# Clinical Immunology & Research

## Effective Cancer Treatment and Prevention with Plant-Based Immunotherapy

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#### ABSTRACT

Cancino<sup>®</sup>, a dietary supplement registered with the Thai FDA, was developed to prevent cancer, which has been increasing at an alarming rate, by building on the previous success of the formula of Mylife/Mylife100<sup>®</sup> in cancer treatment. The formulation is modified from that of Mylife/Mylife100<sup>®</sup> by using a different concentration of active ingredients from five edible plants, namely pennywort leaves, black sesame seeds, soybeans, guava fruit, and mangosteen aril. Consumers with low immunity who took 3 capsules of Cancino<sup>®</sup> per day before breakfast over a study period of 8 weeks experienced increased immunity as evidenced by the sharp increases in lymphocytes, CD4 and CD8 T cells. Most participants also demonstrated significant increases in their absolute mean telomere length simultaneously as they increased their immunity. In addition, they all exhibited autophagy induction, as indicated by the increase in the expression of Atg12 and LAMP1. All these results demonstrated that Cancino<sup>®</sup> is effective in cancer prevention, achieved through the combined effects of increased T cells, telomere elongation in previously existing and newly increased T cells, and autophagy induction.

## **Keywords**

Age reversal, Anti-aging, Black sesame seeds, Cancer, Cancino<sup>®</sup>, CD4, CD8, Extract, Food supplement, Guava, Killer T cell, Mangosteen, Mylife/Mylife100<sup>®</sup>, Pennywort leaves, Soybeans, T cell, Telomere.

#### Background

Mylife/Mylife100<sup>®</sup>, a Thai innovation, is a dietary supplement made from mangosteen aril, pennywort leaves, guava fruit, black sesame seeds, and soy protein. It has been used as an alternative treatment for cancer patients in Thailand for over a decade. This supplement has been shown to significantly increase CD4 and CD8 counts and promote telomere lengthening. Many cancer patients in Thailand have turned to Mylife/Mylife100<sup>®</sup>, which stimulates killer T cells to specifically target cancer cells, as an alternative to conventional treatments with severe side effects, enabling them to enjoy prolonged, higher-quality lives [1,2].

recent studies in the U.S. and the U.K. indicating that one in every two people will develop cancer in their lifetime [3]. The unpreventable rise in cancer cases can be attributed to hereditary factors [4], the current rapidly aging population [5], and the growing number of individuals vaccinated with mRNA vaccines [6]. Therefore, our research group set a goal to develop an innovative formulation for global cancer prevention, improving on our previous success in the efficacy of T-cell stimulation, telomere elongation, and the additional positive effects of autophagy, which has been proven to help prevent the occurrence of cancer cells. The formulation has now been successfully developed and registered with the Thai FDA as Cancino<sup>®</sup>. This article describes the effects of increased immunity and telomere length from the consumption of Cancino<sup>®</sup> in 17 consumers.

## **Materials and Methods**

#### Preparation of Cancino<sup>®</sup> from Five Edible Plants

The dietary supplement Cancino<sup>®</sup> is formulated based on Mylife/ Mylife100<sup>®</sup>, with a different concentration of active ingredients

The number of cancer cases has been rising continuously, with

from five edible plants, namely pennywort leaves, black sesame seeds, soybeans, guava fruit, and mangosteen aril [2]. Mangosteen aril juice powder is prepared by grinding, centrifuging, filtering, and spray-drying the juice. Pennywort leaf powder is obtained by heating and centrifuging an extract from the dried leaves. Guava juice powder is produced by grinding, filtering, and spray-drying the juice, while black sesame and isolated soybean protein powders are processed similarly with grinding, centrifuging, and drying steps. Each capsule contains specific doses of these powders, and the product is registered with the Thai FDA.

#### **Subjects**

The study included 17 participants (2 males and 15 females) with an age range of 35 to 83 years. All participants were generally healthy, non-drinkers, non-smokers, and had no chronic illnesses requiring regular medication. Their diet, exercise, and daily routines remained consistent throughout the 8-week study period.

## **Study Design**

The study spanned 8 weeks, with each participant attending four scheduled visits: at week 0 (first visit), week 2 (second visit), week 4 (third visit), and week 8 (fourth visit). Body composition data and blood samples were collected at each visit. Participants were instructed to maintain their usual lifestyle, including their daily dietary intake and exercise habits, throughout the entire 8-week period.

#### **Blood Sample Collection**

Blood samples were collected by venipuncture after a 12-hour fasting period to measure various biomarkers. Serum was obtained from clotted blood. An aliquot of EDTA blood was used for a complete blood count and to assess T-lymphocyte subpopulations. Another EDTA blood sample was used to isolate peripheral blood mononuclear cells (PBMCs) for measuring absolute telomere length [2] and autophagy protein expression [7].

#### **Measurement of Blood Biochemistry and Body Composition**

Serum aspartate transaminase (AST), alanine transaminase (ALT), blood urea nitrogen (BUN), and creatinine were analyzed with specific assay kits using an automatic analyzer. Body weight, height, and body mass index (BMI: kg/m<sup>2</sup>) were measured using a Tanita BC-420MA segmental body composition analyzer (Tanita Corporation, Tokyo, Japan) [2].

## Measurement of Leukocyte Telomere Length

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood by density gradient centrifugation. Genomic DNA was extracted from 2-5 million PBMCs using the DNeasy kit (Qiagen), and its purity and quantity were checked via UV spectrophotometry. PBMC telomere length was measured by quantitative PCR, comparing telomere repeat copy number to the single-copy gene 36B4, based on the qPCR method of O'Callaghan and Fenech (2011). Each 10- $\mu$ L qPCR reaction included 20 ng DNA, PowerUp SYBR Green, and telomere primers, with 40 cycles and a dissociation curve for verification. A standard curve ensured reaction linearity (R<sup>2</sup>>0.99) and allowed conversion to absolute telomere length (aTL) in kb per diploid genome. Triplicate runs were used, with a 1% interassay variation [2].

### Measurement of Autophagy Protein Expression Western Blot Analysis

The study spanned 8 weeks, with each participant attending four scheduled visits: at weeks 0, 2, 4, and 8. The western blot analysis was used for protein expression detection according to the methodology in a previous study [7]. Briefly, PBMCs were extracted using RIPA buffer with a protease inhibitor cocktail. The protein mixtures were then separated via SDS-PAGE and transferred onto a PVDF membrane. Blocking was performed by incubating the membrane with 5% BSA at room temperature for 1 hour. The blots were then incubated overnight at 4°C with antibodies against autophagy-related gene 12 (Atg12), and lysosomal-associated membrane protein 1 (LAMP1) (Cell Signaling Technology, Beverly, MA, USA), as well as β-actin (Merck Millipore Corp., Darmstadt, Germany). Afterward, the bands were detected using HRP-conjugated secondary antibodies and visualized with an enhanced chemiluminescent substrate (ECL) (Merck Millipore Corp., Darmstadt, Germany).

## **Statistical Analysis**

The statistical analyses were performed using GraphPad Prism 6.0, with a significance level of 5% (p < 0.05) for all analyses [2].

## Results

The initial body composition of the 17 participants is shown in Table 1. The sample group consisted of 17 participants (2 males and 15 females), aged 35 - 83 years (average age:  $62 \pm 12$  years). Their average body mass index (BMI) was  $23.8 \pm 5.1$  kg/m<sup>2</sup>. All participants were generally healthy, non-drinkers, non-smokers, and had no chronic conditions requiring regular medication. Their daily living activities (diet, exercise, and routines) remained consistent throughout the 8-week data collection period.

**Table 1:** Initial Body Composition of 17 Participants.

| No | Sex | Age | Height, cm | Weight, kg | BMI, kg/m <sup>2</sup> |
|----|-----|-----|------------|------------|------------------------|
| 1  | F   | 35  | 160        | 67.5       | 26.4                   |
| 2  | F   | 45  | 160        | 55.8       | 21.8                   |
| 3  | F   | 49  | 165        | 54.7       | 20.4                   |
| 4  | F   | 60  | 150        | 84.8       | 37.7                   |
| 5  | F   | 61  | 150        | 48.6       | 21.6                   |
| 6  | F   | 64  | 157        | 48.1       | 19.5                   |
| 7  | F   | 64  | 159        | 60.2       | 23.8                   |
| 8  | F   | 64  | 151        | 58.1       | 25.5                   |
| 9  | F   | 65  | 166        | 54.4       | 19.7                   |
| 10 | F   | 66  | 150        | 51.0       | 22.7                   |
| 11 | F   | 68  | 160        | 48.0       | 18.8                   |
| 12 | F   | 70  | 153        | 56.8       | 24.3                   |
| 13 | F   | 70  | 150        | 45.4       | 20.2                   |
| 14 | F   | 71  | 151        | 56.8       | 24.9                   |
| 15 | F   | 74  | 152        | 50.4       | 21.8                   |
| 16 | М   | 48  | 169        | 95.9       | 33.6                   |
| 17 | М   | 83  | 162        | 62.5       | 23.8                   |

During weeks 1-8, 3 capsules of Cancino<sup>®</sup> were taken before breakfast. All participants experienced no side effects. They reported better sleep, improved skin health, a refreshed mood, enhanced concentration, no fatigue, increased energy, and less exhaustion. Food intake and energy expenditure remained consistent throughout the study, as assessed by body mass index (BMI), which remained stable during the entire study.

#### Safety of Cancino®

## Effects of Cancino® on Liver and Renal Function

Kidney function was assessed by measuring blood urea nitrogen (BUN) and creatinine levels, while liver function was evaluated using SGOT and SGPT enzyme levels. Throughout the study, the levels of serum BUN, creatinine, SGOT, and SGPT, which indicate liver and kidney function, remained within normal ranges (Table 2).

| Table 2: Liver and Renal Function Tests of 17 | Participants I | During the Study. |
|---|----------------|-------------------|
|---|----------------|-------------------|

| Parameter            | Week | Mean ± SD      | Normal value        |
|----------------------|------|----------------|---------------------|
|                      | 0    | $13.8\pm3.0$   | 7.8 - 20.3          |
| Blood urea nitrogen, | 2    | $13.4\pm3.9$   |                     |
| mg/dL                | 4    | $13.1 \pm 2.8$ |                     |
|                      | 8    | $13.7 \pm 2.5$ |                     |
|                      | 0    | $0.80\pm0.13$  | Female: 0.65 – 1.08 |
| Creatining mg/dI     | 2    | $0.81\pm0.13$  | Male: 0.81 – 1.43   |
| Creatinine, mg/dL    | 4    | $0.80\pm0.13$  |                     |
|                      | 8    | $0.78\pm0.12$  |                     |
|                      | 0    | $23 \pm 5$     | Female: 0 – 31      |
| SGOT, U/L            | 2    | $27 \pm 6$     | Male: 0 – 35        |
| 5GU1, U/L            | 4    | $24\pm 6$      |                     |
|                      | 8    | $26 \pm 7$     |                     |
|                      | 0    | $22 \pm 13$    | Female: 0 – 34      |
| SCDT U/I             | 2    | $24 \pm 14$    | Male: 0 – 45        |
| SGPT, U/L            | 4    | $26 \pm 17$    |                     |
|                      | 8    | $23 \pm 12$    |                     |

#### Effects of Cancino® on Leukocyte Telomere Length

**Table 3:** Leukocyte Telomere Length of 17 Participants During the Study.

| Parameter             | Week | Mean ± SD                     |
|-----------------------|------|-------------------------------|
|                       | 0    | $6,355 \pm 1,139$             |
| Telomere, base pairs  | 2    | $6,420 \pm 1,062$             |
| reioniere, base pairs | 4    | $6,510 \pm 1046^{a, c}$       |
|                       | 8    | 6,550 ± 1,012 <sup>a, c</sup> |
|                       | 0    | $51 \pm 16$                   |
| Talamana nanaantila   | 2    | $52 \pm 15$                   |
| Telomere, percentile  | 4    | $54\pm14^{b,d}$               |
|                       | 8    | 55 ± 13 <sup>a, c</sup>       |

Significant difference from week  $0 : {}^{a}p < 0.02$ ,  ${}^{b}p < 0.05$ Significant difference from week  $2 : {}^{c}p < 0.02$ ,  ${}^{d}p < 0.05$ 

An increase in absolute telomere length of 70 base pairs is equivalent to a 1-year age reversal [3]. As shown in Table 3 above, the mean  $\pm$  SD of absolute telomere length for all 17 participants was  $6,355 \pm 1,139$  base pairs at week 0, increasing to  $6,420 \pm 1,062$ base pairs at week 2,  $6,510 \pm 1,046$  base pairs at week 4, and  $6,550 \pm 1,012$  base pairs at week 8. The telomere lengths at both week 4 and week 8 increased significantly from week 0 and week 2. The absolute mean telomere lengths at weeks 2, 4 and 8 increased from week 0 by 65, 144, and 195 base pairs, respectively, equivalent to an age reversal of 1, 2, and 3 years. The percentiles of telomere lengths at weeks 0, 2, 4, and 8 were  $51 \pm 16$ ,  $52 \pm 15$ ,  $54 \pm 14$  and  $55 \pm 13$ , respectively. The percentile telomere lengths at both week 4 and week 8 increased significantly from week 0 and week 2.

## Effects of Cancino® on T Cells

Table 4 shows absolute CD4 and CD8 T-cell counts of 10 participants with low immunity during the study. Three participants with low lymphocyte counts, < 1,500 cells/ $\mu$ L, experienced increases of 11.2%, 35.4%, and 28.2% at weeks 2, 4, and 8, respectively, from week 0.

**Table 4:** Lymphocytes, Absolute CD 4 and CD 8 T-Cell Counts of 10Participants with Low Immunity at Baseline.

| Parameter  | Week  | Mean ± SD   |  |  |  |  |
|--|---|---|--|--|--|--|
| 3 participants with low lymphocyte (< 1500 cells/µL) |   |   |  |  |  |  |
|  | 0   | $1,059 \pm 476$                                       |  |  |  |  |
|  | 2   | $1,178 \pm 357$ increased 119 cells or 11.2% of       |  |  |  |  |
| Lymphocyte,  |   | week 0  |  |  |  |  |
| cells/µL   | 4   | $1,434 \pm 47$ increased 375 cells or 35.4% of week 0 |  |  |  |  |
|  | 8   | $1,358 \pm 154$ increased 299 cells or 28.2% of       |  |  |  |  |
|  |   | week 0  |  |  |  |  |
| 2 participant<br>cells/µL)                           | 2 participants with low CD4 (< 470 cells/µL) and low CD8 (< 360 cells/µL) |   |  |  |  |  |
|  | 0   | $300 \pm 171$   |  |  |  |  |
| CD 4,  | 2   | $347 \pm 120$ increased 47 cells or 15.7% of week 0   |  |  |  |  |
| cells/µL   | 4   | $490 \pm 59$ increased 190 cells or 63.3% of week 0   |  |  |  |  |
|  | 8   | $514\pm135$ increased 214 cells or 71.2% of week 0    |  |  |  |  |
|  | 0   | $220\pm91$  |  |  |  |  |
| CD 8,  | 2   | $236 \pm 44$ increased 16 cells or 7.3% of week 0     |  |  |  |  |
| cells/µL   | 4   | $385 \pm 136$ increased 165 cells or 75.0% of week 0  |  |  |  |  |
|  | 8   | $366\pm146$ increased 146 cells or 66.4% of week 0    |  |  |  |  |
| 9 participants with low CD8 (< 360 cells/µL)         |   |   |  |  |  |  |
|  | 0   | $268\pm58$  |  |  |  |  |
| CD 8,  | 2   | $304 \pm 72$ increased 36 cells or 13.4% of week 0    |  |  |  |  |
| cells/µL   | 4   | $332 \pm 99$ increased 64 cells or 23.9% of week 0    |  |  |  |  |
|  | 8   | $310 \pm 79$ increased 42 cells or 15.7% of week 0    |  |  |  |  |

Two participants with low CD4 counts,  $< 470 \text{ cells/}\mu\text{L}$ , and low CD8 counts,  $< 360 \text{ cells/}\mu\text{L}$ , experienced CD4 increases of 15.7%, 63.3%, and 71.2% at weeks 2, 4, and 8, respectively, from week 0 whereas the increases in their CD8 counts were 7.3%, 75.0%, and 66.4% at weeks 2, 4, and 8, respectively, from week 0. Nine participants with low CD8 counts,  $< 360 \text{ cells/}\mu\text{L}$ , experienced CD8 increases of 13.4%, 23.9%, and 15.7% at weeks 2, 4, and 8, respectively, from week 0.

#### Effects of Cancino<sup>®</sup> on Autophagy Induction

Autophagy is a process which can also help prevent cancer cell formation [8]. To examine whether autophagy was affected by Cancino<sup>®</sup> treatment in PBMCs, Atg12 and LAMP1 were investigated at different time points, including week 0 (baseline), and after treatment with Cancino<sup>®</sup> at weeks 2, 4 and 8. The results

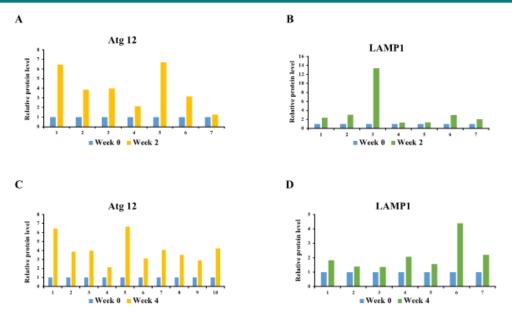


Figure 1: Expression of Autophagy-Related Proteins in Participants (The x-axis represents the number of participants).

showed that Cancino<sup>®</sup> induces autophagy process in participants by increasing the expression of Atg12 and LAMP1 at weeks 2 and 4 (Figure 1).

PBMC samples were collected at week 0 (the baseline) and at weeks 2 and 4, then analyzed by western blot. Histograms represent the relative band intensities of Atg12 and LAMP1 at week 2 (A and B) and week 4 (C and D) compared to week 0 (baseline).

## Discussion

The results of this study clearly show an increase in all immune cells, including lymphocytes, CD4 and CD8 T cells. Cancino<sup>®</sup> is therefore effective in preventing the occurrence of cancer. In addition, all participants experienced an increase in their telomere lengths, which improved their health condition, including antiaging/age-reversal effects. It is noteworthy that the telomere elongation process occurs at the same time as the increase in T cells. These previously existing T cells, as well as the newly increased T cells, will have telomeres that are longer than their normal telomere lengths. These T cells with longer-than-normal telomeres will be more effective in providing cancer prevention.

The study also shows that Cancino<sup>®</sup> induces autophagy, which suppresses tumorigenesis in abnormal cells. This further enhances the efficacy of Cancino<sup>®</sup> in cancer prevention.

## Conclusion

Mylife/Mylife100<sup>®</sup>, which was successful in treating cancer, was modified into Cancino<sup>®</sup>. Our study demonstrated that Cancino<sup>®</sup> is effective in preventing cancer through the combined effects of increased T cells, telomere elongation in previously existing and newly increased T cells, and autophagy induction. While Mylife/ Mylife100<sup>®</sup> offers an effective cancer treatment, Cancino<sup>®</sup> provides a method for cancer prevention.

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