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# Glyco-Polypeptides (Comosain) in Treating of Various Types of Late-Stage Refractory Solid Carcinoma in Humans - A Double-Blind Study-Case Report of 126 Patients

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**ABSTRACT**

Glyco-polypeptides (Comosain, Bromelain) induced leucocyte binding ability to tumor surface antigens, such as interleukin 2, 6, 8, and TNFs, is known as an immuno-target therapy. Using different 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 were carried out in a double-blind study: low dose cohort was on 10 mg/kg/day and a high dose cohort which was on 50 mg/kg/day for a period of over six months. A total of 83 patients with 3rd and 4th stage of refractory solid tumors were enrolled, whom at least previously failed on two regimens of chemotherapy and/or failed on radiation therapy. The rates of Complete Response (CR) and Partial Responses (PR) in 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 with in view of the reported anti-metastatic activity of orally administrated Bromelain.

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## Introduction

Administration of glycol-polypeptides (bromelain) in cancer treatment in nonclinical trials has been reported as early as 1968 by Wolf M, & Ransberger K. 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. In recent years, Batkin & Taussig (1988) suggested the antitumor mechanisms are due to fibrinolytic effects in Bromelain. Taussig & Batkin in 1988 discovered that bromelain has anti-platelet aggregation effects. Taussig and Batkin in 1985 also discovered the inhibition growth of tumor cells such as Lewis lung carcinoma, V-8 lymphoma, MC1-1 acites, KATO-gastric carcinoma cells. Maurer, & Hozumi, in 1994 (6) discovered bromelain-induced differentiation in leukemic cells. 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. 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 [1-15].

From the experimental studies above, we hypothesize that activation of bromelain proteinases in lymphocytes and T-cells have 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. 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, hematopoetic 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 were administered in high dose cohort patients.

**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 [10]. Monosaccharides fraction are L-fucose, D-galactosamine, D-glucosamine, D-xylose, D- mannose, D-glucose, D-galactose, D-fructose, and Deoxyribose.

**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. The bromelain (comosain) extract derived from the stem and fruit of ananas comosus will be administered orally each day.

**Patient Eligibility and Selection**

1. 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.
2. 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 regiments 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 for hormonal therapy.
3. 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.
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.
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.

4. Patients with 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.
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.
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 low dose 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.

**Drug Dosage and Schedule**

The dose of gylco-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 (see Section VII-A and HR Maurer's study in 3000 patients; Bromelain Complimentary Tumor Therapy: Journal of Oncology, 31: 66-73, 1989).

For this clinical investigation, the high dose group will be given bromelain at 50 mg/kg/day (at a body weight of 50- 60 kg) to a maximum of 2400 mg (5000 GDU) /day and divided into 2 doses/day of 1200 mg/dose [42].

In both high dose group and 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 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 [43-50].

**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.

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 base, the low dose group patients will be transferred to the high dose group due to lack of efficacy in the treatment [51-60].

**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 CT scan and/or PET scan, 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.

c) 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 24Hrs.

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, Need ICU Care. Fever and Temperature: > 40° C for more than 24 Hrs.

d) 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 facilitate location of related adverse events.

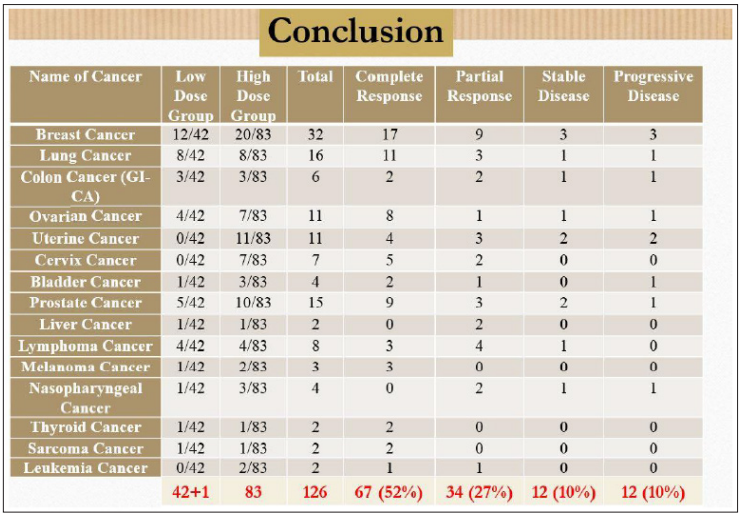
e) Grades of Adverse Events For each adverse event, grades are assigned and defined using a scale from 0 to 5. With 0 representing no adverse event within normal limits and 5 representing death related to an adverse event.

f) Documenting Related Adverse Events

| 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**

**Study Endpoints**




| 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%) |

**Study Endpoints**

At the end of six months, an assessment of the therapy results for each study patient will be 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. The study endpoints for both groups are:

3-a.Use of Standard response Criteria: (NCI Chapter Standard Response Criteria 11.1.1 through 11.1.7 and 11.2, 11.3,).

I. Evaluation of Target Lesions

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

b) Partial Response (PR): At least a 30 % decrease in the sum of the diameters of target lesions, 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 study). In addition to the sum to the relative increase of 20%, the sum must 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 sufficient increase to qualify for PD, taking as reference to the smallest sum diameters while on study.

II. 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) (if tumor makers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

b) Non-CR/ Non-PD: Persistence of one or more non-target lesion(s) and /or maintenance 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, not a single lesion increase.

III. Evaluation of Best Overall Response

A). For Patients with Measurable (Target Disease) Disease

| Target Lesions | Non-Target Lesions | New Lesions | Over all Response | Confirmation |
|---|---|---|---|---|
| C R | C R | No | C R | ≥4 wks |
| C R | Non-CR/Non-PD | No | P R | ≥4 wks |
| C R | Not evaluated | No | P R | ≥4 wks |
| P R | Non-CR/Non- PD Not evaluated | No | P R | ≥4 wks |
| SD | Non-CR/Non- PD Not evaluated | No | SD | ≥4 wks |
| PD | Any | yes | PD | |

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 |

**Data Analysis**

Data collected from all patients will be analyzed to determine overall efficacy of Bromelain to treat advanced cancers. Statistical analysis such as Student t-test will be used. The results of the ongoing analyses will be reported to the FDA in annual report.

a) Adverse events are mild to moderate anemia, leukopenia, thrombo -cytopenia and / or liver and renal impairment.

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.

**Results and Conclusions**

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

1. Age distribution: Both in high dose group and low dose group patients are mainly ages 65 and above. 68% and 81% respectively of participants were over the age of 65 (See table I below). And the participants that are male gender are 75 % and 67% respectively.
2. The disease classification and distribution are as following: breast carcinoma account for 25% in high dose group and 28% in the low dose group, in lung carcinoma the incidence 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 high dose group is about 13% etc.

Please see table II for overall disease distribution. Table II 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 both 2.5%. In the incidence of liver cancer are 2.4% and 1.2% respectively. In 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.

The overall clinical response rate in high dose group patient and low dose group patient are as following: the Complete Response (CR) rate are 52% and 0% respectively, the Partial Response (P R) rate are 27% and 0% respectively. In the patients of low dose group there were no stable disease (SD) and in the patients of high dose group is 13.6%. The progressive disease (PD) in the high dose group is 9%, and in the low dose group is 100%. (Please see Table IV.)

The overall adverse effects and toxicities are shown in Table III-A to Table III-D which all concluded that there were no hematological, renal, and hepatic toxicities in patients of all group.

The primary target lesion size is less than or equal to 2 cm in 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 (Please see table VII).

The tumor markers such as CEA,CA-125,CA-153, CA-199, PSA, TSH, and alpha-feto- protein are being monitored, their value corresponds to the tumor masses, they return to normal value when tumor have been complete responded (CR), and when the tumor progress the tumor marker value are elevated (Please see Table VIII).

The serious adverse effect in toxicity in the both low dose group and high dose group are not observed as seen in Table III-A to Table III-D. There are 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 the patients with average body weight are effective and non-toxic (Please see Table VIIII and Table X).

The following figures are self- explanatory for the above results.

**Table I: Baseline Characteristics – Age, Gender**

| 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 II: Disease Classification**

| 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 III: Lab Test WBC, Hb, Platelets, Survey, Labs Test**
**Table III-A 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 | 5.3 ± 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 | 33 ± 18 | |

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

| | 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 III-C: 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 | 5.6 ± 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 III-D: Miscellaneous Ca. Patients Number: 16/81 (20%)**

| | Pretreatment Value | During Treatment 6 Weeks | During Treatment 12 Weeks | During Treatment 18 Weeks | During Treatment 24 Weeks | During Treatment 30 Week |
|---|---|---|---|---|---|---|
| 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 | |

Tumor Measurement in Phase -2 Clinical Trial Study

a) Low dose cohort with measurable disease were either by direct measurement, X-ray, CT scans and /or PET scans (See Table IV). Table IV 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 transferr/ed to the high dose cohort to continuing therapy for humanitarian reason.

b) High dose cohort measurable disease was either by direct measurement, X-ray, CT scans and/or PET scans (see Table V). Table V showed tumor regression in 80% of patients in the high dose group.

**Table VI: Overall response rate**

| | 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 VII: Outcome Measurement- Primary Target Lesion Size**

| 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—10cm | 12/42 (28.6 %) | 20/124 (16.1%) |

**Table VIII: Outcome Measurement- Tumor Markers**

| Target Lesion Size | Low Dose Group | High Dose Group |
|---|---|---|
| CA 125 | 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 IX: Outcome Measurement- Serious Adverse Outcomes in Toxicity**

| Target Lesion Size | 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 X: Non-serious outcome toxicity**

| | | |
|---|---|---|
| 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 %) |

## Discussion

In summary, throughout the 6 to 10 months course of the double-blind study of bromelain administration for high and low dose group, 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.

The glycopetitdes of sten Bromelai was 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 (Ishihara et al 1979).The amount of the glycopeptide was calculated from its content of glutaminic acid as determinate by amino acid analysis. The average molecular weight was assumed to be 1.5x10^3 DA. Bromelain contains nine different glyco-polypeptides. Each polypeptides contains amino acids in double benzene ring structure and one of twelve different monosaccharides fraction (Harrach et al 1994). Specifically, breakthrough fraction such as Comosain (F9) account for 80%, ananase account for 10%, the rest of 10% were derived from Bromelain F1, F2, F3, F5, F6, and so forth (Batkin et al 1988). They mainly comprise of 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 proteinase, F4 and F5, were further characterized and shown to have molecular masses of 24397 Da and 24472 DA, respectively (Harrach and Haynes et al 1994&1989). 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 197 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 282mu [61-70].

The remarkable cancercidal effects (we designate as Chimeric WBC Immuno-Therapy in cancer) (Cantrell et al-5) (Mott-57) probably due to massive production of Interleukin-II, VI, VIII, and tumor necrotizing factors (TNF) (Wajant–58) from CD-2, CD-3 (Cell Device 2&3) in monocytes and lymphocytes (T- cells). The fibrinolitic effects on tumor surface antigens of CD-44, CD-44V, CD-44S, CD-45, and CD-47 (Denning-8) (Eckert & Maurer-12) (Harrach & Maurer–21) (Hoffman-28) (Matsumoto-41), which induce dehydration, necrosis, and possible calcification in the tumor cells. This action mechanism of Bromelain is mainly due to the inhibition of following two kinases (1) MMAPK (Major Mitogen Activating Protein Kinases) (Cantrell et al-5) TPK (Tyrosine Phosphorylation Kinases) (Cantrell et al -5). 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 time/$10^6$ WBC, and the TNF by 42 times / $10^6$ WBC (Barnes et al–2) (Desser et al–9) (Garbin & Maurer et al–17) [17-80].

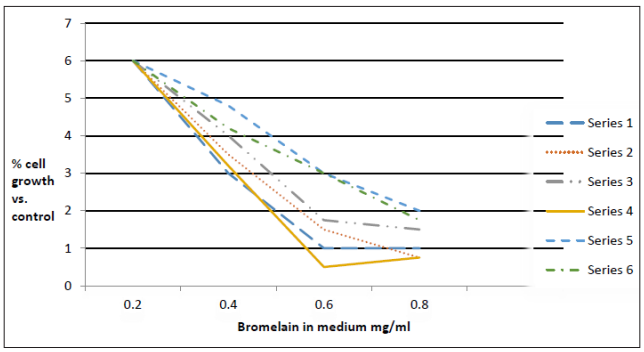



**Figure IA:** Growth inhibition of various types of tumor cell lines in vitro

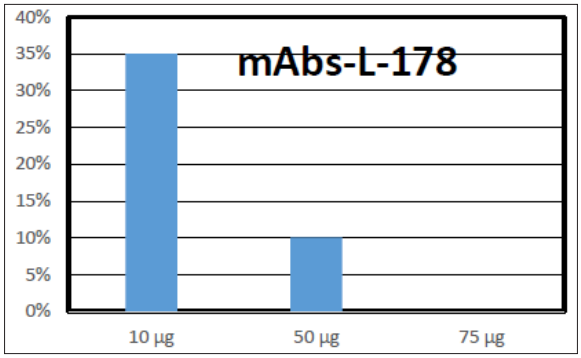



**Figure IB:** Depicts that detection of the CD44s modulation with two different mAbs clones, L-178, J-173. Breast carcinoma cells were incubated for 1 hr at 37 C with 10, 50, 75 ug/ml of Bromelain (Comosain) treatment. The CD44s become 35 %, 10 %, and 1% of Bromelain (Comosain) treated cells (Birch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

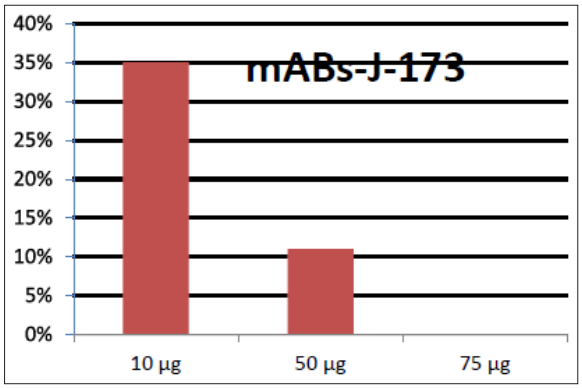
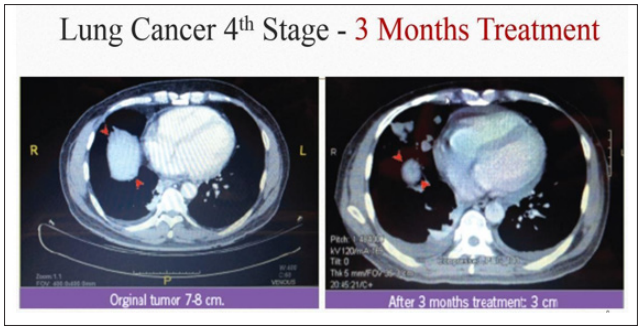



**Figure IC:** Depicts the Interlukin –IB, II-6, TNF production by the monocytes from healthy donors. (Birch et al- 4)

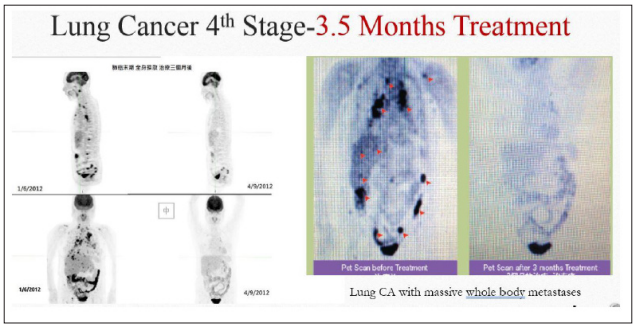
According to the outcome measure, the results in the patients in the high dose group showed remarkable complete response rates of 52%, partial response rates of 27%, stable disease of 11%, progressive diseases of 10 % in these late-stage refractory solid carcinomas by using student T statistical analysis $P<0.05$ which showed statistical significance. Dr. HR Maurer in his complimentary tumor therapy, he 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 of any severe side effects nor had any life threaten events [42].

In the present investigation we conclude that high dose of bromelain therapy (comosain) in an amount of 2500 mg to 3000 mg is a lifesaving regimen and hoping to save thousands of lives in the future.

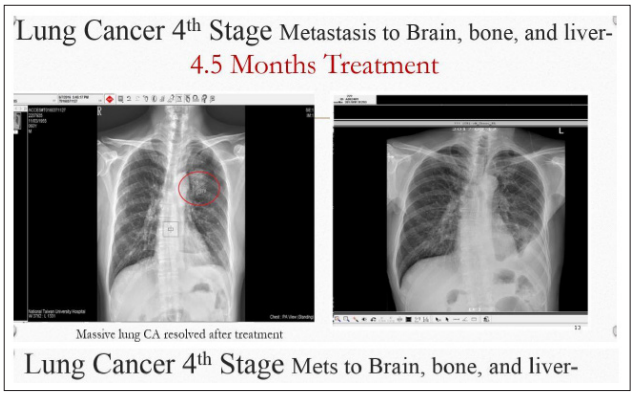


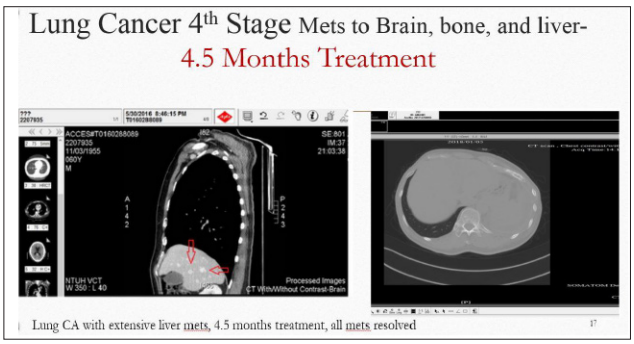

**Case 1: Lung cancer pre and post treatments**

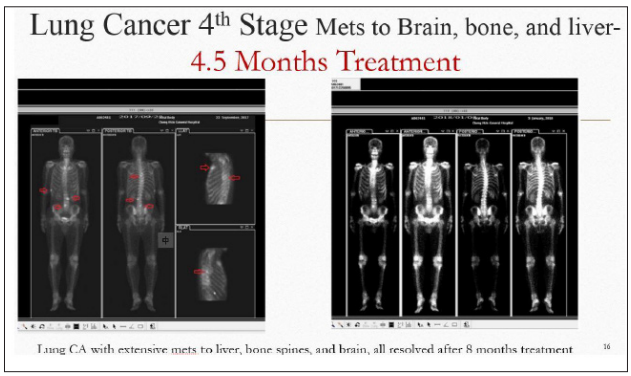
**Citation:** Benedict S Liao, Elizabeth Harvowitz, Michael Fishbein, Austin Liao, Alex Liao, et al (2021) Glyco-Polypeptides (Comosain) in Treating of Various Types of Late-Stage Refractory Solid Carcinoma in Humans - A Double-Blind Study-Case Report of 126 Patients. Journal of Cancer Research Reviews & Reports. SRC/JCRR-156. DOI: doi.org/10.47363/JCRR/2021(3)150



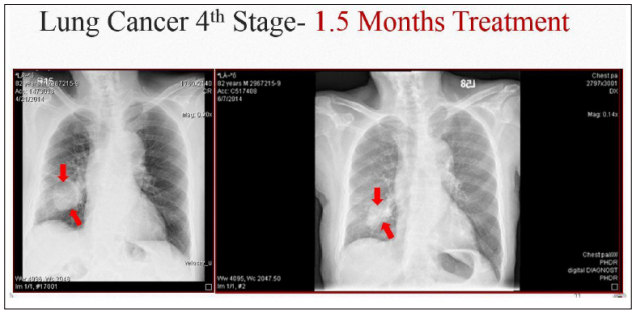

**Case 2: Lung cancer pre and post treatments**



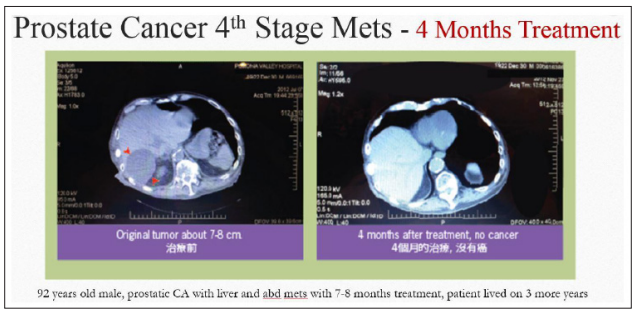



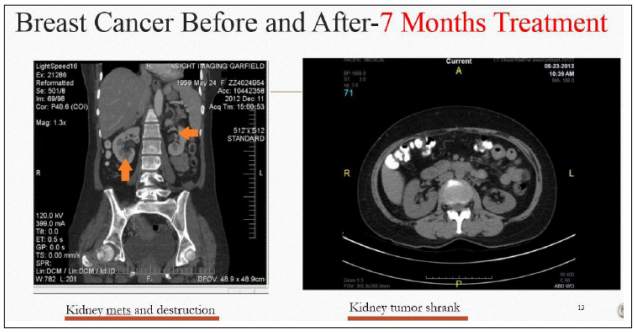

**Case 3: Lung cancer pre and post-treatments**



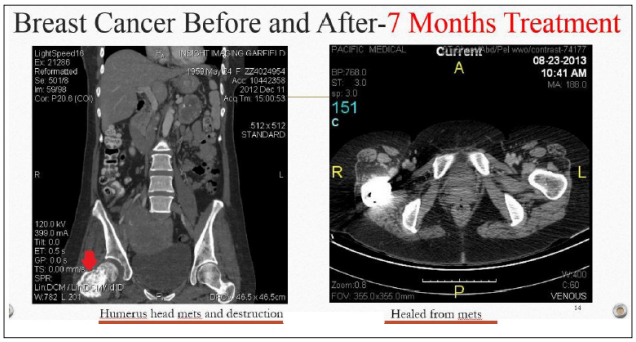

**Case 4: Lung cancer pre and post-treatments**



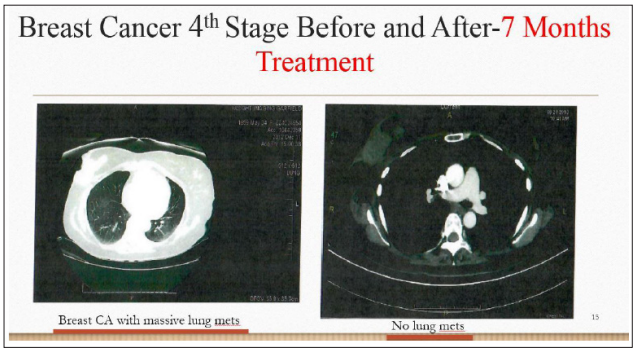

**Case 5:** Prostatic cancer pre- and post-treatments



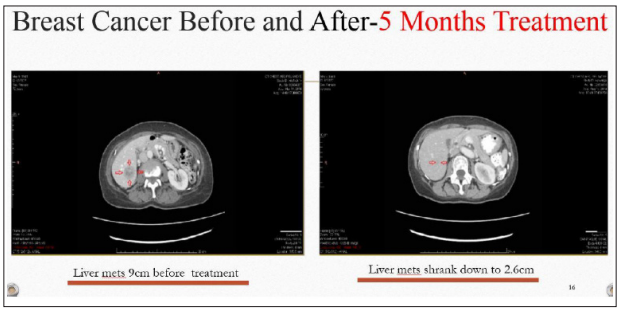

**Case 6:** Breast cancer pre- and post-treatments



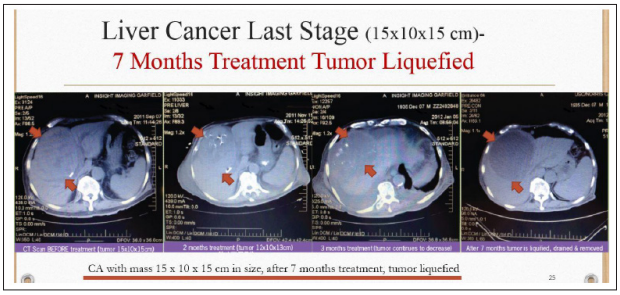

**Case 7:** Breast cancer pre- and post-treatments



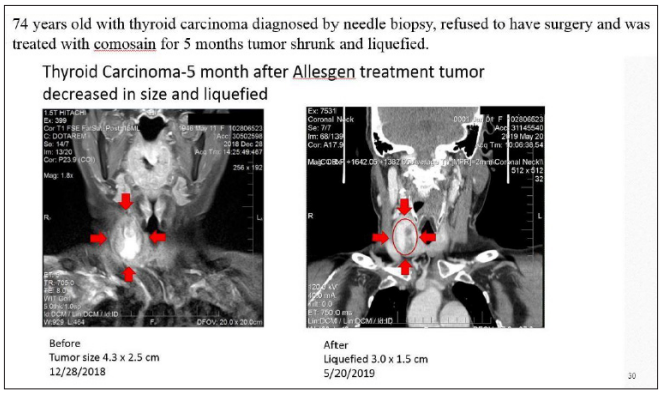

**Case 8:** Breast cancer pre- and post-treatments




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




**Case 11: Liver cancer pre- and post-treatments**




**Case 12: Thyroid cancer pre- and post-treatments**

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