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Managing immunity in resistant cancer patients correlates to survival: results and discussion of a pilot study

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Abstract

Many cancer patients do not die due to impaired organ functions, but as a result of reduced general conditions, such as cachexia, sarcopenia, depression, infections, or stress. Reduced general health may be caused by immune modifying cytokines released from the tumor into the body. Improvement of immunity would not only reduce cancer side effects through inhibiting cytokine release from the tumor into the blood, but also, according to a new hypothesis, modify the cancer stem cells (CSC) in the tumor, which are believed to drive cancer growth and metastasis. We reported previously several investigations with a dietary fermented soy formulation (FSWW08) in cancer patients, where we saw a) strong reduction of cancer symptoms, b) broken resistance to chemotherapy, and c) a strong reduction of chemotherapy's toxic side effects, when taken in combination. This publication reports two new findings from a pilot study with post-surgical, treatment resistant patients conducted over four years. First, neither treatment resistance nor side effects were observed. Second, more patients have survived than expected. The improved health and immunity is detected together with increased CSC differentiation, suggesting lower aggressiveness, which was corroborated by increased gene expressions, particularly of steroidal hormones, *MAPkinase*, *NF-κB*, and *tumor suppressor factor p53*, a typical marker of "stemness" or cell differentiation. Although limited by its small, homogenous sample size, the results of this pilot study illustrate the relationship between CSCs differentiation, and the clinical symptoms of immunity, which influence survival outcomes and raise the clinical potential of measuring CSCs in ovarian, prostate, and breast cancers. The improved survival rates are also seen in larger cohort studies, which show similar gene expression profiles, which were induced by FSWW08 in the treatment resistant cancer patients in this study.

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Introduction

It has been shown in several epidemiological meta-analyses that consumption of soy foods protects against various types of cancers, particularly breast cancer [1, 2]. A recent epidemiologic finding is that soy's protection against cancer is independent of the estrogen receptor (ER) status, and irrespective of whether a breast cancer is ER-positive (ER+) or ER-negative (ER–) or whether patients are Oriental [3–5] or Caucasian women [6], or ER-negative breast cancer patients, where cancer cells show very low or no estrogen receptor expression, and experience more aggressive cancers with lower survival rates for which no effective chemoprevention or treatment has been developed.

Chemotherapy or radiotherapy treatment resistant cancer patients suffer from aggressive cancer cells, which have very low hormone receptor expression and resemble the cancer cells in ER-negative breast tumor [7–9]. Because soy protects against ER-negative breast cancer, the question arises as to whether it may have protective effects in chemotherapy resistant cancers because they are similar to the ER-negative cancer cells. To investigate whether soy's anti-cancer effects could be expanded to treatment resistant cancer patients, we conducted several studies with a special fermented dietary nutritional soy formulation in cancer patients (FSWW08) [10–13]. We reported previously that FSWW08 reduced immunity-related side effects and reduced the aggressiveness of in-blood circulating cancer cells, even in treatment resistant cancers [10–14].

This report discusses the long-term results of a pilot study with breast, ovarian and prostate cancer patients: FSWW08 continued to improve immunity-related side effects of cancer patients without causing any resistance and or other adverse side effects. Surprisingly most patients are still alive, even though almost all were considered treatment resistant to chemotherapy and radiation, which was a requirement for enrollment in this study. FSWW08 prevented progression of metastatic liver and bone tumors, with the exception in one patient suffering from an adrenal tumor.

As the pilot study serves as a basis for larger clinical studies, the data has to be carefully examined. At present, it cannot be precisely determined, which factor or factors caused the increase in survival. We assume the increased survival is a result of the synergistic combination of a) improved nutritional status, b) improvement in immunity and reduction of

inflammatory cytokines and their immunity-related cancer side effects, and c) the prolongation of previously ineffective chemotherapy. Clinical laboratory investigations, however, suggest a direct impact of FSWW08 on cancer cells, in vitro and in vivo [10–14], where particularly the cell differentiation is increased, judged by increased hormone receptors and particularly the increase of the "stemness" marker *tumor suppressor factor p21* and *p53*. Patients with increased cancer cell differentiation have increased survival rates, so we conclude that the increased cell differentiation induced by FSWW08 may have contributed to the increased survival of the treatment resistant cancer patients in this pilot study. FSWW08 showed strong simultaneous improvements in immunity (reduction of pollen allergy and infections, particularly viral infections) and cancer symptoms directly related to immunity like cachexia, stress, joint pain, stomatitis, and depression (Figures 1 and 2) [10–12]. It is interesting to note that a new hypothesis on how to survive cancer focuses on improvement of immunity [7, 8].

As the US government asked Goodman and Gilman in 1942 to identify several toxins to fight cancer, chemotherapy has saved many lives [16]. However, many patients do not benefit, because they develop resistance to chemotherapy with time [17–19]. Second, many cancer patients do not die as a result of impaired organ functions, but to cancer-related side effects, which are immunity-related, such as cachexia, sarcopenia, depression, infections, or stress (Figure 1) [12, 18].

An improvement of immunity in cancer patients would therefore a) not only reduce cancer side effects through inhibition of cytokines released from the tumor into the peripheral circulation (Figure 1) [20], but also b) as Wicha et al. hypothesized, reduce treatment resistant cancer cells, the so-called cancer stem cells (CSC) in the tumor, which are believed to be the driving force of cancer and cause of metastasis

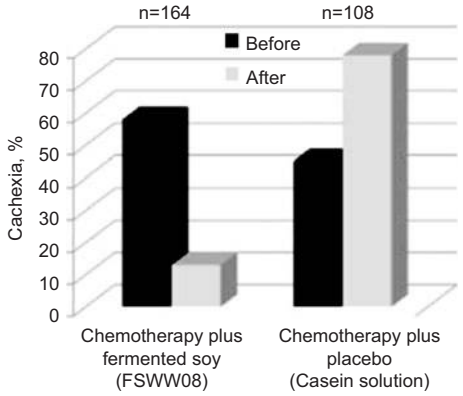

Figure 2 Influence of daily consumption of a fermented soy formulation (FSWW08) on appetite loss (cachexia) in cancer patients under chemotherapy compared to a group receiving placebo solution containing casein. This was a double-blind study. Reproduced from [12].

(schematically depicted for fermented soy in Figure 3) [7, 8, 15, 22]. More than 99% of all cancer cells in a tumor are differentiated cells with high hormone receptor expressions, which do respond to chemotherapy: CSCs are treatment resistant to radio- and chemotherapy [9]Wicha and co-workers suggested that the success of chemotherapy being toxic to differentiated cancer cells (the white cancer cells in Figure 3) and initially smaller tumor sizes, release at the same time immune active cytokines from these dying cells, like IL-8, into the tumor which inhibits the differentiation of stem cells resulting in their continued undifferentiated state which we now identify as a treatment resistant CSC. As "stemness" is not defined, CSCs are frequently circulating tumor cells (CTC). The transformation of CTC into more differentiated cancer cells

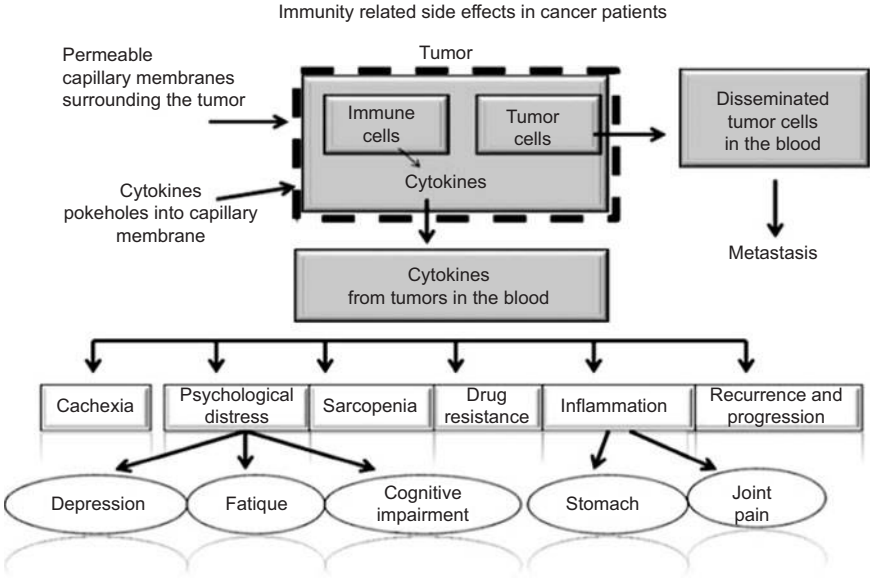

Figure 1 Conceptual model of immunity-related side effects in cancer caused by cytokines. Tumor and immune cells are sources of cytokines, which support the growth of cancer and lead to psycho behavioral symptoms (fatigue, depression, and cognitive impairment), drug toxicity, drug resistance, anorexia and cachexia, pain, and cancer recurrence and progression. Genetic background, cancer treatment and psychological distress can corroborate the production of cytokines [15].

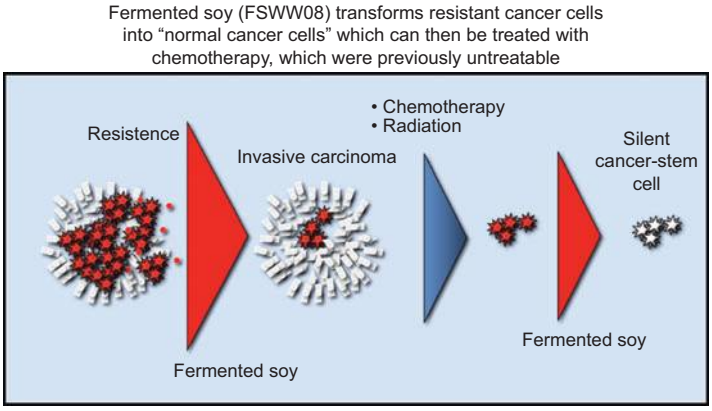

Figure 3 Schematic depiction of invasive breast cancer. Primary breast cancer consists of two types of cancer cells. Cancer stem cells have only low estrogen receptor expression, whereas primary cancer consists of cancer cells expressing 68% estrogen receptor. Chemo- or radiotherapy cannot reduce cancer stem cells [8, 9, 21]. Therefore, cancer recurrence is difficult to predict: schematic development of cancer therapy through chemotherapy.

may therefore reduce treatment resistance to chemotherapy and radiation (Figure 3). Therefore strengthened immunity in cancer patients should inhibit CTC increases and reduce treatment resistance [20], while at the same time reduce cachexia, depression, stress and infections (Figure 1) [23].

Co-administration of immune modifying agents, which increase cell differentiation in cancer cells, together with chemotherapy is established for leukemia [24–30] and myeloma (Figure 4) [32–37]. Dexamethasone and radiation therapy are established modalities in single and multiple myeloma [32–37]. It was discovered recently that some of the newly developed chemotherapeutic agents have stem cell differentiating properties themselves, e.g., rapamycine induces differentiation of glioblastoma cells [38].

Naturally occurring dietary compounds can directly or indirectly affect CSC self-renewal pathways [39–41]. The dietary compounds, including curcumin, sulfuraphanes, soy isoflavones, epigallocatechin-3-gallate, resveratrol, lycopene, piperine and vitamin D(3) are discussed for their direct or indirect effect on these self-renewal pathways [40] and have demonstrated ability to target breast CSCs. Epidemiologic studies revealing soy's protection against ER-negative tumors support the assumption of a direct impact of soy on breast CSCs. FSWW08's impact on cell differentiation of in-blood circulating CTC and survival is discussed.

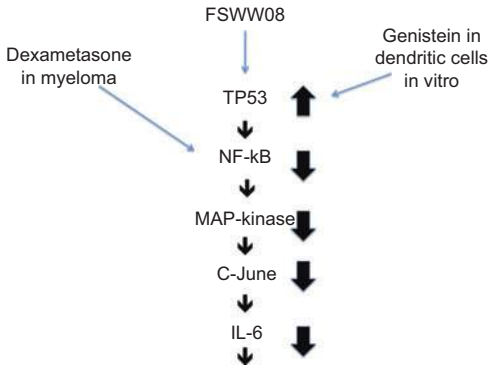

Figure 4 Dexametasone, FSWW06 (fermented soy), or genistein in vitro, share changing gene expression cascade within cancer tissue or circulating tumor cells, or dendritic cells. TP53 denotes for tumor suppressor factor p53 gene expression, which is a key cell differentiation marker and is reduced in 96% of all cancer patients [31]; *NF-κB* denotes for nuclear factor kappa B, MAP-kinase denotes for mitogen activated protein kinase, c-june is a key immunity factor, IL-6 denotes for interleukin-6.

Materials and methods

Study medication

A fermented soybean product (Haelan Research Foundation, Woodinville, WA, USA), FSWW08, investigated in the USA qualifies under the FDA Orphan Drug category as a Medicinal Food or Food For Special Medical Purposes. This status was also achieved in Europe by EMAS. The phytochemical substances contained in soybeans are broken down in smaller molecular units by a patented fermentation process [42]. This fermentation of soybeans transforms proteins into smaller units to make it palatable and to minimize the risk of protein allergies. The fermentation process is particularly optimized as protein cleavage into amino acids is facilitated, and the full integrity of Bowman Birk Inhibitory factor is maintained: FSWW08 contains 900 c.i. units of the Bowman Birk Inhibitory factor [43].

FSWW08 contains among other ingredients the bioactive isoflavones genistein and daidzein [42]. The active agent MDT-13 (13-methyl-tetradecanoic acid), patented as an ingredient of FSWW08, has been shown to induce apoptosis (i.e., programmed cell death) in human tumor cell lines [44].

Analysis of gene expression

Extraction of circulating stem cells and further processing was reported elsewhere [14]. Cancer Quantitative mRNA expression was determined by real-time RT-PCR using 5′-nuclease ("TaqMan") chemistry (Applied Biosystems, CA, USA). Each TaqMan run was accompanied by a serial log dilution of a control cell line for generation of the standard curve. Hence, all values in the following schedules are given in the unit of cell equivalents according to the standard

curve. To make the expression values comparable, the values were normalized to the housekeeper GAPDH, which is expressed in each cell line equally. All gene expression measurements were done in duplicate.

We did not previously report gene expression of MAP-kinase cascade or inhibitors and promotors of matrix metalloproteinases. MAP-kinase cascade and *NF-κB* are linked to immunity, which may be an important mechanism explaining the survival of cancer patients [45]. MCF7 breast cancer cells were spotted with blood of breast cancer patients and breast cancer patients under consumption of FSWW08.

Clinical trial with prostate, ovarian and breast cancer patients

We previously reported a clinical pilot trial for method development with treatment resistant cancer patients [10]. Key parameters like, age and weight can be found in the literature [10]. Patients undergoing evaluation for suspected ovarian malignancy (initial diagnosis or recurrence) from January 1, 2007 to Febuary 28, 2011 were recruited. Blood samples were collected after written informed consent was obtained. Patients with pathologically or cytologically confirmed epithelial ovarian cancer or primary peritoneal cancer were included in the study, as well as cytologically confirmed breast cancer, and cytologically confirmed prostate cancer. Disease staging was defined for ovarian cancer using the FIGO classification system. The TNM staging system was used for breast cancer staging, and the Gleason Score for prostate cancer. All participants of that pilot trial were monitored for the next 4 years and clinically evaluated. Survival data, as well as gene expression of *tumor suppressor factor p53* are reported in Kaplan-Meier plots.

Clinical data collection

In this retrospective cohort study, all clinical data was abstracted from patient medical charts using a frozen file date of February 1, 2011. Tumor grade and histology were collected from pathology and cytology reports. Staging was conducted according to accepted standards: FIGO for ovarian cancer, TNM grading for breast cancer, Gleason scale for prostate cancer.

Survival length was calculated from the date of blood sample collection to date of death (for overall survival) or recurrence/progression, or most recent documented contact, if the patient was still alive with no evidence of disease. Patients were judged as: SD for stable disease; RE: remission; PR: partial remission if tumor shrunk up to 50% compared to time of enrollment; CR: complete remission. A standard questionnaire (EORTC QLQ-C30 V1), describing quality of life, and side effects of therapy of cancer patients was used to describe clinical symptoms [10].

It was shown in a small-scale prospective study in prostate, breast and ovarian cancer patients that a fermented soy product could reduce depression, stress and cachexia, which were unresponsive to conventional therapy. Reduction of cachexia and a significant increase of body weight were achieved [10].

Gene expression studies with circulating tumor cells

We previously reported changes of gene expression studies with circulating CSCs after consumption of FSWW08 [10–14], and expanded those investigations to 4 years. The method of extraction of CSC from the blood, tumor cell enrichment, and processing can be found in the literature [46, 47]. Prospects and limitations measuring circulating CSC in cancer patients, which is not a standard method in cancer treatment and therefore accepted ranges have not been defined, can be found in the literature [46, 47].

It was previously reported that FSWW08 did alter *NF-κB* in CSC as well as in vitro in cell culture experiments [12]. Apoptosis marker in disseminated tumor cells like *BAX/Bcl2-ratio*, cell cycle inhibitor p21 and anti-proliferation factor *Estrogen Receptor beta* (*ER-β*) gene expressions were increased in prostate and ovarian cancer patients. Breast cancer patients did show increased cell cycle inhibitor p21 and p53, however no increase in *ER-β*-expression [12]. Leakiness is one of multiple abnormalities of tumor vessels that influences angiogenesis, tumor growth and metastasis but also affects drug delivery and present novel targets for therapeutic intervention in cancer [48–53].

Results

In vivo gene expression studies in-blood circulating cancer tumor cells

It has been reported that soy isoflavones reduce inflammatory cytokines, such as TNF-α and IL-6, via silencing of the MAP-kinase/*NF-κB* cascade of immune cells (Figure 4) [45, 54] what was corroborated in-blood CTC (Figures 5–8, Table 1). It has been reported that FSWW08 alters gene expression of cell cultures in the in vitro situation [14], particularly increased gene expression of hormone receptors (Figures 5 and 6), PPAR receptors (Figure 6) and *tumor suppressor factor p53* (TP53) (Figure 8) and reduction of *NF-κB* (Table 1). Also, gene expression of *tumor suppressor factor 21* [10] and *tumor suppressor factor p53* (TP53) were increased in CTC in long-term application up to 4 years in the enrolled patients, regardless of the type of cancer (Figure 8). In total, 50% of all cancer patients showed no sign of TP53 gene expression when investigated untreated. Therefore, the increase of TP53 by soy (Figure 8) could help to explain mechanistically together with the change in *NF-κB* prevention of recurrence of breast cancer by FSWW08. Most importantly, TP53 is a marker of cell differentiation, which may explain the reduction of resistance to chemotherapy by FSW08 or even survival.

Estrogen receptor beta and alpha were differently increased in CTC (Figure 7). Whereas estrogen receptor alpha was not increased, regardless of the type of cancer, *estrogen receptor beta* was increased in prostate and ovarian cancer. No increase of *estrogen receptor beta* was increased in CTC of breast cancer patients.

As can be seen in Figure 5 also, the gene expression of androgen receptor was increased. This was corroborated in a follow-up trial, where testosterone and "androstenes" or adiols like androstenediol and androstanediol, were increased, which are by definition zwitter steroids with estrogenic and androgenic properties [12].

It is also worth mentioning that the gluco-corticoid receptors, which have immune modulating properties, are increased (Figure 5).

CTCs, tumor cells leaving the local tumor compartment into bone marrow or peripheral blood, are of utmost clinical importance for the establishment of distant metastasis during the metastatic cascade [55–57]. The advantage of CTC diagnosis over cancer cells from a local tumor

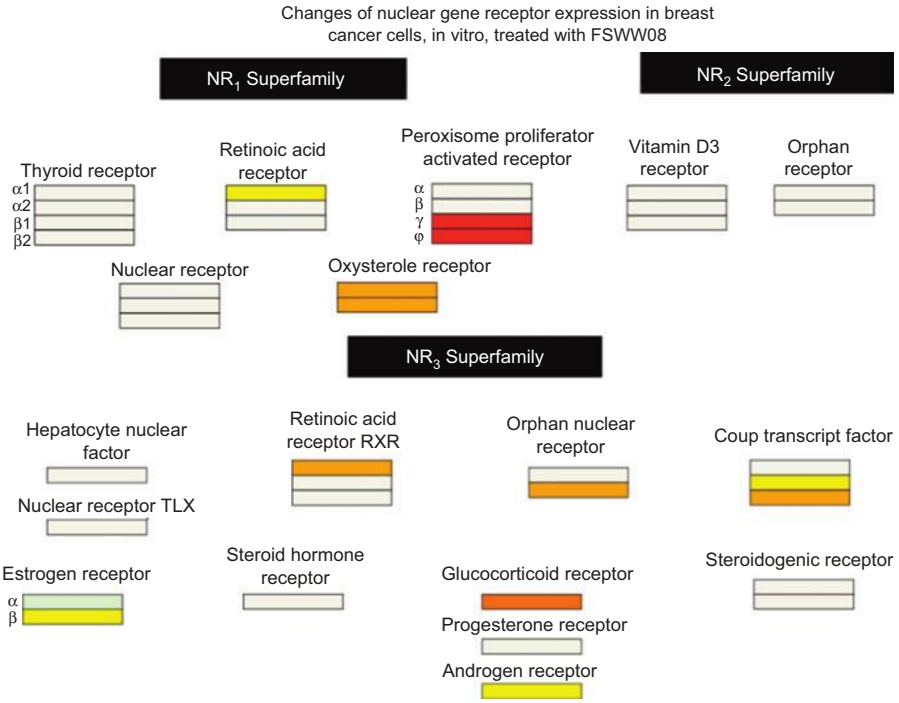

Figure 5 Nuclear receptor gene expression of pooled blood of cancer patients spotted with FSWW08 in-blood circulating tumor cells (CTC) compared to CTC in blood of untreated cancer patients. Following colors denote for gene expression changes on a log scale: ■ >2, ■ 1.5<x<2, ■ 1.0<x<1.5, □ no change, □ 1>x>0.66, ■ 0.66>x>0.5, ■ >5.

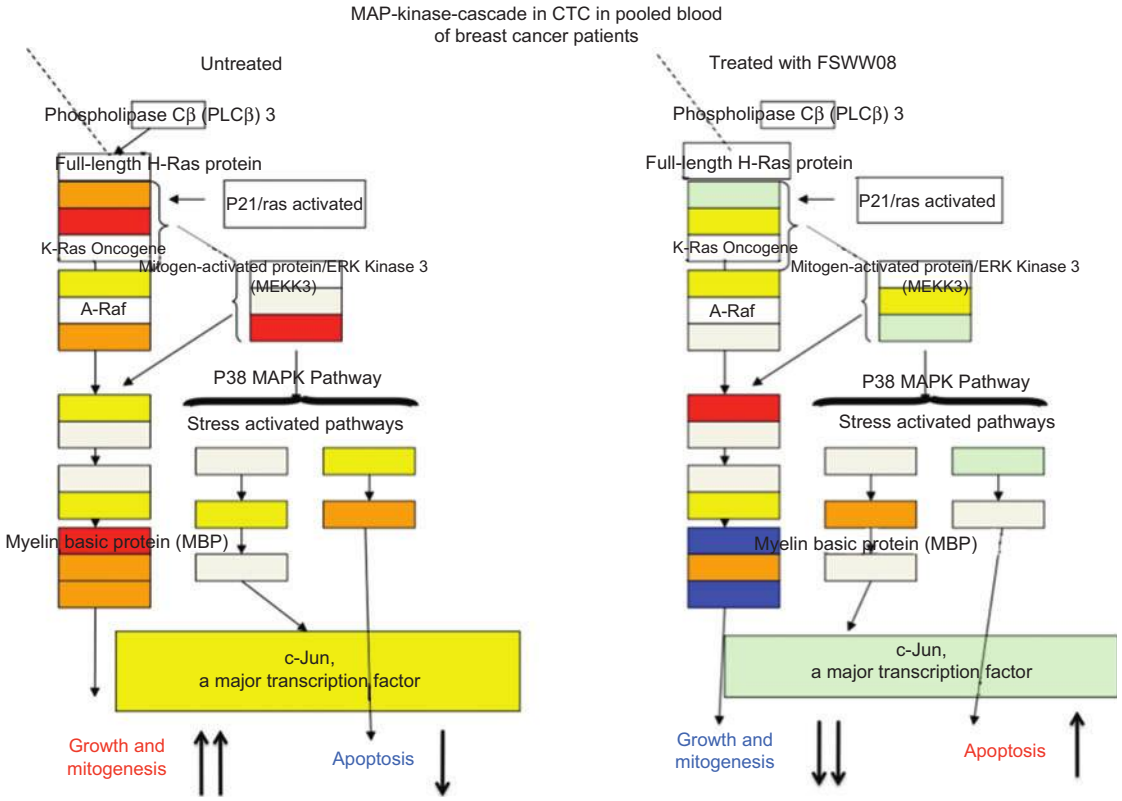

Figure 6 Gene expression changes by fermented soy (FSWW08) on blood circulating tumor cancer from breast cancer cells, before and after consumption of FSWW08. ■ >2, ■ 1.5<x<2, ■ 1.0<x<1.5, □ no change, □ 1>x>0.66, ■ 0.66>x>0.5, ■ >5.

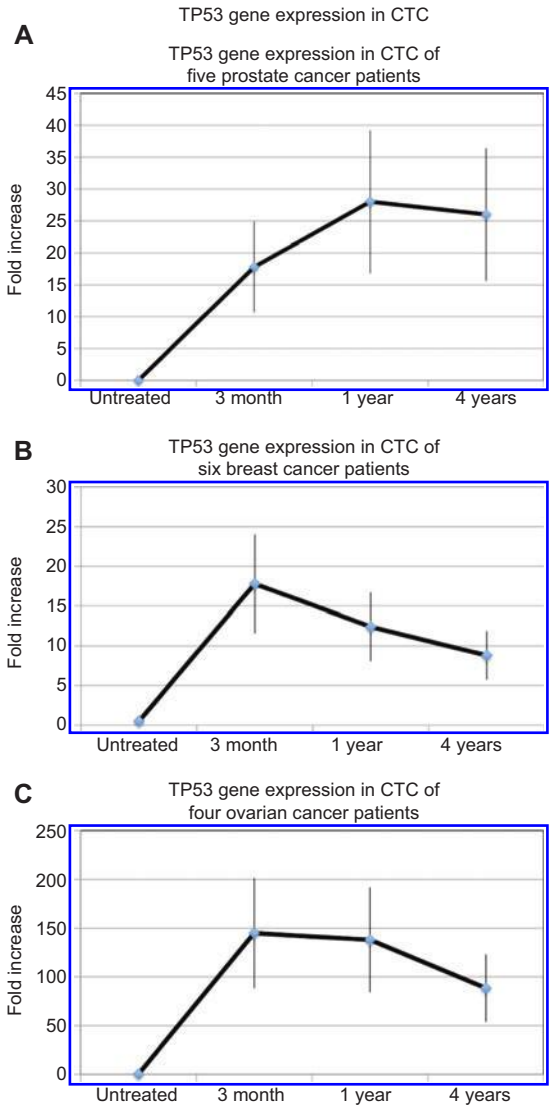

Figure 7 Relative gene expression changes of in-blood circulating cancer cells ovarian cancer patients compared to baseline, compared to expression after 1.5 months, after 3 months and after 4 years (A) prostate cancer patients, (B) breast cancer patients (C) ovarian cancer patients. Numbers denote individual patients.

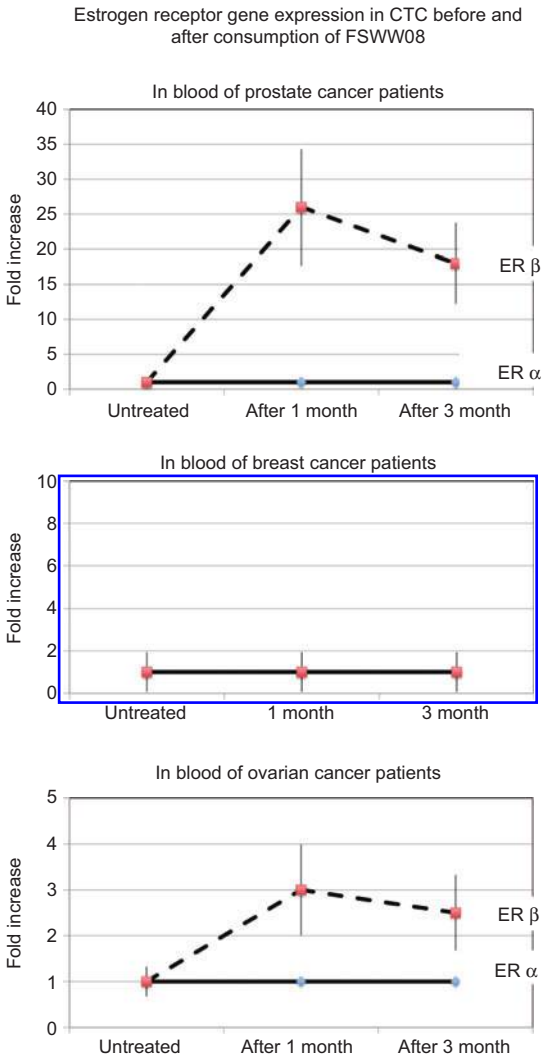

Figure 8 Relative gene expression of estrogen receptor alpha and beta in-blood circulating tumor cells during a pilot trial after consumption of FSWW08, a fermented soy product. X-axes denotes time, during 3 months, measured after 1 and 4 years. Prostate cancer circulating tumor cells was determined in 5 min, breast cancer was determined in six patients, ovarian cancer was determined in four patients, one patient was not reachable for testing.

compartment is first, an easy detection, second, reduces the risk of local efflux of cytokines and cells into the periphery by the needle, and third, they consist of cell forms from which metastasis can develop. Prospects and limitations of this method are discussed in the literature [57]. Although a reduction of CTC was detected in this trial, despite high variability, the number of patients is too small to make general conclusions.

Immunity and gene expression

Patients suffering from pollen allergy or frequent flu infections reported complete remission of these symptoms when FSW08 was consumed during spring season (Table 2).

It was reported in the literature that intracellular release of specific cytokines in asthma or other allergic reactions is linked to increased c-Jun kinase, p38 MAP-kinase, and *NF-κB* in local immune cells [58, 59]. First-generation antihistamines, such as diphenhydramine and chlorpheniramine reverse cytokine afforded eosinophil survival by enhancing apoptosis [60]. First-generation antihistamines and soy isoflavones share similar immune modulating responses to FSWW08. It is of importance that dexamethasone shares similar ability to alter gene expression of p38 *MAPkinase*, particularly c-Jun and *NF-κB* in myeloma cells, which may help to explain the increased cell differentiation and increased survival in prostate, breast and ovarian cancers as well. As can be seen in Figure 7, MAP-kinase is altered when FSWW08 was consumed.

Table 1 BAX/Bcl, what is a surrogate marker for *NF-κB*, determined in-blood circulating human cancer cells extracted from blood of cancer patients. Baseline levels were compared with those found after 3 months of treatment with FSWW08 (taken from [12]).

Type of cancer	Breast cancer (n=7)	Ovarian cancer (n=5)	Prostate cancer (n=5)
Number of patients	7	5	5
In vivo, from human cancer patients with blood circulating tumor cells. In vivo, from human cancer patients with blood circulating tumor cells, after 3 months consumption of FSWW08	–20% (±8%)	–94% (±11%)	–80% (±22%)

Clearly, the anti-viral effects of reducing herpes labialis infection and the sustained reduction of bacterial infections and the absence of yeast infections, was unexpected.

Increase of well-being and immunity

It was reported that FSWW08 had significant clinical effects reducing stress, depression [determined with standard questionnaires EORTC QLQ-C30 (v1)], and improves immunity documented by changes in circulating cytokines, particularly reducing Th1 and increases Th2 cytokines [10].

The improvement in well-being was directly related to the application of FSWW08 (Table 2). Immunity was improved, documented by a reduction of pollen allergies, reduction of flu-like symptoms, reduction of cystitis, stomatitis and rheumatic pain and pain in joints (Table 2). Particularly viral infections were diminished.

Increase in survival

All enlisted patients besides a women suffering from adeno-carcinoma are still alive (Figures 9–11). At the time of enlistment it was not clear whether the adeno-carcinoma developed independently from a previously surgically removed breast cancer. The women died due to cancer-related complications. Cancer growth did not continue in all of the other patients (Table 2).

In a few patients, particularly liver cancer, FSWW08 reduced tumor size in vital organs [10]. In three cases (two prostate cancer, one breast cancer) general health increased so surgeons attempted a surgical removal of the liver cancer metastasis (Table 2). These patients are disease-free until today and have to be judged as tumor-free (Table 2). Interestingly surgeons reported that the tumors had lost their attachment to the tissue.

Survival of ovarian cancer after FSWW08 consumption

Ovarian cancer is the second most commonly diagnosed gynecologic malignancy and the leading cause of death from a gynecologic malignancy in the USA [64]. One reason that ovarian cancer is so lethal is because over 75% of cases are stage III/IV by the time of diagnosis. The preponderance of advanced stage is associated with poorer survival outcomes [65]. Furthermore, although a majority of optimally cyto reduced, late stage disease patients achieve a complete clinical response after completing chemotherapy, most of those patients eventually relapse [66]. Any attempts by practicing physicians to help diagnose ovarian cancer earlier and detect metastasis sooner have the potential to improve survival [67].

In the current study it is observed that regular consumption of FSWW08 was related to improved immunity in a previously described study. A significant decrease in disease-free survival was observed in patients with detectable CSCs (Figure 9). It was reported in the literature that CSC-positive patients demonstrated a median disease-free survival time of 15 months, vs. 35 months in CTC-negative patients (Figure 9) [67].

It may be difficult to compare a very small number of patients with larger trials, but it can be stated that consumption of FSWW08 caused a higher survival than expected and that this may be caused by the increased cell differentiation, documented by the increase of *tumor suppressor factor p53* (Figure 9).

Survival of prostate cancer patients

It is reported in the literature, that the Gleason score has an impact on survival in prostate cancer patients [61]. Treatment resistant prostate cancer patients with a Gleason score between 8 and 10 were enlisted to consume FSWW08. As can be seen in Figure 10, all patients are alive presently. Although the number of patients is small, immunity was increased, as well as *tumor suppressor factor p53* (Figure 10) by FSWW08. Metastasis to the liver did not increase. Most patients showed an increase in well-being and physical activity, fatigue was abolished and they continued a normal life. Two men suffering from pollen allergy in spring showed complete remission as long as FSWW09 was consumed.

Survival of breast cancer patients

As can be seen in Figure 11, the expression of *tumor suppressor factor p53* has a strong impact on survival of breast cancer patients. The survival of breast cancer patients increased, when gene expression of *tumor suppressor factor p53* was expressed (here marked as "wild type") [62]. Consumption of FSWW08 did increase gene expression of *tumor suppressor factor p53* in almost all cancer patients (Figure 11). Findings are supported by strong increase in immunity (Table 2).

Table 2 Individual cancer parameter and accompanying diseases and treatment-related findings.

Time of first diagnosis	Situation at time of enlistment at 1/1/2007	Accompanying diseases	Immunity-related diseases before FSWW08	Clinical observation after application FSWW08	Current status
Breast cancer					
4/2003 Surgical removal of primary breast cancer	2 years detection of liver metastasis with 10 cm diameter	Sleeping disorders, mild depression	Constant flu, cystitis, stomatitis	Patient was referred to chemotherapy again Strong improvement in well-being: reduction of stress and depression	After removal surgery of liver metastasis was performed after 8 months of FSWW08 CR
7/2005 Bone metastasis Surgical removal of breast cancer	Bone metastasis	Depression, stress, diabetes As a consequence of diabetes, ulcerosa of the leg since 3/2004	–	Strong improvement in well-being, depression, stress Complete wound healing of a diabetic ulcer of the leg	SD
12/2003 Surgical removal	Adeno-carcinoma Relationship to primary tumor?	Impaired hair growth Hemorrhoids	Pollen allergy, viral infections	CR of pollen allergy, however as the patient died due to cancer-related side effects	
4/2003	Bone metastasis	Stress, depression, complete healing of hemorrhoids	Pollen allergy, colitis ulcerosa, viral infections	Complete removal of colitis ulcerosa, stress, depression, and viral infections	SD
9/2004	Bone metastasis		Krupp syndrome, stress, depression	Improvement of Krupp syndrome	SD
7/2002 Primary tumor was surgically removed	Metastasis in the lung	Edema in the leg and in the lung Stress and depression	Edema was removed	Strong increase of well-being increased After FSWW08 application, patient did receive chemotherapy	SD
1/2004 Primary tumor was surgically removed	Metastasis of the skin in the neck Tumor could not be removed	Ulcer of the skin		Tumor size was smaller and the ulcer was closed, which was a relief for the patient, as it did reduce infection Patient could not be surgically treated, but SD	SD
Prostate cancer					
9/2001 Gleason Score: 9 No lymph node infiltrated Bone metatstasis	Bone metastasis		Pollen allergy	Strong increase of activity, patients rides the bike every afternoon, which he did not do before	SD
11/2004 Gleason Score 10 Surgical removal	Liver metastasis	Sleeping disturbances, stress, chronic fatigue, edema in the lung therefore breathing problems		Three liver metastasis were surgically removed; surgeons reported that metastasis had lost tissue attachment and could easily be removed	CR
11/2001 Gleason Score 9	Lung metastasis	Sleeping disturbance, edema in the lung	Colitis ulcerosa stomatitis, herpes labialis infection	Edema in the lung disappeared. Patient was referred to chemotherapy CR of stress, depression, viral infections	SD

(Table 2 continued)

Time of first diagnosis	Situation at time of enlistment at 1/1/2007	Accompanying diseases	Immunity-related diseases before FSWW08	Clinical observation after application FSWW08	Current status
2/2000 Gleason Score 10 Surgical removal of prostate tissue	Liver metastasis	–	–	Patient did not report improvement in well-being, or any other clinical effect, physicians recommended continuing the treatment, because gene expression of CTC was improved, also PSA reduced from 235 to 14	SD
10/2002 Gleason Score 9	–	Thrombosis, stress, lupus	Stomatitis, viral infections, gingivitis	Since 2007 no lupus attack, reduction of stress	SD
Ovarian cancer					
10/2006 IIIA-Microscopic peritoneal metastases	Surgical removal of the left side Recurrence at right side	Bone metastasis, depression, constipation, stress	Pollen allergy, Crohn's disease	Improved well-being, and reduction of Crohn's disease, reduction of stress, patient did receive chemotherapy, but was considered stable disease Patient gained normal body weight	SD
9/2006 IIIA	–	Liver metastasis, cachexia	Flu-like symptoms		SD
3/2006 IIIA-Microscopic peritoneal metastases beyond pelvis	–	–	–	Patient was referred back to chemotherapy Although patient did not report any improvement in well-being or immunity-related disease, gene expression study did continue due to improvement in gene expression Chemotherapy was conducted without side effects like cachexia, stress	SD
2/2006 Tumor limited to both ovaries	–	Cachexia	Eye infections	Co-treatment with chemotherapy improved reduction of the tumor Weight gain was documented	PR
6/2006 IV-Distant metastases to the liver		Fatigue, cachexia, stress, depression	Flu-like symptoms; herpes labialis; wound-healing disturbances	Patient was referred back to chemotherapy, CR of cachexia, and stress	SD

CR, complete remission; PR, partial remission; SD, stable disease.

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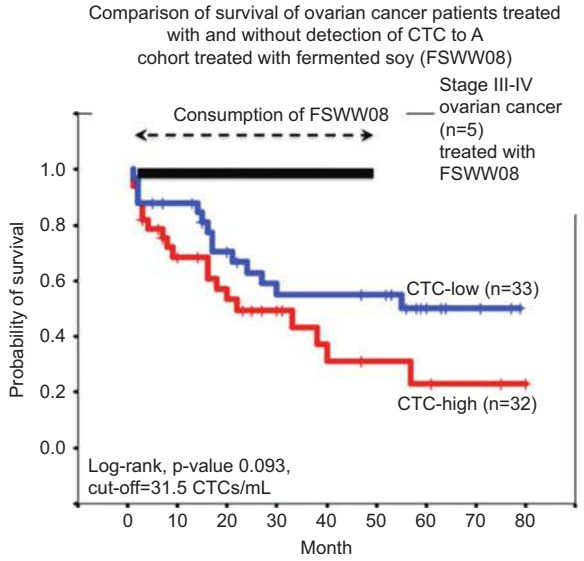

Figure 9 Overall survival of ovarian cancer patients taken for the literature (thin lines) with circulating tumor cells (CTC) or without detection of CTC: taken form the literature [61]. Comparison is made to a cohort tested in a pilot study where treatment resistant ovarian cancer patients were enlisted and consumed a fermented soy product (FSWW08). Dotted line denotes time of consumption of FSWW08.

One patient succumbed to the disease. She was suffering from adrenal cancer and it was not possible to decide whether this was a metastasis from the surgically reduced breast cancer. But it is interesting to note that the survival of breast cancer patients consuming FSWW08 is similar to those patients, which show higher survival rates, due to higher cell differentiation, judged by the increase of hormone receptor status (Figures 5 and 8) and TP53 (Figure 8).

We did report earlier that one patient stopped taking FSWW08 after 3 months because this was the official end of the pilot study and the tumor in the liver did shrink

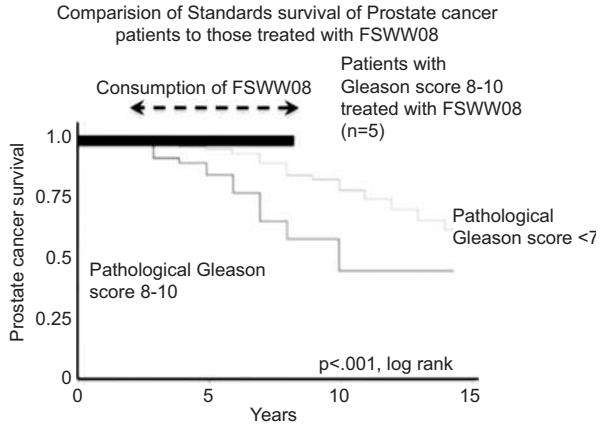

Figure 10 Risk of prostate cancer-specific mortality following biochemical recurrence after radical prostatectomy, taken from [62]. Bold line represents survival of patients (n=6) treated with a fermented soy product (FSWW08). Dotted line denotes time of consumption of FSWW08.

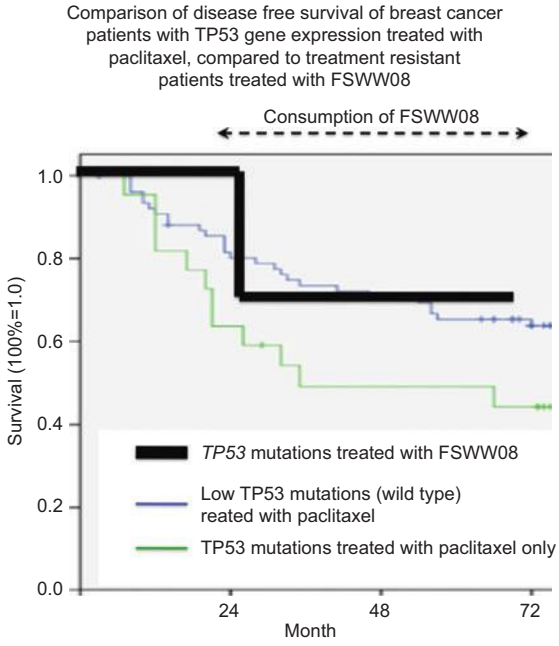

Figure 11 Kaplan-Meier curves of relapse-free survival of breast cancer patients. Difference in respect to TP53 mutation status in paclitaxel treatment (taken from [63]). Bold line, patients received FSWW08, a fermented soy beverage indicated by a dotted line (n=7). All enlisted patients were treatment resistant at time of enrollment. Consumption of FSWW08 inceased tumor suppressor factor p53 (TP53) in these patients (thick black line) were taken from a published study [63]. Patients with *tumor suppressor factor p53* expression, which is a cell differentiation marker, had significant improved survival (thin blue line) [63].

substantially [10]. After discontinuation of the therapy, the size of her liver tumor did increase immediately and was accompanied by a dramatic increase in depression and stress. After FSWW08 was applied again, the size of the tumor reduced, as well as depression and stress [10]. After 6 months the patient experienced surgical removal of the shrunk tumor by a surgeon. Since that time the patient has been tumor-free and is judged by CR (complete remission), however, CTCs are still present.

Discussion

The main finding of this long-term pilot study is the possible increase of survival time for treatment resistant cancer patients after consuming FSWW08, a fermented soy beverage (Figures 9–11), accompanied by a substantial sustained increase in mental well-being and immunity (Table 2). No general claims can be deduced from these results because the cohort of enrolled patients was too small. But this investigation may be particularly interesting for researchers and may be the basis of follow-up studies. Clinical findings are corroborated by gene expression investigations in vitro and from in-blood CTCs, which had left the tumor, extracted from the blood of cancer patients, where a substantial increase of cell differentiation of CTC is detected. It is known that patients

with higher cell differentiation of CTC like in prostate, breast and ovarian cancers, have higher survival rates [61, 62, 67]. Therefore, the increase of cell differentiation by FSWW08 (Figures 6, 9 and 10) may have contributed to the increase in survival (Figures 9–11).

The standard treatment of most cancers is aggressive surgery followed by chemotherapy, in the case of Ovarian Ca, platinum-taxane chemotherapy [63]. After therapy, platinum resistant cancer recurs in approximately 25% of patients within 6 months [31, 63], and the overall 5-year survival probability is 31% [68]. One reason that ovarian cancer is so lethal is because over 75% of cases are stage III/IV by the time of diagnosis [67]. In contrast to this finding, all five ovarian cancer patients, who were previously resistant to chemotherapy and were enlisted in the pilot trial, after consumption of fermented soy product (FSWW08) (Figure 9) were not only able to break the resistance of the ovarian cancer patients to paclitaxel, it also increased survival in breast and prostate cancer patients, supporting earlier single case reports [11].

The lack of successful treatment strategies in cancer led many researchers to measure comprehensively genomic and epigenomic abnormalities on clinically annotated samples to identify molecular abnormalities that influence pathophysiology, affect outcome and constitute therapeutic targets [63]. For the fist time ever, we display genetic investigations in CTCs, which have left either the primary tumor or metastasis, showing that FSWW08 shares gene alterations seen by dexametasone treatment in myeloma tissue (Figure 4), however, without raising the risk of side effects like infections.

Approximately 13% of ovarian cancer is attributable to germline mutations in BRCA1/2 [69, 70], and a smaller percentage can be accounted for by other germline mutations. Most ovarian cancer can be attributed to a growing number of somatic aberrations [71]. Only very recently it was discovered that tumor suppressor factor p53 gene (TP53) was mutated in 303 of 316 tissue samples of ovarian cancer patients, compared to BRCA1 and BRCA2, which had germline mutations in 9% and 8% of cases, respectively, and showed somatic mutations in a further 3% of cases [63]. That TP53 mutations may have tremendous importance in other cancers too was seen in clinical trials where women with and without TP53 mutations were women with TP53 expression who had a higher survival cancer rate [62].

We identified in CTCs in cancer patients significant sustained changes of gene expression in CTC related to immunity, which were also described by ingredients of soy in vitro in cell culture work. The soy isoflavone genistein significantly reduces expression of various *NF-κB*-mediated [54] genes and suppresses global dendritic cells maturation in a p53-dependent manner [45]. These effects were seen in monocyte-derived dendritic cells as well as bone marrow-derived dendritic cells [45]. Interestingly, TP53 has the ability to decrease (TNF-α-induced) *NF-κB* reporter gene activity in vitro [45]. Authors of in vitro studies already speculated that this opens up new perspectives for dietary (by phytochemicals like soy) or therapeutic, by TP53, interventions in transplantation or immune disorders, such as allergy, asthma, or autoimmunity, because it could dampen unwanted or excessive immune responses [45]. This assumption is corroborated in this pilot trial in human patients.

Currently it is unknown whether TP53 will develop into a general marker for severity and outcomes of cancer, however, scientific publications are numerous showing that *tumor suppressor factor TP53* is a representative marker of: a) cell differentiation; b) apoptosis, as a marker for severity of breast, prostate and ovarian cancer; and c) is reduced in many immune diseases in cancer patients like allergies, infections, etc.

It would be interesting to ascertain whether other cells of the innate or adaptive immune system show a similar p53-dependent inhibition of LPS-stimulated *NF-κB*-driven gene expression in response to genistein (Figure 4) [45].

Apart from in vitro studies on TP53-*NF-κB* crosstalk, TP53 also appears to act as a repressor of *NF-κB* in vivo, because *NF-κB* dependent cytokines are elevated in macrophages, which have no TP53 expression [72] and thymus/spleen tissue [73]. Furthermore, TP53 mutations have been found to elicit hyper-inflammatory conditions, which increase the severity of chronic diseases [74] and promote cancer progression in many tumors like breast, prostate, ovarian, colon and kidney cancers [75].

Sustained modification of immunity was observed in this pilot trial, particularly the reduction of inflammation, allergies, and viral infections (Table 2). This is an important finding, as yeast, bacterial and viral infections are quite common among cancer patients. Additionally, over a period of 4 years cancer patient experienced substantial sustained improvement in well-being, particularly stress reduction [10].

Clearly, the detected gene expression effects were similar to those reported by dexamatosone in myeloma (Figure 4). However, there are two significant differences to dexametasone. Although FSWW08 shares many similarities on the genetic level, like reduction of *NF-κB*, *MAPkinase*, c-June and reduction of cytokines over a 4-year period, patients did not detect any side effects normally seen with cortisones in long-term application.

Improvement of cancer therapy in humans by increased cell differentiation through immunity modifying agents is documented in myeloma. Dexamethasone is anti-inflammatory, however, unfortunately it reduces immunity and results in increased viral infections and is a drug that inhibits the activation of the redox-regulated prosurvival transcription factors nuclear factor κB (*NF-κB*) and activator protein 1 (AP-1) that govern cellular radio-sensitivity [34]. Dexamethasone has been shown to inhibit the cytokine IL-6 expression [35], and an *NF-κB*– and c-Jun-mediated IL-6 expression has been reported in myeloma cells, similar to our findings [36]. Dexamethasone was also found to inhibit the release of interleukin-6 from irradiated bone marrow stem cells, which is an established myeloma cell pro-proliferative cytokine (Figure 4). The combination of dexamethasone with skeletal targeted radiotherapy prolonged median survival time in humans and inhibited radiation-induced myelo-suppression [37].

We reported previously in our first report that FSWW08 modified *NF-κB* and *MAPkinase*, similar to dexamethasone or antihistamines, because the gene modifications are similar to nasal and general allergy treatments. We hypothesized in our

earlier publication that FSWW08 modifying efficacy cannot be described via estradiol, testosterone, progesterone, or glucocorticosteroids receptors. Therefore the question has to be answered, what ingredient of FSWW08 caused the clinical effects, as fermented soy is composed of many ingredients. Also the question has to be answered as to why FSWW08 suppresses inflammation and increases antiviral activity (Table 2).

There are 10,000 reports in the literature found in PUBMED that soy ingredients have anti-cancer activities in vitro, in cell culture, and in animal studies, and show cancer prevention in epidemiological studies [1–6]. However, there are only a few reports that soy is involved in improving cancer therapy. It has to be critically commented that in most in vitro and animal studies very high doses of soy ingredients are employed, coined as pharmacological doses, which cannot be reached in the in vivo human situation. In vitro anti-cancer experiments of FSWW08 were reported, mechanistically and kinetically [14]. The doses of FSWW08 in the vivo human situation are much lower compared to those in the vivo human situations.

Normally, fermentation processing of soy is used to improve bioavailability of amino acids of soy and increases acceptance of soy proteins [43]. FSWW08 was particularly biotechnologically engineered to maintain the integrity of the Bowman-Birk-Inhibitory-Factor, which is normally destroyed by the fermentation process [43]. The soybean derived protease inhibitor known as the Bowman-Birk inhibitor (BBI) has been evaluated in several different human trials for different purposes and achieved Investigational New Drug status (IND) from the FDA in 1992 [76]. The studies involved six different INDs from the FDA in oral leukoplakia, benign prostate hyperplasia, prostate cancer, ulcerative colitis, gingivitis, and esophagus/lung cancer. Other indications could be multiple sclerosis and muscular dystrophy [76]. BBIC is known to be a powerful cancer preventive agent in vivo and in vitro models [77, 78].

FSWW08 contains 900 c.i. units of the BBI, which is greater than those levels of BBI tested previously in clinical human cancer trials by the NIH [78]. This may explain local anti-inflammatory effects in the gastrointestinal tract, like reductions of stomatitis, colitis ulcerosa, and anticancer efficacy of the mouth and gingivitis, as was seen in some patients in clinical trials. However, the oral bioavailability of BBI in humans has to be considered low, because only a few molecules of that size are able to penetrate the gastrointestinal tract to reach the blood stream, which is the reason why insulin is injected. BBI is partially orally absorbed and its contribution to cancer therapy has to be discussed and further investigated.

The active ingredient MDT-13 (13-methyl-tetradecanoic acid), patented as an ingredient of FSWW08, has shown to induce apoptosis in human tumor cell lines [44].

Due to the stress reducing effects and improvement in well-being of FSWW08 in cancer patients, we conducted a follow-up study in treatment resistant veteran soldiers suffering from combat related post-traumatic stress disorder (PTSD) [13]. We did see a complete reduction of stress and symptoms related to stress, although the soldiers were resistant to pharmaco- or psychotherapy. Also a dramatic reduction of viral and bacterial infections in PTSD patients was seen similar to those patients in the FSWW08 cancer trial. Analysis of steroidal hormones in PTSD patients revealed that particularly adrenal hormones, metabolites of DHEA, like androstenediol and androstenetriol were increased by FSWW08, which are a part of the HPA-axis (hypothalamus pituitary adrenal axis). This observation may help to explain three clinical effects in the cancer pilot trial: An increase of adrenal hormones causes a) improvement of the HPA axes which reduces stress and depression, b) a strongly modified immunity, which elicits both anti-inflammatory, anti-viral, and anti-bacterial effects, and c) most important of all, the increased adrenal hormones increase cell differentiation of stem cells, preventing cancer radiation damage [36] and rheumatic arthritis [79].

As outlined by Loria et al., adrenal hormones modify immunity broader than glucocorticoids, like dexamethasone, because adrenal hormones improve anti-inflammation, anti-viral, and anti-bacterial infections [80]. Gluco-corticoids like dexamethasone, however, modify only inflammation, and may cause bacterial and viral infection. Unfortunately, we did not determine steroidal hormones in the cancer pilot study, which should be conducted in follow-up cancer trials. Nevertheless, there is an indirect proof that adrenal hormones may be involved in modifying immunity, because we detected an increase of Th2 cytokines [12] and a reduction of Th1 cytokines like TNF-alpha, which are directly related to adrenal hormones [80].

It may be also discussed that soy isoflavones are *estrogen receptor beta* agonists, like DHEA, and its metabolite androstenediol, and its further metabolite androstenetriol [12]. Wicha et al. recently indicated that natural products like soy, curcumin and Vitamin D may indeed be capable to prevent cancer by inducing cell differentiation of CSCs, the driving force of cancer [20, 40]. We saw in our in vivo human experiments changes in gene expression of hormone receptor genes in CSC, the *tumor suppressor factor p21* (not shown here but reported in [10, 12]) and TP53, which are direct markers of cell differentiation. *Tumor suppressor factor TP53* has recently been linked to stem cell phenotype [81–87]. However, the molecular mechanism underlying and regulation of "stemness" remains elusive. Nevertheless, loss of *tumor suppressor factor p53* correlates with a decrease in the level of miR-200c, but an increase in the expression of stemness markers, and development of a high tumor grade in a cohort of breast tumors [62, 84]. It is speculated that an increase in TP53 gene expression in circulating cancer cells would increase survival, and our reported results support this assumption that the increase of p53 has to be considered as a transfer from a poor prognosis to an increasingly better prognosis [81].

Androstenediol has been discussed and shown to increase growth of prostate cancer cells, because it is a "Zwitter-Hormone", which has androgenic and *estrogen receptor beta* agonistic receptor affinity [88]. Moreover, androstenediol has strong CSC differentiating properties [89], which may explain, at least partially, the reduction in resistance and increase in survival, because CSCs are the driving force of cancer and metastasis. This has to be further investigated in vitro as well as in vivo.

Although limited by its small, homogenous sample size, the results of this pilot study illustrate the relationship between invasive CTCs and cancer disease staging and survival outcomes and raise the clinical potential of measuring CTCs in ovarian, prostate and breast cancer. Currently, no consensus has been reached on the clinical relevance of CTCs in cancer patients [85, 86]. For example, traditionally it was believed in the case of ovarian cancers that epithelial ovarian cancers spread primarily by direct extension into the abdominal cavity [87]. While studies have established the presence of tumor-like epithelial cells in the peripheral blood of ovarian cancer patients [67], the clinical significance of these studies' findings is unclear. Limitation of measuring CTC, like measuring viable and non-viable cells, can be found in the literature [90, 91]. However, the tendency is heading towards measuring CTC even in routine testing.

Clinical studies with larger cohorts have to be conducted, incorporating cancer stem cell investigation. Our pilot study suggests that the concept of improving immunity in cancer patients may ultimately have a strong impact not only in survival but improve the well-being and general health in cancer patients. It is worth mentioning that chemotherapy in neoadjuvant therapy does not alter reduced TP53 gene expression [21] and this study may point into another possibility of therapy.

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