Viscum album (L.) in experimental animal tumors: A meta-analysis

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Abstract. Mistletoe (Viscum album L.) has been used as complementary anticancer treatment for ~100 years. Although the clinical efficacy of mistletoe in cancer and associated survival benefits remain contested, several studies point to its effectiveness and others have reported antitumor and immunomodulatory properties. In the present review, a search was conducted for original articles reporting the outcomes of treatments for experimental animal tumors with mistletoe. The inclusion criteria were: Publication in English, from 1996 onwards and in peer-reviewed journals included in the database PubMed. The parameters analyzed were: Provenance and time of publication, rationale, methods (animal species used, mistletoe preparation, treatment protocol, tumor lineage, blinding, randomization, controls and concomitant treatments), outcomes and investigated mechanisms of action. A total of 37 studies met the inclusion criteria. The quality of the studies was adequate in the terms of sample size and use of controls, and the only animal species employed were mice and rats. However, few studies reported having performed random allocation and none reported blinding. There was wide variation in the type and preparation of mistletoe used, route of administration, regimen, tumor type and the mechanism of action assessed. A temporal trend was identified; earlier studies sought to establish the antitumor effect of mistletoe and its possible mechanisms, cytotoxicity and immunomodulation in particular, whereas the later ones tended to focus more on biologically active principles, genomics and oxidative stress. A total of 32/37 studies reported an antitumor effect, 3 of which had mixed results. A total of 2 studies did not detect any antitumor effect and a further 2 found stimulation of tumor growth in the treated groups. One study did not assess antitumor effects, investigating immunomodulation action instead. The quality of the studies was satisfactory and the majority reported positive outcomes. Nevertheless, there is a great deal of methodological heterogeneity among the studies, which precludes conclusive comparisons. Based on these results, the present authors strongly suggest developing guidelines for reporting in vivo mistletoe cancer treatment experiments.

Introduction

Mistletoe (Viscum album L.) has been used for the treatment of various illnesses for centuries (1); however, modern use of mistletoe in cancer treatment was introduced in the 1920s by Rudolf Steiner, the founder of anthroposophy (2). As Kröz et al (3) reported, in 2017 mistletoe will have been used for the treatment of cancer for almost a century, and the significance of this is corroborated by >1,200 citations in the PubMed database, which correspond to a multitude of studies demonstrating the immunomodulatory, cytotoxic and proapoptotic, anti-angiogenesis and DNA stabilizing properties of mistletoe. There have also been a number of studies and reviews concerning quality of life and patient perspectives (4,5), evidence-based benefits in human patients (6,7), ongoing clinical studies in humans (8,9) and adverse effects (10).

The mechanism of mistletoe's anticancer action has been of continued interest since its cytotoxic and immunomodulatory qualities were documented in 1990 (11). In the following decade, several studies were concerned with identifying the most effective therapeutic dosage, time and regimen of treatment (12-14). Recently, it has been suggested that mistletoe exerts a potent anti-inflammatory effect via selective inhibition of Cox protein expression, which may contribute to the antitumor action (15,16).

Over time, several biologically active components have been identified, most notably viscosotoxins and lectins (ML-I, ML-II, ML-III) (17-21); eventually a recombinant lectin (viscumin, rML) was developed and tested (22-24). Research has also been conducted into lipophilic components, namely triterpenes, which also exhibit marked cytotoxic effects (25-27).

Mistletoe is a semi-parasitic plant that is able to grow on several host trees; the types most frequently used for therapeutic purposes are the ones grown on fir (A-Abies), apple (M-Malus), pine (P-Pinus), poplar (Po-Populus) and oak (Qu-Quercus) trees (2). Asian subspecies of V. album (Korean and Chinese mistletoe, Viscum album subsp. coloratum Kom.) (28) have also been studied and found to have properties similar to European mistletoe. The concentration of active components and biological activity vary as

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a function of the host tree, harvesting time and extraction procedure (aqueous extraction with/without fermentation, other extraction procedures, pressing) (29), which also remain a notable target of research (30).

The clinical efficacy of and survival benefit associated with the use of mistletoe remains contested (31). As is known, the intrinsic complexity of cancer and the composition of mistletoe, pose a problem for clinical trials (2-4). In vivo tumor models may therefore be particularly elucidative, as features of the tumor microenvironment may be observed and compared with the diverse clinical outcomes. The authors of the present study were able to locate a single review on this topic; however, it was limited to breast and gynecological tumors (29). Another source of relevant information is an NIH/National Cancer Institute overview of the use of mistletoe in anticancer treatment, in which the results of studies involving animal models are discussed (32). As there are no recent reviews of mistletoe treatment in animal models, the current study presents a meta-analysis of the peer-reviewed, published literature on the effect of mistletoe in animal cancer models (in vivo models).

Materials and methods

Literature search. A literature search was performed to locate original research articles reporting the outcomes of mistletoe treatments for experimental tumors in animals. The search was limited to articles published from 1996 onwards, in English, in peer-reviewed journals included in the database PubMed (ncbi.nlm.nih.gov/pubmed/) and with relevant references cited. The aim of this was to facilitate access to the raw data for the interested readerhip. These criteria were adopted as a considerable number of studies are published in German and/or as books or meeting proceedings, and the peer-reviewed criterion ensures a priori the quality of articles. Several searches were performed using the following search terms: ‘(Viscum OR mistletoe) AND cancer’; ‘(Viscum OR mistletoe) AND tumor’; and ‘Viscum album AND tumor AND animal’. Only articles reporting in vivo animal models were considered for inclusion.

Parameters of analysis. The parameters of analysis were as follows: i) Provenance and time of publication; ii) rationale underlying the study; iii) methods used [studied animal species, number analyzed, type of mistletoe/mistletoe-derived preparations used, treatment protocol, grafted tumor lineage (epithelial, mesenchymal, melanoma), blinding, random group allocation, controls (placebo, reference drug) and other combined therapies] iv) results (remission, improvement, worsening and survival) and v) mechanisms of action investigated (antiproliferative activity, cytotoxicity, immunomodulatory, tumor leukocyte infiltration, expression of specific phenotypic markers, and other tumor microenvironment aspects likely to correlate with the reported outcomes).

Data presentation. The data were entered in an ad hoc form and are presented in two tables. Table I lists the articles reporting in vivo studies with detailed descriptions of the substance used, dose and regimen, animal model tested, tumor cell lines, concomitant treatments and outcomes. Table II describes methodological aspects used to determine the quality of studies.

Results and discussion

Initial findings. The literature search located 911 articles. Duplicates were excluded and the remaining studies were analyzed based on their titles and abstracts until 37 remained that met the inclusion criteria and were considered for the present analysis. These 37 studies were written in English, published in peer-reviewed journals from 1996 onward and included in PubMed (mean, 1.85 studies/year; range, 0-7). As mentioned above, only 1 similar review was located for comparison to the present study (29), which assessed 34 studies from 1938 to 2008 (mean, 0.48 studies/year) of which 21 had been published in peer-reviewed journals. The apparently favorable trend detected in the present review should be considered cautiously, as Kienle et al (29) considered breast and gynecological tumors only. However, according to those same authors ~50 articles/year are published on mistletoe, implying that the proportion of studies using in vivo animal models is rather low. Tables I and II describe the results corresponding to the analyzed parameters.

Mistletoe origin. Viscum album (L.) is a parasitic plant whose composition may be influenced by the host plant or the harvest season (2). As a result of this, the commercially available preparations are typically made from mixtures of fresh leafy shoots harvested in both summer and winter, and fruits harvested in winter, when the concentration of the main active principles (lectins and viscotoxins) is highest (28). Only two studies included in the present review reported the harvest season, and this was reported as winter in both (27,33). A total of 32 studies used European mistletoe and 5 studies used Korean/Chinese mistletoe. The host species on which mistletoe was grown were reported as follows: 13 studies on apple, 4 on oak, 4 on pine, 4 on fir, 2 on poplar and 1 on plum (Prunus) trees. A total of 12 studies did not report the host species.

Distribution per date of publication. A total of 25 studies were published between 1996 and 2005 (mean, 2.5 studies/year) and 12 from 2006 to 2016 (mean, 1.2 studies/year). This is a ~50% decrease in the period 2006-2016 compared with the number of studies published in the previous decade. One hypothesis that could explain such a decrease in the number of studies is the trend towards reducing animal protocols in cancer research (34) due to their poor translational features, but this remains to be confirmed.

Country. The majority of studies were conducted in Germany (20 in their entirety, >37 including multicenter studies), followed by Croatia, India and Korea (4 in each), Austria, Belarus, Brazil, Canada, China, France, Norway, Romania, Serbia and the UK (1 in each). Compared with the review by Kienle et al (29), in which studies originated in Germany, Switzerland, Austria, USA, India, Croatia and Serbia only, the distribution reported here represents a favorable trend, as it seems to denote a more global interest in the use of mistletoe.

Animal species. Mice and rats were used in 32 and 5 studies, respectively. This finding is similar to the one reported by Kienle et al (mice, 28; rats, 6) (29); however, as shown herein,
Table I. Study design and outcomes (n=37) according to treatment, animal model, tumor cell line and main outcomes.

| Author/year | Treatments | Animal model | Tumor cell lines | Outcomes | (Refs.) |
|-------------|------------|--------------|------------------|----------|---------|
| Han et al, 2015 | Endragit: Enteric-coated lectin from KM grown on poplar. Toxicity test: 500, 1,000, 2,000 mg/kg, single dose, p.o. Antitumor effect: Added to feed at 1, 2 or 4% concentration. | Toxicity test: ICR mice, both sexes; Antitumor activity: Male GDFI mice | Murine B16.F10 melanoma | POSITIVE | (53) |
| Delebinski et al, 2015 | VA-M; TT (OA 4.0 mg/ml + BA 0.38 mg/ml); ML-1 (3.7 µg/ml); TT/ML-1 (qualitatively positive); 6 groups: (1) dose escalation 20/40/60 mg/kg TT; (2) 0.3/0.4/0.5 µg/kg ML-1; (3) TT/ML-1 combination; VAE given twice every 3rd day, i.v., from day 3 after tumor inoculation; (4) ARA-C 100 mg, i.v., every 3rd day, 4 doses; (5) TT/ML-1 + ARA-C; and (6) control, received equal amounts of cyclodextrins. | Female NOD/SCID/IL-2rg mice in pathogen-free conditions | Human HL-60 cells | POSITIVE | (25) |
| Facina et al, 2014 | VA-Qu AE (Iscador Qu; 5 mg with 0.375 ng/ml of lectins), 0.1 ml, s.c. peritumoral, daily for 10 days | Female C57BL/6N mice | B16F10 melanoma | MIXED | (44) |
| Stan et al, 2013 | VA-M, ethanolic extract, 50 mg/kg, i.p., days 1, 3, 6. DOXO 2.5 mg/kg, i.p., days 1 and 6; 7 groups (VA-M yes/no, DOXO, yes/no, EAC yes/no) | Swiss female mice | EAC | POSITIVE | (58) |
| Strüh et al, 2013 | VA-M AE; STT; CD as control (2.2 g/kg). Treatments and controls injected s.c. peritumorally, every 2nd day starting at day 3 after tumor | Male C57BL/6NrL mice | Murine B6F10 melanoma cells | POSITIVE | (26) |
Table I. Continued.

| Author/year | Treatments | Animal model | Tumor cell lines | Outcomes | (Refs.) |
|-------------|------------|--------------|------------------|----------|---------|
| Podlech *et al*, 2012 | Iscador Qu; (1) 100 µg/ML, culture medium then 2 µg, intratumor, (2) 1 µg, s.c., periodically (NR), (3) 20 µl (100 µg/ML), intratumor, single dose. | Athymic CD1-deficient NMRI nude mice; VMDk mice | LNT-229; SMA560 glioma cells | Pretreatment reduced tumor growth, however this was not due to altered proliferation or cell death. Systemic treatment was associated with slight, non-significant inhibition of tumor growth, whereas intratumor administration had significant effect. | (52) |
| Li *et al*, 2011 | CM-1 of Chinese mistletoe grown on poplar. Treated groups: 10 µg·kg⁻¹ CM-1 every 3rd day or 20 µg·kg⁻¹ every 10 days, i.v. | Female athymic nude (Balb/c nu nu) mice | CRC CLY and HT-29 cells | CM-1-treated groups exhibited a dose-dependent reduction in tumor growth and high tumor growth inhibition rate. Downregulation of specific miRNA induced by CM-1 was not due to suppression of host gene transcription, but to direct degradation of their precursors. There was direct correlation between miR-135a&b levels and cellular sensitivity to CM-1. | (69) |
| Thies *et al*, 2008 | ML-I from leaves of mistletoe grown on poplar (40%), apple (30%) and red oak (30%) trees collected in the winter. ML stock solution with 963 µg/ML ML-I, ~1% ML-II and <0.5% ML-II. Treatment groups: 30, 150 and 500 ng/kg in 200 µl PBS, i.p., 19 days. | Pathogen-free male Balb/c SCID/SCID mice | Human melanoma MV3 | Primary tumor: Significant reduction of tumor growth with low dose, significant increase of tumor weight with intermediate vs. low dose. The apoptosis rate was significantly higher in all 3 treatment groups, significantly higher with low vs. intermediate and high doses. There was an increased number of infiltrating DC in all treatment groups, significant | (33) |
Table I. Continued.

| Author/year | Treatments | Animal model | Tumor cell lines | Outcomes (Refs.) |
|-------------|------------|--------------|------------------|-----------------|
| Seifert et al, 2008 | VA-A (Helixor), 160 ng/ml ML-II/III and 5-10% ML-I; VA-P, 725 ng/ml ML-II/III; treated groups: 1, 50, 100 mg/kg, i.p., 4 d/wk., 3 wks., 1 day after tumor inoculation. Controls: CPA 100 mg/kg on day 3. | Male CB-17 SCID/SCID mice; specific-pathogen free | Human ALL NALM-6 cells | increase of DC apoptosis with intermediate and high doses vs. low dose; significant increase in the number of infiltrating DCs with low dose vs. controls. Lung metastases: Significant reduction in number with low dose vs. control. Mean number increased with intermediate and high dose vs. low dose. Vascular count increase with high dose vs. controls. POSITIVE (59) |
| Cebovic et al, 2008 | CO₂ extract of leaves of mistletoe grown on plum collected in winter; 2.0 ml/kg/d, i.p., duration NR, several doses tested but NR | Male and female Hann: NMRI mice | EAC | Volatile extract includes TT. Reduction of tumor cell number and viability; reduction of antioxidative enzyme activity in tumor bearing animals. POSITIVE (27) |
| Beuth et al, 2006 | VA-A (Helixor) 3.6 ng/ml ML-I, 354 ng/ml ML-III; VA-M, 230 ng/ml ML-I, 500 ng/ml ML-III. Treated groups: 5 mg/µl/injection, i.p., of VA-A or VA-M on days 5, 6, 7, 12, 13 and 14 | Male Balb/c mice | BT474 human ductal breast carcinoma | Both extracts induced a significant reduction in tumor weight; reduction of tumor cell proliferation rate; increased cell necrosis and apoptosis rates. POSITIVE (68) |
| Duong Van Huyen et al, 2006 | Iscador Qu FrF, 20 µg/dose, once/day, i.p., 7 days starting from the day of tumor inoculation | C57BL/6 and B16.129-II2b (IL-12 deficient) mice | B16F1 melanoma | The chosen dose was 20 µg, which was an effective dose that did not promote lethality. Reduced tumor weight and restoration of proliferative capacity of splenocytes in tumor-bearing animals, associated with increased secretion of IL-12. POSITIVE (67) |
| Pryme et al, 2004 | Pure ML-I 10 mg/day as dietary supplement in different concentrations | Female NMRI mice | Krebs II NHL cells adapted to grow | Reduction in the number of mitotic figures; POSITIVE (70) |
Table I. Continued.

| Author/year | Treatments | Animal model | Tumor cell lines | Outcomes | (Refs.) |
|-------------|------------|--------------|------------------|----------|---------|
| Yoon et al, 2003 | Aqueous extract (KM-110) and lectin (KML-C) of KM, KM-110 100 µg/animal; KML-C 0.05, 0.02, 0.005 µg/animal, i.v.; lung metastasis: 1 and 2 days before tumor inoculation; liver and spleen metastasis 1 day after tumor inoculation | Experimental lung metastasis: Balb/c; C57BL/6 mice | Experimental lung metastasis: colon26-M3.1 carcinoma; B16.BL6 melanoma, 2.5x10⁴ Experimental liver and spleen metastasis: f CDF1 mice Spontaneous lung metastasis: C57BL/6 mice | intra-tumor lymphoid infiltrate doubled, with a corresponding increase in tumor necrosis; accelerated cell turnover inside tumor, and increased number of apoptotic cells. 11% of treated animals no longer had histological evidence of viable tumors on day 11. **POSITIVE** | (66) |
| Braun et al, 2002 | sME Iscador Spezial, 2, 20, 100 µg/animal, s.c., 3 times per wk., 24 h after tumor inoculation | Inbred male Balb/c mice RAW-117 P (liver); L-1 (lung) lymphosarcoma | | | (49) |
| Elsässer-Beile et al, 2001 | rML 300 or 1,500 ng/ml in solution (EDTA, NaCl, PBS, PVP); 0.1 ml per intravesical catheter. Control group: NMU on wks. 0.2,4,6 Treated groups: 30 or 150 ng on weeks 8 to 13 or 14 to 19 | Inbred female Fischer 344 rats | NMU-induced urinary bladder carcinoma | **POSITIVE** | (22) |
| Author/Year       | Treatments                                                                 | Animal Model          | Tumor Cell Lines          | Outcomes                                                                 | Refs. |
|------------------|----------------------------------------------------------------------------|-----------------------|----------------------------|--------------------------------------------------------------------------|-------|
| Braun et al., 2001 | Helixor ME-A 160 ng/ml, Helixor ME-P® 725 ng/ml of ML-II/III (both >95%), 5 and 50 µg/mouse, s.c., i.p., 3 times/wk, 24 h after tumor inoculation | Inbred male Balb/c mice | RAW-117 P (liver); L-1 (lung) lymphosarcoma | POSITIVE; Dose-dependent reduction of liver and lung colonies; i.p. significantly greater effect. Dose-dependent increase of leukocyte, lymphocyte and monocyte count. | (48)  |
| Schaffrath et al, 2001 | rML, 0.3, 3, 30, 150 ng/kg, s.c., every 2nd day, starting 2 days after tumor inoculation | Inbred male Balb/c mice | RAW-117 P (liver); L-1 (lung) lymphosarcoma | POSITIVE; Increased survival rate; reduced number of liver and lung tumor colonies. Increased number of leukocytes in RAW-117 P | (23)  |
| Burger et al., 2001 | Lektinol, 405 µg/ml of ML, from poplar, 0.3, 3, 30, 300 ng/kg/d, i.p. or s.c., once per day, 5 days/wk. for up to 4 wk.; positive controls: DOXO, 8 mg/kg, i.v., on days 0 and 14; 5-FU, 100 mg/kg, i.p., on days 0, 7, 14 | C57BL/6, Balb/c mice, both genders | B16.F10 melanoma, C8 38 colon carcinoma, F9 testicular carcinoma, Lewis lung carcinoma or Renca renal cell carcinoma. | MIXED; Dose-dependent tumor growth inhibition (Renca, colon, testicle) borderline effect; greatest inhibitory effect in Renca model with dose 300 ng, however, the sensitivity of the 3 tumor types to VA was similar. In Renca model VA as effective but less toxic than DOXO; colon carcinoma responded better to 5-FU (comparable toxicity). Lewis carcinoma and melanoma did not respond to VA. | (45)  |
| Timoshenko et al, 2001 | ML-I 1 ng/kg dissolved in PBS and 50 µg/ml mouse albumin to concentration 0.22 ng/ml, 100 µl injection, s.c., days 7, 10, 13, 16, 19 after tumor inoculation; IL-2, i.p., 10⁵ Cetus Units/ml, 100 µl injection, every 8 h, 5 days. Experiment 1: 2 rounds with 4-day interval. Experiment 2: 1 round. 5 groups: (1) healthy mice, (2) tumor inoculated (3) IL-2 alone (4) ML-I alone and (5) IL-2 + ML-I | Female C3He/Hej mice | C3L5 murine mammary adenocarcinoma, 1 x 10⁶ | NEGATIVE; ML-I alone increased primary tumor growth and development and growth of metastases. In combination, ML-I did not modify the tumor growth suppressive action of IL-2. ML-I did not cause capillary leakage neither inhibited the one caused by IL-2 and associated nitric oxide changes. | (47)  |
| Park et al, 2001 | KML, 10, 30, 50 ng/animal; (1) Tumor growth: 10-50 ng/mouse, i.p., 3 times per wk. (2) Antimetastatic effect: 10-50 ng/mouse 2 days before and 1 day after tumor inoculation | Female C57BL/6 mice | Melanoma B16.BL6 | MIXED; No effect on tumor growth; increased survival. Dose-dependent inhibition of tumor cell proliferation via apoptosis, dose-dependent anti-angiogenesis effect. No effect on cell cycle. | (46)  |
Table I. Continued.

| Author/year | Treatments | Animal model | Tumor cell lines | Outcomes | (Refs.) |
|-------------|------------|--------------|------------------|----------|---------|
| Zarkovic *et al.*, 2001 | Isorel, 50 µl, every 2nd day, 3 consecutive wks., s.c. or s.c. contralateral to tumor (local vs. systemic) | Female CBA/HZgr mice | Murine mammary carcinoma | POSITIVE | (65) |
| Mengs *et al.*, 2000 | AE, 30 or 300 ng/ml ML, i.v., 3 days/wk., 4 consecutive wks., total volume 0.1 ml | Female C57BL/6J mice. | Urinary bladder carcinoma MB49 | POSITIVE | (54) |
| Schumacher *et al.*, 2000 | rML, 20, 150, 500 ng/kg, Monday to Friday until day 83 or 120% of initial body weight | SCID/SCID mice | SoTü 3 human ovarian cystoadenoma | POSITIVE | (24) |
| Kunze *et al.*, 2000 | ML-1, 1 ng/kg, twice/week, s.c., 6 or 15 months | Pathogen-free female Wistar rats | BBN-induced bladder carcinoma | NEGATIVE | (40) |
| Antony *et al.*, 1999 | Iscador M: (1) For spleen cell activation, 1.66 mg/dose/animal, i.p., 5 days. (2) For treatment: 1.66 mg/dose, i.p., daily, 10 days | C57BL/6 mice | B16.F10 melanoma | POSITIVE | (64) |
| Lenartz *et al.*, 1998 | ML-1. (1) Systemic: 1, 10 ng/kg, s.c., 2 wk. (2) Local: 10, 100 ng, single dose, i.p. | Adult female Fischer 344 rats | F98 glioma | POSITIVE | (50) |
| Yoon *et al.*, 1998 | KM AE grown on oak. Survival: 100 µg single dose i.v. Metastasis inhibition: 100 µg i.v., 2 and 4 days | Specific-pathogen free female C57BL/6, Balb/c, CDF1 mice | B16.BL6 melanoma; 26-M3.1 colon carcinoma; spleen | POSITIVE | (51) |
| Author/year | Treatments | Animal model | Tumor cell lines | Outcomes (Refs.) |
|-------------|------------|--------------|-----------------|-----------------|
| Weber et al, 1998 | Lektinol, 30 ng/ml of bioactive MLs; 4 groups: (1) Vehicle, (2-4) 3, 30, 150 ng/kg ML, respectively, i.v., 1 hour after tumor injection then 4 days/wk., 3 wk. | Male Balb/c mice | B16.F10 melanoma | POSITIVE: Dose-dependent inhibition of metastasis; reduction of melanoma cell count in lungs. In vitro: stimulation of macrophages, CD4⁺CD8 lymphocytes; significant enhancement of anti-CD8, no effect on anti-CD4. (55) |
| Zarkovic et al, 1998 | Isorel M; pretreatment of tumor cells with whole extract, ML-1 and 2 fractions-high and low molecular weight, 0.1 µg/ml | Male C57BL; GoZgr mice | Melanoma | POSITIVE: All tested preparations inhibited metastasis formation. (21) |
| Kunze et al, 1998 | Lectin, 1 ng/kg, s.c., twice per wk., 15 months | Female Wistar rats; specific pathogen-free | MNU-induced bladder carcinoma | NO EFFECT: The incidence of bladder carcinoma did not differ between treated and control groups. There was no difference in the population of inflammatory cells infiltrating the tumor. (41) |
| Zarkovic et al, 1997 | Isorel M, 100 mg/kg, single dose, i.p. | Male C57BLGoZgr mice | B16F10 melanoma | POSITIVE: Tumor growth reduced in treated animal, with a reduction of tumor viable cells. Tumor necrosis and abundant inflammatory response. In vitro tests: Strong stimulation of lymphocytes, with increased cytotoxic activity; increased sensitivity of tumor cells to cytotoxic activity of lymphocytes; inhibition of growth of tumor cells more than normal cells. (63) |
| Jurin et al, 1997 | Isorel M; strength 60, 2 protocols: (1) Single dose, 1 day before, simultaneous, 1 day after sheep RBC injection; 140 or 1,400 mg/kg/animal. (2) Repeated doses: a) normal mice and tumor-bearing | Male and female CBA/Hzgr mice | Murine methylcholanthrene-induced fibrosarcoma | IMMUNOMODULATING EFFECT: 1) Isorel single dose before sheep RBC injection restored the immune response; 140 mg/kg had greatest effect. 2) Dose-dependent immunostimulating effect; prolonged application (5 wk.) had dose-dependent (62) |
## Table I. Continued.

| Author/year | Treatments | Animal model | Tumor cell lines | Outcomes (Refs.) |
|-------------|------------|--------------|-----------------|------------------|
| Antony et al, 1997 | Iscador M, prophylactic, 5 doses, 1.66 mg/kg/dose, 4 groups: Control, before tumor inoculation, with tumor then 10 consecutive days, 5 days after tumor, 10 consecutive days | C57BL/6 mice | B16.F10 melanoma | POSITIVE Reduction of lung metastasis formation; increased survival rate of metastasis-bearing animals; lifespan increased in all treated groups. Markers: reduction of hydroxyproline (fibrosis), sialic acid (melanoma development). (61) |
| Kunze et al, 1997 | Purified lectin, 50 µg/ml, 1 ng/kg, s.c., twice per wk., 7 h after tumor induction and then for 6 months | Pathogen-free adult, female Wistar rats | NMU-induced urinary bladder carcinoma | NO EFFECT No significant differences in tumor rates, characteristics or histology. (42) |
| Kuttan et al, 1997 | 5% Iscador M, 1.66 mg/dose, route NR; 2 experiments: Carcinogenesis, twice/wk., 15 wks. Metastasis inhibition: 5 days before, simultaneous with and after tumor inoculation, 10 days in each group | Female Swiss albino and C57BL/6 mice | Methylcholanthrene-induced sarcoma; B16.F10 melanoma for metastasis induction | POSITIVE Reduction of tumor size and development; reduction of metastasis formation; increased survival. Markers: Reduction of hydroxyproline and sialic acid. (60) |
| Kuttan et al, 1996 | Iscador; (1) 1 mg/dose, i.p., twice/wk., 15 wks. (2) dilutions 0 to 1:3,000 (1.66 mgІ.66 µg), i.p., twice/wk., 15 wks. | Female Swiss albino mice | Methylcholanthrene-induced sarcoma | POSITIVE Iscador fully inhibited sarcoma development; 100% of animals survived; effect was dose-dependent. (56) |

KM, Korean mistletoe; p.o., oral route; VA-M, mistletoe grown on apple tree; TT, triterpenes; OA, oleanolic acid; BA, betulinic acid; ML-1, lectin-I; VAE, *Viscum album* extract; wk(s), week(s); i.v., intravenous; ARA-C, cytarabine; NOD, non-obese diabetic; SCID, sever combined immunodeficiency; VA-Qu, mistletoe grown on oak tree; AE, aqueous extract; s.c., subcutaneous; Th, T-helper; IL, interleukin; CAD, caspase-activated DNase; i.p., intraperitoneal; DOXO, doxorubicin; EAC, Ehrlich ascites carcinoma; STT, soluble TT; CD, cyclodextrins; NR, not-reported; CM-1, Chinese mistletoe lectin >90% identical with European mistletoe ML-1; CRC, colorectal cancer; miR, microRNA; DC, dendritic cells; VA-A, mistletoe grown on fir; VA-P, mistletoe grown on pine; CPA, cyclophosphamide; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; FrF, extract prepared without fermentation; NK, natural killer cell; rML, recombinant lectin; 5-FU, 5-fluorouracil; BBN, N-butyl-N-(4-hydroxybutyl) nitrosamine; GGTP, gamma-glutamyl transpeptidase; PVP, polyvinylpyrrolidone; NMU, N-methyl-N-nitrosourea; RBC, red blood cells.
Table II. Methodological characteristics of studies (n=37), considering animal species, tumor cell origin, quality criteria (blindness, randomization, sample size) and incidence of toxic effects.

| Author/year | Animal species | Tumor type | Blinded study | Control | Random allocation | Number of animals/group | Toxicity |
|-------------|----------------|------------|---------------|---------|------------------|------------------------|----------|
| Han et al, 2015 | Mice | Melanoma | NR | Negative: 0.85% saline Positive: VAE + PKA | Yes | Toxicity test: 5; Antitumor effect: 9 | Toxicity test: No deaths or clinical morbidity, no significant effect on body weight. No significant change in food intake. |
| Delebinski et al, 2015 | Mice | Mesenchymal (Leukemia) | NR | Negative: CD; Positive: ARA-C | NR | 10 | Very good tolerability; no evidence of toxicity measured by body weight and autopsy with liver, spleen histology; some spleen atrophy in group ARA-C. |
| Facina et al, 2014 | Mice | Melanoma | NR | Negative: Distilled water | NR | 6 | NR |
| Stan et al, 2013 | Mice | Epithelial | NR | Negative: Vehicle Tumor control (EAC) Positive control: DOXO | NR | 8 | NR |
| Strüth et al, 2013 | Mice | Melanoma | NR | Negative: CD vehicle | NR | 8-9 (additional 4 animals/group for histology) | Higher dose: STT caused skin necrosis, apathy and weight loss, with 3 dropouts. All treatments caused local skin inflammation and inflammatory peritumoral infiltrates. Lower dose: No toxic effects; reduced inflammatory infiltrates. |
| Podlech et al, 2012 | Mice | Mesenchymal (Glioma and astrocytoma) | NR | No treatment; vehicle | NR | 6 | NR |
| Li et al, 2011 | Mice | Epithelial | NR | Negative control: PBS | NR | 10 | NR |
| Thies et al, 2008 | Mice | Melanoma | NR | Negative control: PBS | NR | 20 | Vitality score, behavior, physiological response and water and food intake assessed: No animals exhibited changes; no treatment-related lesions were found on biopsy. |
| Scifert et al, 2008 | Mice | Mesenchymal (Leukemia) | NR | Negative control: PBS Positive control: CPA | NR | 8 (treated groups) 10 (control groups) | Tolerability was very good; no changes in body weight or assessed hematological parameters. VA-P was more toxic than VA-A in vitro, especially at the higher concentrations (50 mg/kg). |
| Cebovic et al, 2008 | Mice | Epithelial | NR | Placebo; tumor control | NR | 6 | NR |
| Author/year       | Animal species | Tumor type       | Blinded study | Control                             | Random allocation | Number of animals/group | Toxicity                                                                 | (Refs.) |
|------------------|----------------|------------------|---------------|-------------------------------------|-------------------|-------------------------|--------------------------------------------------------------------------|---------|
| Beuth et al., 2006 | Mice           | Epithelial       | NR            | Negative control: PBS               | NR                | 8                       | All animals free from side effects (behavior, appearance).              | (68)    |
| Duong Van Huyen et al., 2006 | Mice | Melanoma         | NR            | Untreated; vehicle                  | NR                | 6                       | NR                                                                       | (67)    |
| Pryme et al., 2004 | Mice           | Mesenchymal (lymphoma) | NR       | Standard diet                        | NR                | 5                       | NR                                                                       | (70)    |
| Yoon et al., 2003 | Mice           | Epithelial and mesenchymal | NR     | Negative control: PBS               | NR                | 5                       | NR                                                                       | (66)    |
| Braun et al., 2002 | Mice           | Mesenchymal (Lymphoma and sarcoma) | NR     | PBS                                 | NR                | 10                      | NR                                                                       | (49)    |
| Elsässer-Beile et al., 2001 | Rats | Epithelial       | NR            | Tumor control                       | NR                | 8                       | NMU-induced carcinoma was accompanied by high mortality associated with the tumor or procedure (15/71 in the control group and 40/123 in the treated groups). All treated animals exhibited weight loss over the 6 weeks of treatment. | (22)    |
| Braun et al., 2001 | Mice           | Mesenchymal (Lymphoma and sarcoma) | NR     | PBS                                 | NR                | 8                       | NR                                                                       | (48)    |
| Schaffrath et al., 2001 | Mice | Mesenchymal (Lymphoma and sarcoma) | NR     | PBS                                 | NR                | 10                      | No toxic effects (agility, food intake, body weight).                    | (23)    |
| Burger et al., 2001 | Mice           | Epithelial and melanoma | NR     | Negative control: PBS; positive control: DOXO, 5-FU | NR                | 5-7                      | VA less toxic than DOXO (body weight; mortality); not different from 5-FU. | (45)    |
| Timoshenko et al., 2001 | Mice | Epithelial       | NR            | Healthy mice; tumor control         | NR                | 10 (2 rounds), 8 (1 round) | Nephrotoxicity.                                                         | (47)    |
| Park et al., 2001 | Mice           | Melanoma         | NR            | PBS; no treatment                   | NR                | NR                      | NR                                                                       | (46)    |
| Zarcovic et al., 2001 | Mice | Epithelial       | NR            | Saline                              | NR                | 7                       | NR                                                                       | (65)    |
| Mengs et al., 2000 | Mice           | Epithelial       | NR            | Vehicle                             | Yes               | 13                      | NR                                                                       | (54)    |
Table II. Continued.

| Author/year   | Animal species | Tumor type                  | Blinded study | Control            | Random allocation | Number of animals/group | Toxicity                                                                 |
|---------------|----------------|-----------------------------|---------------|--------------------|-------------------|-------------------------|--------------------------------------------------------------------------|
| Schumacher et al, 2000 | Mice           | Epithelial                 | NR            | PBS + Tween-80     | NR                | 20                      | NR                                                                      |
| Kunze et al, 2000   | Rats           | Epithelial                 | NR            | No treatment       | Yes               | 60/70 in tumor-bearing groups; 20 in untreated and non-tumor bearing groups | NR                                                                      |
| Antony et al, 1999  | Mice           | Melanoma                    | NR            | No treatment       | NR                | 14                      | NR                                                                      |
| Lenartz et al, 1998 | Rats           | Mesenchymal (glioma)        | NR            | PBS                | NR                | 5                       | NR                                                                      |
| Yoon et al, 1998    | Mice           | Epithelial, melanoma, mesenchymal | NR            | PBS                | NR                | 5                       | NR                                                                      |
| Weber et al, 1998   | Mice           | Melanoma                    | NR            | PBS + povidone     | Yes               | 5                       | No toxic effects (mortality; clinical signs; food intake; body weight; no significant findings on necropsy). |
| Zarcovic et al, 1998| Mice           | Melanoma                    | NR            | Culture medium     | NR                | 5                       | NR                                                                      |
| Kunze et al, 1998   | Rats           | Epithelial                 | NR            | No treatment       | NR                | 75                      | NR                                                                      |
| Zarkovic et al, 1997| Mice           | Melanoma                    | NR            | Saline             | NR                | 14 (total number, allocation NR) | NR                                                                      |
| Jurin et al, 1997   | Mice           | Mesenchymal                 | NR            | Healthy mice; saline | NR         | 8-12                    | NR                                                                      |
| Antony et al, 1997  | Mice           | Melanoma                    | NR            | No treatment       | NR                | 8                       | NR                                                                      |
| Kunze et al, 1997   | Rats           | Epithelial                 | NR            | No treatment       | NR                | Controls: 57; Treated: 61 | 43/100 controls and 59/120 treated mice died by causes associated with the tumor-induction procedure. |
in recent decades mice have become the model used in almost all studies of antineoplastic drugs. This fact raises critical questions as to the translational aspects of such studies and the real possibility of extrapolating experimental findings to clinical practice with humans and other animal species (34,35). Such issues notwithstanding, veterinary clinical studies have demonstrated that mistletoe extracts elicit effective responses in various other animal species (36-39).

### Number of animals per group

The number of animals included in each study ranged from 5 to 20 (median, 8), excluding 4 studies that induced autochthonous urinary bladder carcinoma (22,40-42), which included a large numbers of animals in the control and treated groups (range, 20-100). The possible reason for this may be the notably high procedure-related mortality, as is discussed below. These findings agree with those of Kienle et al (n=5 to 20) (29) and can be rated adequate according to the current standards (43); however, it should be noted that none of the studies presented a justification for the sample size used.

### Tumor type

There was an even distribution of tumor types in the studies, including the following: 14 in melanoma cell lines, 15 in epithelial cell lines and 13 in mesenchymal cell lines. Only 7 studies reported negative or no effects relative to models of melanoma (44-46) or epithelial tumors (40-42,45,47). The toxic effects reported were rare and unrelated to the type of tumor. One study reported skin inflammation and necrosis, mainly peritumoral (26) in mice bearing melanoma, whereas 2 studies revealed weight loss in rats and nephrotoxicity in mice bearing epithelial tumors (22,47).

### Preparations

A total of 23 studies used whole plant extracts, 13 used lectins alone, 4 used recombinant lectin I, 3 used triterpenes and 1 used a high and low molecular fraction (> and <30 kDa; total number is >37 as some studies tested more than one substance either alone or in combination). These findings differ from the ones reported by Kienle et al (29): Whole plant extracts=24; lectins=2; and recombinant lectins=2. There is a clear tendency towards increased use of isolated active components, lectins in particular, and the previous interest in other isolated proteins and polysaccharides (4 each) appears to have been replaced by lipophilic components.

### Route of administration

A total of 36 studies tested mistletoe administered via various systemic routes (intraperitoneal, oral, intranasal, intravenous, subcutaneous) and 5 at the local tumor site. Three studies pretreated the tumor cells and 4 did not report the route used. These results show a tendency towards systemic administration compared to the results of the study by Kienle et al (29), in which the frequency of systemic and local administration was similar (17 vs. 15 studies, respectively).

A total of 3 studies compared the subcutaneous (SC) and intraperitoneal (IP) routes of administration. In the Braun et al study (48), a reduction in the number of lung and liver colonies of RAW-117 P (liver) and L-1 (lung) lymphosarcoma was obtained only with IP administration of aqueous extract standardized for ML-II/III; however, in a later study they used the SC route only and obtained less positive results (49). Lenartz et al (50) reported a reduction in F98 glioma volume.

| Animal species | Tumor type | Control Toxicity | Random allocation | Number of animals/group | Toxicity (Refs.) |
|----------------|------------|------------------|-------------------|-------------------------|-----------------|
| Mouse          | Mesenchymal, melanoma | Saline Yes | NR                | Yes                     | 15 (carcinogenesis) |
| Mice           | Mesenchymal, melanoma | NR               | NR                | No treatment            | 15 (cancer induction) |

**Table II. Continued.**
for both the SC and IP routes, but tumor necrosis was observed in the animals treated with IP administration only. This supports the hypothesis that IP administration leads to better pharmacokinetics of the main active compounds. Nevertheless, Burger et al (45) compared the SC and IP routes in F9 testicular carcinoma and did not find a difference in the antitumor effect.

Yoon et al (51) compared a variety of dosages (1-100 μg) of aqueous extract of Korean mistletoe via various routes-systemic (intravenous, subcutaneous) and mucosal (oral and intranasal). Systemic administration induced a dramatic inhibition of colon carcinoma even at a very low dose (2 μg). Furthermore, mucosal routes were associated with metastasis inhibition at a low dose and significantly, no antitumor effect was detected with higher doses via the intranasal route. One study (52) used lectin-rich extract (Isacodr Qu) to investigate the effects of pretreatment on tumor cells, systemic and local treatment. Pretreatment mitigated tumor growth, indicating that the antitumor effects mistletoe extract were transferred in vivo to growing tumors; however, this effect was not due to reduced cell proliferation or increased cell death. While intratumor treatment significantly reduced tumor growth, systemic administration had no significant effect.

Taken together, the results are highly variable and divergent, and no definite conclusions can be drawn. With that proviso, there do appear to be indications that local administration may be more effective (see below).

Reported random allocation. Only 5 of the 37 studies included reported having randomly allocated the animals to the experimental groups (40,53-56), whereas in the Kienle et al (29) review, 6 of 31 studies reported randomization. Randomization is a crucial step in any experimental protocol, particularly in the case of complementary medicine as the risk of false positive or false negative results is very high (57). It may therefore be beneficial if a methodological guideline were formulated, as it has been for homeopathic studies (57). In the present review, as well as in the Kienle et al review (29), none of the included studies reported whether blinding was performed or not. It has been suggests that this is not reported as it is a standard procedure (29). However, unlike in clinical studies, the use of blinded protocols in pharmacological basic research is very rare. For example, in the present review, searching the key works ‘animal model’ and ‘blind protocol’ in PubMed only identified 94 articles. This is another point to be considered in a putative guideline.

Controls/combination therapy. All 37 studies included control groups, as follows: 26 studies used a placebo-saline/distilled water (5), vehicle (18) or culture medium (1); 12 compared to untreated (10) and/or healthy mice (3). A total of 5 studies compared mistletoe with standard chemotherapy/adjuvant drugs (polysaccharide K, cytarabine, doxorubicin, cyclophosphamide, 5-fluorouracil) alone or in combination (25,45,53,58,59); and 1 study compared with interleukin-2 alone and in combination (47).

Aims of studies. The objectives of the studies exhibit clear temporal trends. Studies conducted between 1996 and 2005 sought to establish the antitumor effect of mistletoe and its possible mechanisms, cytotoxicity and immunomodulation in particular (21,46,54-56,60-68), eventually with comparison to or in combination with standard chemotherapeutic agents. By the end of the 1990 s, the focus of studies shifted towards the study of lectins, in particular ML-1 (21,50), also including Korean mistletoe (46,51,53,66). Between 2000 and 2001, novel studies concerning recombinant lectin were beginning to be published (22-24); however, the specific effects of lectins II and III began to be explored later (48,49). The overall profile of studies performed in the past decade is notably different to previous studies, tending to investigate biologically active principles (33,53,59,69) including lipophilic ones, in particular triterpenes (25-27). A shift can be also noticed in investigations of the putative mechanism of action, with a stronger focus on genomics (44,52,69) and oxidative stress (27,28).

Side-effects/toxicity. A total of 12 of 37 studies (~30%) specifically investigated side effects as variables of interest, including mortality (45,53,55,56), clinical morbidity (26,53,55), body weight (22,23,25,53,55,56,59), food intake (23,33,53,55,56), water intake (33), histology on necropsy (25,26,33,55), behavior (26,33,68), vitality score (33), physiological response (33), hematological parameters (59), overall appearance (68) and agility (23). The majority of studies reported no significant toxic findings, the exception being a study by Strüh et al (26) in which the highest tested dose of solubilized triterpenes administered SC peritumorally resulted in skin necrosis and apathy, which led to 3/13-14 dropouts. Furthermore, animals in all the treated groups exhibited skin inflammation and peritumoral inflammatory infiltrating cells. In the Seifert et al study (59), mistletoe grown on pine trees proved to be more toxic compared to that grown on fir. In the Burger et al study (45) mistletoe was demonstrated to be considerably less toxic than doxorubicin, but showed no differences compared with 5-fluorouracil.

It is worth noting that in the studies using N-methyl-N-nitrosourea (NMU)-induced autochthonous bladder urinary carcinoma the tumor-induction procedure and complications caused high mortality, ranging from 20 to 50% (22,41,42). In a later study, Kunze et al (40) employed N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) as a tumor inducer; although the procedure-related mortality was significantly lower (0 to 1%), the number of animals that developed tumors was also low, as is discussed below.

Outcomes. A total of 32/37 studies reported some antitumor effect, 3 of which had mixed results. Facina et al (44) did not detect any changes in tumor volume due to mistletoe treatment, however they did demonstrate evidence of genetic regulation of apoptosis. Burger et al (45) reported a dose-dependent reduction in tumor growth for 3 of the tested cell lines, but no effect on the other 2 lines tested. In Park et al study (46), mistletoe was demonstrated to have no effect on tumor growth; however, it did induce dose-dependent inhibition of metastasis and led to a slight increase in survival rates. Two studies reported no antitumor effect (41,42) and 2 reported an overt negative effect in the treated groups (40,47). Finally, 1 study assessed immunomodulatory activity only, with no investigation of antitumor effects (62); the results from this study indicated that treatment with mistletoe restored the immune response,
however prolonged application also had a dose-dependent immunosuppressing effect on normal cells. The relationship between the concentration of active molecules and the balance between cytotoxicity and immunomodulatory effects requires further investigation; the combination of both factors in the same protocol may be a useful tool to treat aggressive tumors with mistletoe components.

A total of 21 studies assessed tumor growth inhibition. Of these, 17 found a reduction in tumor size, 7 of them in a dose-dependent manner and with eventual inversion of the effect at a higher dose (33), and one of these studies (45) indicated a reduction in tumor size in only 3 out of the 5 tested cell lines. In 1 study, remission was reported when mistletoe was administered inside the tumor, whereas there was no such effect when it was administered via the systemic route (52). Two studies reported an unexpected increase in tumor size in the treated groups (40,47). Future studies investigating the underlying molecular mechanisms may explain these paradoxical results.

Survival was analyzed in a total of 12 studies, and was higher in the treated groups in all cases, most of them in all studied doses but dose-dependence was reported in 2 studies (24,56).

Inhibition of primary or metastatic tumor formation was assessed by 1 (56) and 12 studies, respectively. Tumorigenesis was inhibited in all studies with the exception of Timoshenko et al (47). Based on these findings, the authors of the present study suggest that the action of mistletoe active principles in the tumor microenvironment should be investigated further, as the onset of the metastasisformation process depends on it. Additionally, the variability of results among the different studies may also be attributed to the fact that most protocols were based on transplantable tumors, in which tumorigenesis cannot be evaluated, since tumor cells are inoculated directly in the animals, being immunogenic stimuli. The cases that are most illustrative of this are the 4 studies that assessed the effect of mistletoe lectin on autotchronous urinary bladder carcinoma. Kunze et al (41,42) and Elsässer-Beile et al (22) induced tumors using NMU, and Kunze et al (40) induced tumors with BBN. In all 3 studies by Kunze et al, a lectin isolated from unspecified mistletoe was used. Of these, the 2 studies that induced tumors with NMU had a high procedure-related mortality, and no significant difference was found between the control and treated groups. In contrast, the study that used BBN had a very low procedure-mortality rate, but also a low number of induced tumors in the control and treated groups (n=6 and 4, 10.2 and 6.7%, respectively, in the 6-month experiment; n=16 and 13, 25.8 and 19.7%, respectively, in the 15-month experiment). In this case, at 15 months, the tumor size was twice larger, on average, in the lectin-treated group. Elsässer-Beile et al (22) used recombinant lectin starting 8 or 14 weeks following tumor induction and reported significantly lower rates of atypical hyperplasia and neoplastic transformation in all treated groups compared with the control. The main difference between these two groups of experiments is that Kunze et al used the subcutaneous route for long periods of time (6 and 15 months), whereas Elsässer-Beile et al (22) applied shorter 6-week treatments via the local route of administration (intravesical instillation). It could therefore be that the divergence in these results is due to the methodological differences between the studies. Mengs et al (54) used the intravesical route to administer a lectin-rich aqueous mistletoe extract to mice implanted with urinary bladder carcinoma MB49 cells in a short 4-week course of treatment; the results of this study indicated a dose-dependent statistically significant tumor growth inhibition in the treated groups.

Several putative mechanisms for the anticancer action of mistletoe were tested, including tumor cell viability [reduced, (27,53,63)] and number [reduced, (37)]; tumor cell turnover [increased, (70)]; antiproliferative effect [4 studies, no effect in 1 (26)]; proapoptotic action (increased in 8 studies); increased tumor necrosis (6 studies); increased macrophage cytotoxicity (66); reduction of mitotic figures in tumor cells (65,70); cycle cell regulation [deregulated in one study (53), and no effect in another (46)]. Furthermore, 1 study reported degradation of specific miRNAs, this being an indirect effect (affection of precursors) (71) and 2 studies reported a reduction in oxidative stress (27,58).

Reduced tumor invasiveness was reported by 1 study (65), whereas in another (70), 11% of the treated animals exhibited no histological evidence of viable tumors on day 11 after the onset of treatment. Reduced angiogenesis was identified in 2 studies (26,46); however, in 1 study, the vascular count increased when high doses were used (33). The possible underlying antineoplastic mechanisms require further investigation using specific molecular methods in animal models, as developing an effective mistletoe anticancer agent may on the addition of different mechanisms, possibly associated with a mixture of active molecules in different concentrations and their interactions with the tumor microenvironment. Few studies have been performed from this perspective; a total of 5 studies analyzed the role of local cell infiltrates in tumor development, with an increase in absolute numbers of infiltrating leukocytes being found in 3 studies (23,63,70) and no significant effect found in 2 studies (40,41).

For the immunomodulatory action of mistletoe, induction of the Th-1 response was assessed and detected in 1 study (44) and cytokines were assessed in 2, of which 1 reported no effect (22) and the other reported upregulation of IL-12 only (67). An increase in natural killer cell activity was detected in 2 studies (51,66). Macrophage stimulation was reported in 1 study (55) and 2 studies found an increase in white cell count in the peripheral blood, but not of granulocytes (48,49). Upregulation of the number of thymocytes (49), activation of spleen cells (64), lymphocyte stimulation (CD4+ and CD8+, but only anti CD8+ activity was enhanced) (55) and finally stimulation of cytotoxic population (63) were also reported.

In conclusion, the majority of studies investigating the effects of mistletoe in animal cancer models analyzed in the present review reported positive outcomes in terms of the inhibition of tumor formation and growth. However, there is considerable methodological heterogeneity among studies, which precludes performing conclusive comparisons and explains the variation in results. A great deal of research is still required with standardization of mistletoe preparation, dose, concentration, regime of administration, length of treatment, targeted cancer type, and other aspects. Similarly, while it is universally assumed that mistletoe has cytotoxic and immunomodulatory effects, there is a wide variation among studies in terms of the mechanisms assessed. The present review found little or no continuity within
and among research groups, with little progress being made in the past 20 years of studies with experimental animal models. Based on these results, the present authors suggest that the community of interested researchers should develop a guideline for reporting in vivo mistletoe cancer treatment experiments.

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