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(54) **METHODS USING TCF -II**

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(57) **ABSTRACT**

The present invention provides an agent for preventing and/or treating cachexia comprising TCF-II as an effective ingredient. An agent for preventing and treating cachexia caused by cancer, acquired immunodeficient syndrome (AIDS), cardiac diseases, infectious disease, shock, burn, endotoxemia, organ inflammation, surgery, diabetes, collagen diseases, radiotherapy, chemotherapy is provided by the present invention.

Fig. 1

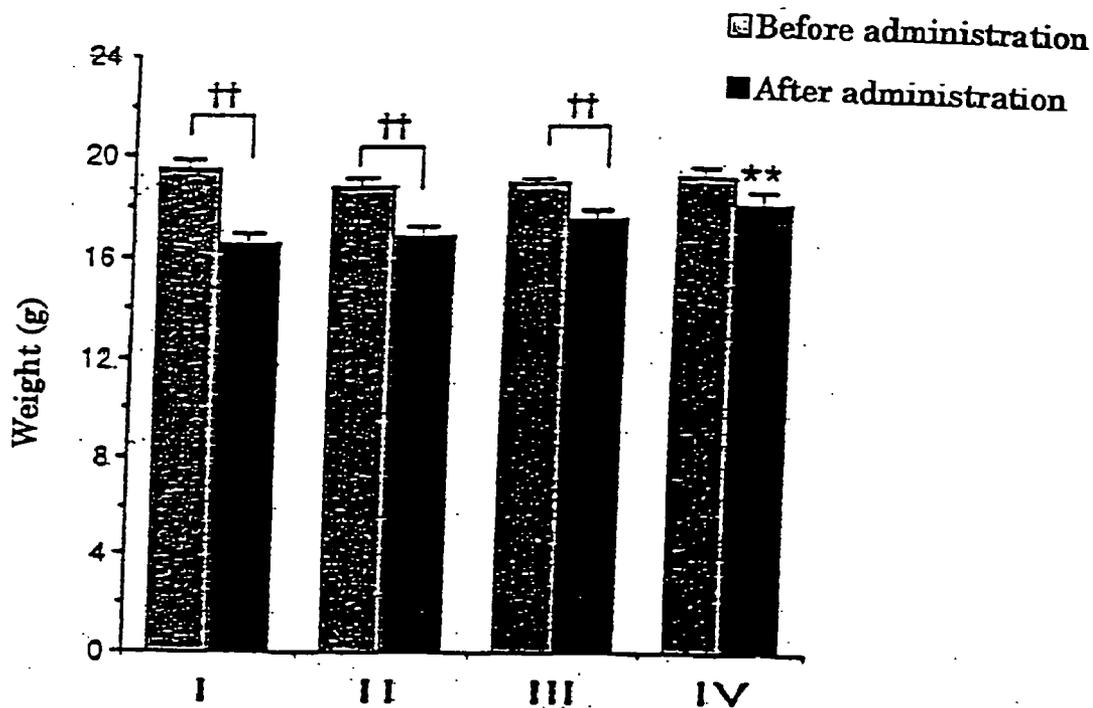


Fig. 2

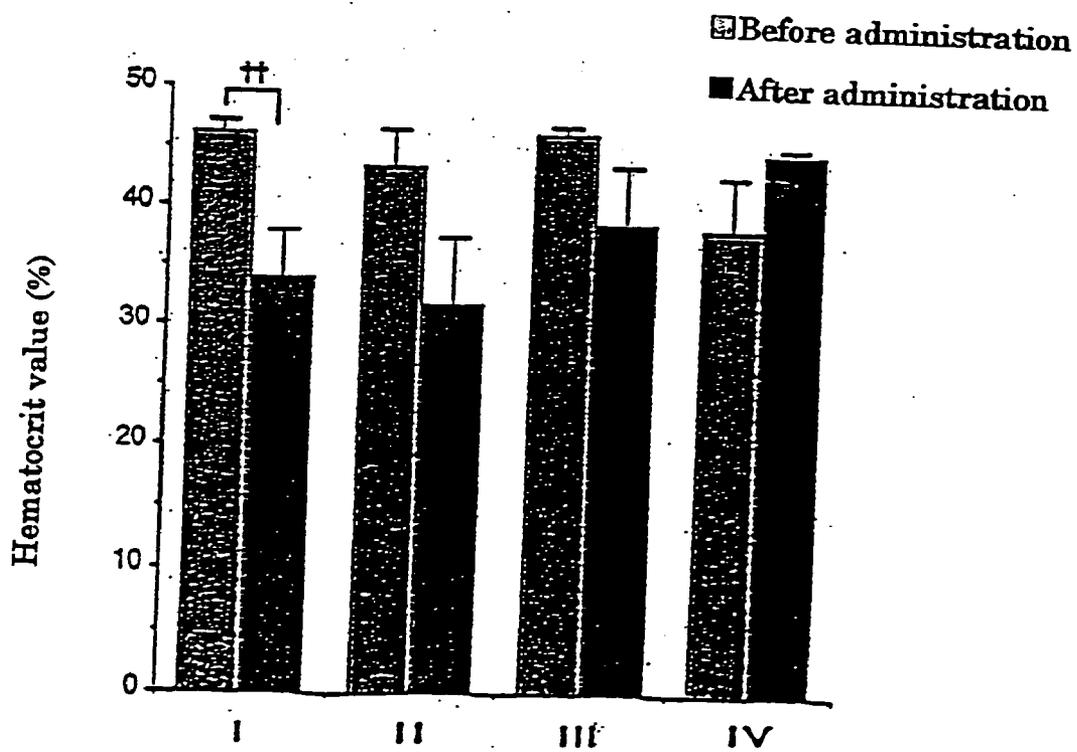
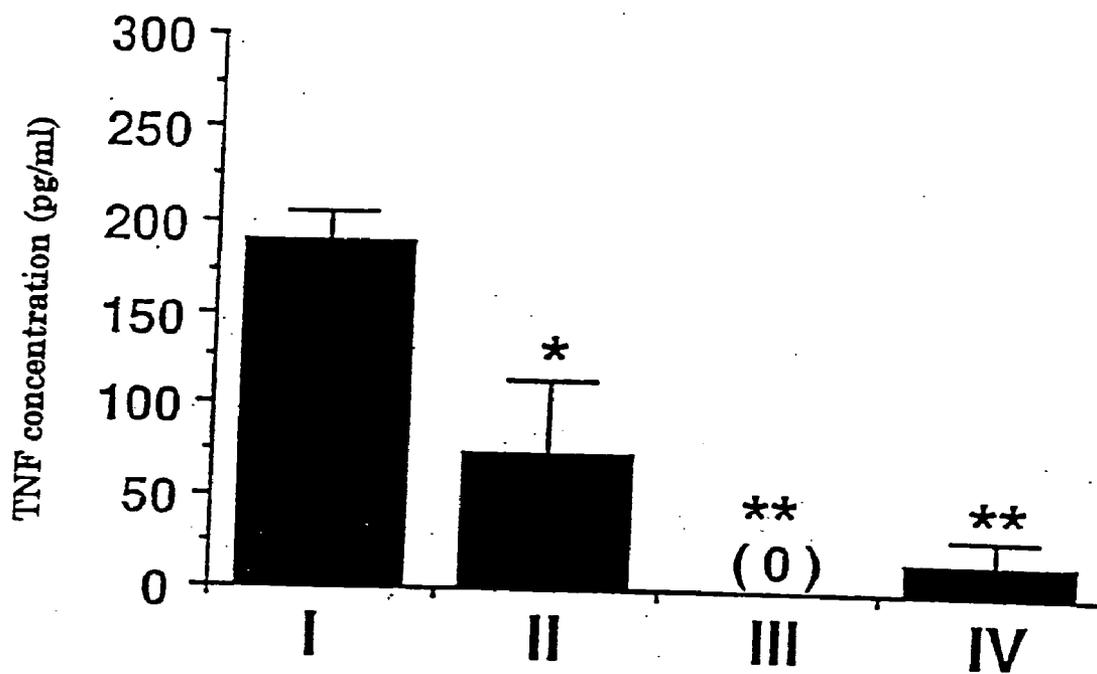


Fig. 3



METHODS USING TCF -II

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional application of U.S. patent application Ser. No.10/267,906, filed on Oct. 9, 2002, which is a continuation of U.S. patent application Ser. No. 09/180,586, filed on Jul. 29, 1999, which claims priority to International Patent Application No. PCT/JP98/00999, filed on Mar. 11, 1998, which claims priority to Japanese Patent Application No. 9-82162, filed on Mar. 14, 1997, the entire disclosures of which are incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The present invention relates to an agent for preventing and/or treating cachexia comprising Tumor Cytotoxic Factor-II (TCF-II) or hepatocyte growth factor (HGF) as an effective ingredient. In particular, the present invention relates to the use of TCF-II or HGF as an agent for preventing and treating cachexia caused by one of the factors selected from the group consisting of cancer, acquired immunodeficient syndrome (AIDS), cardiac diseases, infectious disease, shock, burn, endotoxemia, organ inflammation, surgery, diabetes, collagen diseases, radiotherapy, and chemotherapy.

BACKGROUND OF THE INVENTION

[0003] Generally, a disease such as cancer, acquired immunodeficiency (AIDS), or cardiac disease will be accompanied by anorexia, weight loss, physical exhaustion, marasmus, dermatophthia, xerosis, anemia, edema, and/or abnormal blood coagulation-fibrinolysis. This pathology is defined as cachexia. After suffering from this systemic marasmus, a patient will eventually die (Tamaguma, M. et. al., Igakuno-ayumi, 149, 371-373 (1989)). Further, if radiotherapy and/or chemotherapy is carried out for a patient with progressive or terminal cancer for whom a curative operation can not be expected, it may lead to extremely lowered biological body defensive functions, such as immunological function due to specific malnutrition, resulting in a decreased life expectancy. Therefore, there are serious problems in practical treatment of nutritional equilibrium resulting from lowered nutrition intake combined with increased nutrition consumption, along with humoric factors mobilized from the cancer or the lesion having an effect on systemic metabolism. In the above situations, positive alimentation is carried out using total parental nutrition in order to supplement an extreme nutritional or energetic deficiency and to enhance immunological function in the treatment of cachexia. However, in cachexia, intake of energy will be used not for saving a patient's life but for proliferation of tumor cells, so that alimentation can not be sufficient for a cachexic patient.

[0004] Recently, monokines or cytokines, such as Tumor Necrosis Factor (TNF) mobilized from macrophage, have been implicated in the pathogenesis of cachexia. TNF was found to be a factor affecting tumor cells and was elucidated to be secreted by macrophages, immunocytes that have phagocytic action. Though it was originally studied as a potential anti-cancer drug because of its direct cytotoxic effect and strong anti-tumor activity, recently, various actions of TNF have been investigated since it was found

that TNF may cause cachexia, that is marasmus including weight loss of a patient with cancer, severe infectious disease, or a ringleader cytokine induced inflammation. The main actions of TNF are: (1) osteoclastic action, (2) induction of hyperlipidemia by inhibition of uptake of lipid into cell, (3) induction of production of interleukin 1 and colony stimulation factor, (4) impairment of angioendothelial cells, and (5) intervening in the reaction of exotoxin shock in grave infectious disease.

[0005] Though an agent for treating cachexia accompanied with cancer, acquired immunodeficient syndrome (AIDS), cardiac diseases, infectious disease, shock, burn, endotoxemia, organ inflammation, or these diseases themselves or various kinds of inflammatory diseases including chronic rheumatoid arthritis and inflammatory gut disease are expected to be developed, in fact, there is no satisfactory agent available at present.

SUMMARY OF THE INVENTION

[0006] The present inventors found that TCF-II, known as tumor cytotoxic factor, has an excellent effect of preventing and treating cachexia. Accordingly, the present invention relates to an agent for preventing and/or treating cachexia comprising TCF-II as an effective ingredient.

[0007] An agent for preventing and treating cachexia caused by one of the factors selected from the group consisting of cancer, acquired immunodeficiency syndrome (AIDS), cardiac diseases, infectious disease, shock, burn, endotoxemia, organ inflammation, surgery, diabetes, collagen diseases, radiotherapy and chemotherapy is provided by the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] FIG. 1 shows an improving effect of TCF-II on weight loss in mice with transplanted KYN-2 cell as described in Example 1. (++) in the figure means significant difference ($p < 0.01$) between before and after administration and (**) means significant difference ($p < 0.01$) from Group I (the Control Group) after administration.

[0009] FIG. 2 shows an improving effect of TCF-II on lowered hematocrit value in mice with transplanted KYN-3 cell as described in Example 1. (++) in the figure means a significant difference ($p < 0.01$) between before and after administration.

[0010] FIG. 3 shows a suppressing effect on elevated TNF in ascites of mice with transplanted KYN-3 cell as described in Example 1. (*) in the figure means a significant difference ($p < 0.05$) from Group I (the Control Group) and (**) means a significant difference ($p < 0.01$) from Group I (the Control Group).

DETAILED DESCRIPTION OF THE INVENTION

[0011] TCF-II which is an effective ingredient of the present invention is a known protein derived from human fibroblasts having the following characteristics:

[0012] 1) Molecular weight (by SDS electrophoresis)

[0013] under non-reducing conditions: $78,000 \pm 2,000$ or $74,000 \pm 2,000$

[0014] under reducing conditions: 52,000±2,000 (common band A) 30,000±2,000 (band B) 26,000±2,000 (band C)

[0015] 2) Isoelectric point: 7.4-8.6

Isolation of TCF-II

[0016] The above TCF-II can be obtained by adsorbing culture medium of human fibroblast on an ion exchange column then purifying the elute by affinity chromatography (WO 90/10651) or by genetic engineering manipulation (WO 92/01053). TCF-II which is an effective ingredient of the present invention can be derived from fibroblasts or produced by genetic engineering manipulation using microbial organism or other cell as based on the genetic sequence described in WO 90/10651. Further, TCF-II obtained by genetic engineering manipulation described in WO 92/01053 can be also used. TCF-II with different carbohydrate chain or without carbohydrate chain due to difference of host cell or microbial organism can be also used. However, TCF-II with a carbohydrate chain(s) can be preferably used. TCF-II obtained by these methods can be concentrated and purified by usual isolation and purification methods, for example, precipitation with organic solvent, salting out, gel permeation, affinity chromatography using monoclonal antibody, or electrophoresis. Purification by affinity chromatography can be carried out using the monoclonal antibody described in a Japanese unexamined laid open patent application No.97 (1993). The purified TCF-II can be lyophilized or kept frozen.

[0017] Substances having the same activity as TCF-II can be used as the agent of the present invention. For example, hepatocyte growth factor (HGF; Japanese unexamined laid-open patent application No.22526 (1988)) which is formed by insertion of 5 amino acids into TCF-II protein or purified Scatter Factor (SF; Gherardi and Stocker, Nature, 346, 228 (1990)) may be used as the agent of the invention.

[0018] The agent of the present invention for preventing and/or treating cachexia can be administered intravenously, intramuscularly or subcutaneously. These pharmaceutical preparations can be prepared according to a known pharmaceutical preparation method and, if necessary, pH conditioner, buffer and/or stabilizer can be added thereto. Dosage of the present agent may vary depending on the severness of symptom, health conditions, age, body weight of a patient. Though the dose will not be restricted, a pharmaceutical preparation comprising 0.6mg-600mg-TCF-II/day, preferably 6mg-60 mg-TCF-II/day, for one adult person can be administered in a single dose or in multiple doses.

[0019] The present invention will be described below in detail by the following examples. However, these are only examples and the present invention will not limited therewith.

Purification of TCF-II

[0020] According to a method described in WO 90/10651 and a method of Higashio et al (Higashio, K. et. al, B.B.R.C., vol. 170, pp 397-404(1990)), fibroblast cells were cultured and purified TCF-II was obtained. That is, 3×10⁶ human fibroblast IMR-90 (ATCC CCL-186) cells were placed in a roller bottle containing 100 ml of DMEM medium including 5% calf fetal serum and cultured by rotating it at the rate of 0.5-2 rpm for 7 days. When the total number of cell reached 1×10⁷, cells were detached from the wall by trypsin diges-

tion and collected at the bottom of bottle. 100 g of ceramic with the size of 5-9 mesh (Toshiba Ceramic) was added to the roller bottle. Culturing was continued for 24 hours. After then, 500 ml of the above culture medium was added and the culture was continued. The total volume of culture medium was recovered every 7- 10 days and fresh medium was supplemented. Production proceeded for 2 months under these conditions, after which 4 liters of culture medium was recovered per roller bottle. Specific activity of TCF-II in culture medium obtained as above was 32 µg/ml. Culture medium (750 L) was concentrated by ultrafiltration using membrane filter (MW 6,000 cut; Millipore, Bedford, Mass.) and purified by 4-steps chromatography, that is, CM-Sephadex C-50 (Pharmacia, Peapack, N.J.), Con-A Sepharose (Pharmacia), Mono S column (Pharmacia), Heparin-Sepharose (Pharmacia) to yield purified TCF-II. This TCF-II had the same molecular weight and isoelectric point as described before.

Production of Recombinant TCF-II

[0021] According to the method described in WO 92/01053, cells transformed with TCF-II gene were cultured and purified TCF-II was obtained. That is, transformed Namalwa cells were cultured and 20 µL of culture medium was obtained. This culture medium was treated by CM-sphadex C-50 chromatography, Con-A Sepharose CL-6B chromatography and finally HPLC equipped with a Mono S column to yield about 11 mg of recombinant TCF-II.

Manufacturing of Pharmaceutical Preparations of TCF-II

[0022] An example of manufacturing injections of TCF-II obtained as described above.

(1)	TCF-II	20 µg
	human serum albumin	100 mg

[0023] The above composition was dissolved in citric acid buffer solution with pH 6.03 so that the total volume would be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(2)	TCF-II	40 µg
	Tween 80	1 mg
	human serum albumin	100 mg

[0024] The above composition was dissolved in physiological saline solution for injections so that the total volume would be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(3)	TCF-II	20 µg
	Tween 80	2 mg
	Sorbitol	4 g

[0025] The above composition was dissolved in citric acid buffer solution with pH 6.03 so that the total volume would

be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(4)	TCF-II	40 µg
	Tween 80	1 mg
	Glycine	2 g

[0026] The above composition was dissolved in physiological saline solution for injections so that the total volume would be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(5)	TCF-II	40 µg
	Tween 80	1 mg
	Sorbitol	2 g
	Glycine	1 g

[0027] The above composition was dissolved in physiological saline solution for injections so that the total volume would be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(6)	TCF-II	20 µg
	Sorbitol	4 g
	human serum albumin	50 mg

[0028] The above composition was dissolved in citric acid buffer solution with pH 6.03 so that the total volume would be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(7)	TCF-II	40 µg
	Glycine	2 g
	human serum albumin	50 mg

[0029] The above composition was dissolved in physiological saline solution for injections so that the total volume would be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(8)	TCF-II	40 µg
	human serum albumin	50 mg

[0030] The above composition was dissolved in citric acid buffer solution with pH 6.03 so that the total volume would be 20ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

EXAMPLE 1

Effect of TCF-II Administration Against Cachexia in Mice with Transplanted Human Hepatocellular Carcinoma

[0031] A transplanted human hepatocellular carcinoma, KYN-2 cell line or KYN-3 cell line which show proliferation or cell dispersion in preliminary experiment in vitro was used. Static culture of either cell line was carried out using Dulbecco's MEM medium (Nissui-seiyaku) containing 100 U/ml penicillin, 100 µg/ml streptomycin (Gibco Life Technologies, Rockville, Md.), 12 m mol/L sodium bicarbonate, 20% heat-inactivated calf serum (Whittaker Bioproducts Walkersfield, Md.) at 37° C., 5% CO₂ and 100% humidity. After trypsin-EDTA was added to both of the cultured cell lines, cells were separated, washed twice with phosphate buffer solution (PBS) and resuspended at a concentration of 2.0×10⁷ cell/ml.

[0032] After shaving hair on skin of transplantation site in 4-5 weeks old female SCID, disinfecting the site with 70% ethanol and ether anesthesia, tumor cell prepared beforehand was transplanted into the mice using 23 G syringe needle. KYN-2 cell line (1.0×10⁷) was transplanted subcutaneously into animal's back and KYN-3 (1.0×10⁷) was transplanted intraperitoneally. On 3 weeks after subcutaneous transplantation of KYN-2 cell line when the diameter of tumor became 5 mm and on 5weeks after intraperitoneal transplantation of KYN-3 cell line, these mice with transplanted tumor cells were divided into 4 groups, respectively. Mice in I, II, III and IV groups were administered with vehicle (i.e., no TCF-II), 0.3 mg-TCF-II/kg/day, 3.0 mg-TCF-II /kg/day and 30 mg-TCF-II/mg/kg, respectively. Twice a day for 2 weeks, TCF-II was intraperitoneally administered in mice with transplanted KYN-2 cell line and was subcutaneously administered in mice with the transplanted KYN-3 cell line.

[0033] Body weight was determined before and after administration in mice with subcutaneous transplanted KYN-2 cell line. Hematocrit-was measured before and after administration of TCF-II by taking blood sample with hematocrit heparinized capillary (Terno) from ocular fundus artery under ether anesthesia in mice with KYN-3 and centrifuging it in a usual method. Then, TNF level in ascite was measured using Factor-Test-X™ Mouse TNF ELISA kit (Genzyme, Cambridge, Mass.) after TCF-II administration and autopsy under ether anesthesia. Easy Reader EAR 400 (SLT Laboinstruments) was used in absorptiometry.

[0034] Body weight change in vehicle-administered group and TCF-II administered groups are shown in **FIG. 1**. In the vehicle-administered group (Group I), body weight was significantly lost (about 20% loss) during 2 weeks. On the other hand, body weight loss was suppressed in a dose dependant manner in TCF-II administered groups. Especially in Group IV, there was no significant difference before and after administration and, furthermore, body weight in Group IV was clearly higher than that in Group I after administration.

[0035] Hematocrit change in vehicle-administered group and TCF-II administered groups was shown in **FIG. 2**. By administration of TCF-II, the hematocrit level in mice with cancer was improved and the progress of anemia accompanying with tumor proliferation was suppressed. Further, the suppressive effect of TCF-II on TNF elevation was shown in

FIG. 3. TNF, which was a cause of cachexia in animals with cancer, was significantly lowered in a dose dependant manner by TCF II administration and significant suppression was observed even in Group II which was administered with the minimum dose of 0.3 mg/kg/day. The results shown in **FIGS. 1-3** demonstrate TCF-II administration significantly improved cachexia, that is body weight loss caused by cancer proliferation, progress of anemia and elevation of TNF.

[0036] From what was described as above, a useful agent for preventing and treating cachexia caused by one of the factors selected from the group consisting of cancer, acquired immunodeficient syndrome (AIDS), cardiac diseases, infectious disease, shock, burn, endotoxemia, organ inflammation, surgery, diabetes, collagen diseases, radiotherapy and chemotherapy is provided by the present invention.

1-2. (canceled)

3. A method for increasing hematocrit levels in a patient having anemia comprising the step of administering to a patient with anemia a therapeutically effective amount of

Tumor Cytotoxic Factor II, wherein the Tumor Cytotoxic Factor II increases hematocrit levels in the patient.

4. The method of claim 3, further comprising the step of determining the level of hematocrit in the patient after the administration of Tumor Cytotoxic Factor II.

5. The method of claim 3, wherein the therapeutically effective amount of Tumor Cytotoxic Factor II is a dosage between about 0.3 mg/kg body weight/day and about 30 mg/kg body weight/day.

6. The method of claim 3, wherein the therapeutically effective amount of Tumor Cytotoxic Factor II is a dosage between about 0.6 mg/day and about 600 mg/day.

7. The method of claim 3, wherein Tumor Cytotoxic Factor II is dispersed in a composition comprising saline or citric acid.

8. The method of claim 3, wherein Tumor Cytotoxic Factor II is administered by injection.

9. The method of claim 3, wherein the patient is a human patient.

* * * * *