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Review

Augmentation of cell numbers and function in the immune system by *in vivo* administration of North American (NA) ginseng (*Panax Quinquefolium*): Assessment in normal and cancer-bearing infant, juvenile, adult and elderly mice

Sandra C. Miller*
Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, H3A OC7, Canada.

ABSTRACT

In this review, data have been assembled which demonstrates the profound and indisputably positive effect that North American (NA) ginseng (*Panax Quinquefolium*) has on the immune system *in vivo* in mice. The mouse was chosen because of the numerous parallels of this immune system to that of the human. Moreover, it has been shown that this immune-enhancing influence extends across all age groups from pre-weaning to old age and is gender independent. This consistently positive influence occurs in normal animals, which, moreover, maintain their elevated immune system even after discontinuing exposure to this agent. A distinct prophylaxis has been established thereby, such that ginseng-consuming animals, challenged with either tumor cells, or carcinogenic agents, have a level of resistance to the impending pathology not seen in parallel control animals. Moreover, it has been shown that in both leukemia-afflicted infant and adult mice, given this extract of NA ginseng, tumor abatement and significantly prolonged life span occurred. Thus, as an agent so effective in enhancing the mechanisms responsible for disease defense, its potential for debuting along side conventional, mainstream medicine, cannot be ignored.

KEYWORDS: *Panax Quinquefolium*, age, immunoenhancement, pre-clinical, *in vivo*.

*Email id: sandra.miller@mcgill.ca

1. Introduction

The immune system consists of various cell lineages, all with specific functions in the disease defense process. Cells which function in the combat process are: (i) T lymphocytes, which although generated *de novo* as T "stem" cells in the bone marrow, undergo, upon transfer to the thymus, full maturation into functional T lymphocytes of assorted sub-types, (ii) B lymphocytes, generated and maturing functionally in the bone marrow, and, (iii) natural killer (NK) cells, also generated and maturing functionally in the bone marrow, however their numbers are far fewer than those of either the T or B lymphocyte lineages. This review is predominantly, although not exclusively, concerned with NK cells.

These lineages of the immune system are dynamic and sensitive to both stimulatory and suppressive agents, both endogenous and exogenous. These agents include a whole range of molecules such as pharmaceuticals, nutraceuticals, carcinogenic agents, endogenous and exogenously administered cytokines and steroids (sex hormones or adrenal gland-derived), or live pathogens which can enter *via* gastrointestinal, respiratory or cutaneous routes. All these molecular influences are dependent upon dose and exposure time, and can either (a) increase or decrease mature cell functional capabilities of one or more of these lineages, (b) influence circulation of one or more of these lineages among the various organs through which they regularly circulate, or (c) influence the production rate(s) of

one or more of these lineages in the organ in which they are generated *de novo,* i.e., the bone marrow.

The ageing process alone is a powerful and inescapable negative regulator of all 3 lineages of the immune system. The immune system is at its peak performance in the life span of all mammalian species during young-to-middle adulthood. This is logical since this age range represents the reproductive phase of mammalian species, and species survival depends upon peak performance of the immune mechanisms in disease defense during this period. In other words, the very existence of a species could be compromised if life-threatening disease processes were to be rampant during the reproductive periods.

The 3 lineages of immune cells (above) usually have low numbers of functional, pathogen-combatting cells until puberty. The immune system progressively declines from middle to old age, a period which, in nature, is defined as the inability to reproduce. The various lineages of the immune system decline at species-specific rates. Inversely related to this age-related decline among the immune cells, is the increased frequency, in mammalian species, at least, of various diseases and cancer.

NK cells are well-established as being generated in the bone marrow and tightly regulated by a microenvironment inside that organ [1-10]. The stromal cells constituting various niches in this microenvironment can stimulate or withhold new cell production signals (molecular factors emitting from the stromal cells). Specific niches govern NK cell production and it has been well established over the past several decades that other specific niches drive the various hemopoietic and immune cell lineages generated within the bone marrow. The cytokine interleukin-2 (IL-2), is capable of stimulating new NK cell production. When IL-2 levels are increased by either exogenous administration of the interleukin itself, or when mice are given an agent to stimulate endogenous levels of IL-2, the result is a quantitative increase in, i.e., absolute numbers of, NK cells. CVT-E002 is the proprietary, standardized extract of NA ginseng (Afinity Life Sciences, Inc., Edmonton, AB, Canada), capable of inducing IL-2 *in vitro* [11, 12]. CVT-E002 is the active ingredient in the product with the marketing label Cold fX©. Over the past few years, the effect of this agent in infant, juvenile, young adult and elderly animals, both in health, and during affliction with assorted cancers has been investigated.

CVT-E002, throughout these studies, has been used only where batch-to-batch consistency was fully ensured. Reproducibility was thus ensured in the data in all assays. CVT-E002 consists of specific polysaccharides (poly-furanosyl-pyranosyl-saccharides), and *in vitro* when this extract is mixed with lymphocytes [11, 12], the result is the production of several cytokines, some of which are powerful stimulants of NK cells. NK cells represent the first line of defense against neoplasms in mammals including humans, and it is when NK cell numbers are suppressed, especially chronically as in old age, that the window of opportunity is open for the development of neoplastic growths of assorted types.

All the animals used in the studies to be described below have been approved by the McGill University committee on animal use in research and the CCAC (Canadian Council on Animal Care).

2. Effect of NA ginseng on the immune system of normal, young adult mice

Young adult (8-9 wk of age), virgin female mice of the inbred C3H strain were fed CVT-E002 (80 mg/6 gm full nutrition chow/day) [13] for 4 wk at which time these mice were converted to the regular diet for the next 8 wk. At this time, they were 20-21 wk old (5½ mo old). The daily dose of 80 mg of CVT-E002 was selected as the one producing the best results based on earlier dose/response studies. Eighty mg of CVT-E002/ day/mouse produced no signs of clinical or physiological/immunological toxicity in these young adult mice after assessing several doses which were both lower and higher than the selected optimal dose of 80 mg. Parallel control mice were fed untreated chow throughout, and euthanized also at 20-21 wk of age.

The results in the control organs (spleen, bone marrow) of mice which never received dietary CVT-E002 were very different from the results obtained in the CVT-E002-fed mice. Indeed, NK cells in the spleens of these CVT-E002-fed mice

numbered 730,000 – statistically significantly more plentiful than those in the spleens of their identically aged control (no CVT-E002) counterparts at 20-21 wk of age, i.e., 450,000. Thus, the 4 wk interval of daily dietary CVT-E002 was able to significantly augment and then retain super normal levels of NK cells in the spleen through the 8 wk period from 12-13 to 20-21 wk of age during which the mice had been transferred to the control (untreated chow) diet [13]. In the bone marrow of these same mice, there was also an increase in the numbers of NK cells at 20-21 wk: 40,000/2 femurs vs control at 34,000/2 femurs. In the bone marrow, this meant that there was a retained ability to continue to produce super-normal numbers of NK cells long after cessation of the dietary extract. Based on ever-increasing evidence for a fundamental role of the microenvironment-based stromal cell network in the bone marrow to govern new NK cell production, it is believed that CVT-E002 is acting upon specific niches of NK cell-governing stromal cells. Upon their stimulation in the presence of CVT-E002, stromal cells may subsequently produce cytokines in greater than normal quantities, and these, in turn, increase NK cell production. This phenomenon would account for the observed quantitative increase in NK cells in their bone marrow-generating site. It is this bone marrow-based, augmented production of new NK cells which is then responsible for the significantly elevated, absolute numbers of NK cells observed in the spleen. It is known [2] that the vast majority of bone marrow-derived NK cells migrate to the spleen, *via* the blood, the latter being their only exit route from the bone marrow.

Non-NK lymphocytes were also positively influenced *in vivo* by this extract [14]. Non-NK lymphocytes (collectively T and B cells) from the CVT-E002-consuming mice (4 wk) were quantitatively enumerated at 20-21 wk of age. In both the spleen and bone marrow, the absolute numbers of non-NK lymphocytes, were significantly more numerous in CVT-E002-fed mice than those found in identical control mice consuming untreated chow (spleen: $p < 0.0001$; bone marrow: $p < 0.0032$). The bone marrow is the sole generator of the vast quantities of newly produced, virgin B lymphocytes. The generation of this population of cells is well known to be regulated by B cell-governing niches of stromal cells in the bone marrow [15-18]. It is this bone marrow-based, significant increase in new B lymphocyte production which contributes largely to the significant increase in non-NK lymphocytes in the spleen. As with NK cells, B lymphocytes also use the only exit route from the bone marrow, i.e., the blood.

3. Effect of NA ginseng on the immune system and life span of tumor-bearing, young adult mice

The next step was to explore the role of this agent in tumor-bearing mice [19]. In the first study of its kind, the efficacy of CVT-E002, in leukemia-afflicted, young adult (9 wk), male DBA/2 mice was assessed by (i) defining the size of the NK cell lineage in CVT-E002-administered mice, and by (ii) measuring any change in life span (Kaplan-Meier software) resulting from daily, long-term consumption of CVT-E002. These assays were performed employing 3 doses of CVT-E002 (2 mg, 40 mg, 120 mg/6 gm chow/mouse per day). Daily feeding in each of the 3 CVT-E002 dosing groups began one hour after leukemia cell injection. Parallel, control, leukemia cell-injected mice were identical in every way except that their chow did not contain CVT-E002. The results showed that whereas a dose of 2 mg/day of CVT-E002 was able to double the absolute numbers of NK cells in both the spleen and bone marrow of leukemic mice, it was not able to extend life span and in fact, the life span of mice consuming this dose of CVT-E002 was almost identical to that of control leukemic mice consuming untreated chow. Thus, 100% of mice consuming chow containing 2 mg CVT-E002 succumbed to their leukemia at 18 days post leukemia cell injection, paralleling that of control leukemic mice fed only the untreated chow. It appeared therefore, that although the low dose of 2 mg/day of CVT-E002 was able to double the absolute numbers of NK cells, it was inadequate to improve life span over that of the control mice not fed the extract. However, a dose of 40 mg CVT-E002/6 gm chow/mouse/day, was able to increase the absolute numbers of NK cells to more than 4 times the control numbers in both the spleen and bone marrow and, moreover, this dose significantly extended life span such that

50% of all mice consuming 40 mg CVT-E002 were not only still alive by 2 months after leukemia cell injection, but were healthy/normal as determined by the clinically defined criteria of body weight, level of activity, food/water consumption, and coat quality at the time of euthanasia. Finally, when a dose of 120 mg CVT-E002 was provided daily in the chow, 100% of the mice in this dosage group succumbed to their leukemia between 1 and 2 mo. (28-55 days) after leukemia cell injection. It appears, thus, that there is a narrow window, i.e., > 40 mg but < 120 mg, during which CVT-E002 is optimally effective in leukemia combat and life span extension. It is believed that the optimal dose of CVT-E002, to achieve maximum life expectancy after leukemia cell injection in the mouse, and under the present protocol, would be in the range of 80 mg/day. This dose falls within the "narrow window" referred to above. Regardless, however, of what may be the optimal dose in leukemia combat, it may be quite different from a dose of CVT-E002 which may be optimal for prophylactic purposes or from a dose which may be optimal for amelioration of other kinds of neoplasms. It was with this latter possibility in mind that the following set of experiments was conducted.

Young adult, male C3H mice (7-8 wk of age) were injected [20] with diethylnitrosamine (DEN), a known carcinogen which produces primary liver cancers. Indeed, the mechanisms whereby DEN induces hepatocarcinogenesis have been identified [21-25]. This particular neoplasm was of interest because of the ubiquitous presence of DEN in the environment in modern civilization. DEN is found in vehicular exhaust, tobacco smoke, alcoholic beverages, meats and other foods preserved with nitrite pickling salt. In the following studies, CVT-E002 was provided *via* the diet daily (80 mg/ 6 gm chow/day/mouse), beginning 4 hr after DEN injection and continuing until the mice were 82 wk (1 yr + 8 mo) of age. Control, parallel mice were identical in every way with the exception of CVT-E002 in their daily chow. Mice in the control group developed hepatocarcinoma between 52-66 wk of age after having been injected with the hepatoma inducer, DEN, when they were 7-8 wk of age. These mice (100%) had macroscopic, palpable hepatomas, signaling compulsory euthanasia at that time, in keeping with federal regulations governing animal care in research laboratories. The NK cells and non-NK lymphocytes in the spleen and bone marrow (both femurs) were assayed at euthanasia when their tumor first became palpable (52-66 wk of age). In contrast, 100% of DEN-injected mice which had been given daily CVT-E002 in their chow were alive, and healthy by all clinical criteria. They were purposely euthanized at age 82 wk, at which time their spleen and bone marrow (both femurs) were assayed for their content of NK cells and non-NK lymphocytes. At the time of euthanasia (82 wk of age), all CVT-E002-consuming mice were tumor free. These mice had statistically significant, quantitatively elevated (absolute numbers) NK cells in their spleen and bone marrow over the corresponding control organs ($p < 0.005$; $p < 0.001$) from mice euthanized between 52-66 wk of age. Non-NK lymphocytes were also statistically significantly elevated in the spleen and bone marrow ($p < 0.0001$; $p < 0.0001$, respectively) [20].

In a parallel study, another group of CVT-E002-consuming mice, at age 82 wk, were transferred to the control diet for the purpose of assessing any life span change or potential tumor development after removal of dietary CVT-E002. However, even after converting these mice to the control diet for the next 16 wk (4 mo) after withdrawing CVT-E002, they remained healthy by all clinical criteria and were still tumor-free at the time of euthanasia at 98 wk of age (82 + 16 wk) [20]. Euthanasia in these elderly mice was carried out simply in the interests of concluding this study.

4. Effect of NA ginseng on immune cells of the NK cell lineage of normal, juvenile mice

The immune system of juvenile mammals is in a "growing" phase and is not fully competent until puberty is attained. Because of this juvenile immaturity in the immune system *vs* the adult, it was of considerable interest to learn if the immuno-stimulant, CVT-E002, had any effect on the NK cell lineage before puberty; the hypothesis being that during this development phase of life, the NK cell lineage may be sheltered from/resistant to exogenous interference, i.e., CVT-E002. Functional immaturity of the NK cell lineage - the first line of defense against developing neoplasms - may be

the reason for neoplasms appearing relatively frequently in young mammals, including children.

To test this hypothesis, newly weaned, normal 4 wk old C3H mice were fed CVT-E002 in graded doses of chow daily, through this pre- and para-pubertal period for the next 6 wk into young adulthood (10 wk) at which time the mice were converted to the control diet for a further 8 wk [13]. All these mice were euthanized when they were 18 wk of age (4 wk + 6 wk + 8 wk). Parallel, control mice were identical in every way, except that they were placed on the control diet (without CVT-E002) from 4 wk of age until 18 wk. The results revealed that at 18 wk of age, NK cells were quantitatively, statistically significantly elevated over control, in both their spleen and the bone marrow. Thus, the developing, immature NK cell lineage had proven to be responsive to the presence of CVT-E002 *in vivo*.

While it has been well established that the production/generation of new NK cells is under the control of stromal cell products of the bone marrow microenvironment (referenced above), stromal cells themselves are also under regulatory influences, i.e., soluble factors (cytokines) such as IL-1, IL-6 and TNF-α [26-31]. Moreover, the production of these cytokines is augmented in the presence of CVT-E002 [11, 12]. This further supports the probability that the mechanism by which CVT-E002 increases NK cells is an indirect one, acting by stimulating the NK cell-governing stromal cell network (the microenvironment) in the bone marrow. Since NK cells, themselves, have only a 1-2 day life span [2], they need continuous replacement to maintain their numbers. The only mechanism by which NK cells in the bone marrow can achieve elevated numbers, in the presence of CVT-E002, is *via* their precursor response to elevated levels of the appropriate precursor-driving cytokines. An elevated number of stromal cells themselves, occurring in the presence of CVT-E002, could logically give rise to elevated levels of NK-driving cytokines.

In the developing bone marrow, i.e., that of the normal, newly weaned, juvenile, pre-pubertal mouse, stromal cells are themselves proliferating, i.e., increasing their own numbers, while those of the adult no longer proliferate [32, 33]. The net result of such a pre-pubertal, stromal cell proliferation, potentially further augmented in the presence of CVT-E002, would account for a super-normal number of stromal cells relative to those found in the bone marrow of juvenile, pre-pubertal mice not exposed to CVT-E002. Such super-normal levels of bone marrow-based stromal cell numbers would remain in the adult – an age where stromal cell numbers are fixed and no longer capable of proliferating. These conceivably could be the source of correspondingly elevated levels of NK cell-stimulating cytokines, and could explain the observations of significantly elevated, absolute numbers of NK cells in adult bone marrow long after daily exposure to CVT-E002 has been terminated. This phenomenon would also then account for the significantly elevated numbers of NK cells in the spleen, the known major destiny for newly generated NK cells arriving from the bone marrow.

Collectively, these observations may be interpreted to mean that normal, juvenile animals, potentially including humans, when stimulated, i.e., *via* CVT-E002, to produce sustained, super-normal numbers of NK cells, may be fortified against the development of leukemia and lymphomas – these neoplasms being relatively common in youth.

5. Effect of NA ginseng on immune cells of the NK cell lineage in normal and leukemic, pre-weaned, infant mice

In the following study, employing groups of normal, infant mice [34], CVT-E002 was administered *via* the intraperitoneal route. This necessitated establishing the most appropriate dose for these infants. This was done by "downsizing" on a body weight basis, the most effective dose based on CVT-E002 administration studies in adult mice. Thus, 7-day-old infant mice were injected with sterile saline in which CVT-E002 was dissolved (20 mg/6.5 gm b.wt., i.e., that of a 7-d-old mouse). As the pre-weaned infants (gender indiscriminant) grew daily, thus increasing their body weight throughout the next 14 days, the dose of CVT-E002 was adjusted upward from the 7 day old "starting" dose of CVT-E002. One group of these mice was euthanized at age 21-26 days of age, i.e., just prior to puberty. This age represents 0-5 days after terminating the last of the 14 daily CVT-E002 injections. Parallel, control, normal infants

were sham-injected with vehicle only. The results indicated that at 21-26 days of age, the spleens and bone marrow of these CVT-E002-injected infants did contain statistically significantly elevated absolute numbers of NK cells both in the spleen ($p < 0.018$) and bone marrow ($p < 0.039$), relative to their respective control organs [34]. These results in the infant organs indicated that, like the juvenile mice which received their CVT-E002 *via* the diet, these infants also responded positively to CVT-E002 even when it was given *via* intraperitoneal injection.

A second, parallel group of identical, CVT-E002-injected, normal infants (with their sham-injected controls) was allowed to live on, after the 14 day CVT-E002 exposure regimen, having been transferred to control chow at weaning, until age 7-8 wk. At that age, the absolute numbers of NK cells in their spleen and bone marrow were assessed. In spite of these mice, now young adults at 7-8 wk of age, having had no exposure to CVT-E002 since those 14 pre-weaning days, the NK cell content of their spleen and bone marrow was significantly elevated relative to the control organs. This study further supports the postulated mechanism of CVT-E002-mediated increase in stromal cell proliferation/numbers in the bone marrow of these infant mice. It would be the bone marrow-based, elevated stromal cell numbers, and their correspondingly augmented, NK cell-stimulating cytokine output which, in turn, would have resulted in the observed elevated NK cell numbers. Subsequent export of these bone marrow-derived NK cells to the spleen, as normally occurs, would account for the elevated NK cell numbers in the spleen.

A follow-up study [35] was then undertaken in which infant mice, beginning at 7 days of age (body weight 6.5 gm), were injected with 500,000 viable, sterile leukemia cells intraperitoneally and 3-4 hr later, graded "starting" doses (5, 10, 20, 30, 40 and 50 mg) of CVT-E002 were also intraperitoneally injected daily for the next 14 days for a final age of 21 days. As the infants grew through these 14 days, the level of each of the above "starting" doses of CVT-E002 was adjusted upward according to body weight. The data revealed that only one dose level of CVT-E002 best extended the life span of these leukemic infants. Doses of 5 or 10 mg proved to be ineffective altogether, with all mice succumbing to their cancer by age 16 days. This was comparable to control mice receiving only leukemia cells. Similarly, the higher doses (30, 40, 50 mg) also proved ineffective, with all mice deceased between age 16 -18 days. The most appropriate "starting" dose appeared to be 20 mg, with all these mice living somewhat longer but nevertheless succumbing by 23 days of age. While 20 mg appeared to be the best dose under the present circumstances, the survival increment was below statistical significance. The intraperitoneal route for the delivery of both CVT-E002, and the leukemia cells could have restricted the ability to optimize life span in these leukemia-afflicted infants. Moreover, other obstacles, involving administration of CVT-E002 *via* the intraperitoneal route, may have been the following: (i) inadequate uptake of CVT-E002 from the peritoneal cavity, (ii) incomplete dissolution of CVT-E002 especially at the higher doses – indeed some clumping was observed inside the peritoneal cavity, and finally (iii) vehicle volume overload especially at doses > 20 mg, when CVT-E002 was delivered intraperitoneally. This could have possibly resulted in high fluid volume-mediated physiological disturbances such as electrolyte dilution incompatible with life. Nevertheless, in higher mammals, including humans, these are surmountable problems, given that CVT-E002 can readily be administered even during the suckling phase or *via* a pre-weaning milk/liquid diet, thus eliminating potential problems involving the intraperitoneal route.

6. Effect of NA ginseng on the immune cells of normal and tumor-bearing elderly mice

Because of the long-standing and well-known phenomenon that the immune system declines progressively with advancing age, it is more than coincidental that various pathologies, not the least of which is cancer, concomitantly increase in frequency with age. Efforts therefore were aimed at assessing the effect of CVT-E002 on the immune cell lineages of elderly mice. The following studies proved that, indeed, it was possible to resurrect the elderly immune system not only in terms of significantly augmenting the population sizes of the major immune cell lineages, but also

in terms of providing significant life span extension in animals prone to neoplasms.

In one study [36], the ability of CVT-E002 to restore the declining immune cell lineages of elderly normal but cancer-prone, female C3H mice of approximately 2 yr old was assessed. CVT-E002 was administered *via* the diet, at 80 mg/6 gm chow/day/mouse for 5 wk. Parallel, control mice were identically housed and fed except for the presence of CVT-E002 in their daily diet. At 5 wk, CVT-E002-consuming mice and their controls were euthanized and the spleens, bone marrow and blood were examined for their content of NK cells and other lymphocytes (T/B cells). The data showed that indeed, there was a statistically significant, quantitative increase in the population size of NK cells and of other lymphocytes (T/B) as well, in the spleen and bone marrow *vs* control mice not consuming CVT-E002. The observation that there was such a profound increase in NK cells in the bone marrow reflects the fact that CVT-E002 has stimulated the production of new NK cells, the bone marrow being the generating center for all new NK cells. As indicated previously, once NK cells leave the bone marrow, they never re-circulate back into it, the vast majority of mature NK cells travelling unidirectionally to the spleen. Moreover, virgin B lymphocytes which are also generated exclusively in the bone marrow were responsible for the significant quantitative increase (absolute numbers) in non-NK lymphocytes in that organ. All such newly generated, B lymphocytes exit that organ, *via* the blood. The observation that the blood of CVT-E002-fed mice also had super-normal levels of both NK cells and other lymphocytes (predominantly B) reflects this large CVT-E002-mediated, lymphocyte emigration from the bone marrow.

This study, therefore, has shown that after only 5 weeks of daily feeding of CVT-E002 to these aged, 2-yr-old mice (~ 70 human years), their immune system was, in absolute numbers, fortified to the level of that of the young, adult mouse. It was thus confirmed that the normal, elderly immune system is indeed as sensitive to CVT-E002 as are the immune systems of young adults, juveniles and infants. It is reasonable, therefore, to postulate that an equivalent immune stimulation and fortified disease defense mechanisms may also occur in humans consuming CVT-E002.

In an extension of this latter study, the cancer-prone C3H strain of mice was again used and at 22 mo of age, feeding CVT-E002 (80 mg/6 gm/mouse/day) was initiated. However, this time, the extract was fed daily, for the next 11 mo. [37]. Parallel, control mice, were fed the identical diet for 11 mo, however, without CVT-E002. In keeping with the cancer-prone nature of this strain, all control-diet mice had developed palpable, assorted tumors between 22 and 33 mo of age, and the identity of the tumors was determined at euthanasia which took place immediately upon first detection of palpable tumor. At autopsy, macro- and microscopic analysis of each tumor was carried out. Of all the tumors developing throughout the 11 months in the control group (22-33 mo of age), 47% of them were breast neoplasms. At euthanasia in each case, the spleen and bone marrow were taken and the NK cells and non-NK lymphocytes (T/B cells) were harvested and quantitatively assessed. By contrast, 100% of mice consuming the diet containing CVT-E002 were alive and tumor-free at 33 mo, healthy and normal by all murine clinical standards (above). At 33 mo, these CVT-E002-fed mice indicated a statistically significant quantitative increase, *vs* control, in the population size of both NK cells (spleen: $p < 0.0004$; bone marrow: $p < 0.0001$), and non-NK lymphocytes (spleen: $p < 0.0001$; bone marrow: $p < 0.0001$).

Another group of 22 mo old, C3H mice, were fed CVT-E002 daily for 11 mo, as above, and at aged 33 mo, this group of mice was converted to the control diet [37]. These mice were allowed to live on, clinically healthy and tumor-free, and when they were just over 4 years of age (>120 human years), these mice were purposely euthanized simply in the interests of concluding this study.

7. Conclusion

Throughout the studies described in this review, it has been shown that the standardized, proprietary extract of North American ginseng, i.e., CVT-E002 has enormous prophylactic and therapeutic potential in terms of preventing/treating neoplasm onset and growth. Although this agent is currently

in the marketplace as Cold fX®, at present used exclusively for the amelioration of virus-based respiratory ailments, the described pre-clinical, *in vivo* studies have demonstrated its clear benefits in the prevention and combat against one of the most lethal diseases of mammals, i.e., cancer. The mechanism by which CVT-E002 is so effective in disease abatement is one of profound immune system enhancement. Indeed, it has been possible to resurrect a fundamental immune cell population (NK lineage), such that the onset of tumors was not only prevented but the life span of cancer-prone animals was extended beyond their normal life expectancy. Moreover, CVT-E002 is effective as an immune system stimulant irrespective of gender, age, health status, or route of administration. Although the effective dose range has been established in mice, doing so in humans would not be an insurmountable task. Moreover, given that this ginseng extract has already proven to be non-toxic in humans, standardized, and in the marketplace, it would seem imperative that such a powerful disease-ameliorative agent make its debut alongside conventional medicines.

CONFLICT OF INTEREST STATEMENT

The funding organizations supporting the original manuscripts referred to in this review played no role in the design, data collection, analysis, interpretation, or the writing of the original manuscripts. The author of this review (SCM) is Principal Investigator and Laboratory Head for all the original manuscripts referred to in this review. The author has no conflicts of interest to declare.

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