Double-Blind, Placebo-Controlled, Randomized Clinical Trial Demonstrates Telomerase Activator TA-65 Decreases Immunosenescent CD8⁺CD28⁻ T Cells in Humans

Gunasekaran Singaravelu 1, Calvin B Harley 2, Joseph M Raffaele 3, Pratheesh Sudhakaran 4, Anitha Suram 5.*

1. Independent consultant, Edison, NJ, USA; E-Mail: singaraguna@gmail.com
2. Independent consultant, Murphys, CA, USA; E-Mail: calvinbruceharley@gmail.com
3. PhysioAge, New York, NY, USA; E-Mail: jmr@physioage.com
4. Texas State University, City, TX, USA; E-Mail: pratheesh81@txstate.edu
5. T.A. Sciences Inc., New York, NY, USA; E-Mail: anitha@tasciences.com

* Correspondence: Anitha Suram; E-Mail: anitha@tasciences.com

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Abstract
TA-65 is a small molecule telomerase activator extracted from Astragalus species. A previous observational study suggested that TA-65 decreased the number of immunosenescent cells in healthy subjects. Here we examined the impact of TA-65 in a much larger randomized, double-blind, and placebo-controlled study. This study aims to evaluate the effects of TA-65 on senescent CD8⁺CD28⁻ T cells in healthy subjects. This was a randomized, double-blind, placebo-controlled, and multi-arm parallel trial in 500 healthy subjects. Subjects, clinical staff, and primary outcome assessors were blinded until the database lock. A total of 500 healthy volunteers were randomly allocated, 100 subjects each, into one of the five groups; placebo, TA-65 (100 Units) qd, TA-65 (250 Units) qd, TA-65 (500 Units) qd, or TA-65 (250 Units) b.i.d. Change in the immunosenescence biomarker after nine months was measured. The intention-to-treat analysis of the primary outcome measure included all the randomized subjects (n = 500). Per-protocol analysis of the primary outcome measured included 93% of the
randomized subjects ($n = 424$). Multilevel analysis revealed a significant decrease in the CD8$^+\text{CD28}^-$ T cells with TA-65 intervention compared to the placebo group ($p < 0.05$). Intervention by 100 units and 250 units of TA-65 qd led to a decrement of CD8$^+\text{CD28}^-$ T cells by 28 cells/μl, while the intervention by 500 units of TA-65 led to a decrement of CD8$^+\text{CD28}^-$ T cells by 22 cells/μl; the placebo intake led to an increment of CD8$^+\text{CD28}^-$ T cells by 4.38 cells/μl. None of the serious adverse events (9) were deemed related or were unlikely to be related to the product. Adverse events (AEs), ranging from mild to moderate severity were, observed in 34.6% of the subjects. Oral intake of TA-65 significantly decreased CD8$^+\text{CD28}^-$ T cells.

**Keywords**
Telomeres; CD8$^+\text{CD28}^-$ T cells; TA-65; CMV

### 1. Introduction

Telomeres are a stretch of repetitive DNA sequences with telomere binding proteins, which protect the ends of each chromosome. Telomeres form an unique higher order structure by sequestering the 3’ overhang with the telomere DNA resulting in a telomere loop (T-loop) that contributes to the capping of the chromosomal ends. Attrition of telomere length underpins the aging process itself and the maladies associated with aging [1]. Telomerase is an enzyme that counteracts the attrition of telomere length by adding telomere repeats to the ends of chromosomes [2].

TA-65 is a single chemical entity purified from the *Astragalus membranaceus* species and demonstrated to increase telomerase activity and lengthen telomeres in mice and humans [3-6]. The safety of TA-65 has been well documented [7], and TA-65 has been granted generally recognized as safe (GRAS) status. No product-related toxicity was reported in three randomized placebo-controlled studies over a one-year duration [3, 8, 9].

A series of *in vitro* studies suggest that TA-65 increases telomerase activity, which in turn results in the cell proliferative effects. Richardson *et al.* demonstrated the proliferative effect of TA-65 on cultured splenocytes [10]. Fauce *et al.* showed that TA-65, which was previously named TAT2, improved immune function by increasing the replicative capacity of CD8$^+$ T lymphocytes [11] through telomerase activation and increased telomere length. Another independent study also demonstrates treatment with TA-65 leads to increased proliferation of T cells *in vitro* [12], implying the ability of TA-65 to improve immune function.

Cytotoxic T cells (CD8$^+$) play an important role in eliminating cells infected with intracellular pathogens and cancer cells [13]. The effectiveness of the immune response by CD8$^+$ T cells depends on the presence or absence of its co-receptor, CD28. Downregulation of CD28 receptor is a hallmark of T cell senescence and thus T cells expressing CD28 better proliferate upon antigen stimulation compared to T cells that lack CD28 [14]. As a consequence of senescence the CD8$^+$C28$^-$ T cells display shorter telomeres compared to CD8$^+$CD28$^+$ T cells and their rate of telomere attrition is more pronounced [15]. Accumulation of CD8$^+$CD28$^-$ enescent T cells is associated with an age-associated
decline of overall immune function [16]. A recent report shows that hyperbaric oxygen therapy decreases senescent T cells [17].

Evidence suggests that CD8⁺CD28⁻ T cells are associated with inflammatory diseases and chronic viral infections; their number increase with age and are correlated with the declining immune function in the elderly [16]. Recently the CD8⁺CD28⁻ T cells were used as important prognosis predictors in inflammatory disease and biomarkers for the antiviral response [18]. An important characteristic of the CD8⁺CD28⁻ T cells is replicative senescence—a phenomenon where the telomeres undergo attrition due to repeated cell division. Due to replicative senescence, these CD8⁺CD28⁻ T cells display short telomeres. Accumulation of CD8⁺CD28⁻ T cells is one of the hallmarks of immunosenescence, and strategies to decrease their number are being actively investigated [19, 20].

A previous observational study indicated that oral intake of TA-65 and other dietary supplements decreased CD8⁺CD28⁻ T cell population by 20% in CMV+ subjects [5], which is associated with improved immune function [11]. In a double-blind, placebo-controlled study, a net increase of telomere length of 530 ± 180 bp/year was observed in the subjects who were on TA-65 for one year[3].

Here we report the results of a placebo-controlled study on the effect of oral intake of TA-65 on CD8⁺CD28⁻ T cells in humans. We present intention-to-treat (ITT) analysis, as well as per-protocol analysis of the data. Additionally, we present a sub-analysis of the CD8⁺CD28⁻ T cells in the subjects seropositive for human cytomegalovirus (CMV).

CMV is wide-spread, infecting 60% to 70% of adults in industrialized countries and up to 100% in developing countries [21]. CMV-seropositivity is estimated to be prevalent among 50% of the United States population [22]. CMV infection leads to multifarious clinical manifestations in immunocompromised individuals [21], but in immunocompetent individuals, the infection is largely asymptomatic. However, while asymptomatic, one of the significant hallmarks of CMV infection is increased senescent T cells [23]. Given that more senescent T cells are present in CMV-positive subjects [24], and the senescence of T cells is associated with shorter telomeres [15], we hypothesized that TA-65, a telomerase activator [3], might offer benefits to CMV-positive subjects by decreasing senescent T cells.

2. Materials and Methods

2.1 Trial design

This was a single-center, randomized, double-blind, and placebo-controlled study. Full details of the trial protocol are available upon request. The study was conducted by a contract research organization (CRO) in accordance with Good Clinical Practice (GCP) and approved by the Institutional Review Board (IRB), Bio-Kinetic Clinical Applications, Springfield, MO. The study was registered prior to subject recruitment on ClinicalTrials.gov (NCT02766790).

2.2 Participants

Healthy adults aged between 45-75 years whose BMI ranges from 18 to 40 kg/m² were recruited for this study. Subjects who consumed TA-65 within 30 days before screening visits were excluded from participating. Subjects with a medical condition that might affect the safety or impact the
validity of the study results in the opinion of the investigator were also excluded. Eligible subjects were recruited from May 2016 to December 2016. All participants visited the study center at the screening, baseline, and the end of the study (after nine months).

2.3 Intervention

A total of 500 healthy volunteers were equally and randomly allocated into one of the five groups: placebo, TA-65 (100 Units) qd, TA-65 (250 Units) qd, TA-65 (500 Units) qd, or TA-65 (250 Units) b.i.d. (bis in die or ‘twice a day’). All subjects took two capsules per day for nine months, one in the morning and the other in the evening. The placebo group took two placebo capsules per day; the TA-65 (100 Units) qd, TA-65 (250 Units) qd, and TA-65 (500 Units) qd groups took one placebo capsule and one TA-65 capsule per day; the TA-65 (250 Units) b.i.d. group took two TA-65 (250 Units) capsules per day. Nine months of treatment duration was chosen based on the previous randomized study, at which the telomere length increase was significant [3]. Blood samples were collected at baseline and the end of the study. The status of CMV seropositivity was examined at baseline.

2.4 Outcomes

The primary endpoint was to assess the changes in immunosenescent cells. Immune cells were analysed by UCLA Immunogenetics Center, which is accredited by the American Society for Histocompatibility and Immunogenetics (ASHI) and Clinical Laboratory Improvement Amendments (CLIA). Secondary endpoints include safety markers.

2.5 Randomization, Allocation Concealment, and Blinding

The computer-generated sequence of numbers was used to randomly assign subjects to one of five groups. The details of the series were unknown to participants of this study, the investigators who recruited and monitored the study participants, and the investigators who measured or analyzed the study outcome. Volunteers, investigators, UCLA Immunogenetics Center and T.A. Sciences Inc. remained blind until CRO collected all the data. All doses of TA-65 capsules and placebo capsules were identical in appearance and were given to subjects in blister packages.

2.6 Analysis of Senescent CD8^+CD28^- T Cells

UCLA Immune Assessment Core performed the analysis of immunosenescent cells. Total CD3^+ T cells, CD4^+ T cells, CD8^+ T cells, CD19^+ B cells, and CD56^+/CD16^+ NK cells were enumerated in EDTA whole blood with the BD Multitest 6-color TBNK reagent and BD Trucount tubes following the manufacturer’s instructions, acquired on a BD FACSCanto II and analyzed with the BD FACSCanto Software. CD8^+ T cell sub setting was performed by staining 50 μl of EDTA whole blood with CD3 FITC, CD8 PerCP, CD28 PE, and CD95 APC (BD) for 10 minutes, followed by BD FACS Lysing used according to the manufacturer’s instructions. At least 10,000 lymphocyte events per sample were acquired and analyzed using DIVA 8.0 software on BD FACSCanto II. The gating strategy used in the enumeration of CD8^+CD28^- is summarized in Figure S1.
2.7 Statistical Methods

Intention-to-treat (ITT) analysis of data from all 500 subjects randomized in this study was performed by Student’s t-test in spreadsheet. Missing data of the subjects \( n = 44 \) who dropped out from this study were filled in by imputation strategy described by Sainani [25] before performing ITT analysis. Briefly, missing data of the subjects were filled in by the baseline values for ITT analysis. We aimed to assess whether data provided evidence of superiority of TA-65 to placebo in per protocol analysis using multilevel model. Additionally, a sub-analysis of CMV-positive subjects was also performed using Student’s t-test and multilevel model. The multilevel model analysis was performed to model any variation that might exist between groups. Controlling the variation between groups would be essential to have a more reliable model result. The student’s t-test was performed using spreadsheet. Multilevel model was performed using SAS software (version 9.0).

3. Results

3.1 Subjects

A total of 681 subjects were screened for eligibility to participate in this study. 500 of them met the eligibility criteria and were randomized; 456 subjects completed the study; 44 subjects didn’t complete the study due to either early exit or drop out from the study (Figure 1).
All randomized subjects (n = 500) were included in the intention-to-treat (ITT) analysis of the primary outcome measure (change in the % of CD8+CD28- T cells from baseline to the end of the study). Of the total 456 subjects who completed the study, 93% of the subjects (n = 424) were included in the per-protocol (PP) analysis of the primary outcome measure. The remaining 32 outliers did not meet the criteria of healthy volunteers based on the medical history and/or deviations of their biomarkers’ values from the normal ranges. This report mainly focuses on the CD8+CD28- T cells.

### 3.2 Baseline Characteristics

Table 1 summarizes baseline characteristics of subjects participated in this study.

**Table 1** Baseline characteristics of subjects participated in the trial.

| Parameter                      | Placebo qd | TA-65 100 Units qd | TA-65 250 Units qd | TA-65 500 Units qd | TA-65 250 Units b.i.d. |
|-------------------------------|------------|---------------------|---------------------|---------------------|-----------------------|
| N                             | 100        | 100                 | 100                 | 100                 | 100                   |
| Age (mean ± SD)               | 57 ± 8     | 57 ± 8              | 57 ± 8              | 58 ± 8              | 58 ± 7                |
| Gender                        |            |                     |                     |                     |                       |
| Men (n)                       | 35         | 35                  | 36                  | 36                  | 37                    |
| Women (n)                     | 65         | 65                  | 64                  | 64                  | 63                    |
| BMI (kg/m^2, mean ± SD)       | 28 ± 5     | 29 ± 5              | 28 ± 4              | 29 ± 5              | 28 ± 4                |
| CMV-positive (n)              | 63         | 70                  | 59                  | 60                  | 59                    |
| **Race**                      |            |                     |                     |                     |                       |
| Caucasian (n)                 | 96         | 92                  | 94                  | 94                  | 93                    |
| African American (n)          | 2          | 5                   | 3                   | 5                   | 5                     |
| American Indian or Alaska native (n) | 1 | 2        | 2                   | 1                   | 0                     |
| Asian (n)                     | 1          | 1                   | 0                   | 0                   | 0                     |
| Other (n)                     | 0          | 0                   | 1                   | 0                   | 2                     |
| **Ethnicity**                 |            |                     |                     |                     |                       |
| Hispanic or Latino (n)        | 5          | 3                   | 5                   | 1                   | 1                     |
| Not Hispanic or Latino (n)    | 95         | 97                  | 95                  | 99                  | 99                    |

### 3.3 CMV-Positive Subjects Have More CD8+CD28- T Cells at Baseline

Among the 500 subjects enrolled in this study, 62% were CMV-positive, and 38% were CMV-negative. The average number of CD8+CD28- T cells for CMV-positive subjects in this study was 221 ± 165 cells/μl (mean ± SD). For CMV-negative subjects, the average was 66 ± 57 cells/μl (mean ± SD). The difference between the two groups (155 cells/μl) is statistically significant (p<0.05), indicating that CMV infection increases the number of senescent T cells in humans. This observation is consistent with previous studies demonstrating that CMV infection increases senescent T cells [23, 24].
3.4 Intention-To-Treat (ITT) Analysis of CD8⁺CD28⁻ T Cells

Intention-to-treat analysis of senescent T cells at baseline and at the end of the study for the group that took placebo or TA-65 for nine months was performed using a prespecified two-tailed, paired t-test (Table S1). The change in the mean number of senescent cells was borderline-but not statistically significant-in the placebo group (p = 0.05), indicating that placebo treatment does not significantly influence the senescent T cells’ abundance. In contrast, most of the groups on TA-65 exhibited significant decrements in the abundance of senescent T cells. The mean number of senescent cells decreased from 189 ± 15 cells/μl at baseline to 170 ± 14 cells/μl at the end of the study in the group that took TA-65 (100 Units), and the resultant decrement (10%) was statistically significant (p<0.001). Likewise, intake of 250 Units or 500 Units of TA-65 also led to a decrement of the mean number of senescent T (11% and 9% respectively) cells in a statistically significant manner (p values <0.001 and 0.01, respectively). The mean number of senescent T cells decreased from 167 ± 8 cells/μl at baseline to 153 ± 8 cells/μl at the end of the study in the group that took any dose of TA-65 [TA-65(all)], and the resultant decrement (8%) was statistically significant (p<0.05). Taken together, ITT analysis indicates that 100, 250, or 500 Units of TA-65 significantly decreased senescent T cells in humans.

3.5 Per Protocol Analysis of CD8⁺CD28⁻ T Cells

Per-protocol analysis of senescent T cells at baseline and at the end of the study for the group that took placebo or TA-65 for nine months was performed using prespecified two-tailed, paired t-test (Table S2). The change in the mean number of senescent cells was not statistically significant in the placebo group (p = 0.62), indicating that placebo treatment does not significantly influence the abundance of senescent T cells. In contrast, all groups on TA-65 exhibited highly significant decrements in the abundance of senescent T cells. The mean number of senescent cells decreased from 191 ± 17 cells/μl at baseline to 167 ± 15 cells/μl at the end of the study in the group that took TA-65 (100 Units) qd, and the resultant decrement (13%) was statistically significant (p<0.05). Likewise, groups that took 250 Units, 500 Units qd, and 250 Units b.i.d. of TA-65 also led to a significant decrement of the mean number of senescent T cells (14%, 13%, and 13%, respectively) in a statistically significant manner (p values p<0.05). Taken together, per-protocol analysis indicates that 100, 250, or 500 Units of TA-65 can significantly decrease senescent T cells in humans. As fewer senescent T cells reflect improved immune function [16], it follows that the intake of TA-65 improves immune function in humans.

Table 2 Multilevel model analysis of senescent CD8⁺CD28⁻ T cells change compared to baseline in the indicated groups (n = 424) in the per protocol population.

| CMV Status & CMV-negative | Group                  | N  | Change in CD8⁺CD28⁻ T cells (cells/μl) | SE* | p value†  |
|---------------------------|------------------------|----|----------------------------------------|-----|-----------|
|                           | Placebo qd (Reference) | 72 | 4.38                                   | 6.93| 0.52      |
|                           | TA-65 (100 Units) qd   | 86 | -28.40                                 | 9.39| <0.001    |
|                           | TA-65 (250 Units) qd   | 94 | -28.18                                 | 9.20| <0.001    |
3.6 Analysis of CD8^+CD28^- T Cells Using Multilevel Model

Table 2 shows the multilevel model estimate of CD8^+CD28^- T cells for the difference between the end of the study and the baseline for subjects on TA-65 in compared to the placebo. In the pooled data that includes both men and women, CMV-positive and CMV-negative subjects, the estimate of change from baseline to the end of the study for the placebo group was an increase in the mean (4.38 ± 6.93, SE), and this change is not statistically significant (p = 0.52). This result indicates that placebo treatment does not significantly alter the number of circulating senescent T cells. In contrast, the number of senescent T cells significantly decreased in subjects on 100 Units of TA-65 (mean ± SE, -28.40 ± 9.39; p < 0.001; Table 2). Similarly, the estimate of change for senescent CD8^+CD28^- T cells significantly decreased in subjects on other doses of TA-65 (Table 2) as well. Taken together, these results indicate that TA-65, and not placebo, significantly decreased senescent CD8^+CD28^- T cells in all TA-65 groups, regardless of their gender and CMV status.

3.7 Subgroup Multilevel Model Analysis of CD8^+CD28^- T Cells in CMV-Positive Subjects

Next, in the light of prior studies showing the influence of CMV status on circulating senescent T cells [23, 24], and our observation in this study that CMV-positive subjects have significantly more senescent T cells (see above), we sought to sub-analyse the number of senescent cells in CMV positive subjects in this study.

As shown in the Table S2, the mean number of CD8^+CD28^- T cells did not change significantly from baseline to end of the study in the placebo group (t test; p = 0.44). However, groups that took TA-65 exhibited a significant reduction of CD8^+CD28^- T cells from baseline to end of the study (p values <0.03). This result indicates that TA-65 significantly decreased CD8^+CD28^- senescent T cells in CMV positive subjects.

The multilevel model also yielded a similar conclusion (Table 2). As shown in Table 2, the estimate of the change from baseline to end of the study for CMV-positive subjects on placebo was not statistically significant (mean ± SE, 11.24 ± 11.04; p = 0.30), whereas those on any dose of TA-65 were statistically significant. These results indicate that TA-65 - and not placebo-decreases the mean number of CD8^+CD28^- T cells in CMV-positive subjects. Relatively fewer subjects enrolled in this study were CMV-negative, rendering it difficult to understand the impact of TA-65 on CMV-negative subjects.
Furthermore, a comparison of combining all four TA-65 treatment groups with the placebo group also revealed significant decrement in CD8⁺CD28⁻ (13% reduction, \( p < 0.001 \) as assessed by ANOVA) in CMV-positive subjects.

Finally, independent analysis of senescent T cells using different gating strategies (CD3⁺CD8⁺CD28⁻ T cells) also yielded results like the one that used CD8⁺CD28⁻ T cells (Table 3), further validating the trustworthiness of the flow cytometry results obtained from this study.

**Table 3** Multilevel model analysis of change from baseline to end of study in the number of CD3⁺CD8⁺CD28⁻ T cells in the indicated groups in the per-protocol population.

| CMV Status          | Group                        | \(-N\) | Change in CD3⁺CD8⁺CD28⁻ T cells (cells/μl) | SE   | \( p \) value³ |
|---------------------|------------------------------|--------|---------------------------------------------|------|----------------|
| CMV-positive &      | Placebo qd (Reference)       | 72     | -11.53                                      | 12.87| 0.37           |
| CMV-negative        | TA-65 (100 Units) qd         | 86     | -46.59                                      | 16.66| <0.001         |
|                     | TA-65 (250 Units) qd         | 94     | -42.58                                      | 16.66| 0.01           |
|                     | TA-65 (500 Units) qd         | 92     | -7.08                                       | 16.75| 0.67           |
|                     | TA-65 (250 Units) b.i.d.     | 79     | -26.01                                      | 17.48| 0.13           |
| CMV-positive only   | Placebo qd (Reference)       | 42     | 2.92                                        | 12.23| 0.81           |
|                     | TA-65 (100 Units) qd         | 59     | -43.02                                      | 16.00| <0.001         |
|                     | TA-65 (250 Units) qd         | 54     | -53.31                                      | 16.30| <0.001         |
|                     | TA-65 (500 Units) qd         | 55     | -37.72                                      | 16.24| 0.02           |
|                     | TA-65 (250 Units) b.i.d.     | 43     | -33.05                                      | 17.19| 0.05           |

N = number of subjects; SE = Standard error; † \( p \) values <0.05 are indicated in bold fonts. Statistical analysis was performed using multilevel model

### 3.8 Serious Adverse Events (SAE) and Adverse Events (AE)

No product related toxicity or serious adverse events (SAEs) were observed for this study. Adverse events (AEs) ranging from mild to moderate severity were observed in 34.6 % of the subjects, and these subjects are almost evenly distributed across TA-65 and the placebo group (Table 4).

**Table 4** Summary of Adverse Events (AEs) observed in this study.

| Group                        | Not related | Unlikely | Probable | Possible | Total |
|------------------------------|-------------|----------|----------|----------|-------|
| Placebo qd                   | 14 (2.8%)   | 10 (2%)  | 8 (1.6%) | 6 (1.2%) | 38 (7.6%) |
| TA-65 (100 Units) qd         | 20 (4%)     | 13 (2.6%)| 8 (1.6%) | 2 (0.4%) | 43 (8.6%) |
| TA-65 (250 Units) qd         | 13 (2.6%)   | 9 (1.8%) | 5 (1%)   | 3 (0.6%) | 30 (6%)   |
| TA-65 (500 Units) qd         | 13 (2.6%)   | 9 (1.8%) | 8 (1.6%) | 0 (0%)   | 30 (6%)   |
| TA-65 (250 Units) b.i.d.     | 11 (2.2%)   | 7 (1.4%) | 9 (1.8%) | 5 (1%)   | 32 (6.4%) |
| Total                        | 71 (14.2%)  | 48 (9.6%)| 38 (7.6%)| 16 (3.2%)| 173 (34.6%)|

† % corresponds to the total number of subjects randomized (\( n = 500 \)) in this study.
4. Discussion

There is an increasing interest in telomerase therapies and pharmacological interventions that can rescue the telomere dysfunction caused by attrition [26, 27]. Recently, hyperbaric oxygen therapy (HBOT) has been reported to significantly increase telomere length in T cells, B cells, and NK cells, along with a significant decrease in CD8^+CD28^- T cells in healthy volunteers [17]. In a previous observational study with a one-year health maintenance program consisting of TA-65 and other dietary ingredients senescent CD8^+CD28^- T cells dropped by about 3% at 12 months in the overall population consisting of both CMV-positive and CMV-negative subjects [5]. However, a prominent 20% drop in the number of CD8^+CD28^- T cells was observed in CMV-positive subjects at 12 months, demonstrating the positive remodelling of the immune system in CMV positive subjects [5].

The current study reports that telomerase activator TA-65 significantly decreases the CD8^+CD28^- T cells in CMV-positive subjects. The decrease in CD8^+CD28^- T cells by TA-65 is consistent with the previous observational data indicating TA-65 as the primary driver in the telomere maintenance thereby reducing the CD8^+CD28^- T cells [5]. However, the previous observational study enrolled a small number of subjects (n = 114), preponderance of men (72%), and did not have a placebo group. The current randomized, placebo-controlled study addressed those shortcomings by recruiting a larger number (n = 500) of relatively more women (64%) subjects.

In the current study, there were more CMV positive subjects (62%) than CMV-negative subjects (38%), which is consistent with the previous observation that CMV-seropositivity is more prevalent among older people [28]. Likewise, we show that CMV-positive subjects harbour more senescent T cells than CMV-negative subjects, consistent with previous reports [23, 24]. All doses of TA-65 (100 units, 250 units, and 500 units) significantly reduced the number of CD8^+CD28^- T cells (p values<0.001) in the overall population. A more pronounced decrease was seen in lower doses (28.48 cells/μl and 28.18 cells/μl for 100 units and 250 units, respectively) compared to the higher dose (22 cells/μl 500 units). Although the reason for this is unclear, telomere length was shown to be significantly increased in the low dose group (250 units), while higher dose (1000 units) did not cause any significant increase in an earlier study [3]. The significant decrease in the CD8^+CD28^- T cells is also demonstrated in CMV-positive subjects (p value<0.05).

Induction of telomerase by TA-65 in cells can compensate for telomere loss and restore the cells’ proliferative potential [10, 11]. A recent placebo-controlled clinical study demonstrated that TA-65 increases telomere length in lymphocytes in a one year period [3]. Based on the above findings, we propose that TA-65, by increasing telomerase and lengthening telomeres, might mitigate T cells’ replicative senescence, thereby decreasing the CD8^+CD28^- T cells.

Since the study recruited both men and women of a broad age range (45 to 75) and included both CMV-positive and CMV-negative subjects, the results from this study indicate that TA-65 may benefit the general population by decreasing senescent T cells. Although TA-65 has been shown to induce telomerase levels, lengthen telomeres, and reduce senescent cells, this study’s limitations are the lack of the telomere length and telomerase activity assessments in the CD8^+CD28^- T cells to directly correlate to the senescence phenotype.
5. Conclusion

Oral intake of TA-65 for 9 months significantly decreased CD8⁺CD28⁻ T cells in healthy male and female volunteers with age ranging from 45 to 75 years. The majority of the AEs were determined to be either not related or unlikely to be related to the test products used in this study.

Acknowledgments

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Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Figure S1: Gating strategy for CD8⁺CD28⁻ T cells.
2. Table S1: ITT analysis of changes in the number of senescent T cells (CD8⁺CD28⁻) in subjects on placebo or TA-65 for nine months (n = 500).
3. Table S2: Per-protocol analysis of changes in the number of senescent T cells (CD8⁺CD28⁻) in subjects on placebo or TA-65 for nine months (n = 424).

Author Contributions

T.A. Sciences Inc. was involved in the design of the study but played no role in the conduct of the study; T.A. Sciences’ consultant, Dr. Sudhakaran did statistical analyses involving multilevel models and ANOVA. The manuscript was prepared by Drs. Singaravelu and Suram, and reviewed by Drs. Harley and Raffaele.

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Competing Interests

The authors have declared that no competing interests exist.

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