

## Usefulness of Our Newly Designed Supplements (Deriskool A<sup>®</sup> & B<sup>®</sup>) for Reducing the High Risk of Eleven Kinds of Solid Cancer

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### Abstract

**Introduction:** An analysis covering small molecule RNA in blood samples was developed (MIA test-platinu ; Miltel<sup>®</sup>= platina test) to further increase diagnostic accuracy [1,2,3]. Since the comprehensive analysis of small molecule RNA of 11 solid cancers enabled early detection of these solid cancers, we examined whether our developed supplements (Deriskool A<sup>®</sup>, and B<sup>®</sup>) significantly reduced the risk of cancer for these 11 solid cancers.

**Material and Methods:** The subjects were 99 pre-diseased adults. More than 10 million species of small molecule RNA containing miRNA in the blood was extracted and comprehensive analysis was performed with a next-generation sequencer [2,3]. In order to detect various solid cancer patients at an early stage, 11 solid cancers were studied with a responsible cancer risk using the algorithm of biomarkers specific to cancer patients. By diagnostic imaging, if the tumor was not visualized, the cancer carrier reducing supplement of Deriskool A<sup>®</sup> and Deriskool B<sup>®</sup> was taken for 3 months. Qualitative changes in the information of small molecule RNAs of the subject and changes in the base sequence were quantified as test values by adapting the risk to the optimal algorithm for each disease. The test value of the carrier risk of each solid cancer was measured by the platina test, before and after supplement intervention was done.

**Results:** By taking our newly designed supplements, the risk of cancer carrier was significantly reduced in all 11 solid cancers before and after taking them ( $p < 0.05$ , Wilcoxon test).

**Conclusion:** From the risk assessment of the cancer carrier risk of analysis by the next generation sequencer of comprehensive small molecule RNA in the blood, therapeutic intervention was done with a supplement developed by our hospital, and in all 11 solid cancers, it was possible to significantly improve the cancer carrier risk. This is an effective practice of pre-emptive medicine.

**Keywords:** High Risk; Methyl-Resveratrol; microRNA; Newly Designed Supplements; Next Generation Sequencer; Solid Cancer; Pre-Emptive Medicine; Prevention; Beta-Glucan; Small RNAs

**Abbreviations:** MIA Test Premium=Premium Test; MIA Test Platinum=Platina Test

## Introduction

Primary prevention through lifestyle and environmental interventions remains the main way to reduce the burden of cancers [4]. Improvements in lifestyle behaviour's to reduce cancer risks include a healthy diet, calorie restriction, and regular physical activity [5,6]. Changes in the metabolism of nutrients such as glucose, amino acid, and fatty acid are associated with cancer risk. Luckily, this can be controlled with lifestyle modification [7]. In addition to lifestyle and environmental interventions, our pre-emptive treatment using newly designed supplement represented effective practice of pancreatic cancer prevention [1]. The supplement we developed consists of beta-glucan; Deriskool A, which activates all immune system cells [6], and Methyl resveratrol; Deriskool B, which activates the Sirtuin gene and also activates cancer-suppressing microRNAs [9]. Taking these supplements for 3 months significantly reduced the risk of pancreatic cancer.

The development of platina test has enabled early cancer detection with high accuracy by small molecule RNA analysis [2] for solid cancers in addition to pancreatic cancer. Therefore, we examined whether our developed cancer risk reduction supplement was effective for newly enabled to examine solid cancer. We report here and emphasize that primary prevention using our newly designed supplements is particularly effective way to prevent for a variety of solid cancer.

## Materials and Methods

The approval to perform this study was obtained from the ethical committee of Southern Tohoku General Hospital (acceptance No.; 360). Documented informed consent was obtained from each subject. Blood samples were obtained from 99 healthy adults for small RNA and miRNA extraction. The ratio of males to female subjects who tested for 11 types of cancer risk was as shown in Table 1.

	Male	Female	Total NO.	Mean age(Y)
pancreas	9	5	14	64.4
lung	11	8	19	64
stomach	6	4	10	63.8
liver	5	6	11	65.4
esophagus	4	3	7	64.1
breast	0	5	5	62
colon	4	2	6	63.5
Thyroid	4	3	7	64.6
gall bladder	7	2	9	65.6
kidney	4	2	6	69
ovary	0	5	5	64.4

**Table 1:** Composition of Male to Female Subjects

The presence of tumors was evaluated by radiographic images such as CT (320 channels, GE, USA), 3T -MRI (Signa, GE, Japan). After confirming the absence of visible tumor, the patients took our newly designed supplements orally for 3months. The supplements consisted of beta-glucan [8] (Deriskool-A<sup>®</sup>, 2 g/day) and methyl-resveratrol [9] (Deriskool-B<sup>®</sup>, 2caps/day). Risk determination was then carried out repeatedly by analyzing small molecule RNA from the patients' blood samples.

Because small molecule RNA has the function of controlling the protein's being made in the cell, the pattern changes early even in the early stage when the state of the function of the cell and the organ changes by the sickness etc. platina test targets small molecule RNA, which changes patterns from the early stages of disease, and considers the presence of early-stage cancer cells that are

difficult to find in images as risks. In order to detect various solid cancer patients at an early stage, 11 solid cancers (i.e., pancreatic cancer, lung cancer, gastric cancer, liver cancer, esophageal cancer, breast cancer, colorectal cancer, thyroid cancer, gallbladder cancer, kidney cancer, ovarian cancer) with a responsible cancer risk using the algorithm of biomarkers specific to cancer patients obtained from the results of next-generation sequencers) (commissioned by Miltel®). [2,3].

### RNA sequencing for miR Test

Total RNA was isolated from 200 ml serum using a miRNeasy mini kit (Qiagen) according to the manufacturer's protocol. A cDNA library for small RNA sequencing was prepared by an Ion Total RNA-Seq Kit v2 ((Thermo Fisher Scientific). The size and concentration of base pairs of the cDNA library were measured with an Agilent 2100 Bioanalyzer (Agilent Technologies). The Synthesized templates were sequenced on an Ion S5–XL sequencer (Thermo Fisher Scientific) using an Ion 540-chip.

### Data Analysis

The data quality was checked, and analyzed using a CLC genomics work bench 7(CLC bio). Small RNAs expression levels were estimated using RPM values. The normalized reads were annotated to miRBase version 21 with high priority. Remaining reads were annotated to GtRNadb and GRCh38.p12 as references.

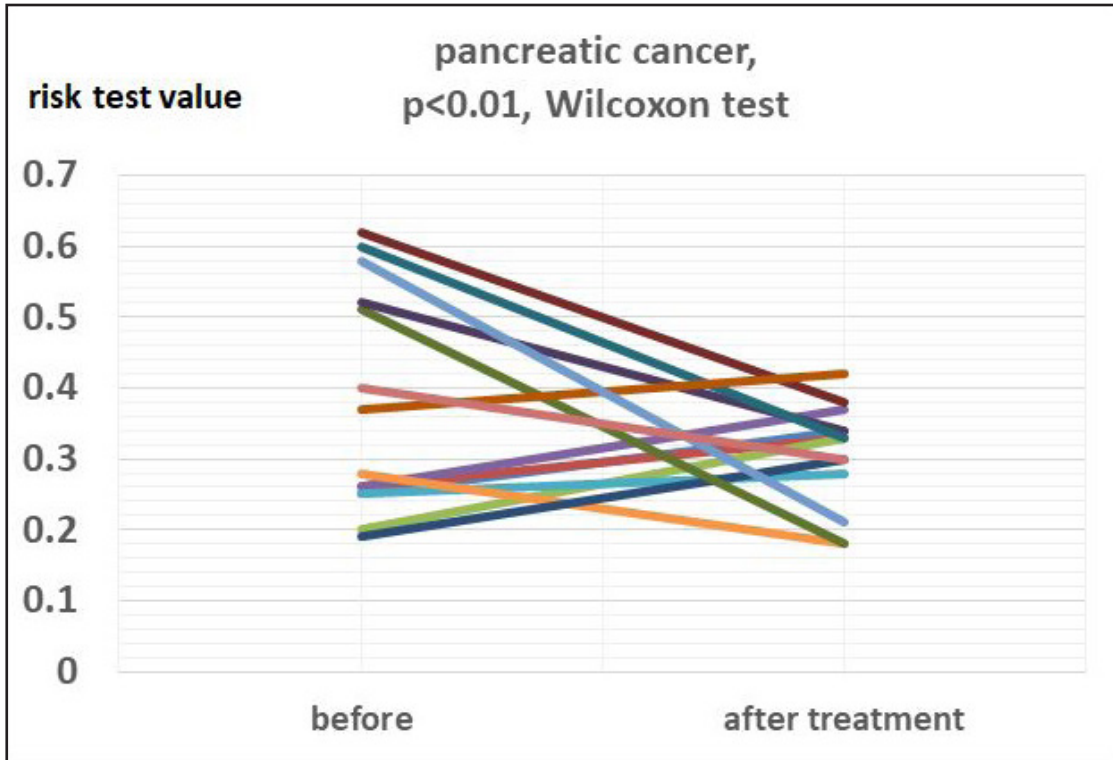
The biomarkers for miR Test were identified by analyzing normalized read numbers of small RNAs between healthy control and each disease group using the Student t-test. A miR Test index was calculated by assigning the read number of some candidates of small RNAs using previously reported method. [2,3] (details of candidate small RNAs of miR Test are not disclosed). All data were statistically analyzed using JMP1 14 (SAS Institute Inc.).

The risk test value distribution was set so that the risk values fluctuate between 0.00 and 1.00. Based on the threshold (test value = 0.5), which accurately separates healthy and disease groups, the test value of less than 0.5 is a low-risk group, and the test value of 0.5 or more is a high-risk area. The low-risk group is divided into 2 minutes based on the intermediate value = 0.25 of healthy subjects, and the high-risk region is divided into 2 minutes based on the intermediate value = 0.75 of the disease group. In this study, it was recommended to take supplements as disease groups and high-risk groups with a risk value exceeding 0.35. Changes in the tumor-bearing risk after treatment with our tumor risk reduction supplements were evaluated by the risk test value as calculated by next generation sequencing, just before and after the intervention then the results were examined by the Wilcoxon test. Significance threshold was set at  $p < 0.05$ .

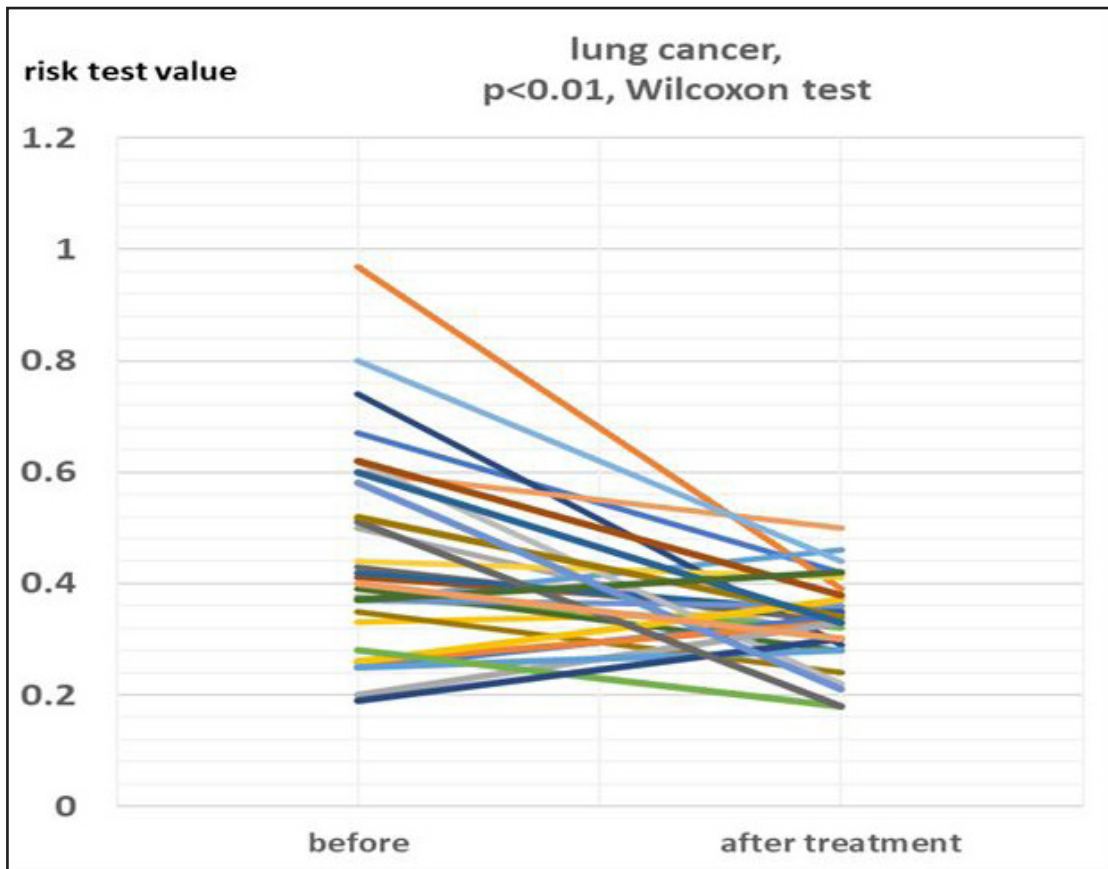
### Results

A total of 99 subjects were composed of 54 males and 45 women. The average age of the subjects was 64.6 years old (Table 1). Table 1 shows the average age of the subjects in each solid cancer.

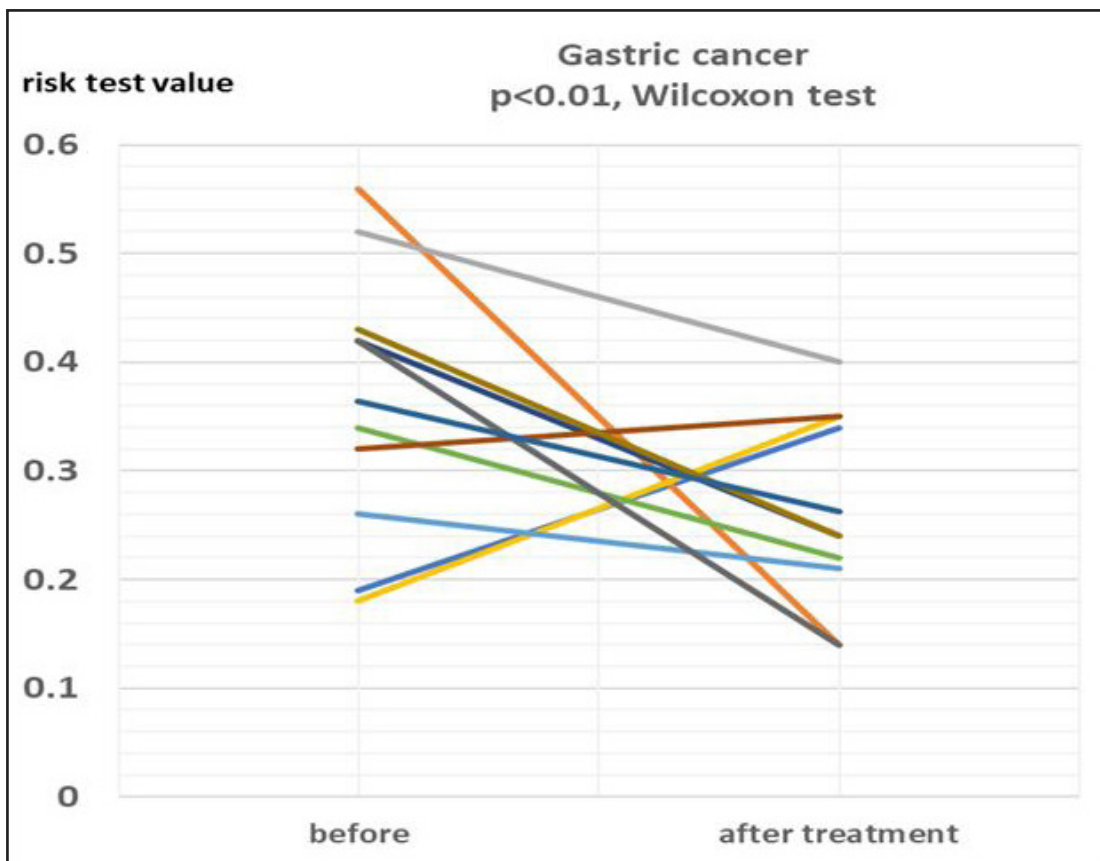
By taking Deriskool A® and Deriskool B® for 3 months, the risk of cancer carrier was significantly reduced in all 11 solid cancers before and after taking. (Figure 1: a~k).



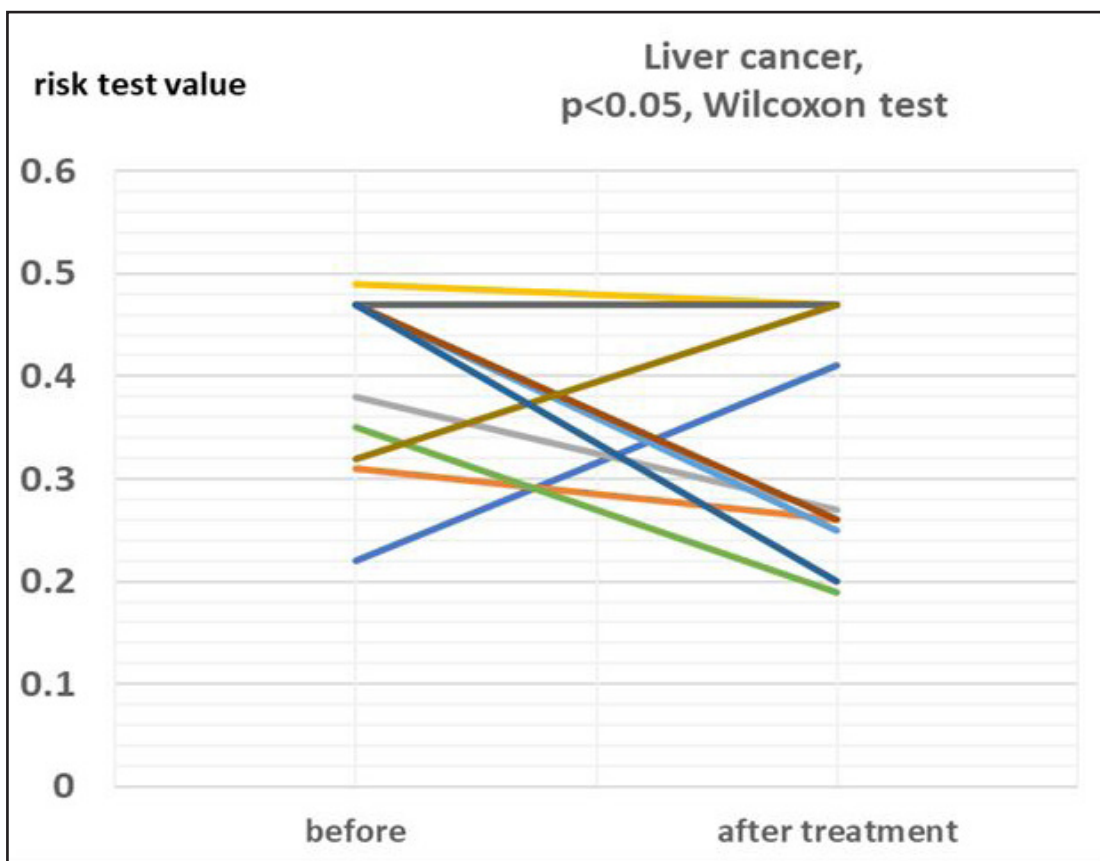
a) Pancreatic cancer



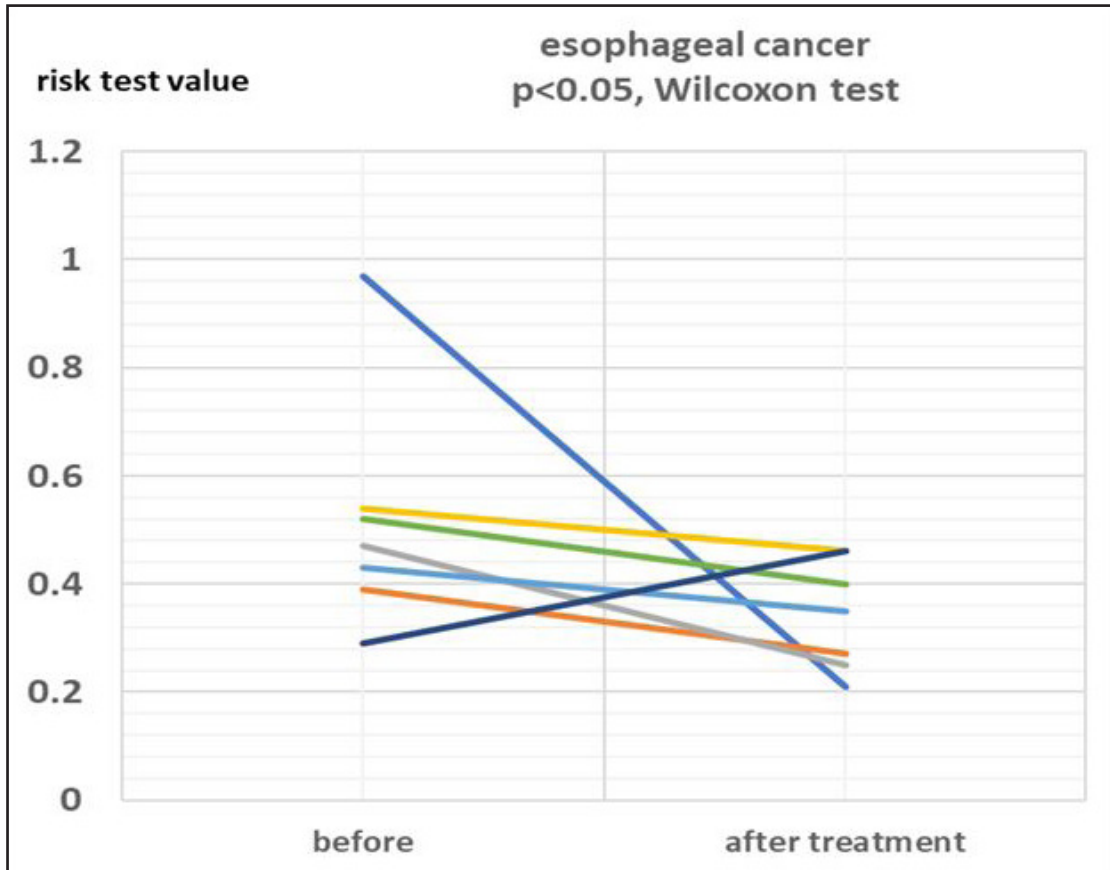
b) Lung Cancer



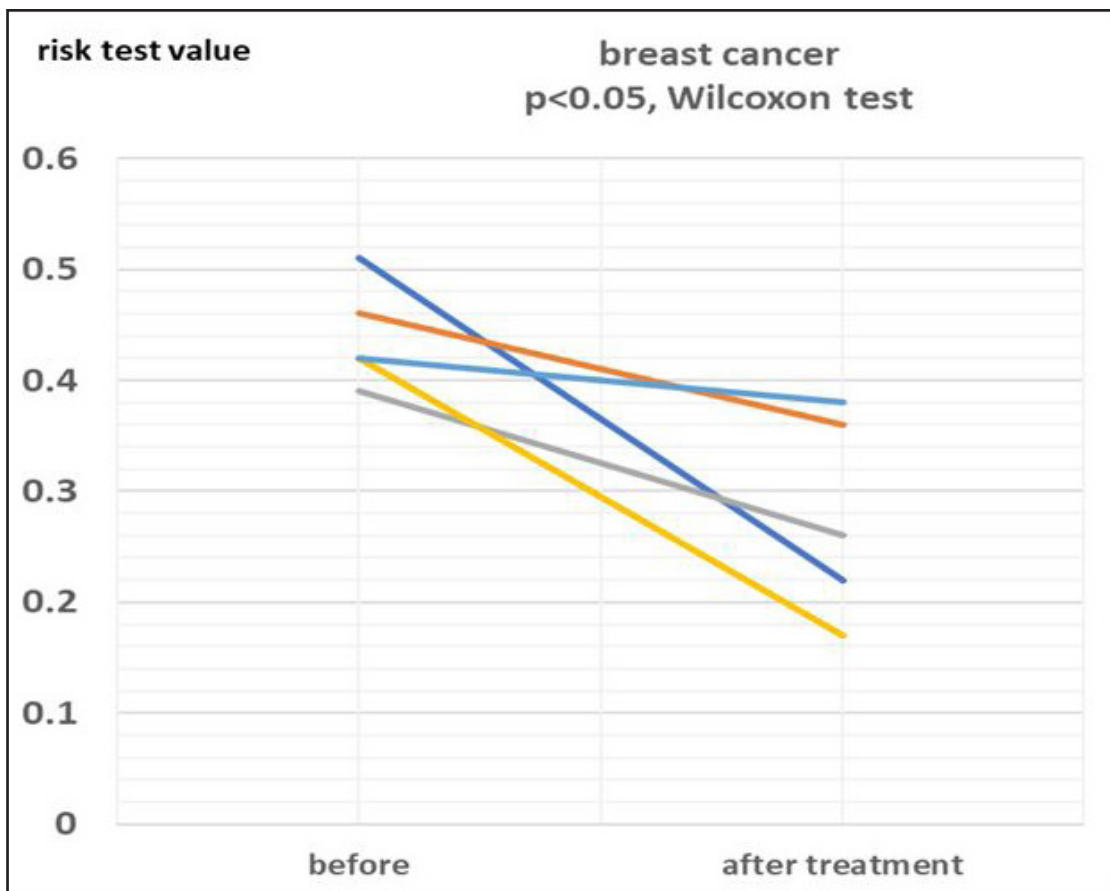
c) gastric cancer



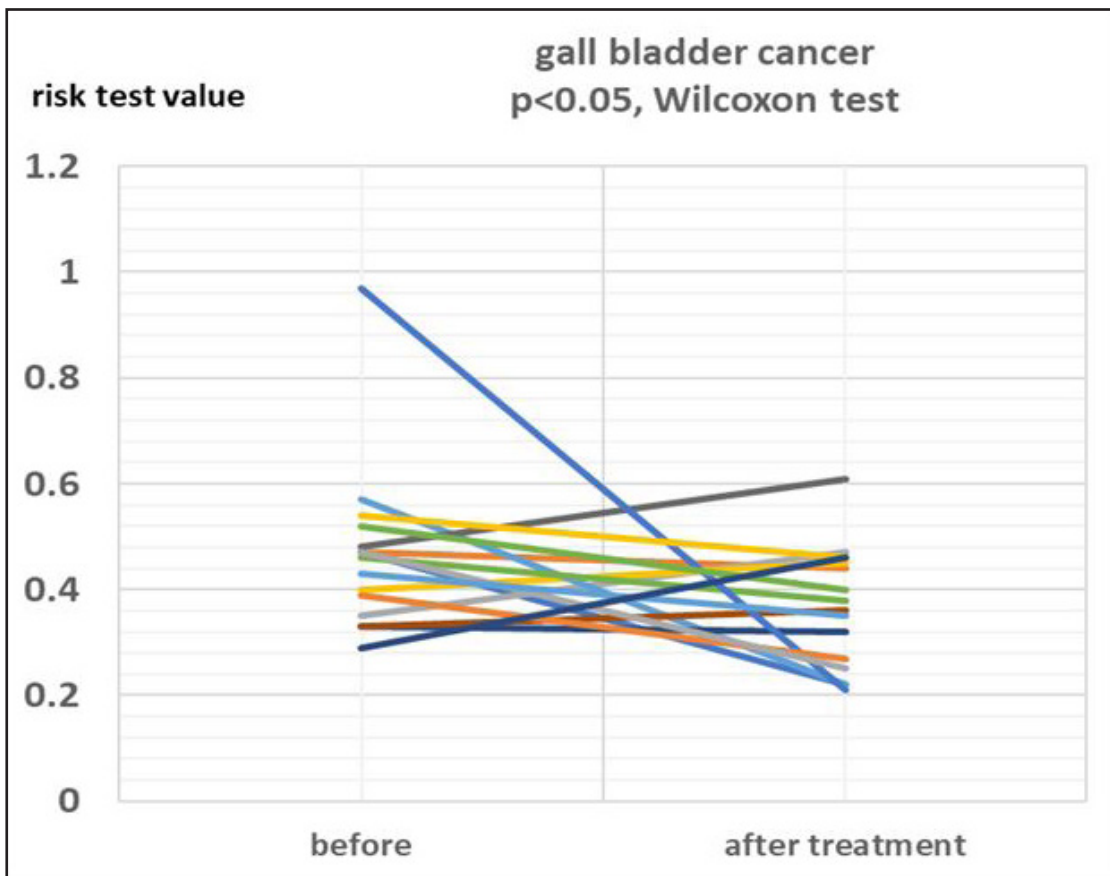
d) Liver Cancer



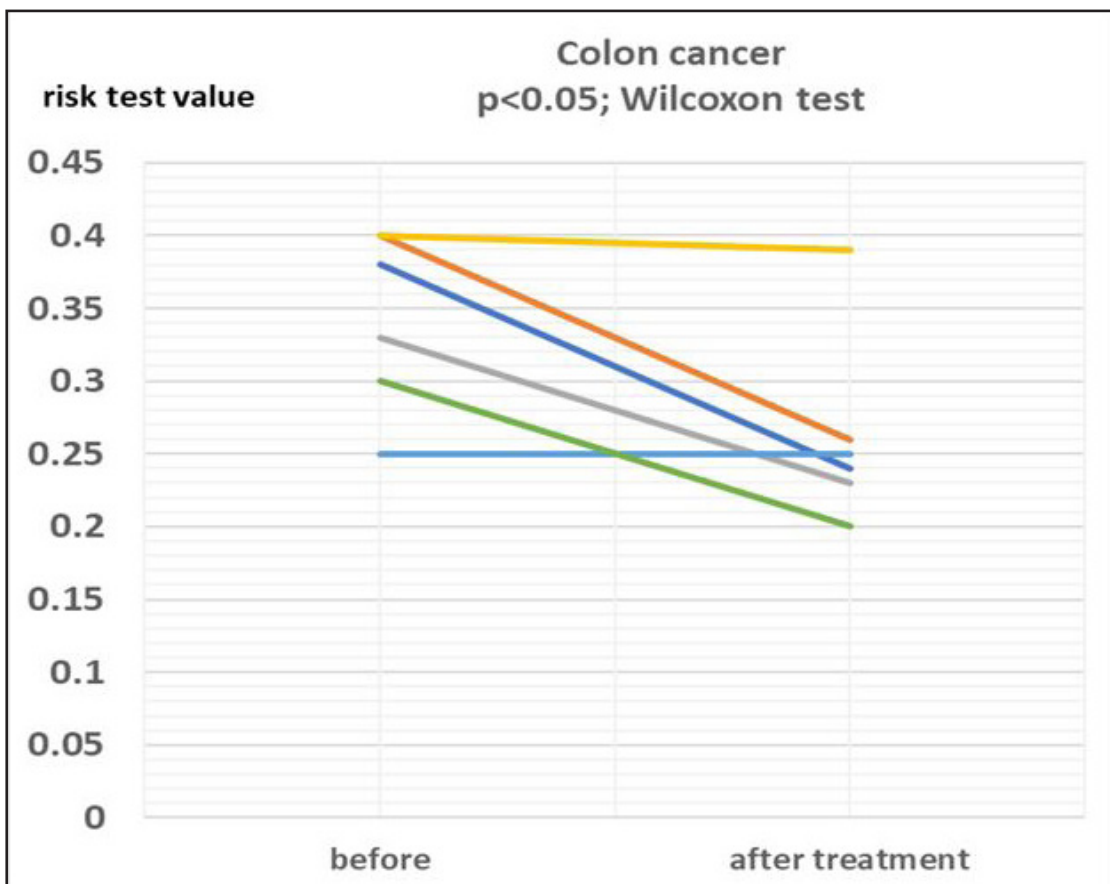
e) esophageal cancer



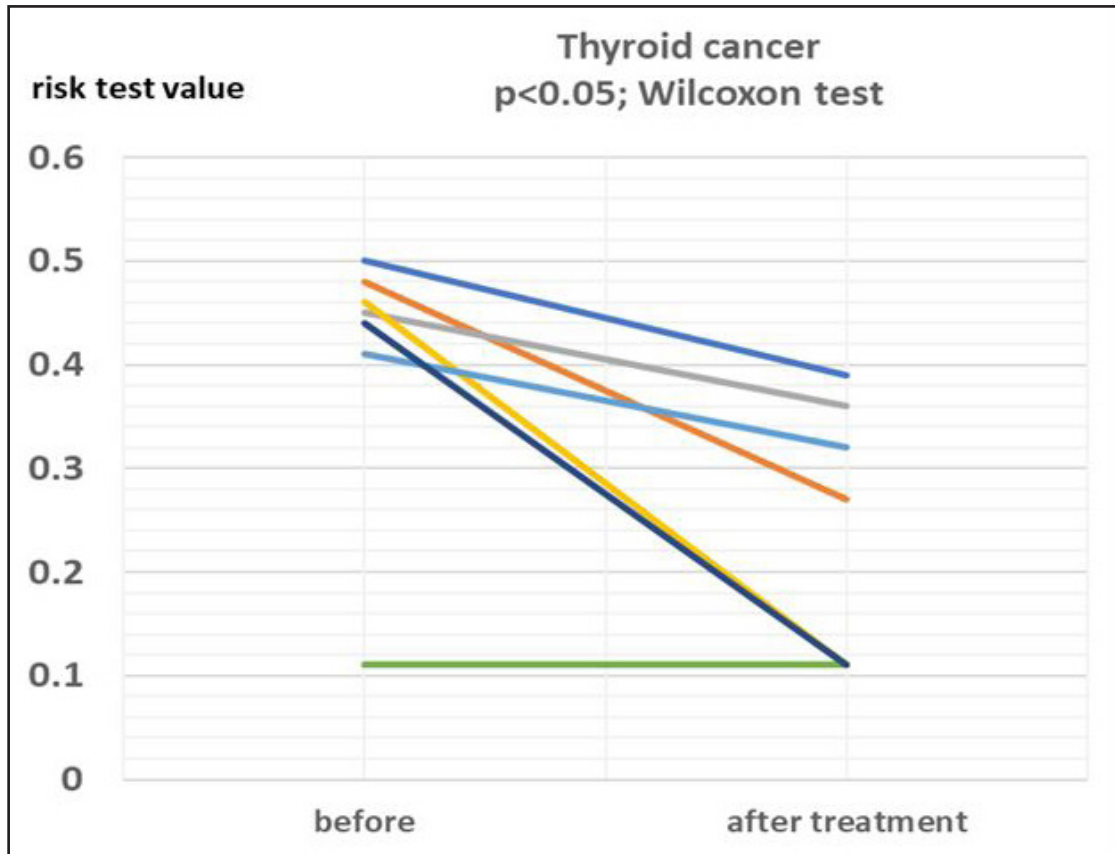
f) Breast cancer



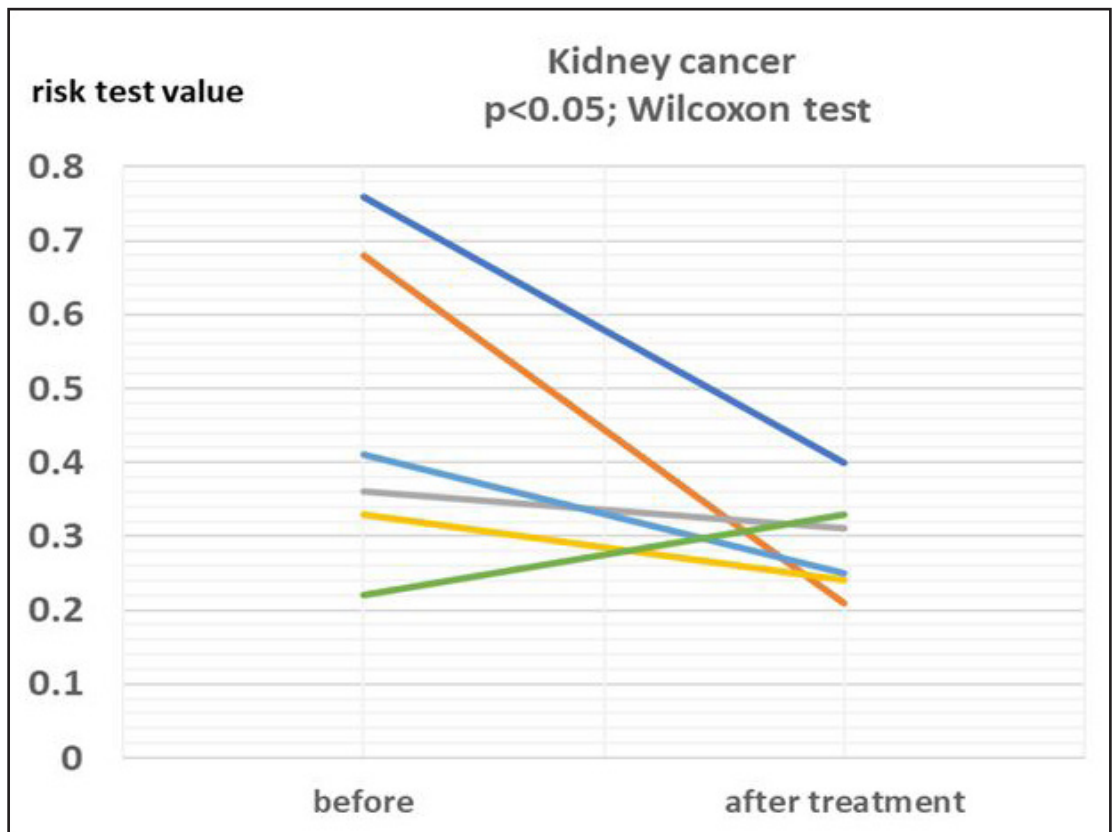
g) Gall bladder cancer



h) Colon cancer

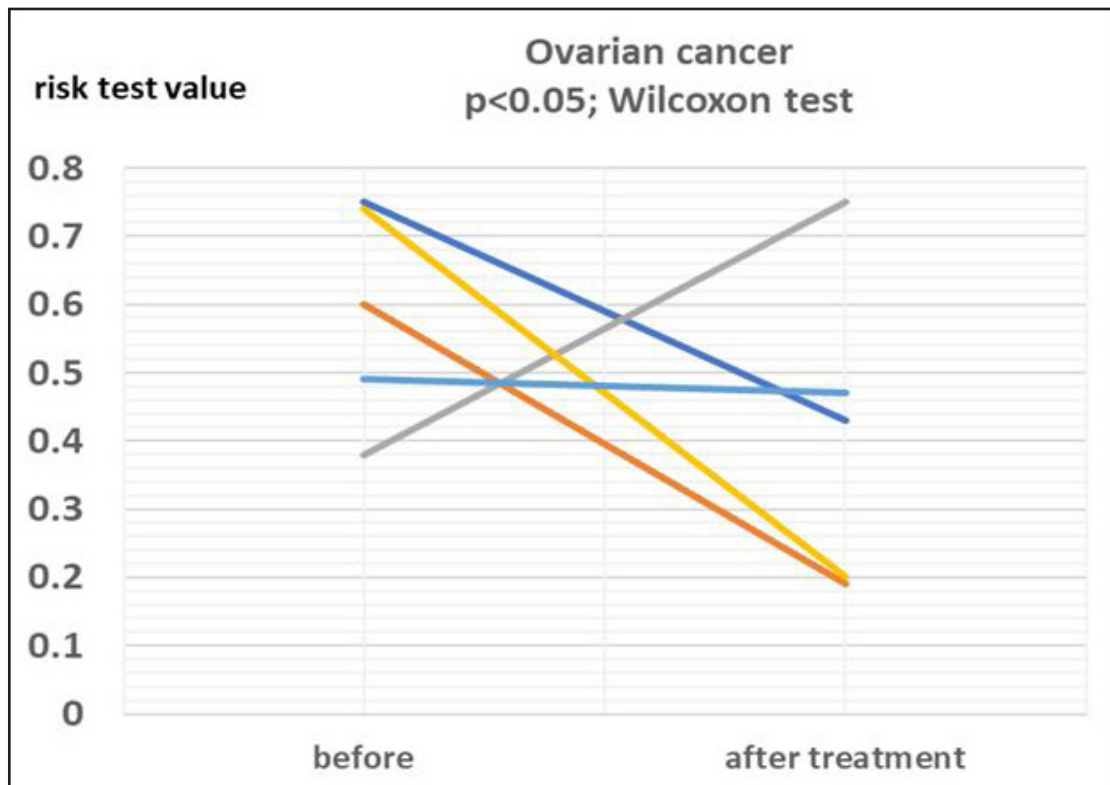


i) Thyroid cancer



j) Kidney cancer





k) Ovarian cancer

Figure 1: Change in risk test value before and after intervention in all cases

## Discussion

Cancer is the top leading cause of death in Japan. Despite the advancement in screening, early diagnosis, and development in treatment technology in last several decades, cancer incidence overall is far from being controlled. To avoid important causes of cancers such as smoking, alcohol use, overweight and obesity, irregular physical exercise, and low fruit and vegetable intake, is important [4]. It is also essential to take preventive measures other than to correct these lifestyles, that is, to catch the risk of cancer at an early stage and to picking buds.

In recent years, it has been reported that microRNAs are useful for early diagnosis and treatment of cancer [10,11]. Earlier, we used premium test to determine the risk of pancreatic cancer and find that our developed supplement (Deriskool A<sup>®</sup> and B<sup>®</sup>) significantly lowers the risk of pancreatic cancer. The premium test was only evaluated for the expression level of microRNAs (several types) specific for pancreatic cancer. Then, the result was compared with that of the pre-diseased person, and the cancer carrier risk was calculated.

*Platina test*, which evolved the *premium test*, has made it possible to determine the risk of responsible cancer for 14 solid cancers. This comprehensively performs next-generation sequencer analysis of all data of small molecule RNA in the blood (more than 10,000 types) and obtains sequence information of all small molecule RNAs present in the blood. miRNA, isomiR, t-RNA fragments, ncRNA (other non-coding RNA) using all vast amounts of data to match a genetic database is analyzed. Since the expression level and sequence information are also evaluated, *platina test* is a test with higher accuracy and improved disease specificity. Both sequence information and quantity are analyzed based on the obtained information, and the risk is scored by comparing it with various disease-specific databases [2,3]. In this paper, the cancer carrier risk was examined for 11 kinds of solid cancers in which the number of samples which can withstand the statistical analysis was obtained using *platina test*.

In precision diagnostic imaging (3Tesla-MRI, 64-channel helical CT, and gastrointestinal endoscope), we conducted treatment interventions with our supplements when cancer could not be confirmed. The diagnostic method using small RNA used in this study is epoch-making because it has high specificity and sensitivity [2,3] and can detect early lesions as small as 0.1 mm<sup>3</sup>. Therefore, it is considered that there is a high possibility that minute lesions that are not understood by diagnostic imaging are found.

The intention of our supplements was to reduce or eliminate the risk of cancer by increasing immunity to cancer [8] and supplementing the progression to cancer triggered by microRNA instability with cancer-suppressive microRNAs [9].

*Deriskool A*<sup>®</sup> (Meshimas) are mainly composed of beta-glucan obtained from mushroom hyphae. Beta-glucan is known to activate natural killer T cells, T cells, B cells, and macrophages several times [8]. *Deriskool B*<sup>®</sup> (Pterostilbene) also increases the activity of inhibitory miRNAs and exhibits anticancer effects [9]. *Deriskool A*<sup>®</sup> (9 tablets/day) and *Deriskool B*<sup>®</sup> (2 tablets/day) were taken after meal and continued for three months.

As a result all 11 cancers, after taking our developed supplements, significant ( $p < 0.05$ , Wilcoxon test) improvement of the cancer risk was obtained.

In conclusion, the paradigm of preventive medicine in the future is to use miRNA diagnostics to identify disease risk and, if there is no visible cancer, to take our cancer-reducing supplement for 3 months. After confirming that the cancer risk has decreased, it may be important to observe the five lifestyle behaviors such as quitting smoking, drinking moderately, eating a balanced diet, physical activity, and keep proper weight, and to protect your body so that the cancer risk does not rise again. This is what preemptive medicine should be and is a goal.

## Conclusion

For patients at high risk of a variety of solid cancer, we found it possible to efficiently reduce the risk value of cancer by means of our newly designed supplies. Early evaluation of cancer risk is important and spinning its buds are powerful measures to prevent cancer. The supplements we developed proved to be one of the most powerful means to prevent cancer.

## Conflict of Interest

We have no conflict of interest.

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