancer activity of Culevit Drink Powder in humans, a randomized, blinded, combination clinical trial should be conducted.

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Disclosure Statement

Gyula Kulcsár owns a 15% share in Culevit Ltd.

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