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**Article**

# CVT-E002 Stimulates the Immune System and Extends the Life Span of Mice Bearing a Tumor of Viral Origin

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## ABSTRACT

The present study evaluated the dose-related effects of CVT-E002, a proprietary extract of *Panax quinquefolius* (CV Technologies Inc., Edmonton, AB), in the treatment of a tumor of viral origin, that is, erythroleukemia, in mice. Three treatments including ingestion of 2, 40, and 120 mg/d were compared. The study revealed that the dose of 40 mg/d was particularly effective in stimulating cells mediating nonspecific immunity and extending the life span of tumor-bearing mice. This study represents the first in vivo demonstration of the anticancer efficacy of CVT-E002 in an animal model. CVT-E002 treatment significantly elevated the absolute numbers of natural killer cells and monocytes and reduced the number of tumor cells in the bone marrow and spleen. This study has shown that (1) approximately 30 to 50% of tumor-bearing mice administered CVT-E002 at a dose of 40 mg/d achieved a significantly extended life span, and (2) dosage is critical in producing these ameliorative effects.

**Keywords**
cancer.ginseng.mice.natural killer cells.

It has been known for several decades that many of the cancer-causing viruses are ubiquitous and that only the appropriate stimuli are needed for the production of overt tumor.**1** Murine leukemia virus is found in many strains of mice and needs only to be chemically induced. In humans, Burkitt lymphoma cells harbor viruses (Epstein-Barr virus) of the herpes group of deoxyribonucleic acid (DNA) viruses, and a viral (adenovirus) etiology of Hodgkin's lymphoma has also been revealed., **1,2** Moreover, it is becoming increasingly evident that viruses are behind other cancers, such as gastric, cervical, and prostate cancer, hepatocellular carcinoma, Kaposi sarcoma, and, of course, the long-known human T-lymphotropic virus–induced T-cell lymphomas. Given that at least 100,000 segments of human DNA are identical to retroviruses, it is not surprising that, with the appropriate triggering stimulus, initially latent viruses become oncogenic and manifest as tumors.

Immunotherapy is one of several approaches that, for a decade during the 1980s, was touted as the final answer to cancer combat. What is clear, based on experimental and clinical evidence, is that it is indeed possible to mobilize the immune system toward effective combat and ultimately eliminate the offensive growth. However, current immunotherapeutic approaches have met with little success. For instance, antitumor vaccines have either been weakly or transiently effective because cancer cells and viruses are unstable and mutate frequently.

Appropriate modulation of the endogenous immune responses is emerging as a potential new approach in the battle against cancer. A number of alternative and complementary therapies involving a host of natural, immuno-stimulating products are also becoming popular as adjunct supplements. For example, products containing CpG DNA or synthetic CpG oligonucleotides have been found to stimulate the activity of natural killer (NK) cells and macrophages, the cells

involved in the first line of defense against cancer, and to induce rejection of various kinds of tumors in experimental animals.**3,4** Systemic administration of these oligonucleotides has also been found to increase the survival of leukemia-bearing mice., **5**

Occasionally, drugs or natural products that have been on the market for some time and demonstrated effectiveness for one ailment can prove to be an effective therapeutic for an entirely different condition. For instance, valproic acid has been used to stop epileptic seizures for years; however, it now turns out that valproic acid may have a fundamental role in the treatment of human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS). It is this type of "new thinking" that we applied to the present study. That is, we hypothesized that a ginseng derivative, CVT-E002 (CV Technologies Inc., Edmonton, AB), marketed as COLD-fX for use in the amelioration of virus-induced upper respiratory infections, may prove to be effective against virus-mediated cancers as well.

CVT-E002 is composed of polysaccharides isolated from the root portion of *Panax quinquefolius* (North American ginseng). The immunoenhancing properties of CVT-E002, the source material *Panax quinquefolius*, and the related species, *Panax ginseng*, have been demonstrated in vitro and are well established.**6–16** The present study has extended existing science on CVT-E002 in demonstrating the efficacy of this agent in vivo and, for the first time, in murine hosts bearing a tumor of viral origin. Our results have shown that daily dietary administration of CVT-E002 extends the life span of leukemic mice.

## Materials and Methods

### Animals

Eight to 9-week-old male DBA/2 strain mice (Charles River Laboratories, St. Constant, QC) were housed on arrival, one/cage, and maintained under pathogen-free conditions (microisolator cages) in a temperature- and humidity-regulated facility with a 12-hour day/night cycle at the Animal Care Facility of McGill University. The facility is under continuous veterinary surveillance and strictly adheres to the regulations of the Canadian Committee on Animal Care. Animals were provided with water and food ad libitum and remained undisturbed until 10 weeks old, the age of experiment initiation. Regular assessment of sentinel mice contained in the facility consistently demonstrated the absence of all common mouse pathogens.

### Tumor Cells: Maintenance and Administration

The tumor chosen as a murine example of a cancer of viral origin is Friend leukemia virus–induced erythroleukemia cells. This tumor is a well-established susceptible target for NK cell–mediated killing.**17–19** Moreover, this murine tumor shows many similarities with the human erythroleukemia (K562) cell line,, **20** which is also a known target for NK cell–mediated killing., **21–23** The tumor was purchased from American Type Culture Collection (Manassas, VA), and the cells were maintained in vitro by methods well established in our laboratory., **24–26** Each mouse was aseptically injected with $3 \times 10^6$ viable tumor cells in 0.1 mL of phosphate buffered saline at pH 7.2 via the lateral tail vein. Injecting the tumor cells provides a precise, and known, starting point from which to assess the effect of any agent, such as, CVT-E002. The tumor cells settle into their destiny organs (bone marrow, spleen) after one to two circuits through the blood—all tumor cells being in location within a few hours. The tumor is thus well "established" before the immune system becomes functionally capable of antitumor combat under the stimulation of CVT-E002.

### In Vivo Administration of CVT-E002

CVT-E002 consists of polysaccharides (poly-furanosyl-pyranosyl-saccharides) derived from the roots of North American ginseng (*Panax quinquefolius*). It is standardized using a patented dual-fingerprinting technique called ChemBioPrint. This technology ensures both chemical and pharmacologic consistency of a developed product. Chemical consistency of CVT-E002 is determined by measuring the relative proportions of various sugars, whereas pharmacologic consistency is verified through a lymphocyte proliferation assay.**6** Routine microbial screening for endotoxin and *Escherichia coli* is also performed on every batch of CVT-E002. The CVT-E002 used in the present study was proven negative for microbials. The agent was administered via the diet as a powder homogenized in finely ground standard Purina Laboratory mouse chow (Labchow, Agribrands, Woodstock, ON), the standard diet for all mice in the facility. All feedings, described below, began immediately after leukemia cell injection. All such leukemia-bearing mice were provided each morning (8:00–10:00 am) with freshly ground chow with or without (control) the ginseng extract. Other mice (normal, nonleukemic) consumed regular fresh but untreated chow. For all mice, the nutritional content was identical. Each leukemic, experimental mouse was provided with 2, 40, or 120 mg of CVT-E002 in 6 g of ground chow/day, whereas leukemic, control mice consumed

untreated ground chow only. The lowest of these doses (2 mg) was within a range already demonstrated**6** to produce an immune response in normal mice, after in vivo injection. We therefore surmised that the excessive burden placed on the immune system in vivo, by the developing tumor, would necessitate considerably larger doses—hence 40 and 120 mg were used in the present study. Extensive previous studies in our laboratory have revealed that male mice of this strain and age regularly consume 6 g of chow/day, virtually all of which is consumed during the dark phase of the 24-hour cycle. Moreover, we have consistently shown in studies in which nutritive agents were added via the daily chow that there was no difference in consumption of the daily total provided by leukemic mice with or without the additive. Second, animal use protocols strictly prohibit the survival of any mouse deemed to be clinically unhealthy—a condition that obviously would lead to differences in total daily food consumption, but a condition that was not an issue in the present study. All mice were active, sleek of control weight, and exhibiting normal food and water consumption. All food containers in all cages were equally emptied every 24 hours.

## Preparation of Free Cell Suspensions of Bone Marrow, Spleen, and Blood

Mice were killed by $CO_2$ asphyxiation at 10 days or 6 weeks after beginning CVT-E002 in the diet, as were corresponding control mice receiving untreated chow. Normal mice (above-mentioned) of the same strain, age, and gender were also assayed as described herein. Single-cell suspensions of the bone marrow and spleens were prepared by our standard laboratory methods, and monolayer cytospot preparations were subsequently prepared from those suspensions for both organs.**27–30**

Blood from every mouse (experimental, control, and normal) was transferred onto a Superfrost Plus microscope slide (Fisher Scientific, Ottawa, ON) from a nick (via a sterile needle) in the lateral tail vein while the mouse was alive and immediately prior to euthanizing for harvesting the bone marrow and spleen (see above). Blood smears were then stained with MacNeal's tetrachrome hematologic stain (Sigma Aldrich, Oakville, ON), which permits the ready identification of several morphologically distinct cell types and lineages.**27–30**

## Immunolabeling of NK Cells

The NK cell immunolabeling method (using the ASGM-1 surface marker of NK cell identity) is well established in our laboratory.**24–26,31** After immunolabeling, preparations of monolayer cytospots were prepared and subsequently stained using the tetrachrome staining method. ASGM-1 (asialogangliotetrasyliramide) is a surface molecule that is present on all mature and maturing NK cells,, **32,33** and although T-lymphocyte blast cells also may possess it, the latter are not only rare but are easily distinguishable from NK cells both morphologically, by size, and by the tetrachrome staining method.

## Differential Analysis of Hematopoietic and Immune Cells in the Bone Marrow and Spleen

For both bone marrow and spleen, mature granulocytes, granulocytic precursors (immature granuloid cells), nucleated erythroid cells, NK lymphocytes, non-NK lymphocytes (ie, T and B), and monocytes were identified using light microscopy at ×100 magnification.**24–31** The differential counts were obtained via this method from 1,000 spleen and blood cells/cytospot/mouse and 2,000 bone marrow cells/cytospot/mouse for every experimental (CVT-E002- containing chow), control (untreated chow), and normal mouse. For each organ and for each mouse, the percentages for each cell group (see above) were recorded. The absolute numbers of NK cells and their accessory cells, the monocytes, were then obtained by converting these percentage values per organ via the known total cellularity of that organ, recorded from the hemocytometer at the time of animal death.

## Assessment of CVT-E002-Mediated Survival

Groups of tumor-bearing mice, fed daily with 2, 40, or 120 mg of CVT-E002, as well as a group of control tumor-bearing mice consuming untreated chow, were left unmanipulated to assess the influence of CVT-E002 treatment on life span. Kaplan-Meier survival analysis software was applied to assess the significance of CVT-E002 versus no treatment on the life span of the cancer-afflicted mice.

## Statistical Analysis

The two-tailed Student *t*-test was used to compare the differences between the means of the experimental (CVT-E002 treated) and corresponding control groups. Values of $p < .05$ were considered statistically significant.

## Results

**Table 1** demonstrates the effect of 2 mg/d of CVT-E002 for 10 days on the central generating site of all hematopoietic and immune cells, that is, the bone marrow. Notable is the observation that NK cells had doubled their absolute numbers (borderline statistical significance) in the CVT-E002-consuming group of tumor-bearing mice versus tumor-bearing mice on the untreated diet. A more profound augmentation (statistically significant) in absolute numbers of NK cells was found in the spleens of these animals ($p < .006$), indicating that CVT-E002 alone was responsible for the numerical increment of these vital antitumor cells.

The necessary accessory cells for NK cells are the monocytes. Similar to the observations for NK cells, and in the presence of 2 mg/d of CVT-E002, the absolute numbers of monocytes were also found to be doubled (borderline statistical significance) in both the bone marrow and the spleens of the tumor-bearing animals (see **Table 1**).

The life span of cancer-afflicted mice ingesting 2 mg/d of CVT-E002 from tumor onset was found to be similar to control leukemic mice receiving identical chow without CVT-E002 (**fig1**). We have consistently observed, over the course of the past decade, that control mice afflicted with this particular tumor, left untreated, predictably die, as observed in **fig1**. However, when tumor-bearing mice were given 40 mg/d CVT-E002 from tumor onset, their survival was significantly improved for 30 to 50% of the mice (**fig2**). These mice, in all four groups, remained clinically healthy and active until sacrificed at 6 weeks (the latter having been done solely for the purpose of assessing their hematologic and immune cells). In fact, all CVT-E002-treated mice were indistinguishable from normal mice of the same strain, gender, and age. Four such groups of mice were assayed to confirm the effects of this dosage (40 mg/d of CVT-E002) on survival. Data collected from each of these experiments (see **fig2**) revealed that CVT-E002, at this dose, was consistently effective in enhancing survival. The survival data of all four groups, when averaged together, were also found to be significantly higher than those for the control group ($p < .0212$).

The hematopoietic and immune cell data recorded for tumor-bearing mice receiving 40 mg/d CVT-E002 revealed significant elevations in the proportions of NK cells and monocytes in the bone marrow (**fig3**) and of NK cells in the spleen (**fig4**) at 6 weeks from tumor onset versus healthy normal mice of matched strain, gender, and age. These proportions reflected absolute increases in the numbers of these cells in these organs (**Table 2**). Comparisons with the hematopoietic and immune cell lineage profile of normal mice were made. The proportions of all other hematopoietic and immune cells in the bone marrow and spleen were either at, or close to, normal levels for mice of the same age and gender (see **fig3** and **fig4**). All control tumor-bearing mice (ie, those not receiving dietary CVT-E002) had died considerably earlier (see **fig1**).

A pivotal observation in both the spleen and the bone marrow of the leukemic mice at 6 weeks after receiving 40 mg/d of CVT-E002 was the fact that the numbers of nucleated erythroid cells had returned to levels comparable to those of normal mice (see **fig3** and **fig4**). This suggests the presence of few, if any, remaining erythroleukemic cells indistinguishable from endogenous, nucleated erythroid cells. The barometer of erythroleukemia burden is the presence of excessively and progressively increasing numbers of erythroid blasts in the bone marrow, where they normally and consistently exist in low numbers; the spleen, where they exist again in even lower numbers; and in the blood, where they should not be present at all. At this dose (40 mg/d), at 6 weeks, the total splenic cellularity ($\times 10^6$) in CVT-E002-treated, leukemic mice and normal mice of the same age and gender was 118.05 ± 6.13 and 94.8 ± 3.85, respectively (not significantly different: $p < .10$), and the corresponding values for the bone marrow (total cellularity $\times 10^6$ in two femurs) was 26.66 ± 1.34 and 22.53 ± 0.55, respectively. Again, this was not significantly different at $p < .10$, indicating that the total cellularity of both key organs from leukemic mice given 40 mg/d of CVT-E002 is at or approaching normal levels. The splenic cellularity of untreated, leukemic mice just prior to their death at approximately 2½ weeks of tumor bearing is, by comparison, three to four times that of the values observed (see above) for CVT-E002-treated leukemic mice.

At the 6-week interval, the relative proportions of the various cell lineages in the blood of both CVT-E002-treated and normal mice were also recorded (**fig5**). The CVT-E002 treatment reduced the relative proportion of lymphocytes, which could have been a consequence of increased number of NK cells in the bone marrow and spleen of these mice. The CVT-E002 treatment was found to increase the proportions of immature and mature granulocytes in the blood of leukemic mice, which potentially reflects their higher numbers in their bone marrow–generating site, given that the blood circulation is the only route out of the bone marrow. The proportions of nucleated erythroid cells seen in CVT-E002-treated mice, although statistically elevated (see **fig5**), were found to be very low (0.57 ± 0.09%) and similar to the levels in normal mice (0.17 ± 0.07%). Thus, **fig3**, **fig4**, and **fig5** all reveal a return of the hematopoietic and immune cell values to normal levels, with the exception of the antitumor NK cells and monocytes, whose numbers remained elevated with sustained CVT-E002

administration.

When the daily dose of CVT-E002 was increased to 120 mg/d, an improvement in survival was observed in all groups tested (**fig6**); however, the survival enhancement was considerably less impressive than that seen with 40 mg/d (see **fig2**).

## Discussion

This study represents the first evidence, using a mouse model, of the potential benefits of CVT-E002 in the therapy of virus-induced cancers, employing its established value as an immunoenhancer. CVT-E002, an extract of *Panax quinquefolius*, has an immunostimulating capacity similar to that of *Panax ginseng* in that both induce the immune system–modulating cytokines interleukin (IL)-2 and IL-8.**6,13–16** CVT-E002 is the polysaccharide component of *Panax quinquefolius* and is the immunostimulant**6,14** in that herb, just as the polysaccharide component is the immunostimulating agent from *Panax ginseng*.**7–12** Moreover, macrophages appear to be instrumental in the immunostimulating processes of both the CVT-E002 (polysaccharide) fraction from *Panax quinquefolius* and the polysaccharides from *Panax ginseng*.**6,11,13** Collectively, the present data have shown, for the first time in vivo, that in hosts bearing a tumor of viral origin, CVT-E002 significantly stimulates predominantly those cells involved in the first line of defense against cancer, that is, NK cells and their accessories, the monocytes.

Although the percentages and absolute numbers of NK cells and the monocytes may seem low relative to cell frequencies in the other hematopoietic and immune categories, it must be borne in mind that in normal, healthy, adult mice and other mammals, including humans, NK cell levels are normally relatively low, that is, 1 to 10% of all cells present in the three NK cell–containing organs (bone marrow, spleen, and blood).**34–38** These low levels, nevertheless, are adequate to sustain appropriate immunosurveillance to mount antineoplastic defenses. In fact, NK cells are vigorously cytotoxic to tumor cells generally, and specifically, to the tumor used in the present study., **17–19** Although it is known that CVT-E002 enhances the number of NK cells in blood, the novel and additional observation provided by this study, that is, that the absolute numbers of NK cells are statistically elevated in the bone marrow, is of utmost importance because it necessarily implies that CVT-E002 has stimulated new NK cell proliferation. Decades ago, it was established that NK cells, once generated in the bone marrow, never recirculate back to that organ.

In **fig3**, the decrease in immature (precursor) granuloid cells in the bone marrow of CVT-E002-treated mice correlates with the increase in their progeny (mature granulocytes) in that organ and in the blood (see **fig5**) into which they are dispensed. The blood also contains a slightly elevated level of immature granuloid cells, which would also have been dispensed from the bone marrow. In the spleen (see **fig4**) of CVT-E002-treated mice, the approximate 15% decline in the lymphocytes is almost precisely complemented by an increase in the NK cells, which are themselves morphologic lymphocytes but identified distinct from other lymphocytes by virtue of their ASGM-I surface marker. The presence of CVT-E002 drives some of this mixture of cells, morphologically identified as lymphocytes, into the NK lineage, where they become readily identified by their ASGM-1 surface marker. The non-NK lymphocytes in the spleen include mature and maturing B-lineage cells and mature and maturing T-lineage cells.

With respect to the tumor cells themselves (identifiable as nucleated erythroid blasts), it may be more than coincidental that in the bone marrow, where NK cells were found to be significantly elevated in the presence of CVT-E002, nucleated erythroid blasts (containing normal endogenous and erythroleukemia cells) had returned to normal levels (assessed at 6 weeks of treatment with CVT-E002). The correspondingly reduced to normal levels of nucleated erythroid blasts in the blood of CVT-E002-consuming leukemic mice further support the probability that tumor (erythroblast) cell cytolysis was under way in the bone marrow of CVT-E002-fed leukemic mice, potentially owing to enhanced (vs control diet) numbers of NK cells in that organ. On intravenous injection of the erythroleukemia cells, the latter home to the bone marrow predominantly. By 6 weeks of treatment with CVT-E002, erythroblast proportions had returned to normal levels, indicating few or no remaining tumor cell erythroblasts by this time in the spleen. Furthermore, by 6 weeks of treatment with CVT-E002, the blood levels of nucleated erythroblasts had achieved almost normal levels.

The dose-dependent effects of CVT-E002 treatment on the life span of leukemic mice were also determined in the present study. Although 2 mg/d of CVT-E002 was associated with a doubling of NK cells and monocytes, this dose was found to be insufficient to impact survival. However, the survival of leukemic mice was significantly enhanced by 40 mg/d of CVT-E002, suggesting that adequate levels of the tumor-lytic NK cells had been achieved. Indeed, at this dosage (40 mg/d) of CVT-E002, NK cells were stimulated to levels that were four to five times higher in absolute number than in healthy, normal mice. This enhanced level likely contributed to a successful antitumor defense, which imbued their tumor-

afflicted hosts with extended life span. Previous data, employing CVT-E002,**6** have shown in vitro that CVT-E002 stimulates NK cells. Our study has now extended this observation by demonstrating that NK cells can be stimulated in vivo. The mechanism by which CVT-E002 leads to NK cell enhancement appears to be the following. Gastrointestinal tract enzymes degrade the polysaccharides of CVT-E002 after oral administration, the smallest degradation molecules being galactose, rhamnose, arabinose, glucose, and galacturonic acid. Macrophages have receptors for these monosaccharides., **39–42** Stimulated macrophages produce a cascade of cytokines, many of which are direct stimulants of NK cells.

In contrast to the life span extension observed in leukemic mice receiving 40 mg/d of CVT-E002, a threefold higher dose of 120 mg/d was considerably less effective in promoting survival. CVT-E002 has been shown to stimulate the production of several cytokines, such as tumor necrosis factor a, interferon-g, IL-1, IL-2, IL-8, and IL-18 from cultured macrophages and fresh spleen cells,**6,14** which, depending on the overall cytokine profile and physiologic context, can be either immunoenhancing or immunoinhibitory. Indeed, it is possible that at higher doses of CVT-E002, proinflammatory cytokines may be the predominant factors produced. Thus, the higher dose of CVT-E002 (120 mg/d) may have shifted the beneficial/inhibitory cytokine balance in a manner that was detrimental to survival.

The dosage of any drug is profoundly important in therapy, and clearly, in this study, the dosage of CVT-E002 appears to be important as well. We anticipate that the optimal therapeutic dose of CVT-E002 for leukemic mice may be one that is higher than 40 mg/d but lower than 120 mg/d.

In conclusion, this study has demonstrated for the first time, in vivo, in a laboratory mouse model, the preclinical efficacy of CVT-E002 in a pathologic scenario not hitherto considered, that is, cancer. This study has also shown that dosage is an important parameter when subsequently considering the clinical application of CVT-E002. Further preclinical studies will aim to extend these fundamental, in vivo observations by (1) assessing NK cells for CVT-E002-mediated enhancement of their cytotoxic, antitumor activity in vitro and in vivo; (2) establishing the precise, most effective dose of CVT-E002 for maximum survival; and (3) assessing the effect of CVT-E002 against tumors of nonviral origin.

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## Figures

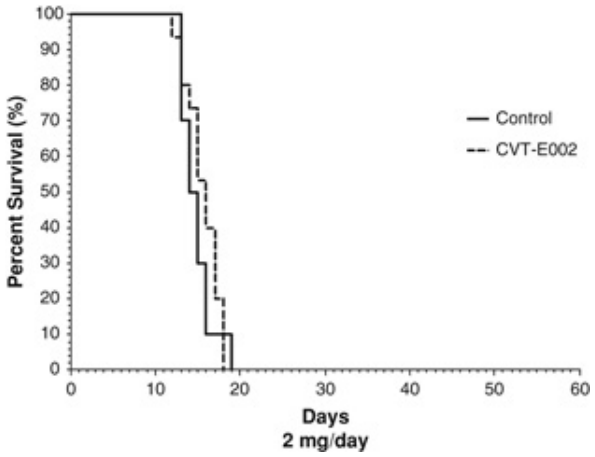


Figure 1

Survival of DBA/2 young, adult, male mice given 2 mg/d of CVT-E002 beginning immediately after intravenous injection of tumor cells at time 0. Control: 10 mice; CVT-E002 treated: 15 mice.

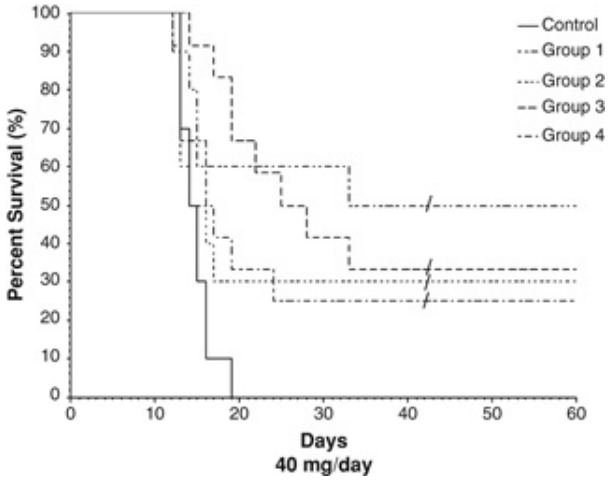


Figure 2

Survival of DBA/2 young, adult, male mice given 40 mg/d of CVT-E002 beginning immediately after intravenous injection of tumor cells at time 0. Control: 10 mice; CVT-E002 treated: four identical groups of 10 to 11 mice each.

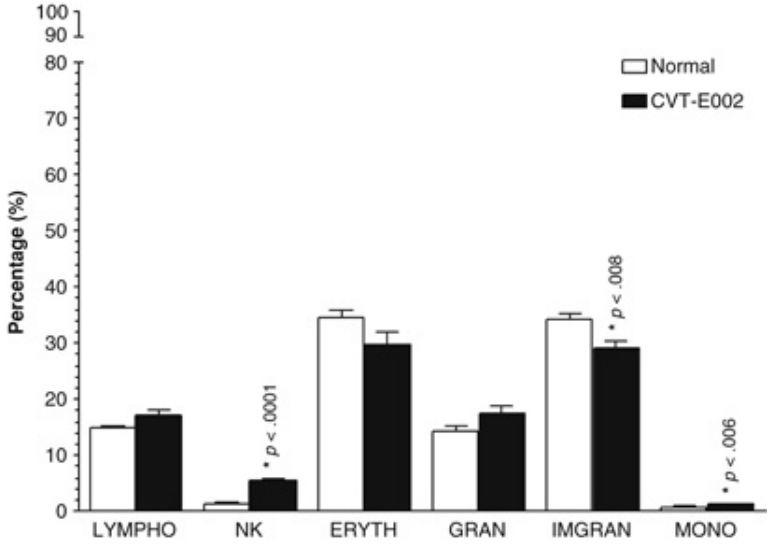


Figure 3

The effect of CVT-E002 on the relative levels of hematopoietic and immune cell populations in the bone marrow of leukemic mice. CVT-E002 (40 mg/d) was administered in the chow for 6 weeks beginning immediately after the onset of leukemia. ERYTH = red blood cell–proliferating precursors; GRAN = mature (functional) granulocytes; IMGRAN = immature granulocytes, that is, proliferating precursors; LYMPHO = lymphocytes, including T and B cells; MONO = monocytes; NK = natural killer cells. *N* = 10 samples (mice)/cell type (10 mice were taken from all four survival groups; see **fig2**); *statistically significant *p* values versus control assessed by means of the two-tailed Student *t*-test. Levels of significance are indicated on the histogram. Given that no control (leukemia without treatment) mice lived until 6 weeks, the experimental values are compared with those for normal mice of the same

strain, age, and gender.

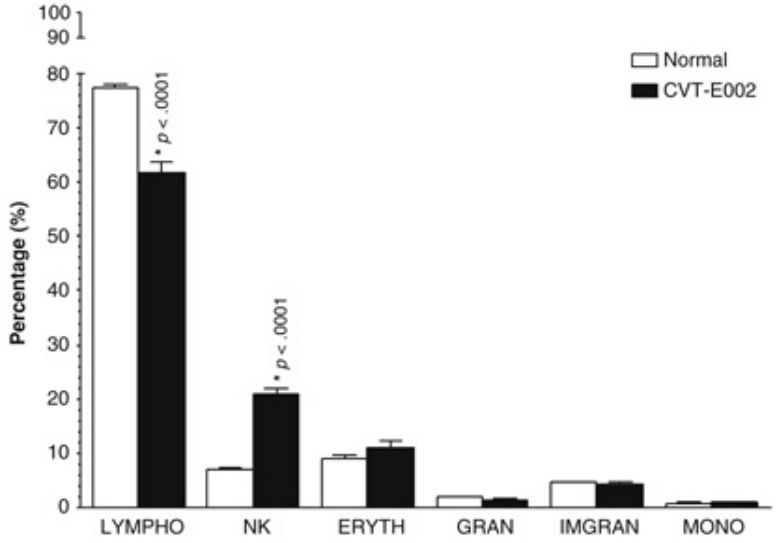


Figure 4

The effect of CVT-E002 on the relative levels of hematopoietic and immune cell populations in the spleen of leukemic mice. CVT-E002 (40 mg/d) was administered in the chow for 6 weeks beginning immediately after the onset of leukemia. ERYTH = red blood cell–proliferating precursors; GRAN = mature (functional) granulocytes; IMGRAN = immature granulocytes, that is, proliferating precursors; LYMPHO = lymphocytes including T and B cells; MONO = monocytes; NK = natural killer cells. *N* = 10 samples (mice)/cell type (10 mice were taken from all four survival groups; see **fig2**); *statistically significant *p* values versus control assessed by means of the two-tailed Student *t*-test. Levels of significance are indicated on the histogram. Given that no control (leukemia without treatment) mice lived until 6 weeks, the experimental values are compared with those for normal mice of the same strain, age, and gender.

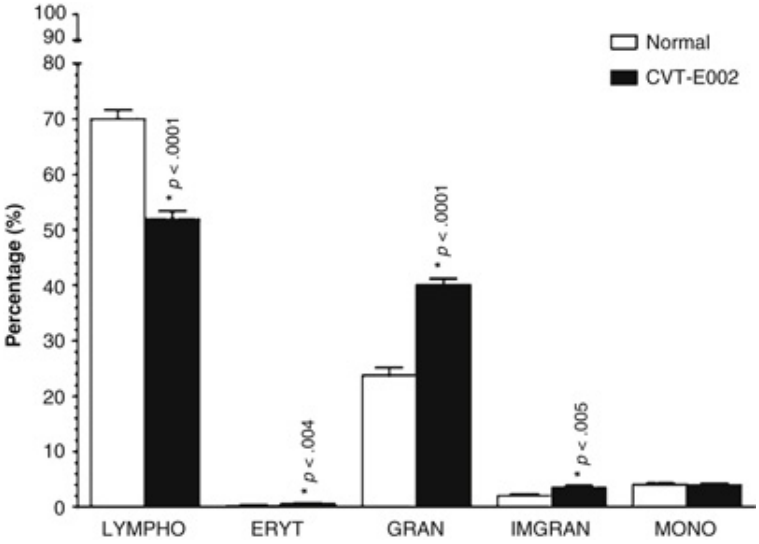


Figure 5

The effect of CVT-E002 on the relative levels of hematopoietic and immune cell populations in the blood of leukemic mice. CVT-E002 (40 mg/d) was administered in the chow for 6 weeks beginning immediately after the onset of leukemia. ERYTH = red blood cell–proliferating precursors; GRAN = mature (functional) granulocytes; IMGRAN = immature granulocytes, that is, proliferating precursors; LYMPHO = lymphocytes (NK, T, and B cells); MONO = monocytes. *N* = 10 samples (mice)/cell type (10 mice were taken from all four survival groups; see **fig2**); *statistically significant *p* values versus control assessed by means of the two-tailed Student *t*-test. Levels of significance are indicated on the histogram. Given that no control (leukemia without treatment) mice lived until 6 weeks, experimental values are compared with those for normal mice of the same strain, age, and gender.

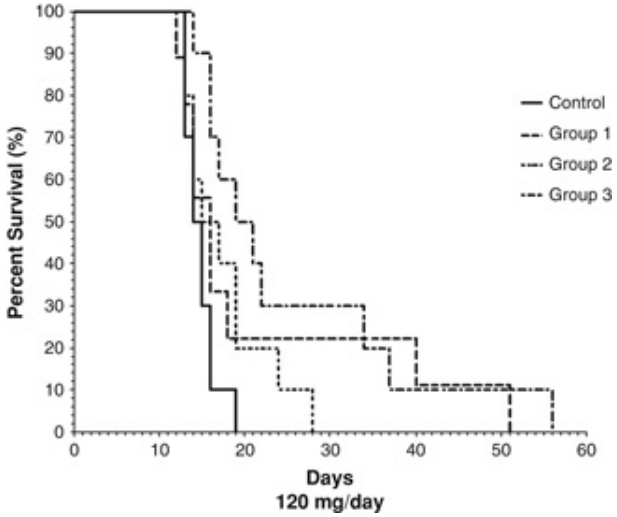


Figure 6

Survival of DBA/2 young, adult, male mice given 120 mg/d of CVT-E002 beginning immediately after intravenous injection of tumor cells at time 0. Control: 10 mice; CVT-E002 treated: three identical groups of 10 to 11 mice each.

## Tables

Table 1

Absolute Numbers ($\times 10^6$) of Natural Killer Cells and Monocytes in the Bone Marrow and Spleen 10 Days Postleukemia Injection of Mice Treated with Daily Dietary CVT-E002 at 2 mg/d

| | NK Cells | | Monocytes | |
|---|---|---|---|---|
| | **Control Mean ± SEM** | **CVT-E002 Mean ± SEM** | **Control Mean ± SEM** | **CVT-E002 Mean ± SEM** |
| Bone marrow | 0.11 ± 0.02 | 0.20 ± 0.06 | 0.20 ± 0.04 | 0.42 ± 0.09 |
| | *n* = 8 | *n* = 8 | *n* = 8 | *n* = 8 |
| Spleen | 11.46 ± 1.49 | 21.79 ± 2.83 | 2.82 ± 1.04 | 5.38 ± 1.05 |
| | *n* = 9 | *n* = 8 | *n* = 8 | *n* = 8 |

NK = natural killer.

Table 2

Absolute Numbers ($\times 10^6$) of Natural Killer Cells and Monocytes in the Bone Marrow and Spleen of Normal Untreated Mice and Leukemic Mice Treated for 6 Weeks with Dietary CVT-E002 at 40 mg/d

| | NK Cells | | Monocytes | |
|---|---|---|---|---|
| | **Control Mean ± SEM** | **CVT-E002 Mean ± SEM** | **Control Mean ± SEM** | **CVT-E002 Mean ± SEM** |
| Bone marrow | 0.32 ± 0.13 | 1.46 ± 0.07† | 0.19 ± 0.02 | 0.36 ± 0.04‡ |
| | *n* = 10* | *n* = 10 | *n* = 10 | *n* = 10 |
| Spleen | 6.44 ± 0.61 | 24.79 ± 1.82§ | 0.70 ± 0.96 | 1.23 ± 0.20‖ |
| | *n* = 10 | *n* = 10 | *n* = 10 | *n* = 10 |

NK = natural killer.

*Sampled from the survivors of all four groups; see **fig2**.

†$p < .0001$.

‡$p < .005$.

§$p < .0001$.

‖$p < .04$.

Article Categories:

- Article

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**Article**

# Systematic Evaluation of the Clinical Effects of Supportive Mistletoe Treatment within Chemo- and/or Radiotherapy Protocols and Long-Term Mistletoe Application in Nonmetastatic Colorectal Carcinoma: Multicenter, Controlled,