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GAVIN PUBLISHERS

Journal of Oncology Research and Therapy

Research Article

Liao BS, et al. J Oncol Res Ther 6: 10116.

DOI: 10.29011/2574-710X.010116

Glyco-polypeptides in Treating of Various Types of Late-Stage Refractory Solid Carcinoma in Humans-A Double-Blind Study-Case Report of 126 Patients: Complete Study Report of Phase II Clinical Trial

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Citation: Liao BS, Harvowitz E, Fishbein M, Liao A, Liao A, et al. (2021) Glyco-polypeptides in Treating of Various Types of Late-Stage Refractory Solid Carcinoma in Humans-A Double-Blind Study-Case Report of 126 Patients: Complete Study Report of Phase II Clinical Trial. J Oncol Res Ther 6: 10116. DOI: 10.29011/2574-710X.010116

Received Date: September 19, 2021; **Accepted Date:** October 18, 2021; **Published Date:** October 22, 2021

Abstract and Summary

Glyco-polypeptides (Comosain) induced leucocyte binding ability to tumor surface antigens, such as interleukin 2, 6, 8, and TNFs, is known as an immuno-target therapy. Using thedifferent concentration of Bromelain proteinases in 6 types of cancer cell, it resulted in hydrolysis, fibrinolysis, necrosis, and anti-metastatic effects in tumor cells. Anti-cancer effects were achieved in carcinoma of lung, breast, colon, ovary, cervix, and uterus. Investigation of anti-metastatic effects in Bromelain was carried out in a double-blind study: low dose cohort wason 10 mg/kg/day and a high dose cohort which was on 50 mg/kg/day for over six months. A totalof 151 patients with 3rd and 4th stage of refractory solid tumors were enrolled, 25 patients were post-surgery within 6 weeks which were excluded from entering this research who at least previously failed on two regimens of chemotherapy and/or failed on radiation therapy. The actualstudy patients were only 126 patients. The rates of Complete Response (CR) and Partial Responses (PR) in the high-dose cohort are astonishing with 52% and 27% respectively. The Progress Disease (PD) was 10%, and the Stable Disease (SD) was 11%. The implications and results of the findings are discussed in view of the reported anti-metastatic activity of orally administrated Bromelain.

Keywords: Glyco-polypeptides; Carcinoma; Clinical trial

Introduction

The administration of glycol-polypeptides (bromelain) in cancer treatment in nonclinicaltrials has been reported as early as 1968 by Wolf M, & Ransberger K [1]. Both *in vitro* and animal studies have suggested anti-metastatic effects through the use of bromelain. Batkin & Taussig in 1988 reported that orally administered bromelain reduced the incidence of pulmonary metastasis in Lewis lung cancer cells in mice [2,3]. In recent years, Batkin & Taussig suggested the antitumor mechanisms are due to fibrinolytic effects in Bromelain [4]. Taussig & Batkin 1988 discovered that bromelain has anti-platelet aggregation effects [5]. Taussig and Batkin in 1985 also discovered the inhibition growth

of tumor cells such as Lewis lung carcinoma, V-8 lymphoma, MC1-1 ascites, KATO-gastric carcinoma cells [5].

Maurer, & Hozumi in 1994 discovered bromelain-induced differentiation in leukemic cells [6]. Hale & Haynes in 1992 and Cantrell, et al. in 1996 have suggested that MMAPT (Major Mitogen Activating Protein Kinase) and TPK (Tyrosine Phosphorylation Kinase) inhibitors were activated by bromelain [5,7-14]. T-cell activation and cascade production of Interleukin II-B, 6, 8,and TNF-a (Tumor Necrotizing Factors) via CD-2, CD-3 surface antigen of WBC. Garbin, Harrach, Eckert, & Maurer in 1994 and Hale & Haynes in 1992 also suggested that bromelain will reduce surface antigens of CD-44, CD-44 v, CD-44s, CD45, & CD 47 in tumor cells of breast carcinoma [7,15].

From the experimental studies above, we hypothesize that activation of bromelain proteinases in lymphocytes and T-cells has anti-metastatic effects both *in vitro* and *in vivo*. In our conducted study, we compared the modulation of low dose cohort and high dose cohort of bromelain administration to the patients with stage 3 and stage 4 refractory solid tumors, which include various types of carcinoma of lung, breast, colon, ovarian, cervix, uterine, prostate, melanoma, lymphoma, and gastrointestinal origins, etc. [16-19]. All patients have previously failed on at least two regimens of chemotherapy and/or failed with radiation therapy. The treatments were carried out for at least 24 to 30 weeks. The complete blood count, liver, renal function, hematopoietic elements, tumor markers were evaluated at an interval of every 4 to 6 weeks. The computerized tomography scans were performed at an interval of every 3 to 4 months. The size of tumors was measured, and the tumor markers were recorded for the evaluation of complete response (CR), partial response, (PR), stable disease (SD), and progressive disease (PD) according to the Standard Response Criteria of National Cancer Institute (NCI). The common toxicity was recorded by using NCI's Standard Toxicity Criteria. The results of CR and PR were promising and astonishing when Bromelain was administered inhigh-dose cohort patients [20,21].

Materials and Methods

Bromelain was purchased from Natural Organics Laboratories, Amityville, N.Y., capsules to contain the bromelain were purchased from Capusugel Co. Greenwood, North Carolina. Bromelain was analyzed by using SDS-Polyacryl-Amide Gel Electrophoresis (SDS-PAGE), Cation Exchange Chromatography (CEC), and/or Multicathodal Polyacrylamide Gel Electrophoresis (MC-PAGE), and Florescence High-Performance Liquid Chromatography (FPLC) to determinate the purity and separation of bromelain fraction of F1, F2, F3, F4, F5, F6,F9 in stem bromelain that were detected by Amperometric detection. Monosaccharides fraction are L-fucose, D-galactosamine, D-glucosamine, D-xylose, D- mannose, D-glucose, D-galactose, D-fructose, and Deoxyribose [10,22].

Clinical Application and Study Protocol

The Phase II Clinical Study investigates the efficacy of low dose and high dose cohort bromelain (comosain) in human subjects diagnosed with advanced late-stage refractory cancers [23,24].The bromelain (comosain) extract derived from the stem and fruit of *Ananas comosus* will be administered orally each day.

Patient Eligibility and Selection

I. Eligible patients are those suffering from late-stage solid cancer of breast, lung, colon, cervical, ovarian, and uterine, prostatic, lymphoma, bladder, etc. They are in stage III or IV with tissue proof of well-documented malignancies, whether by tissue biopsies, laparatomy, or thoracotomy. These individuals have not been cured by conventional methods such as radiation therapy or chemotherapies for at least two separate regimens.

II. Other eligibility requirements also require patients to have no other available therapy known to provide clinical benefit. For example, the breast cancer patients must have failed at least 2 chemotherapy regimens in the metastatic setting. Additionally, if their tumors are HER2 positive or hormone receptor (ER, PR) positive, respectively, they must also have failed several anti-HER2 targeted therapies and no longer be eligible forhormonal therapy [25-27].

III. Additionally, the following conditions must be met:

a) Patient's age is between 18 and above.

b) Patient is not taking anticoagulants or on antiplatelet therapy.

c) Patient does not have a history of abdominal fistula, gastro-enteral perforation, peptic ulcer diseases, or intra-abdominal abscess within 4 months prior to study enrollment [28-30].

d) Patient has not had major surgery within 4 weeks prior to study enrollment. Patients who have not recovered from adverse events due to surgery performed more than 4 weeks earlier are not eligible for this study.

e) Patient does not currently have uncontrolled hypertension, diabetes, or clinically significant cardiac arrhythmia.

f) Patient does not have an allergic reaction to bromelain or bromelain-containing products.

g) Female patients should not be pregnant or breastfeeding [31,32].

h) Patient's platelet counts must be greater than 100,000/uL.

i) Patient's hemoglobin must be greater than 9.0 g/dL.

j) Patient does not have significant abnormal hepatic and/or renal function.

k) Patient's tumors are measurable; between 0.2 – 10 cm in size and number between 1-15. All measurable tumors that have spread to the bones, liver, lung, kidney, and abdomen will be included in the data analysis [33-35].

I. Patients with the following conditions will be excluded from the study:

a) Hemoglobulin < 9 g/dL and WBC < 3.0 K/μL.

b) Platelet count < 100,000/μL.

c) INR < 1.5

d) Patient currently taking therapeutic doses of warfarin or anti-platelet agents.

e) Patient has a history of abdominal fistula, gastrointestinal perforation, peptic ulcer disease, or intra-abdominal abscess within 4 months prior to study enrollment [36-38].

f) Patient currently has uncontrolled hypertension, diabetes, or clinically significant cardiac arrhythmia.

g) Patient who had major surgery performed within 4 weeks prior to entering the study; and patients who have not recovered from adverse events due to surgery performed more than 4 weeks earlier [39,40].

h) Patient with a history of allergic reaction to Bromelain or pineapple-containing products.

i) Female patients who are pregnant or breastfeeding.

j) Patient with tumors that are widely spread in the chest and abdomen that cannot be measured by CT scan.

Patients who are eligible for this study will be randomly assigned to either the lowdose group or the high dose group by a coin toss. Each study subject will be assigned a patient number for the purpose of this study [41].

Drug Dosage and Schedule

The dose of glycol-polypeptide (bromelain) at 50 mg (125 GDU)/kg/day is extrapolated from *in vivo* animal studies. It is determined to be safe by a safety study on healthy human subjects [42,43]. For this clinical investigation, the high dose group will be given bromelain at 50 mg/kg/day (at a bodyweight of 50-60 kg) to a maximum of 2400 mg (5000 GDU)/ day and divided into 2 doses/day of 1200 mg/dose [44].

In both the high dose group and the low dose group, the number of patients suffering from well-documented refractory solid malignancies will be at least 60 and 30 respectively to be assigned to each group. All patients are diagnosed with different types of carcinomas. For example breast, lung, colon, ovarian, cervical, bladder, prostatic and uterine origin, etc. In the high-dose group, patients will be given bromelain at 5000 GDU(2400 mg)/ day divided into two doses of 1200 mg/dose and taken with meals. In the low dose group, patients will be given Bromelain at 1250 GDU (500 mg)/day divided into two doses of 250 mg/dose and taken with meals [45-48].

Duration and Route of Administration

Study subjects will be provided with bromelain for oral administration. The containers will be clearly labeled (see Section V-E). Bromelain will be taken orally twice daily with meals. On their bi-weekly visits to the doctor's office, the study patients will be provided with enough doses for two weeks. The study patients are required to keep a journal of the daily doses they take and any side effects they experience [49-52].

The study patients will be evaluated using blood tests and/or CT scans at the end of each cycle (i.e., 6 weeks) and at six months for signs of disease progression. If the disease did not progress, then treatment will continue and the patient will be evaluated every six months thereafter until the investigator determines otherwise. If the disease did progress, then the patient will be taken off the study. On the humanitarian basis, the low dose group patients will be transferred to the high dose group due to lack of efficacy in the treatment [53,54].

Evaluations to be conducted

A. Blood and Laboratory tests schedule: blood tests will be conducted every 4-6 weeks, Blood tests include CBC, Chemistry-7, Chemistry-24, liver and renal function, CEA, CA125, CA153, CA199, PSA, TSH, alfa-Feto-Protein, and other tumor markers. The test results will be recorded for discussion and evaluation.

B. Radiological tests schedule will be also assessed every 3 months for the result of CTscan and/or PET scan,

C. Each study subject will be also assessed every four weeks for any side effects that they may have experienced during the previous four weeks. These side effects will be recorded for evaluation [55-57].

Use of standard toxicity criteria. (NCI Common Toxicity Criteria Manual Page-1 --20).

Grade I toxicity: WBC>3000/mm^3 (3 k/ ul), Hb>10 gm/dl, Platelets>75,000/mm^3 (75 k/ul) No dehydration, No infection, No transfusion, No renal and liver function impairments. Temperature and Fever: 38-39°C.

Grade II toxicity: WBC 2000-3000/mm^3, Hb 8-10 gram/dl, platelets 50,000-75,000/mm^3, No infection, No transfusion, mild to moderate diarrhea and dehydration, and requires IV hydration, Temperature, and Fever: 39-40°C.

Grade III toxicity: WBC: 1000-2000/mm^3, Hb: 6.5-8.0 gram/dl, platelets 10,000-50,000/mm^3, has infection, Need Transfusion, Moderate Dehydration from diarrhea, need parenteral hydration. Temperature and Fever: >40°C for less than 24 hrs.

Grade IV toxicity: WBC<1000/mm^3, Hb<6.5 gm/dl, Platelets<10,000/mm^3, when WBC<1/ul (1000/mm^3), Life threatening Infection (Sepsis), Need Transfusion, and Need ICU Care. Fever and Temperature: >40°C for more than 24 Hrs.

A. Adverse events and serious adverse events and reporting information:

The NCI listed Adverse Events in CMC (Common Toxicity Criteria) is based on pathological (Allergy/Immunology) and anatomical (Dermatology /Skin) categories to facilitatethe location of related adverse events.

B. Grades of Adverse Events

For each adverse event, grades are assigned and defined using a scale from 0 to 5. With 0representing no adverse event within normal limits and 5 representing death related to an adverseevent [58,59].

Documenting Related Adverse Events (Figure 1)

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4
Diarrhea	< 4 stool/day	4-6	<7	need ICU care
Dehydration	dry mucous membr	need IV	need IV	ICU care
Hypotension	no Rx required	need IV	need IV	ICU care
Bleeding /Grade	3-,4	thrombocytopenia	melena	GI bleeding
	No transfusion	No transfusion	transfusion	transfusion
Platelets	> 75,000/mm^3	50-75,000	10-50,000	< 10,000
Hb	> 10 gm/ dl	8-10 gm/dl	6.5-8 gm	< 6.5gm
WBC	> 3000/mm^3,	2-3000/mm^3,	1-2000/mm^3	<1000/mm^3
Infection/ Fever	(--)/38-39 ^{O}C	(--) 39-40 ^{O}C	(+) > 40 ^{O}C,	(+) >40^{O}C

Figure 1: Documenting Related Adverse Events.

Study Endpoints

At the end of six months, an assessment of the therapy results for each study patient willbe performed to determine whether to continue with this therapy. Individual data sets will be combined to assess the efficacy of the therapy for the cancers studied (Figure 2). The study endpoints for both groups are: 3-a.Use of Standard response Criteria: (NCI Chapter Standard Response Criteria 11.1.1through 11.1.7 and 11.2, 11.3,).

Conclusion

Name of Cancer	Low Dose Group	High Dose Group	Total	Complete Response	Partial Response	Stable Disease	Progressive Disease
Breast Cancer	12/42	20/83	32	17	9	3	3
Lung Cancer	8/42	8/83	16	11	3	1	1
Colon Cancer (GI-CA)	3/42	3/83	6	2	2	1	1
Ovarian Cancer	4/42	7/83	11	8	1	1	1
Uterine Cancer	0/42	11/83	11	4	3	2	2
Cervix Cancer	0/42	7/83	7	5	2	0	0
Bladder Cancer	1/42	3/83	4	2	1	0	1
Prostate Cancer	5/42	10/83	15	9	3	2	1
Liver Cancer	1/42	1/83	2	0	2	0	0
Lymphoma Cancer	4/42	4/83	8	3	4	1	0
Melanoma Cancer	1/42	2/83	3	3	0	0	0
Nasopharyngeal Cancer	1/42	3/83	4	0	2	1	1
Thyroid Cancer	1/42	1/83	2	2	0	0	0
Sarcoma Cancer	1/42	1/83	2	2	0	0	0
Leukemia Cancer	0/42	2/83	2	1	1	0	0
	42+1	83	126	67 (52%)	34 (27%)	12 (10%)	12 (10%)

Figure 2: Study Endpoints.

Evaluation of Target Lesions

A. Complete Response (CR): Disappearance of all target lesions. Any pathologicallymph nodes must have a reduction in short axis to <10mm.

B. Partial Response (PR): At least a 30% decrease in the sum of the diameters of targetlesions, taking as reference the baseline sum diameters.

C. Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on the study). In addition to the sum to the relative increase of 20%, the summust also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions)

D. Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficientincrease to qualify for PD, taking as a reference to the smallest sum diameters while on the study [60-62].

Evaluation of Non-Target Lesions

A. Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis) (iftumor makers are initially above the upper normal limit, they must normalize for a patient to be considered incomplete clinical response.

B. Non-CR/ Non-PD: Persistence of one or more non-target lesion(s) and /ormaintenance of tumor marker level above the normal limits.

C. Progressive Disease (PD): Appearance of one or more new lesions and/ or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, nota single lesion increase [63-65].

Evaluation of Best Overall Response (Tables 1 and 2)

A. For Patients with Measurable (Target Disease) Disease

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Confirmation
CR	CR	No	CR	≥4 wks.
CR	Non-CR/Non-PD	No	PR	≥4 wks.
CR	Not evaluated	No	PR	≥4 wks.
PR	Non-CR/Non-PD Not evaluated	No	PR	≥4 wks.
SD	Non-CR/Non-PD Not evaluated	No	SD	≥4 wks.
PD	Any	Yes	PD	

Table 1: Patients with Measurable (Target Disease) Disease.

B. For Patients with Non-Measurable Disease (No-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/Non-PD	No	Non-CR/ Non-PD
Not All Evaluated	No	Not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

Table 2: Patients with Non-Measurable Disease (No-Target Disease).

Data Analysis

Data collected from all patients will be analyzed to determine the overall efficacy of Bromelain to treat advanced cancers. Statistical analysis such as Student t-test will be used. Theresults of the ongoing analyses will be reported to the FDA in an annual report. (4-a) Adverse events are mild to moderate anemia, leukopenia, thrombocytopenia, and/orliver and renal impairment. (4-b) Severe and serious adverse events are Liver and/or Renal damage or failure,anaphylactic reaction. All serious adverse events will be reported to the FDA [66].

Results and Conclusions

The results of the study will be reported as required to the FDA in an annual report andnow report as following:

1. **Age distribution:** Both in the high dose group and low dose group patients are mainlyages 65 and above. 68% and 81% respectively of participants were over the age of 65 (Table 3). And the participants that are male gender are 75% and 67% respectively.

Age Category	Low Dose Group	High Dose Group
≤18 years	0	0
Between 18 and 65 years	8 (19%)	26 (32%)
≥ 65 years	34 (81%)	55(68%)
Gender Category	**Low Dose Group**	**High Dose Group**
Male	28 (67%）	61 (75%)
Female	14 (33%)	20 (25%)

Table 3: Baseline Characteristics-Age, Gender.

2. The disease classification and distribution are as following: breast carcinoma account for 25% in the high dose group and 28% in the low dose group, in lung carcinoma theincidence are 9.9%, and 19% respectively, in colon & G-I carcinoma the incidence are 3.7% and 7.1% respectively, in ovarian carcinoma the incidence are 8.6% and 9.5% respectively. The uterine and cervical carcinoma in the high-dose group is about13% etc.

Please see Table 4 for overall disease distribution. Table 4 showed breast cancer incidence in low dose group and in high dose group are 28.6% and 25% respectively. In lung cancer, the incidence is 19% and 10% respectively. In colon cancer, the incidence is 7.1% and 3.7% respectively. In Ovarian, uterine, and cervix cancer, the incidence is 9.5% and 30.2% respectively. In bladder and prostate cancer, the incidence is 14.4% and 16% respectively. The incidence of melanoma cancer is 2.5%. The incidence of liver cancer are 2.4% and 1.2% respectively. The incidence of lymphoma are 9.5% and 5% respectively. In the incidence of thyroid cancer and sarcoma cancer, both are 2.4% and 1.23% respectively [67,68].

Name of Cancer	Low Dose Group	High Dose Group
Breast Cancer	12/42	32/124
Lung Cancer	8/42	16/124
Colon Cancer (GI-CA)	3/42	6/124
Ovarian Cancer	4/42	11/124
Uterine Cancer	0/42	11/124
Cervix Cancer	0/42	7/124
Bladder Cancer	1/42	4/124
Prostate Cancer	5/42	15/124
Liver Cancer	1/42	2/124
Lymphoma Cancer	4/42	8/124
Melanoma Cancer	1/42	3/124
Nasopharyngeal Cancer	1/42	4/124
Thyroid Cancer	1/42	3/124
Sarcoma Cancer	1/42	3/124

Table 4: Disease Classification.

The overall clinical response rate in high dose group patients and low dose group patients are as follows: the Complete Response (CR) rates are 52% (66/126) and 0% respectively, the Partial Response (PR) rates are 27% (34/126) and 0% respectively. In the patients of the low dose group there was no Stable Disease (SD) and in the patients of the high dose group is 11% (13/126). The Progressive Disease (PD) in the high dose group is 10% (13/126), and in the low dose group is 100%.

The overall adverse effects and toxicities are shown in Table 5A to Table 5D which all concluded that there were no hematological, renal, and hepatic toxicities in patients of all groups. The primary target lesion size is less than or equal to 2 cm in the low dose group and high dose group are 36% and 38% respectively. The lesion size between 2-5 cm are 38% and 39.5% respectively, the lesion size between 5-10 cm are 28.6% and 25% respectively [69].

	Pretreatment Value	During Treatment6 Weeks	During Treatment12 Weeks	During Treatment18 Weeks	During Treatment24 Weeks	During Treatment30 Weeks
WBC	5.3 ± 1.1	5.4 ± 1.0	5.3 ± 0.9	5.4 ± 1.0	5.6 ± 0.9	
Hb	12.9 ±1.3	12.8 ± 1.2	12.8 ± 1.4	12.9 ±1.3	12.8 ± 1.0	
Platelets	228 ± 56	220 ± 48	220 ± 42	210 ± 48	218 ± 45	
Creatinine	1.0 ± 0.3	1.0 ± 0.2	1.0 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	
AST	20 ± 8	20 ± 7	21 ± 8	21 ± 7	20 ± 8	
Bilirubin	1.0 ±0.3	1.0 ± 0.2	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.4	
CEA	13.0 ± 4.5	13 ± 4.0	12 ± 4.5	12 ± 6	12 ± 7	
CA153	72± 15	50 ±18	40 ± 16	33± 17	32 ± 18	

Table 5A: Breast Ca. Patients Number: 20/81 (25%).

	Pretreatment Value	During Treatment 6 Weeks	During Treatment 12 Weeks	During Treatment 18 Weeks	During Treatment 24 Weeks	During Treatment 30 Weeks
WBC	5.4 ± 1.2	5.4 ± 1.0	5.3 ± 0.9	5.8 ± 1.1	5.9 ± 0.8	
Hb	12.8 ±1.2	12.8 ± 1.3	12.9 ± 1.4	12.9 ±1.5	12.8 ± 1.6	
Platelets	210 ± 58	210 ± 48	220 ± 32	218 ± 32	218 ± 22	
Creatinine	1.0 ± 0.1	1.0 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.1	
AST	18 ± 9	18 ± 8	20 ± 7	20 ± 8	20 ± 9	
Bilirubin	1.1 ±0.3	1.05 ± 0.2	1.1 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	
CEA	12 ± 6	12 ± 5	11 ± 6	11 ± 7	11 ± 8	
CA125	76 ± 11	20 ±9	15 ± 8	11 ± 7	8 ± 5	

Table 5B: Uterine Ca., Ovarian Ca. and Cervical Ca. Patients Number: 25/81 (31%).

	Pretreatment Value	During Treatment6 Weeks	During Treatment12 Weeks	During Treatment18 Weeks	During Treatment24 Weeks	During Treatment30 Weeks
WBC	5.6 ± 1.3	5.6 ± 1.5	5.7 ± 1.4	5.8 ± 1.6	5.8 ± 1.5	
Hb	12.9 ±1.3	12.8 ± 1.3	12.7 ± 1.5	12.8 ±1.4	12.8 ± 1.6	
Platelets	226 ± 46	220 ± 56	220 ± 40	220 ± 38	222 ± 46	
Creatinine	1.0 ± 0.3	1.0 ± 0.4	1.0 ± 0.3	1.1 ± 0.4	1.1 ± 0.4	
AST	20 ± 8	21 ± 7	20 ± 9	20 ± 8	21 ± 9	

Bilirubin	1.0 ±0.2	1.0 ± 0.3	1.0 ± 0.2	0.9 ± 0.3	0.9 ± 0.3	
CEA	28 ± 16	20 ± 12	20 ± 13	28 ± 12	28 ± 11	
CA199	70 ± 18	42± 25	35 ± 16	27± 6	21 ± 8	

Table 5C: Lung and Colon Ca. Patients Number: 11/81 (14%).

	Pretreatment Value	During Treatment 6 Weeks	During Treatment 12 Weeks	During Treatment 18 Weeks	During Treatment 24 Weeks	During Treatment 30 Weeks
WBC	5.7 ± 1.2	5.6 ± 1.4	5.7 ± 1.3	5.8 ± 1.5	5.8 ± 1.6	
Hb	12.8 ±1.2	12.8 ± 1.4	12.7 ± 1.5	12.8 ±1.3	12.9 ± 1.2	
Platelets	210 ± 46	220 ± 42	218 ± 36	216 ± 35	226 ± 56	
Creatinine	1.0 ± 0.4	1.0 ± 0.48	1.0 ± 0.3	1.0 ± 0.4	0.9 ± 0.4	
AST	21 ± 9	21 ± 8	22 ± 8	21 ± 11	21 ± 8	
Bilirubin	1.0 ±0.4	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.3	
CEA	12 ± 18	12 ± 11	11 ± 10.5	11 ± 6	11 ± 6	
CA199	76 ± 23	39 ± 11	38 ± 13	32± 12	20 ± 11	

Table 5D: Miscellaneous Ca. Patients Number: 16/81 (20%).

The tumor markers such as CEA, CA-125, CA-153, CA-199, PSA, TSH, and alpha- fetoprotein are being monitored, their value corresponds to the tumor masses, they return to normal value when the tumor has been Complete Responded (CR), and when the tumor progress the tumor marker value is elevated.

The serious adverse effect in toxicity in the both low dose group and the high dose group are not observed as seen in Table 5A to Table 5D. There is no serious hematopoietic toxicity, no hepato-renal toxicity, no anaphylactic reaction and life threaten events. There were very rarely minor or non-serious side effects such as nausea, vomiting, diarrhea, palpitation, headache, insomnia, pruritus, urticaria, and skin rash. We conclude that bromelain administered in an amount of 2500 to 3000 mg/day to patients with average body weight is effective and non- toxic. The following figures are self- explanatory for the above results.

Tumor Measurement in Phase -2 Clinical Trial Study

A. Low dose cohort with measurable disease was either by direct measurement, X-ray, CT scans, and /or PET scans. Table 4 showed tumors progressions in all low dose group patients without exception. ##Special Note ##: Due to the non-efficacy in this low dose treatment, all patients in this cohort were transferred to the high dose cohort to continuing therapy for humanitarian reasons.

B. High dose cohort measurable disease was either by direct measurement, X-ray, CT scans, and/or PET scans (Table 6).

Table 6 showed tumor regression in 80% of patients in the high dose group.

	Low Dose Group	High Dose Group
Complete response	0/42 (0%)	66/126 (52%)
Partial response	0/42 (0%)	34/126 (27%)
Stable disease	0/42 (0%)	12/126 (10%)
Progressive disease	42/42 (100%)	13/126 (11%)

Table 6: Overall response rate.

Discussion

In summary, throughout the 6 to 10 months course of the double-blind study of bromelain administration for high and low dose groups, only the high dose group patients of 50 mg/kg/day regimen showed effectiveness. The low-dose group patients showed no efficacy at all. Both groups did not show serious adverse effects such as leukopenia, anemia, hepato-renal toxicity, anaphylactic reaction, and life-threaten events. Minor adverse effects such as nausea, vomiting, diarrhea, urticaria, insomnia, palpitation, pruritus, and headache occurred in rare instances [70,71] (Table 7 and Table 8).

Target Lesion Size	Low Dose Group	High Dose Group
Less than ≤2 cm	15/42 (36 %)	31/124 (25%)
2-5 cm	16/42 (38 %)	32/124 (25.8%)
5-10 cm	12/42 (28.6 %)	20/124 (16.1%)

Table 7: Outcome Measurement-Primary Target Lesion Size.

Tumor Markers	Low Dose Group	High Dose Group
CA125	12/42 (29%)	21/124 (16.9%)
CA153	8/42 (19%)	8/124 (6.4%)
CA199	3/42 (7%)	3/124 (2.4%)
PSA	8/42 (19%)	15/124 (12.1%)
α-Fetoprotein	1/42 (2.3%)	1/124 (0.8 %)
CEA	42/42 (100%)	81/124 (65.3%)

Table 8: Outcome Measurement-Tumor Markers.

The glycopeptides of stem Bromelai were obtained from proteolytic digestion with pronase as described by Murachi, et al. in 1967 and later found that there were four kinds of glycopeptides that only differed from each other in the peptide part [29]. The amount of the glycopeptide was calculated from its content of glutaminic acid as determined by amino acid analysis. The average molecular weight was assumed to be 1.5x10^3 DA. Bromelain contains nine different glycol-polypeptides. Each polypeptide contains amino acids in a double benzene ring structure and one of twelve different monosaccharides fractions [22]. Specifically, breakthrough fractions such as Comosain (F9) account for 80%, ananase account for 10%, the rest of 10% was derived from Bromelain F1, F2, F3, F5, F6, and so forth [4]. They mainly comprise glycosylated multiple enzyme species of the papain superfamily with different proteolytic activities, molecular masses between 20 to 31 kDa,and isoelectric points >10 and 4, 8 respectively. Two major basic proteinases, F4 and F5, were further characterized and shown to have molecular masses of 24397 Da and 24472 DA, respectively [22,25]. Napper and Bennett et al in 1994 further purified and characterized multiple forms of bromelainases derived from cysteine proteinases Ananain and Comosain. Lee and Albee in 1997 postulated the complete amino acids sequence of Ananian and comparison with Bromelain and other plant cysteine proteinases. They all have protein electronic density between 272 to 282 mu [32,72] (Table 9) (Figures 1A-1C).

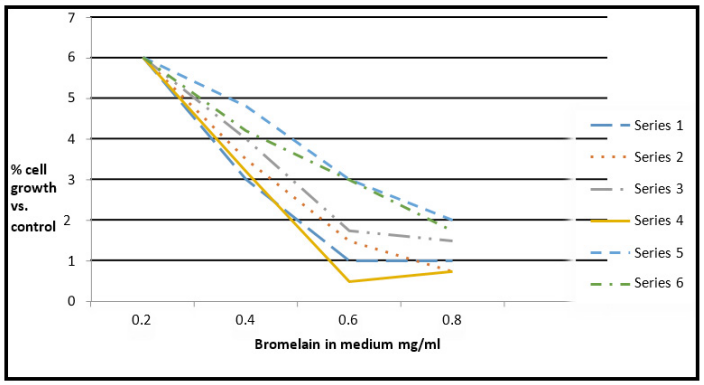


Figure 1A: Growth inhibition of various types of tumor cell lines *in vitro.*

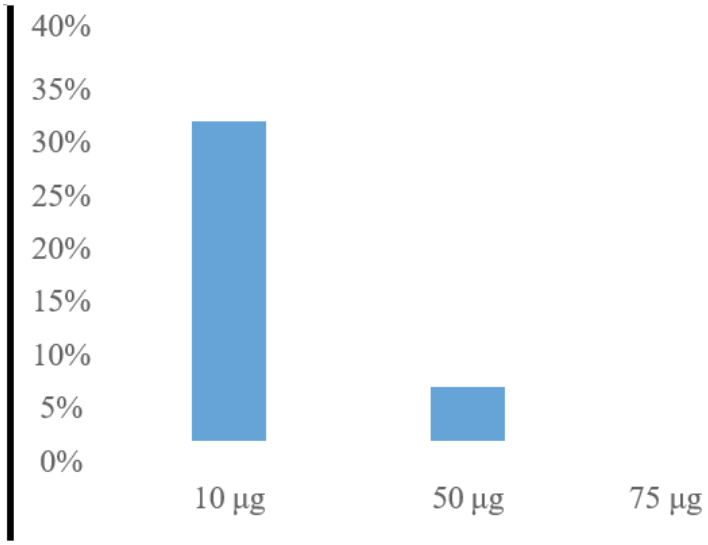


Figure 1B: Depicts that detection of the CD44s modulation with two different mAbs clones, L-178, J-173. Breast carcinoma cells were incubated for 1 hr. at 37°C with 10, 50, 75 ug/ml of Bromelain (Comosain) treatment. The CD44s become 35%, 10%, and 1% of Bromelain (Comosain) treated cells [4].

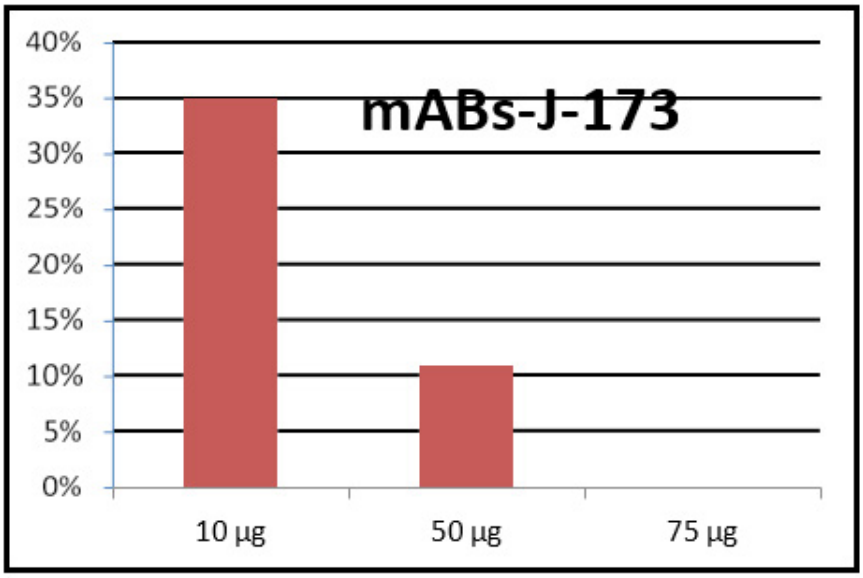


Figure 1C: Depicts the Interleukin–IB, II-6, TNF production by the monocytes from healthy donors [4].

	Low Dose Group		High Dose Group	
Hematological toxicity	0/42	0 %	0/124	0 %
Liver Toxicity	0/42	0 %	0/124	0%
Renal Toxicity	0/42	0 %	0/124	0%

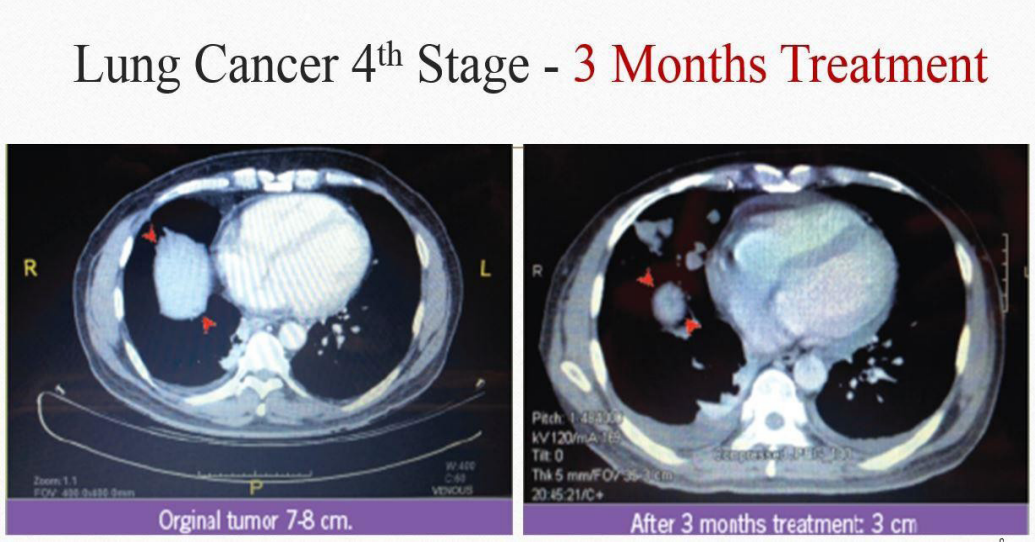
Table 9: Outcome Measurement- Serious Adverse Outcomes in Toxicity(One patient withdraw from testing).

The remarkable cancercidal effects (we designate as Chimeric WBC Immuno-Therapy in cancer) [6,44] are probably due to massive production of Interleukin-II, VI,VIII, and Tumor Necrotizing Factors (TNF) from CD-2, CD-3 (Cell Device 2 & 3) in monocytes and lymphocytes (T- cells). The fibrinolytic effects on tumor surface antigens of CD-44, CD-44V, CD-44S, CD-45, and CD-47 [9,12,22,27,39], which induce dehydration, necrosis, and possible calcification in the tumor cells. This action mechanism of Bromelain is mainly due to the inhibition of the following two kinases (1) MMAPK (Major Mitogen Activating Protein Kinases) [6] (2) TPK (Tyrosine Phosphorylation Kinases) [6]. In the WBC culture test that with the concentration of Bromelain in an amount of 1 mg/ml will increase the production of the Interleukin II by 400 times/10^6 WBC, Interleukin-6 by 650 times/10^6 WBC, and the TNF by 42 times/10^6 WBC [2,10,17] (Table 10).

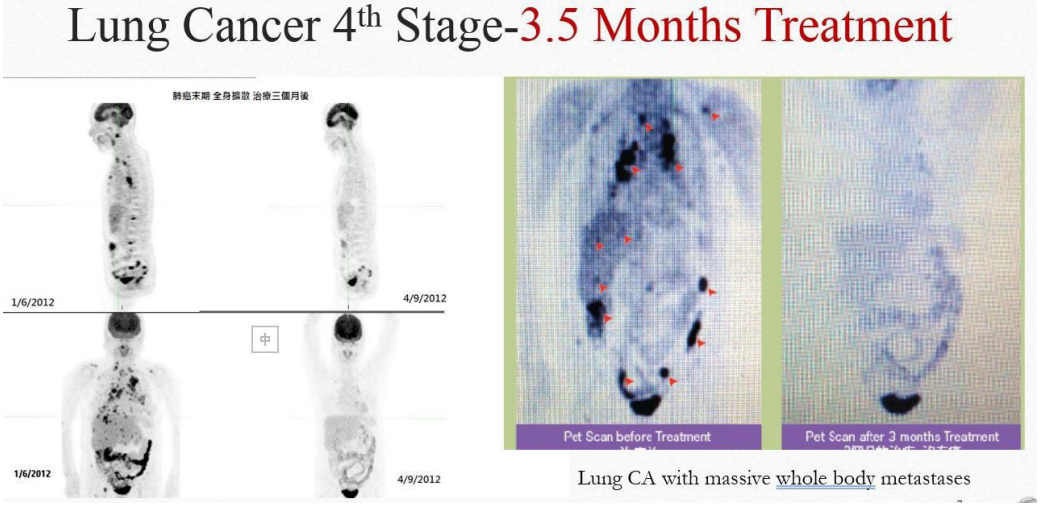
Nausea, gastric upset,diarrhea	2/42 (4.6 %)	2/124 (1.6 %)
Palpitation	1/42 (2.3%)	2/124 (1.6 %)
Insomnia	0/42 (0 %)	1/124 (0.8 %)
Skin rash	0/42 (0 %)	0/124 (0 %)
Urticaria	0/42 (0 %)	0/124 (0 %)
Headache	0/42 (0 %)	0/124 (0 %)
Pruritus	0/42 (0 %)	1/124 (0.8 %)

Table 10: Non-serious outcome toxicity.

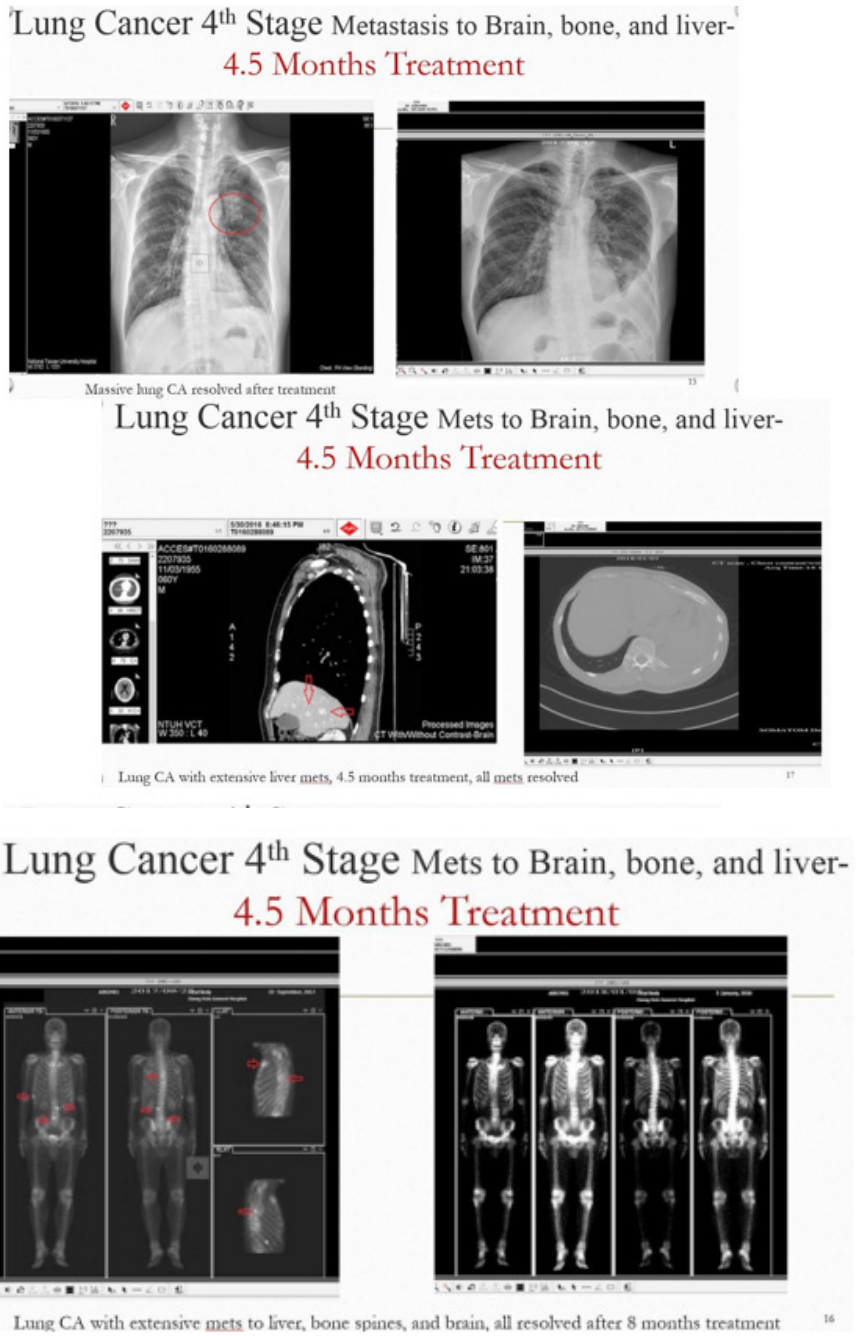
According to the outcome measure, the results in the patients in the high dose group showed remarkable complete response rates of 52%, partial response rates of 27%, stable disease of 11%, progressive diseases of 10% in these late-stage refractory solid carcinomas by using student T statistical analysis $P<0.05$ which showed statistical significance. Dr. HR Maurer in his complimentary tumor therapy, employed more than 3000 patients and treated with bromelain in an amount between 1000-to-3000 mg/day for the period of 1 to 3 years. During this period, he did not discover any severe side effects nor had any life threaten events [41]. In the present investigation, we conclude that a high dose of bromelain therapy (comosain) in the amount of 2500 mg to 3000 mg is a lifesaving regimen and hoping to save thousands of lives in the future [73] (Case 1-11).



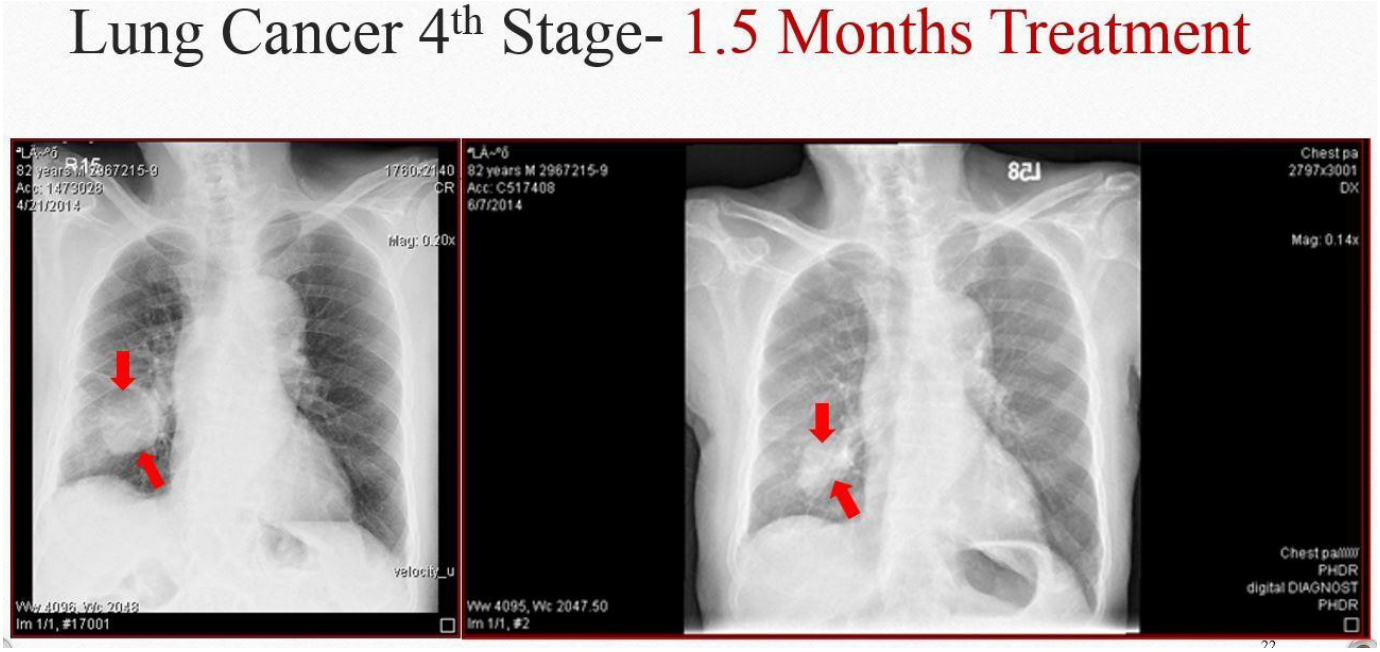
Case 1: Lung cancer pre and post-treatments**.**



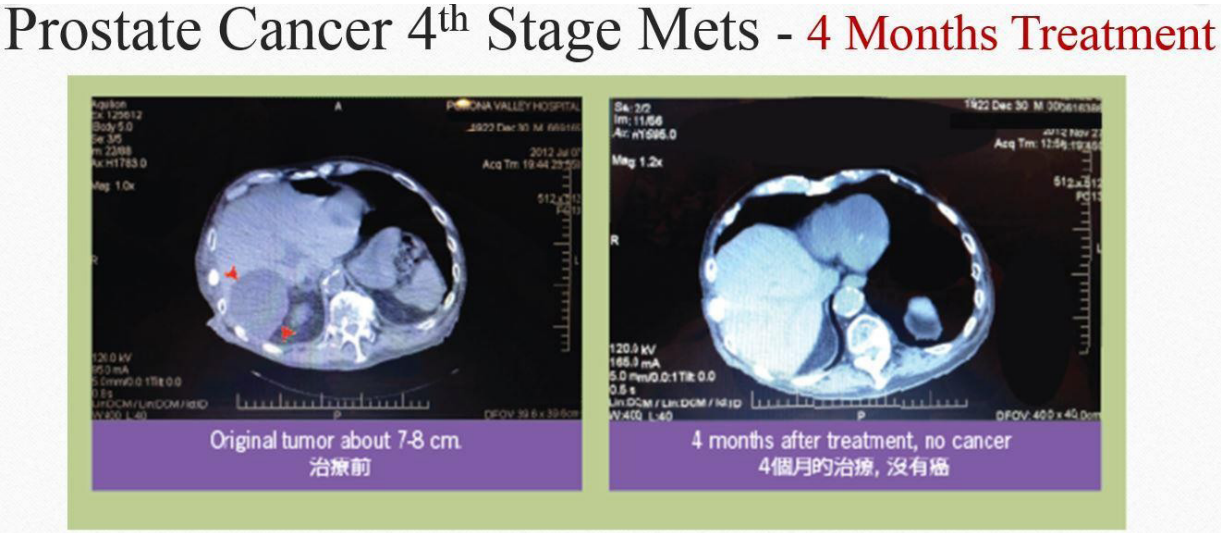
Case 2: Lung cancer pre and post-treatments.



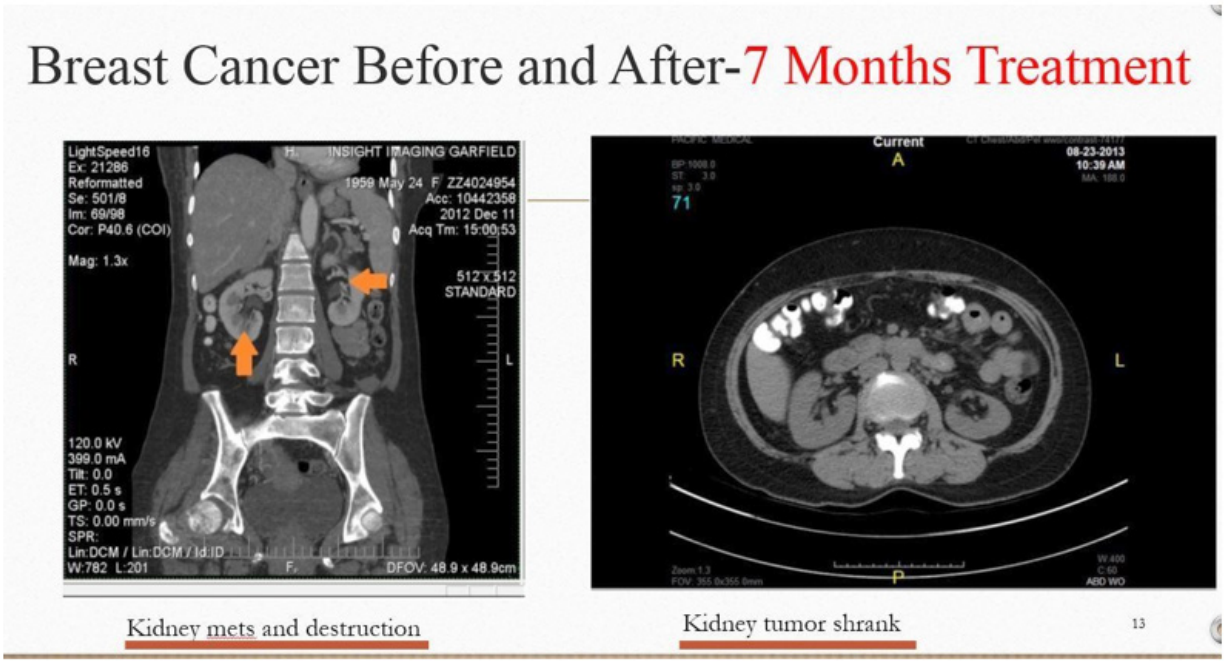
Case 3: Lung cancer pre and post-treatments.



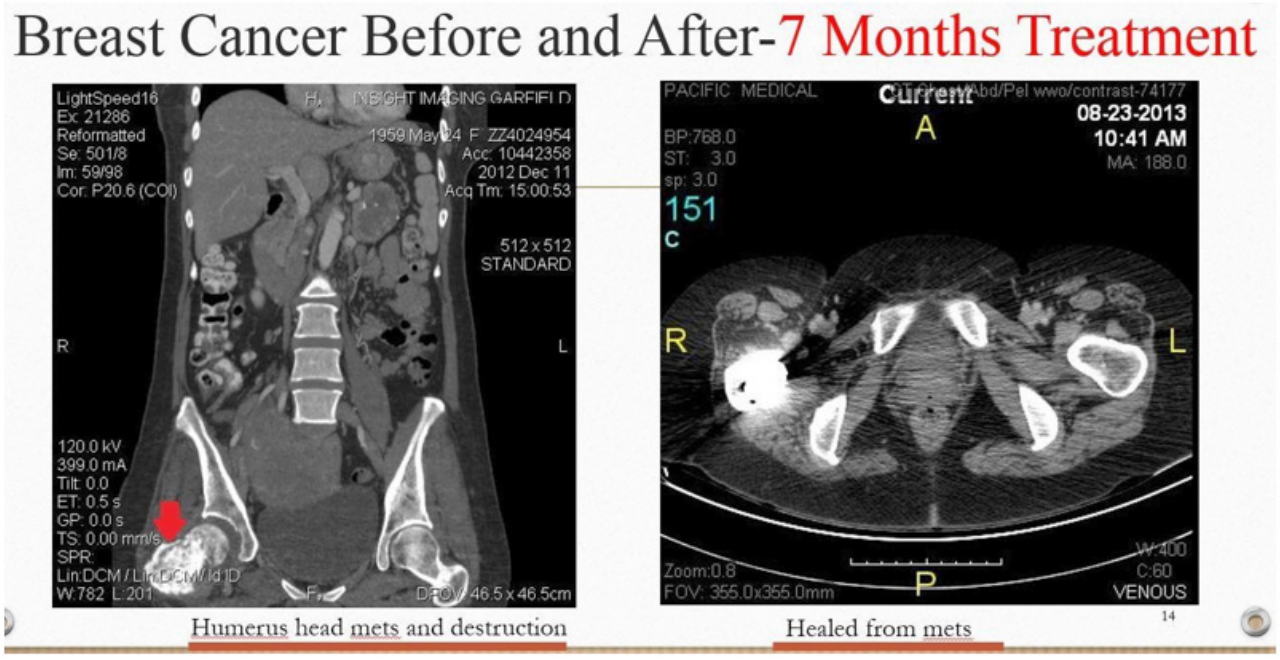
Case 4: Lung cancer pre and post-treatments.



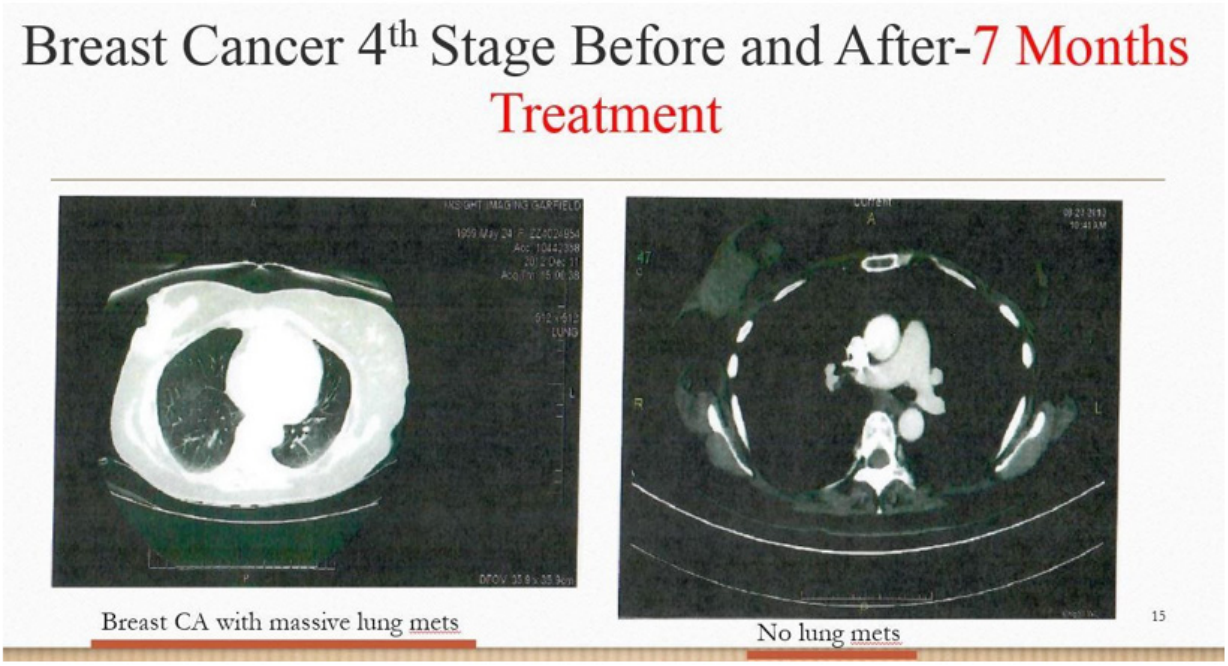
Case 5: Prostatic cancer pre-and post-treatments.



Case 6: Breast cancer pre-and post-treatments.

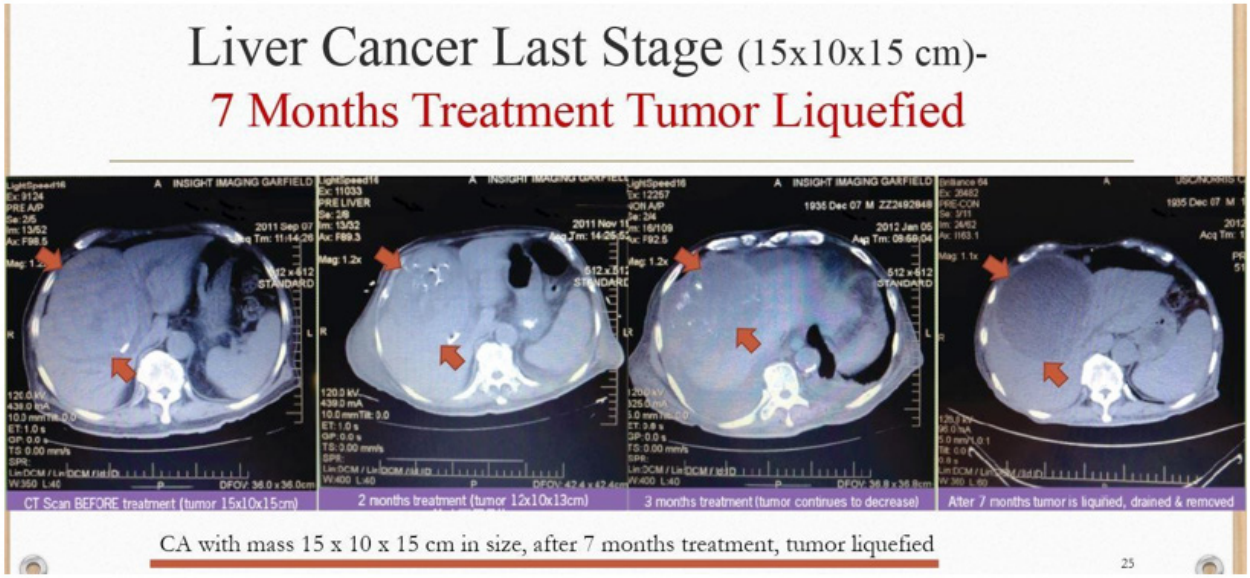


Case 7: Breast cancer pre- and post-treatments.

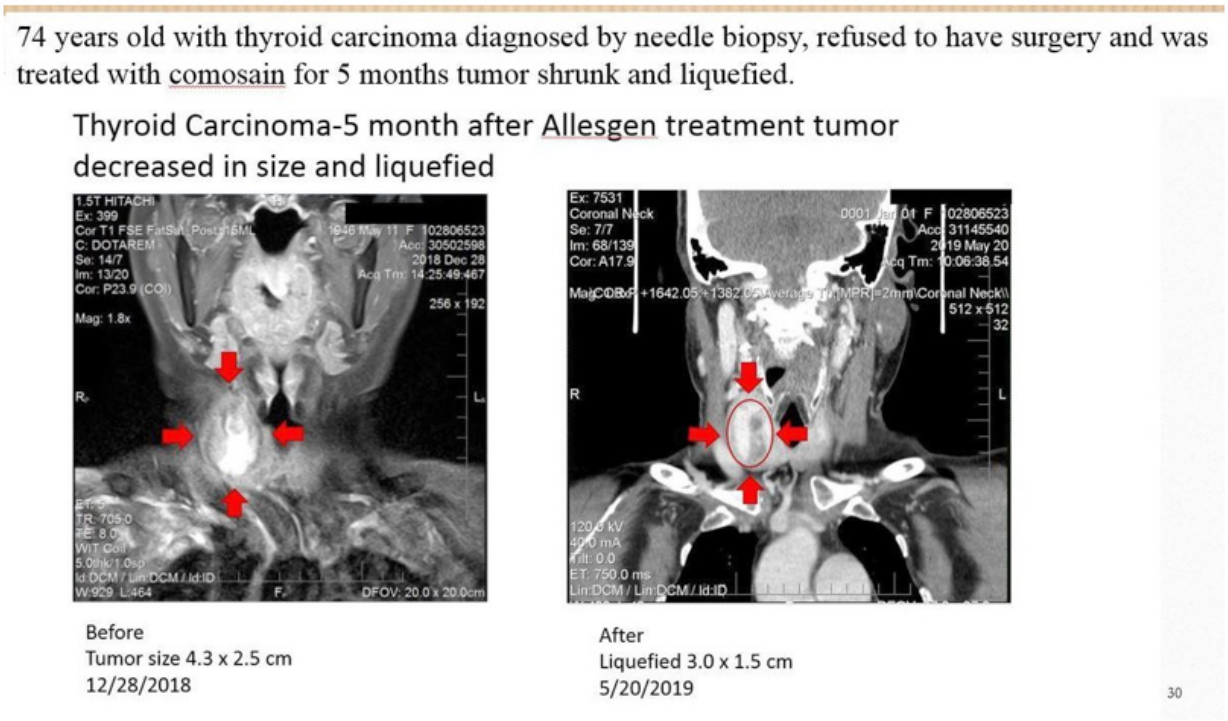


Case 8: Breast cancer pre- and post-treatments.

Case 9: Breast cancer pre- and post-treatments.



Case 10: Liver cancer pre-and post-treatments.



Case 11: Thyroid cancer pre- and post-treatments.

Acknowledgment

The authors sincerely thank Dr. Harvowitz of the Molecular Biochemistry Department at the California Institute of Technology for her expert assistance in molecular protein sequence analysis and assay in SDS-PAGE mass spectroscopy. Dr. Michael Fishbein of the Department of Pathology at the University of California in Los Angeles for his expertise and kind assistance in Pathology. Editorial assistance Miss Beth Shirokawa, Miss Margaret Cheng, Miss Janet Shih, and Miss Oceania Wang are gratefully acknowledged. From the department of molecular biology, biochemistry, surgical oncology, Garfield Medical Group, Oeyama-Moto Cancer Research Foundation, and King-Drew Medical University, Los Angeles, California. This research was supported in part by a grant from the Oeyama-Moto Cancer Research Foundation Grant # (OMCR2002).

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