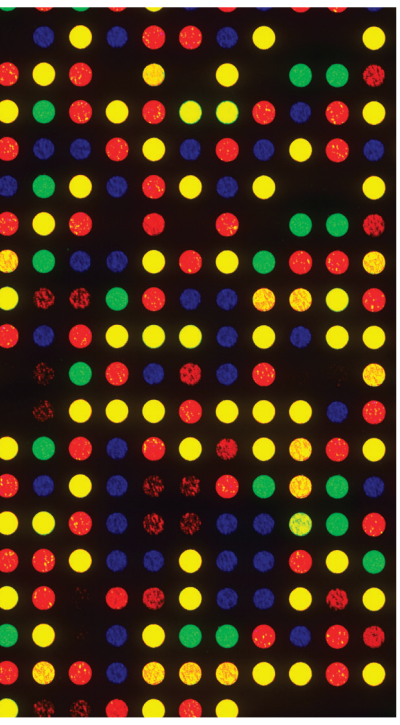
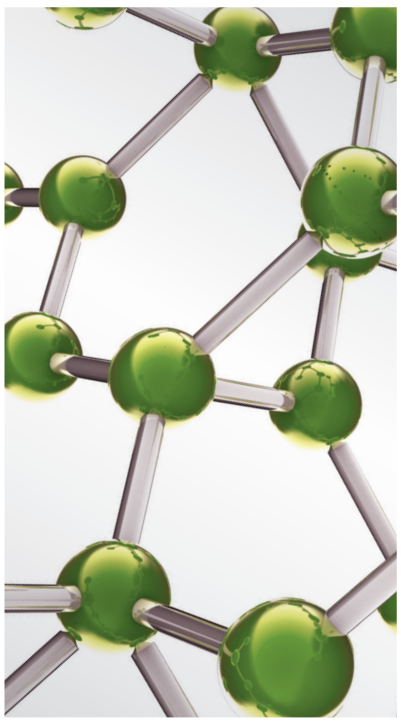
eCAM Evidence-Based Complementary and Alternative Medicine

Mistletoe: From Basic Research to Clinical Outcomes in Cancer and Other Indications

Guest Editors: Matthias Kröz, Gunver Sophia Kienle, Gene Feder, Srini Kaveri, and Steven Rosenzweig





Mistletoe: From Basic Research to Clinical Outcomes in Cancer and Other Indications

Evidence-Based Complementary and Alternative Medicine

Mistletoe: From Basic Research to Clinical Outcomes in Cancer and Other Indications

Guest Editors: Matthias Kröz, Gunver Sophia Kienle, Gene Feder, Srini Kaveri, and Steven Rosenzweig

Copyright © 2014 Hindawi Publishing Corporation. All rights reserved.

This is a special issue published in "Evidence-Based Complementary and Alternative Medicine." All articles are open access articles distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Editorial Board

Mahmood A. Abdulla, Malaysia
Jon Adams, Australia
Zuraini Ahmad, Malaysia
Ulysses Albuquerque, Brazil
Gianni Allais, Italy
Terje Alraek, Norway
Souliman Amrani, Morocco
Akshay Anand, India
Shrikant Anant, USA
Manuel Arroyo-Morales, Spain
Syed Asdaq, Saudi Arabia
Seddigheh Asgary, Iran
Hyunsu Bae, Republic of Korea
Lijun Bai, China
Sandip K. Bandyopadhyay, India
Sarang Bani, India
Vassya Bankova, Bulgaria
Winfried Banzer, Germany
Vernon A. Barnes, USA
Samra Bashir, Pakistan
Jairo Kenupp Bastos, Brazil
Sujit Basu, USA
David Baxter, New Zealand
Andre-Michael Beer, Germany
Alvin J. Beitz, USA
Yong Boo, Republic of Korea
Francesca Borrelli, Italy
Gloria Brusotti, Italy
Ishfaq A. Bukhari, Pakistan
Arndt Büssing, Germany
Rainer W. Bussmann, USA
Raffaele Capasso, Italy
Opher Caspi, Israel
Han Chae, Republic of Korea
Shun-Wan Chan, Hong Kong
Il-Moo Chang, Republic of Korea
Rajnish Chaturvedi, India
Chun Tao Che, USA
Hubiao Chen, Hong Kong
Jian-Guo Chen, China
Kevin Chen, USA
Tzeng-Ji Chen, Taiwan
Yunfei Chen, China
Juei-Tang Cheng, Taiwan
Evan Paul Cherniack, USA
Jen-Hwey Chiu, Taiwan
William C. S. Cho, Hong Kong
Jae Youl Cho, Republic of Korea
Seung-H. Cho, Republic of Korea
Chee Yan Choo, Malaysia
Ryowon Choue, Republic of Korea
Shuang-En Chuang, Taiwan
Joo-Ho Chung, Republic of Korea
Edwin L. Cooper, USA
Gregory D. Cramer, USA
Meng Cui, China
Roberto Cuman, Brazil
Vincenzo De Feo, Italy
Rocío Vázquez, Spain
Martin Descarreaux, USA
Alexandra Deters, Germany
Siva Durairajan, Hong Kong
Mohamed Eddouks, Morocco
Thomas Efferth, Germany
Tobias Esch, Germany
Saeed Esmaeili-Mahani, Iran
Nianping Feng, China
Yibin Feng, Hong Kong
Josue Fernandez-Carnero, Spain
Juliano Ferreira, Brazil
Fabio Firenzuoli, Italy
Peter Fisher, UK
W. F. Fong, Hong Kong
Romain Forestier, France
Joel J. Gagnier, Canada
Jian-Li Gao, China
Gabino Garrido, Chile
Muhammad Ghayur, Pakistan
Anwarul Hassan Gilani, Pakistan
Michael Goldstein, USA
Mahabir P. Gupta, Panama
Mitchell Haas, USA
Svein Haavik, Norway
Abid Hamid, India
N. Hanazaki, Brazil
K. B. Harikumar, India
Cory S. Harris, Canada
Thierry Hennebelle, France
Seung-H. Hong, Republic of Korea
Markus Horneber, Germany
Ching-Liang Hsieh, Taiwan
Jing Hu, China
Gan Siew Hua, Malaysia
Sheng-Teng Huang, Taiwan
Benny Tan Kwong Huat, Singapore
Roman Huber, Germany
Angelo Antonio Izzo, Italy
Kong J., USA
Suresh Jadhav, India
Kanokwan Jarukamjorn, Thailand
Yong Jiang, China
Zheng L. Jiang, China
Stefanie Joos, Germany
Sirajudeen K.N.S., Malaysia
Z. Kain, USA
Osamu Kanauchi, Japan
Wenyi Kang, China
Dae Gill Kang, Republic of Korea
Shao-Hsuan Kao, Taiwan
Krishna Kaphle, Nepal
Kenji Kawakita, Japan
Jong Yeol Kim, Republic of Korea
Cheorl-Ho Kim, Republic of Korea
Youn Chul Kim, Republic of Korea
Yoshiyuki Kimura, Japan
Joshua K. Ko, China
Toshiaki Kogure, Japan
Nandakumar Krishnadas, India
Yiu Wa Kwan, Hong Kong
Kuang Chi Lai, Taiwan
Ching Lan, Taiwan
Alfred Längler, Germany
Lixing Lao, Hong Kong
Clara Bik-San Lau, Hong Kong
Jang-Hern Lee, Republic of Korea
Tat leang Lee, Singapore
Myeong S. Lee, UK
Christian Lehmann, Canada
Marco Leonti, Italy
Ping-Chung Leung, Hong Kong
Lawrence Leung, Canada
Kwok Nam Leung, Hong Kong
Ping Li, China
Min Li, China
Man Li, China

ChunGuang Li, Australia
Xiu-Min Li, USA
Shao Li, China
Yong Hong Liao, China
Sabina Lim, Republic of Korea
Bi-Fong Lin, Taiwan
Wen Chuan Lin, China
Christopher G. Lis, USA
Gerhard Litscher, Austria
Ke Liu, China
I-Min Liu, Taiwan
Gaofeng Liu, China
Yijun Liu, USA
Cun-Zhi Liu, China
Gail B. Mahady, USA
Juraj Majtan, Slovakia
Subhash C. Mandal, India
Jeanine Marnewick, South Africa
Virginia S. Martino, Argentina
James H. McAuley, Australia
Karin Meissner, USA
Andreas Michalsen, Germany
David Mischoulon, USA
Syam Mohan, Malaysia
J. Molnar, Hungary
Valério Monteiro-Neto, Brazil
H.-I. Moon, Republic of Korea
Albert Moraska, USA
Mark Moss, UK
Yoshiharu Motoo, Japan
Frauke Musial, Germany
MinKyun Na, Republic of Korea
Richard L. Nahin, USA
Vitaly Napadow, USA
F. R. F. Nascimento, Brazil
S. Nayak, Trinidad And Tobago
Isabella Neri, Italy
Télesphore Nguelefack, Cameroon
Martin Offenbacher, Germany
Ki-Wan Oh, Republic of Korea
Y. Ohta, Japan
Olumayokun A. Olajide, UK
Thomas Ostermann, Germany
Stacey A. Page, Canada
Tai-Long Pan, Taiwan
Bhushan Patwardhan, India
Berit Smestad Paulsen, Norway
Andrea Pieroni, Italy
Richard Pietras, USA
Waris Qidwai, Pakistan
Xianqin Qu, Australia
Cassandra L. Quave, USA
Roja Rahimi, Iran
Khalid Rahman, UK
Cheppail Ramachandran, USA
Gamal Ramadan, Egypt
Ke Ren, USA
Man Hee Rhee, Republic of Korea
Mee-Ra Rhyu, Republic of Korea
José Luis Ríos, Spain
Paolo Roberti di Sarsina, Italy
Bashar Saad, Palestinian Authority
Sumaira Sahreen, Pakistan
Omar Said, Israel
Luis A. Salazar-Olivo, Mexico
Mohd. Zaki Salleh, Malaysia
Andreas Sandner-Kiesling, Austria
Adair Santos, Brazil
G. Schmeda-Hirschmann, Chile
Andrew Scholey, Australia
Veronique Seidel, UK
Senthamil R. Selvan, USA
Tuhinadri Sen, India
Hongcai Shang, China
Karen J. Sherman, USA
Ronald Sherman, USA
Kuniyoshi Shimizu, Japan
Kan Shimpo, Japan
Byung-C. Shin, Republic of Korea
Yukihiro Shoyama, Japan
Chang Gue Son, Republic of Korea
Rachid Soulimani, France
Didier Stien, France
Shan-Yu Su, Taiwan
Mohd Roslan Sulaiman, Malaysia
Venil N. Sumantran, India
John R. S. Tabuti, Uganda
Toku Takahashi, USA
Rabih Talhouk, Lebanon
Wen-Fu Tang, China
Yuping Tang, China
Lay Kek Teh, Malaysia
Mayank Thakur, India
Menaka C. Thounaojam, India
Mei Tian, China
Evelin Tiralongo, Australia
S. C. Tjen-A-Looi, USA
Michał Tomczyk, Poland
Yao Tong, Hong Kong
K. V. Trinh, Canada
Karl Wah-Keung Tsim, Hong Kong
Volkan Tugcu, Turkey
Yew-Min Tzeng, Taiwan
Dawn M. Upchurch, USA
Maryna Van de Venter, South Africa
Sandy van Vuuren, South Africa
Alfredo Vannacci, Italy
Mani Vasudevan, Malaysia
Carlo Ventura, Italy
Wagner Vilegas, Brazil
Pradeep Visen, Canada
Aristo Vojdani, USA
Y. Wang, USA
Shu-Ming Wang, USA
Chenchen Wang, USA
Chong-Zhi Wang, USA
Kenji Watanabe, Japan
Jintanaporn Wattanathorn, Thailand
Wolfgang Weidenhammer, Germany
Jenny M. Wilkinson, Australia
Darren R. Williams, Republic of Korea
Haruki Yamada, Japan
Nobuo Yamaguchi, Japan
Yong-Qing Yang, China
Junqing Yang, China
Ling Yang, China
Eun Jin Yang, Republic of Korea
Xiufen Yang, China
Ken Yasukawa, Japan
Min H. Ye, China
M. Yoon, Republic of Korea
Jie Yu, China
Jin-Lan Zhang, China
Zunjian Zhang, China
Wei-bo Zhang, China
Hong Q. Zhang, Hong Kong
Boli Zhang, China
Ruixin Zhang, USA
Hong Zhang, Sweden
Haibo Zhu, China

Contents

Mistletoe: From Basic Research to Clinical Outcomes in Cancer and Other Indications, Matthias Kröz, Gunver Sophia Kienle, Gene Feder, Srini Kaveri, and Steven Rosenzweig
Volume 2014, Article ID 987527, 2 pages

Safety of Intravenous Application of Mistletoe (*Viscum album* L.) Preparations in Oncology: An Observational Study, Megan L. Steele, Jan Axtner, Antje Happe, Matthias Kröz, Harald Matthes, and Friedemann Schad
Volume 2014, Article ID 236310, 10 pages

Evaluation of Preclinical Assays to Investigate an Anthroposophic Pharmaceutical Process Applied to Mistletoe (*Viscum album* L.) Extracts, Stephan Baumgartner, Heidi Flückiger, Matthias Kunz, Claudia Scherr, and Konrad Urech
Volume 2014, Article ID 620974, 8 pages

A Randomized Study on Postrelapse Disease-Free Survival with Adjuvant Mistletoe versus Oral Etoposide in Osteosarcoma Patients, Alessandra Longhi, Marcus Reif, Erminia Mariani, and Stefano Ferrari
Volume 2014, Article ID 210198, 9 pages

Additional Therapy with a Mistletoe Product during Adjuvant Chemotherapy of Breast Cancer Patients Improves Quality of Life: An Open Randomized Clinical Pilot Trial, Wilfried Tröger, Zdravko Ždrale, Nevena Tišma, and Miodrag Matijašević
Volume 2014, Article ID 430518, 9 pages

Erratum to "NCCAM/NCI Phase 1 Study of Mistletoe Extract and Gemcitabine in Patients with Advanced Solid Tumors", Patrick J. Mansky, Dawn B. Wallerstedt, Timothy S. Sannes, Jamie Stagl, Laura Lee Johnson, Marc R. Blackman, Jean L. Grem, Sandra M. Swain, and Brian P. Monahan
Volume 2014, Article ID 606348, 2 pages

Adverse Drug Reactions and Expected Effects to Therapy with Subcutaneous Mistletoe Extracts (*Viscum album* L.) in Cancer Patients, Megan L. Steele, Jan Axtner, Antje Happe, Matthias Kröz, Harald Matthes, and Friedemann Schad
Volume 2014, Article ID 724258, 11 pages

Effects of Lipophilic Extract of *Viscum album* L. and Oleanolic Acid on Migratory Activity of NIH/3T3 Fibroblasts and on HaCat Keratinocytes, R. Kuonen, U. Weissenstein, K. Urech, M. Kunz, K. Hostanska, M. Estko, P. Heusser, and S. Baumgartner
Volume 2013, Article ID 718105, 7 pages

NCCAM/NCI Phase 1 Study of Mistletoe Extract and Gemcitabine in Patients with Advanced Solid Tumors, Patrick J. Mansky, Dawn B. Wallerstedt, Timothy S. Sannes, Jamie Stagl, Laura Lee Johnson, Marc R. Blackman, Jean L. Grem, Sandra M. Swain, and Brian P. Monahan
Volume 2013, Article ID 964592, 11 pages

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 987527, 2 pages
http://dx.doi.org/10.1155/2014/987527

Editorial

Mistletoe: From Basic Research to Clinical Outcomes in Cancer and Other Indications

Matthias Kröz,[1,2,3,4] Gunver Sophia Kienle,[5] Gene Feder,[6] Srini Kaveri,[7] and Steven Rosenzweig[8]

[1] *Research Institute Havelhöhe (FIH), Havelhöhe Hospital, 14089 Berlin, Germany*
[2] *Department of Oncology, Havelhöhe Hospital, 14089 Berlin, Germany*
[3] *Institute for Social Medicine, Epidemiology and Institute for Social Medicine, Epidemiology and Health Economics, Charité University Medicine, 14089 Berlin, Germany*
[4] *Institute of Integrative Medicine, University of Witten/Herdecke, 58313 Herdecke, Germany*
[5] *Institute for Applied Epistemology and Medical Methodology, University of Witten/Herdecke, 79111 Freiburg, Germany*
[6] *Academic Unit of Primary Health Care, School of Social and Community Medicine, Bristol BS8 2PS, UK*
[7] *INSERM U1138, Université Paris Descartes, 75006 Paris, France*
[8] *College of Medicine, Drexel University, Philadelphia, PA 19129, USA*

Correspondence should be addressed to Matthias Kröz; mkroez@havelhoehe.de

Received 7 May 2014; Accepted 7 May 2014; Published 27 May 2014

Copyright © 2014 Matthias Kröz et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The importance of integrative medicine in oncological care is increasing in accordance with growth of its evidence base. In central Europe, white-berried mistletoe (*Viscum album*) preparations not only are among the most common types of treatments used in integrative medicine but also have been among of the most commonly prescribed cancer treatments in Germany *per se* in 2010 [1]. By 2017, mistletoe preparations will have been used in the treatment of cancer patients for 100 years. Mistletoe is a historic, folk remedy, but the first recorded use in oncology was by the Dutch physician Ita Wegman who used a mistletoe extraction for the treatment of a breast cancer patient following a recommendation by Rudolf Steiner [2]. The constituents of the mistletoe berry include lectins, viscotoxins, glycoproteins, oligo- and polysaccharides, and membrane lipids [3]. The PubMed database alone lists more than 1,200 citations for "mistletoe," with approximately 50 new entries each year. There are a multitude of laboratory-based studies demonstrating immune stimulation, cytotoxicity, proapoptotic effects, antiangiogenesis, and DNA stabilisation [3–7]; animal experiments have found tumor-reducing effects [8]. Recent observations of a potent anti-inflammatory effect of *Viscum album* via selective inhibition of COX-2 protein expression provide a further rationale for an antitumor role of mistletoe in view of the close relationship between cancer and inflammation [9]. More recent research focuses on new mistletoe extracts that contain lipophilic components, that is, triterpenes, shown to have strong cytotoxic effects in mouse models [10].

Recent years have seen growth in the number and quality of clinical research studies on mistletoe therapy reporting improved patient outcomes, including studies of its coadministration alongside chemotherapy to reduce adverse effects and to improve quality of life in breast cancer, ovarian cancer, and lung cancer patients [11, 12]. Its clinical efficacy regarding tumor control and survival has been contested [13]; other systematic reviews have been more positive, particularly with regard to health-related quality of life outcomes [14]. A 2013 randomised-controlled trial reported an increase in median survival time for patients with pancreatic cancer [15]. Further similar well-designed clinical trials on other cancer types are warranted.

This special issue covers a wide range of research from basic science to clinical outcomes for cancer and other indications. We intend it to provide a scientific forum to promote further research and publication in this field.

We have included three articles dealing with the important subject of safety and quantification of adverse events of mistletoe therapy. P. J. Mansky and colleagues present the results of a National Center for Complementary and Alternative Medicine phase 1 dose escalation study of combined whole mistletoe extract and gemcitabine treatment in cancer patients and interactions between these two agents. In a prospective observational study, M. L. Steele and colleagues report safety data on subcutaneous mistletoe therapy in a large cancer population. Another article of this group focuses on the important and as yet underreported issue of the safety of off-label intravenous *Viscum album* administration.

In a Serbian randomized-controlled study, W. Tröger and colleagues measured the impact of concomitant mistletoe treatment with adjuvant chemotherapy on health-related quality of life in breast cancer patients. In an Italian randomized controlled phase 2 study, A. Longhi and colleagues investigated the effect of *Viscum album* treatment compared with standard epirubicin chemotherapy on survival and health-related quality of life in patients with osteosarcoma.

Shifting upstream to laboratory research, R. Kuonen and colleagues present new *in vitro* findings on the effect of a *Viscum album* lipophilic extract containing triterpenes on fibroblast migration, which serves as a wound healing model. S. Baumgartner and colleagues studied the effects of a traditional (anthroposophical) pharmaceutical process involving high-speed mixing of mistletoe extracts; they used an *in vitro* model to discern tumoricidal from host resistance mechanisms of action.

We hope this special issue contributes to the evidence base of mistletoe therapy and stimulates further robust research to establish its place in oncological treatment.

Acknowledgments

M. Kröz's work was supported by the Humanus Institute, Berlin, and Software AG foundation, Darmstadt, Germany. G. Feder has received support from Helixor in the form of mistletoe and placebo ampoules for a trial and the University of Bristol was paid by Weleda for his chairing of UK Iscador prescribing guidelines.

Matthias Kröz
Gunver Sophia Kienle
Gene Feder
Srini Kaveri
Steven Rosenzweig

References

[1] F. Hoffmann, "Versorgung mit Krebstherapeutika im Jahr 2010," in *Barmer GEK Arzneimittel Report 2011*, G. Glaeske and C. Schicktanz, Eds., Schriftreihe zur Gesundheitsanalyse, Band 8, Schwäbisch Gmünd: Barmer GEK, 2011.

[2] Z. J. van Emmichoven, *Wer War Ita Wegman*, Natura, Heidelberg, Germany, 1992.

[3] G. Kienle and H. Kiene, *Die Mistel in der Onkologie*, Schattauer, Stuttgart, Germany, 2003.

[4] K. Urech, G. Schaller, and C. Jäggy, "Viscotoxins, mistletoe lectins and their isoforms in mistletoe (Viscum album L.) extracts Iscador: analytical results on pharmaceutical processing of mistletoe," *Arzneimittel-Forschung/Drug Research*, vol. 56, no. 6, pp. 428–434, 2006.

[5] A. Büssing and M. Schietzel, "Apoptosis-inducing properties of Viscum album L. extracts from different host trees, correlate with their content of toxic mistletoe lectins," *Anticancer Research*, vol. 19, no. 1, pp. 23–28, 1999.

[6] S. R. Elluru, J.-P. D. van Huyen, S. Delignat et al., "Induction of maturation and activation of human dendritic cells: a mechanism underlying the beneficial effect of Viscum album as complimentary therapy in cancer," *BMC Cancer*, vol. 8, article 161, 2008.

[7] S. R. Elluru, J.-P. D. Van Huyen, S. Delignat et al., "Antiangiogenic properties of Viscum album extracts are associated with endothelial cytotoxicity," *Anticancer Research*, vol. 29, no. 8, pp. 2945–2950, 2009.

[8] M. Rostock, R. Huber, T. Greiner et al., "Anticancer activity of a lectin-rich mistletoe extract injected intratumorally into human pancreatic cancer xenografts," *Anticancer Research*, vol. 25, no. 3, pp. 1969–1975, 2005.

[9] P. Hegde, M. S. Maddur, A. Friboulet, J. Bayry, and S. V. Kaveri, "Viscum album exerts anti-inflammatory effect by selectively inhibiting cytokine-induced expression of cyclooxygenase-2," *PLoS ONE*, vol. 6, no. 10, article e26312, 2011.

[10] C. M. Struh, S. Jager, A. Kersten, C. M. Schempp, A. Scheffler, and S. F. Martin, "Triterpenoids amplify anti-tumoral effects of mistletoe extracts on murine B16.f10 melanoma in vivo," *PLoS One*, vol. 8, no. 4, article e62168, 2013.

[11] V. F. Semiglazov, V. V. Stepula, A. Dudov, J. Schnitker, and U. Mengs, "Quality of life is improved in breast cancer patients by standardised mistletoe extract PS76A2 during chemotherapy and follow-up: A randomised, placebo-controlled, double-blind, multicentre clinical trial," *Anticancer Research*, vol. 26, no. 2, pp. 1519–1529, 2006.

[12] B. K. Piao, Y. X. Wang, G. R. Xie et al., "Impact of Complementary Mistletoe Extract Treatment on Quality of Life in Breast, Ovarian and Non-small Cell Lung Cancer Patients. A Prospective Randomized Controlled Clinical Trial," *Anticancer Research*, vol. 24, no. 1, pp. 303–309, 2004.

[13] M. A. Horneber, G. Bueschel, R. Huber, K. Linde, and M. Rostock, "Mistletoe therapy in oncology," *Cochrane Database of Systematic Reviews*, no. 2, Article ID CD003297, 2008.

[14] G. S. Kienle and H. Kiene, "Influence of Viscum album L (European Mistletoe) extracts on quality of life in cancer patients: A Systematic Review of Controlled Clinical Studies," *Integrative Cancer Therapies*, vol. 9, no. 2, pp. 142–157, 2010.

[15] W. Tröger, D. Galun, M. Reif, A. Schumann, N. Stanković, and M. Milicević, "Viscum album [L.] extract therapy in patients with locally advanced or metastatic pancreatic cancer: randomised clinical trial on overall survival," *European Journal of Cancer*, vol. 49, no. 18, pp. 3788–3797, 2013.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 236310, 10 pages
http://dx.doi.org/10.1155/2014/236310

Research Article

Safety of Intravenous Application of Mistletoe (*Viscum album* L.) Preparations in Oncology: An Observational Study

Megan L. Steele,[1] Jan Axtner,[1] Antje Happe,[1] Matthias Kröz,[1,2,3,4] Harald Matthes,[1,2] and Friedemann Schad[1,2]

[1] *Research Institute Havelhoehe, 14089 Berlin, Germany*
[2] *Hospital Havelhoehe, 14089 Berlin, Germany*
[3] *Institute for Social Medicine, Epidemiology and Health Economics, Charité-University Medical Centre, 10117 Berlin, Germany*
[4] *Institute of Integrative Medicine, Witten/Herdecke University, 58313 Herdecke, Germany*

Correspondence should be addressed to Friedemann Schad; fschad@havelhoehe.de

Received 21 November 2013; Revised 2 April 2014; Accepted 3 April 2014; Published 15 May 2014

Academic Editor: Srini Kaveri

Copyright © 2014 Megan L. Steele et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Traditional mistletoe therapy in cancer patients involves subcutaneous applications of *Viscum album* L. preparations, with doses slowly increasing based on patient responses. Intravenous infusion of high doses may improve therapeutic outcomes and is becoming more common. Little is known about the safety of this "off-label" application of mistletoe. *Methods*. An observational study was performed within the Network Oncology. Treatment with intravenous mistletoe applications is described. The frequency of adverse drug reactions (ADRs) to intravenous mistletoe applications was calculated and compared to ADR data from a study on subcutaneous applications. *Results*. Of 475 cancer patients who received intravenous infusions of Helixor, Abnoba viscum, or Iscador mistletoe preparations, 22 patients (4.6%) reported 32 ADRs of mild (59.4%) or moderate severity (40.6%). No serious ADRs occurred. ADRs were more frequently reported to i.v. mistletoe administered alone (4.3%), versus prior to chemotherapy (1.6%). ADR frequency differed with respect to preparation type, with Iscador preparations showing a higher relative frequency, compared to Abnoba viscum and Helixor. Overall, patients were almost two times less likely to experience an ADR to intravenous compared to subcutaneous application of mistletoe. *Conclusion*. Intravenous mistletoe therapy was found to be safe and prospective studies for efficacy are recommended.

1. Introduction

European mistletoe (*Viscum album* L.) extracts are amongst the most frequently prescribed complementary therapies for cancer patients in Europe, especially in Germany, Switzerland, and Austria [1]. Anthroposophic mistletoe preparations (AbnobaVISCUM, Helixor, Iscador, Iscucin) are most commonly applied via subcutaneous (s.c.) injection starting at a low dose, which is slowly increased over time based on individual patient responses. Increasingly mistletoe preparations are also applied at high initial doses, either by s.c. injection, as an intravenous (i.v.) infusion, or intratumoural (i.t.), depending on the location of the tumour, disease stage, and experience of the physician [2–5]. There appears to be building evidence for safety, improvement of quality of life, and reduction in adverse drug reactions (ADRs) to conventional therapies associated with traditional s.c. mistletoe therapy. It is suggested, however, that high dose i.v. mistletoe infusions might be even more effective and might also reduce the risk of tumour metastasis or recurrence, possibly through modulation of the immune system and antiangiogenesis effects [6–10]. In a study assessing humoral cell responses in cancer patients' serum to mistletoe extracts administered by s.c. compared to i.v. application, a distinctly greater stimulant effect on immune cell populations and granulocyte-colony stimulating factor production was observed in i.v. patients [11]. Therefore, the focus of the current study is on the i.v. administration of mistletoe preparations.

Compared to s.c. mistletoe therapy, there have been relatively few published studies investigating the clinical benefits or safety of i.v. administration of mistletoe preparations. Improvements in well-being and quality of life are the most commonly reported, particularly in frail patients with advanced or end-stage cancer, or beneficial effects regarding cancer-related low mood, fatigue, and pain [3, 6, 12–16]. In clinical practice, i.v. mistletoe therapy is often given concomitantly to conventional chemotherapy [17]. In a phase II study by Büssing et al., i.v. administration of mistletoe preparations (Iscador Mali special) showed significant beneficial effects with respect to chemotherapy-related adverse events. I.v. application of Iscador Mali special at concentrations between 1 and 5 mg was shown to be safe and tolerable [18]. Perioperative i.v. applications of mistletoe preparations (Iscador Mali and Quercus) have been shown to significantly minimise the immune suppression triggered by anaesthesia and operation stress [19–21]. We also found a prospective, randomised, controlled trial in which colorectal cancer patients seemed to benefit in terms of survival from combined postoperative chemotherapy and i.v. mistletoe therapy (Isorel) compared to chemotherapy alone [22]. However, the methodological quality of this and other studies assessing the safety and effectiveness of mistletoe therapy has been criticised [1]. In addition, other studies have involved patients receiving mistletoe preparations via different combinations of s.c., i.v., and i.t. administration but did not assess separately the efficacy or safety of the specific forms of administration.

Although increasingly used in clinical practice, i.v. application of anthroposophic mistletoe preparations is still classified as "off-label" use. While s.c. mistletoe therapy has proven to be well-tolerated [23–25], i.v. mistletoe therapy often involves much higher doses, and limited research has been conducted regarding the safety of this application [19, 20]. The present study describes the use of i.v. mistletoe therapy under standard clinical practice within an integrative oncological setting in Germany [26]. In addition to summarising patient demographics and the mistletoe preparations and doses received, the frequency and severity of i.v. mistletoe-related ADRs are assessed and compared to results from our previous study on the safety of s.c. mistletoe [27].

2. Methods

2.1. Study Design and Data Sources. A multicentre, observational study was carried out within the Network Oncology (NO), a conjoint clinical registry of German hospitals and out-patient practitioners specialised in anthroposophic medicine [26, 28]. As described previously, documentation officers extract patient information, cancer diagnoses, therapies, adverse events, and disease progress from patient files and record data using the QuaDoSta (Quality management, Documentation, and Statistics) software that was developed at Havelhoehe Research Institute [26, 29]. Detailed information about mistletoe therapies received, including dosage data, therapy start and end dates, and related adverse events, is documented. The NO was designed to be compliant with the European Directive on Data Protection, which has implications for record linkage studies, databases, and registers, and the NO-project has received a positive vote from the ethical committee of the Medical Association Berlin [26, 30]. For the present study, data from the medical records of all consenting patients treated between 2003 and 2013 were assessed. Data were recorded in the QuaDoSta between 2010 and 2013 and analysed in 2013. All analyses were conducted and figures were created with R version 2.15.1 [31].

2.2. Selection and Analysis of Patient Data. All patients with a valid identification number, birth date, gender, cancer diagnosis date, ICD-10 code, and at least a start or end date for i.v. mistletoe therapy were included in the final analyses. Descriptive statistics were used to describe patient demographics and the disease stage of patients at diagnosis was described according to Union for International Cancer Control (UICC) staging. The Wilcoxon rank sum (W) test was used to test for differences between groups. Types of mistletoe and conventional therapies received are summarised.

2.3. Analysis of Safety Data. All mistletoe-related adverse events reported by physicians were assessed by the investigators. While increases in body temperatures (especially up to 38°C) are common and often described as a desired or expected effect, an increase in body temperatures >38°C (pyrexia) is internationally regarded as an ADR [32]. In addition, i.v. mistletoe infusions are sometimes accompanied by local reactions surrounding old s.c. application sites. When <5 cm in diameter, these events are considered as expected effects, but when >5 cm, they are recorded as ADRs. Therefore, increased body temperatures >38°C and local reactions >5 cm, along with all other adverse events, were considered as suspected mistletoe ADRs if a causal relationship between i.v. applied mistletoe and an event was described by physicians as at least a reasonable possibility. Decisions of causality were made based on consideration of the dates of mistletoe infusion, of the adverse event, and of other therapies received and on therapy types and doses received, and whether reactions made sense based on known pharmacological activity of therapies and on patient history. I.v. mistletoe is often given on the same day as chemotherapy meaning there is no way to confidently distinguish the causality of some ADRs. However, if given on the same day, mistletoe is almost always administered as the first infusion, before chemotherapeutic premedication (e.g., dexamethasone, prednisolone) and subsequent chemotherapeutic drugs; thus, acute ADRs can be attributed to mistletoe in these cases. All suspected ADRs were classified as MedDRA 15.0 preferred terms (developed under the auspices of the International Conference on Harmonization (ICH)) and grouped by System Organ Class (SOC) [32, 33]. ADRs were evaluated in terms of severity according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 [34] and designated as serious or nonserious according to ICH guidelines [32]. Numbers of ADRs per person were determined and the management and outcome of ADRs are summarised. In order to examine whether experiencing

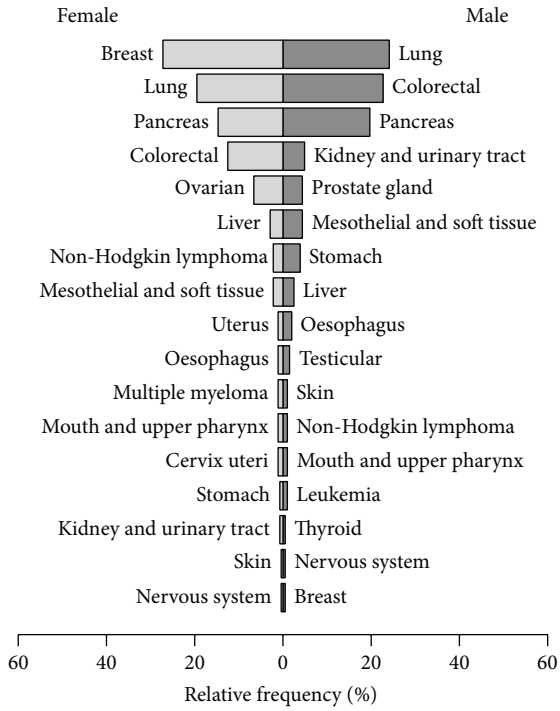


Figure 1: Gender-specific percentages of tumour entities in patients treated with mistletoe preparations via intravenous application.

an ADR might be dose dependent, the doses at which patients experienced ADRs were considered in relation to the doses to which patients were exposed. Basic information about patients treated with i.v. mistletoe and the relative frequency of experiencing an ADR to i.v. mistletoe therapy was compared to results from a previous study on the safety of s.c. mistletoe therapy [27].

3. Results

3.1. Patient and Treatment Characteristics. From a total of 4695 cancer patients treated between July 2003 and June 2013 that were recorded in the NO database, 2805 patients (62.1%) received mistletoe therapy (all forms of administration) and 478 patients (10.2% of all cancer patients and 16.4% of mistletoe patients) received mistletoe via i.v. infusion at least once. Three i.v. mistletoe patients were excluded due to missing information regarding mistletoe preparation types, leaving 475 patients that were analysed in the current study. The patients consisted of 203 males (42.7%) ranging in age from 23 to 86 years and 272 females (57.3%) ranging from 25 to 91 years. The median age of males was 4 years older than that of females (median = 66 : 62, $W = 23945$, $P = 0.01329$) and the overall median age was 63 years. Lung cancer (23% of all patients) was the most frequent cancer disease treated with i.v. mistletoe infusions, followed by pancreatic (18%), colorectal (17%), and breast (17%) cancers (Figure 1). At the time of diagnosis, 46 patients (10%) had a UICC stage of I, 77 (16%) were stage II, 106 (22%) were stage III, and 162 (34%) were stage IV. The UICC stage at diagnosis was not known or applicable for 84 (18%) patients. I.v. mistletoe therapy commenced between 1 day and 16 years (median = 4.3 months) after first diagnosis. At the commencement of i.v. mistletoe therapy, UICC stage was documented for 70% of patients. Of these, 7% were stage II, 18% were stage III, and 75% were stage IV.

Table 1: Characteristics of intravenous mistletoe therapy.

	Patients
Application frequency	
Applied once only	92
2–12 applications	266
>12 applications	117
Duration of therapy	
Less than 1 month	313
1–6 months	83
More than 6 months	79
Other applications of mistletoe	
Intravenous only	123
Intravenous + subcutaneous only	293
Intravenous + intratumoural only	9
Intravenous + subcutaneous + intratumoural	42
Intravenous + other applications*	8

*Other applications were 4 × intrapleural, ascites drainage, intraperitoneal, oral, and inhalation.

Like conventional cancer therapies, mistletoe therapy varies based on cancer type and stage, patient history (and preference), and physician judgement. Application frequencies, durations of therapy, and whether other non-i.v. applications of mistletoe were received are summarised in Table 1. I.v. mistletoe patients mostly received Helixor Mali preparations, followed by Abnoba viscum Fraxini and Helixor Abietis (Table 2). Due to differences in their production, mistletoe dose ranges vary significantly depending on the preparation type. Administered doses of Helixor preparations ranged from 1 mg to 3000 mg with a median dose of 200 mg. Abnoba viscum doses ranged from 0.02 mg to 400 mg with a median of 80 mg and Iscador doses ranged from 0.1 mg to 100 mg with a median of 10 mg (Figure 2).

In addition to mistletoe therapy, 81.5% of patients received a systemic therapy (chemotherapy = 77.5%, immunotherapy = 14.3%, hormonal therapy = 13.1%, bisphosphonates = 11.6% and signal transduction inhibitors = 6.3%), 78.5% of patients had surgery, and 34.1% received radiation therapy. While 445 patients received i.v. mistletoe exclusively (not in combination with another therapy type) at least once, the numbers of patients who received different combinations of therapies within close temporal proximity (very often on the same day) are shown in Table 3. In the cases where i.v. mistletoe was administered alongside chemotherapy (201 from 368 patients who had chemotherapy: 55%), it was documented that 63% received corticosteroids such as prednisolone or dexamethasone as antiemetic or antiallergic medication at least once after administration of i.v. mistletoe and before chemotherapy.

Table 2: Numbers of patients who were exposed to different mistletoe preparations and adverse drug reactions that occurred.

Preparation	Patients	ADRs*	Relative frequency of ADRs (%)#	Types of ADRs
Abnoba viscum (n.s.)	2			
Abnoba viscum Abietis	15			
Abnoba viscum Aceris	2			
Abnoba viscum Amygdali	2			
Abnoba viscum Crataegi	1			
Abnoba viscum Fraxini	133	7 (10)	5.3	Local reaction × 2, pruritus × 2, urticaria × 2, pyrexia, myalgia, hypoaesthesia oral, paraesthesia
Abnoba viscum Mali	2			
Abnoba viscum Pini	4			
Abnoba viscum Quercus	7	1 (1)	14.3	Rash
Total Abnoba viscum	**161**	**8 (11)**	**5.0**	
Helixor (n.s.)	5	0		
Helixor Abietis	106	2 (2)	2.8	Urticaria, pruritus
Helixor Mali	187	8 (12)	4.3	Pyrexia × 5, pruritus × 2, fatigue, infusion site inflammation, headache, hypersensitivity × 2
Helixor Pini	51			
Total Helixor	**321**	**10 (15)**	**3.1**	
Iscador (n.s.)	4			
Iscador Mali	8	1 (2)	12.5	Pyrexia × 2
Iscador Mali c. Arg.	1			
Iscador Mali special	8	2 (3)	25.0	Pyrexia, vomiting, chills
Iscador Pini	2			
Iscador Quercus	9	1 (1)	11.1	Pyrexia
Iscador Quercus special	8			
Iscador Ulmi c. Hg	2			
Total Iscador	**40**	**4 (6)**	**10.0**	
Total	475	22	4.6	

*Number of patients to have an ADR followed by the total number of ADRs in brackets.
#Percentage of patients exposed to that preparation type who had an ADR.
n.s.: not specified.

3.2. Adverse Drug Reactions Attributed to Intravenous Mistletoe Therapy. Of 475 i.v. mistletoe treated patients, 22 patients (4.6%) experienced a total of 32 ADRs (Table 4). Fifteen patients had one ADR, four had two ADRs, and three had three ADRs. The most frequent ADR was pyrexia (1.7% of all patients), followed by pruritus (1.3%) and urticaria (0.6%). In terms of System Organ Class, most ADRs were "general disorders and administration site conditions" (46.9% of ADRs, 2.1% of total patients) or "skin and subcutaneous tissue disorders" (31.2% of ADRs, 2.1% of total patients). The two ADRs described as "local reactions" refer to redness and tenderness with diameters of >5 cm surrounding old s.c. application sites. All ADRs were classified as either mild (59.4%) or moderate (40.6%) and no serious ADRs occurred.

In terms of ADR management, one patient had a pause in therapy, two patients had a reduction in mistletoe dose, the mistletoe preparation was changed for six patients, and therapy was stopped in six patients. One patient with a localised rash was given Tavegil (antihistamine) and Combudoron gel (anthroposophic remedy) was applied. Two patients were treated for pruritus: one received a Fenistil tablet (antihistamine) and Calcium Quercus (anthroposophic remedy) and the other received i.v. Fenistil. A patient with myalgia (muscle pain) was treated with Ibuprofen. All patients completely recovered from i.v. mistletoe-related ADRs without sequelae.

3.3. Investigation of Patients Who Experienced Adverse Drug Reactions to Intravenous Mistletoe Therapy. Of the 22 patients who experienced ADRs, six patients had cancer of the pancreas, three of the breast, two of the colon, two of the rectum, two of the stomach, two of the lung, two of the ovary, one of the uterus, one of the testis, and one patient had non-Hodgkin lymphoma. Of 445 patients who were exposed to

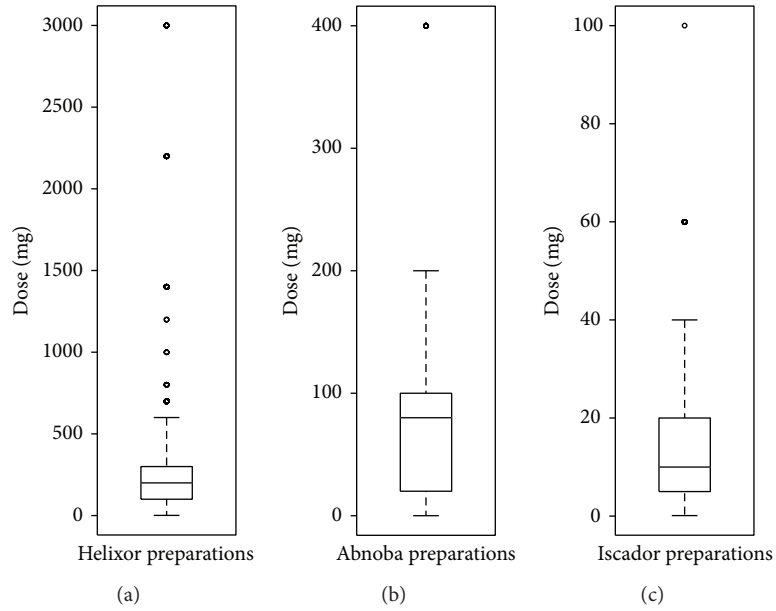


Figure 2: Doses at which intravenous mistletoe applications were given with respect to preparation type. Horizontal lines within boxes show median values, boxes show 25–75% data ranges, the whiskers extend by 1.5x the interquartile range, and circles represent outliers. Note the large differences in scale of the y-axes.

Table 3: Received therapy combinations and adverse drug reactions to those therapies.

Therapy combinations			Patients	Patients who had an ADR (relative frequency)
Intravenous mistletoe therapy alone			445	19 (4.3)
Intravenous mistletoe therapy	+	chemotherapy*	187	3 (1.6)
		chemotherapy* + immunotherapy	17	0
		surgery	13	0
		bisphosphonates	12	0
		chemotherapy* + radiation therapy	12	0
		immunotherapy	9	0
		hormone therapy	5	0
		radiation therapy	3	0
		bisphosphonates + hormone therapy	2	0
		signal transduction inhibitors	2	0
		chemotherapy* + hormone therapy + signal transduction inhibitors	1	0
		chemotherapy* + signal transduction inhibitors	1	0

*A total of 201 patients (55% of all patients that had chemotherapy and 42% of all i.v. mistletoe patients) received i.v. mistletoe and chemotherapy within close temporal proximity (very often on the same day). Of these patients, 63% also received corticosteroids as prechemotherapy medication (i.e., dexamethasone or prednisolone) at least once.

i.v. mistletoe therapy alone, 19 (4.3%) patients experienced an ADR. In comparison, only 3 (1.6%) from 187 patients who received i.v. mistletoe prior to chemotherapy were recorded to have experienced an ADR. Of these, one patient experienced pyrexia (38.5°C) following i.v. mistletoe (Helixor Mali), dexamethasone, and chemotherapy with fluorouracil, docetaxel, folinic acid, and oxaliplatin. One week later the patient had the same treatment minus the mistletoe and reacted in the same way; therefore, it is possible that the initial reaction was also caused by the chemotherapy only and not mistletoe. The second patient experienced pyrexia (40°C) after i.v. mistletoe (Helixor Mali) and gemcitabine (no corticosteroids), while details regarding prechemotherapeutic medications were not available for the third patient who experienced pyrexia (39.5°C) after i.v. mistletoe (Iscador Mali) and doxorubicin.

Table 4: Adverse drug reactions attributed to intravenous mistletoe therapy.

System Organ Class	Preferred term	Total patients	Total events	Incidence (%)
Gastrointestinal disorders	Hypoaesthesia oral	1	1	0.2
	Vomiting	1	1	0.2
Gastrointestinal disorders total		**2**	**2**	**0.2**
General disorders and administration site conditions	Pyrexia	8	10	1.7
	Chills	1	1	0.2
	Fatigue	1	1	0.2
	Infusion site inflammation	1	1	0.2
	Local reaction	2	2	0.4
General disorders and administration site conditions total		**10**	**15**	**2.1**
Immune system disorders	Hypersensitivity	1	2	0.2
Immune system disorders total		**1**	**2**	**0.2**
Musculoskeletal and connective tissue disorders	Myalgia	1	1	0.2
Musculoskeletal and connective tissue disorders total		**1**	**1**	**0.2**
Nervous system disorders	Headache	1	1	0.2
	Paraesthesia	1	1	0.2
Nervous system disorders total		**2**	**2**	**0.2**
Skin and subcutaneous tissue disorders	Pruritus	6	6	1.3
	Rash	1	1	0.2
	Urticaria	3	3	0.6
Skin and subcutaneous tissue disorders total		**10**	**10**	**2.1**
Total		22	32	4.6

For ten out of the 22 patients that experienced an ADR to i.v. mistletoe, it was their first i.v. mistletoe application, and for three of those, their first ever exposure to mistletoe. Eight patients had an ADR after a significant dose increase, one patient had an ADR after a change in preparation, and for three patients there is no plausible explanation. Twelve patients continued i.v. mistletoe therapy with no additional ADRs, seven patients had no further i.v. applications, and three patients had one or more ADRs to further i.v. applications.

In order to investigate whether administration of high doses is associated with an increased risk of experiencing an ADR, doses at which ADRs occurred must be assessed in relation to the doses to which patients were exposed (Figure 2). ADRs to Helixor extracts occurred in response to doses ranging from 100 mg to 400 mg with a median ADR dose of 150 mg (median exposure dose was 200 mg). For Abnoba viscum, ADR doses ranged from 10 mg to 400 mg with a median of 20 mg (median exposure dose was 80 mg) and for Iscador, from 5 mg to 20 mg with a median of 12.5 mg. Therefore, only for Iscador was the median ADR dose (12.5 mg) higher than the median exposure dose (10 mg). Overall, exposure to high doses of mistletoe did not appear to increase the frequency of ADRs.

The absolute and relative frequencies of patients experiencing an ADR and the types of ADRs with respect to preparation type are shown in Table 2. Iscador Mali special, Abnoba viscum Quercus, Iscador Mali, and Iscador Quercus show the highest relative frequencies of all preparation types. The numbers of patients exposed to different preparation types vary largely, however, making it difficult to draw strong conclusions regarding associations of increased ADR risk with certain preparation types.

3.4. Comparison of Intravenous Mistletoe Data to Subcutaneous Mistletoe Data. To assess the safety of i.v. administration of mistletoe extracts in relation to s.c. administration, we compared the present data with results from a previous study on the safety of s.c. mistletoe [27]. There was no significant difference between the ages of patients treated with i.v. or s.c. mistletoe ($W = 478033$, $P = 0.115$). The proportion

of female patients who received i.v. mistletoe applications was lower compared to s.c. mistletoe ($W = 403553$, $P < 0.001$), while a greater proportion of i.v. patients had stage IV cancer at diagnosis compared to s.c. patients ($W = 367656.5$, $P < 0.001$). In terms of ADRs, i.v. mistletoe applications resulted in a lower frequency of ADRs (4.6% of exposed patients) compared to s.c. mistletoe applications (8.4% of exposed patients) (OR = 0.53, CI = 0.33–0.82, $P = 0.005$) [27].

4. Discussion

The present study summarised the use of i.v. mistletoe applications in 475 cancer patients and assessed suspected i.v. mistletoe ADRs. Most patients were exposed to preparations of Helixor Mali, followed by Abnoba viscum Fraxini and Helixor Abietis. Helixor Mali preparations (extracts from mistletoe grown on apple trees) are recommended for tumours of the gastrointestinal tract, lower abdomen, and breast, while Abietis preparations (extracts from mistletoe grown on fir trees) are recommended for lung, prostate, and head and neck cancers [35]. Considering that tumours of the lung, breast, colorectal, and pancreas were the most frequent cancer entities, a high frequency of exposure to Mali and Abietis preparations was expected. Furthermore, the majority of patients were UICC stage IV cancer patients at the commencement of i.v. mistletoe therapy, indicating metastatic disease. According to product information, mistletoe preparations with high concentrations of lectins and viscotoxins (e.g., Abnoba viscum Fraxini) are recommended for treatment of metastatic tumour disease [36].

Only 4.6% of all patients that received i.v. mistletoe applications experienced an ADR. All ADRs were either mild or moderate in severity and most patients only experienced one ADR. "General disorders and administration site conditions" (especially pyrexia) were the most common types of ADRs, occurring in 2.1% of patients. "Skin and subcutaneous tissue disorders" (generalised pruritus and urticaria) were also experienced by only 2.1% of patients. It is not surprising that most ADRs were systemic reactions rather than local reactions since i.v. administration results in rapid distribution of the preparation throughout the systemic circulation. The relative frequency of ADRs to i.v. mistletoe delivered alone was more than double the relative frequency of ADRs to i.v. mistletoe delivered prior to chemotherapy. This is probably partly due to ADRs being less likely to be documented for patients who received i.v. mistletoe prior to chemotherapy, since adverse events might have been attributed to chemotherapy instead. Furthermore, with 63% of patients receiving anti-inflammatory corticosteroids (e.g., dexamethasone, prednisolone) as antiemetic or antiallergic medication at least once after i.v. mistletoe infusion and before infusion of chemotherapeutic drugs, it is likely that ADRs to mistletoe, especially pyrexia, were prevented. We therefore believe that the relative frequency of 4.3% (mistletoe alone) of patients experiencing an ADR to i.v. mistletoe provides a more reliable picture than that of 1.6% (mistletoe and chemotherapy).

Despite the fact that increased dose is commonly considered a major risk factor for experiencing an ADR, an obvious relationship between dose and the frequency of ADRs was not observed in the present study. Only Iscador had a higher median ADR dose compared to the median exposure dose. It is possible that an effect of dose on the risk of patients having an ADR is not clear due to their being too few events for a concise investigation. Nevertheless, an interesting observation is that almost all ADRs occurred in response to a first ever exposure to mistletoe, a first exposure to i.v. administration of mistletoe, or to an abrupt increase in dose. Out of 15 patients that continued i.v. mistletoe applications after an ADR, only three experienced subsequent ADRs, suggesting that ADRs were generally one-off events related to first exposure or dose elevation.

Although it is difficult to draw strong conclusions due to large variations in exposure frequencies of different preparation types, Iscador Mali special, Abnoba viscum Quercus, Iscador Mali, and Iscador Quercus resulted in the highest relative frequencies of ADRs. It has been reported previously that Iscador preparations frequently induce fever [6, 37]. Our results coincide with this observation, since 4 of the 6 ADRs (66.7%) to Iscador preparations were fever reactions (pyrexia). This could be related to the manufacturing process of fermentation, unique to Iscador preparations [6, 37, 38]. In contrast, less frequent but more varied ADRs were observed in response to Abnoba viscum Fraxini and Helixor Mali, including skin and hypersensitivity reactions.

Preliminary results of a qualitative study on physicians applying i.v. mistletoe suggests that the safety of i.v. application of mistletoe preparations strongly depends on the speed of infusion (Gunver Kienle, personal communication). Unfortunately no information was available to assess the relationship between infusion speed and ADR risk in the current study.

In the interest of determining whether "off-label" i.v. administration of mistletoe is as safe as the more common s.c. form of administration, which has marketing authorisation, we compared the present i.v. ADR data with results of a previous study on s.c. mistletoe therapy [27]. Although we previously reported on expected effects of s.c. mistletoe applications in addition to ADRs, we decided not to assess expected effects of i.v. mistletoe infusions for the following reasons: (i) expected effects are less defined for i.v. applications since it is an off-label use of mistletoe, (ii) only ADRs are critical for an analysis of safety, while expected effects might be more important for an analysis of efficacy, and (iii) data regarding expected effects is much more vulnerable to reporting bias (e.g., slight rises in body temperature might not be documented as thoroughly as temperatures >38°C). In regard to ADRs, it was found that patients were almost twice as likely to experience an ADR to s.c. therapy compared to i.v. therapy. Also less ADRs were reported per person in response to i.v. therapy and severe ADRs occurred in response to s.c. but not to i.v. therapy. Whether the lower frequency of ADRs in i.v. patients can be partly explained by differing demography and morbidity is unclear. There was no significant difference in age between the groups, but there was

a lower percentage of females in the i.v. group and a greater percentage of late stage cancer patients. Female gender has previously been identified as a risk factor for experiencing an ADR, while late stage cancer is associated with a suppressed immune system and might have contributed to a lower frequency of ADRs in i.v. compared to s.c. patients [27, 39–41]. The most notable difference between the ADRs that occurred in response to s.c. applications compared to i.v. applications is a much higher frequency of local reactions and pyrexia after s.c. applications and of skin disorders, such as pruritus, after i.v. applications. This makes sense when considering the pharmacokinetics of each of these administration types. I.v. administration involves immediate delivery of a drug into the systemic circulation leading to rapid achievement of the peak drug concentration, known as C_{max}. On the other hand, s.c. administration of a drug involves slow absorption into the circulation taking longer to achieve C_{max}, which by comparison is often lower, but more sustained [42]. Therefore, while i.v. application can rapidly reach a high C_{max}, thereby increasing the risk of systemic, hypersensitivity type reactions (pruritus, urticaria), s.c. application is more likely to induce a local inflammatory response accompanied by cytokine activation of a febrile response [25, 43]. Furthermore, i.v. application not only results in quicker distribution of a drug than s.c. application, but also in a quicker elimination [42, 44]. Studies comparing the half-life of mistletoe lectins and other molecules of comparable size after application by s.c. or i.v. administration have shown that molecules applied i.v. were eliminated up to three times faster than when applied s.c [45–47]. This finding might also help to explain the much higher frequency of pyrexia observed in s.c. patients compared to i.v. patients. Other possible explanations for the difference in ADR frequencies relate to differences in the way i.v. and s.c. mistletoe therapies are delivered. The majority of i.v. patients (70.5%) also received s.c. mistletoe injections. Since patients usually receive mistletoe via the s.c. route earlier in the treatment plan compared to i.v. mistletoe, it is possible that patients who experienced ADRs to s.c. mistletoe were less likely to later receive i.v. mistletoe. Or even if they still received i.v. mistletoe, a tolerance might have developed over time. Furthermore, s.c. injections are often given 2-3 times per week for many months or years, compared to i.v. infusions which are generally given less frequently and for shorter periods of time. Therefore, there is an increased opportunity for patients receiving s.c. applications to suffer an ADR, or for s.c. applications to be wrongly attributed an ADR.

Although there is limited literature available regarding the safety of i.v. mistletoe applications, especially of high doses, our results tend to be in agreement with the few studies that were found. I.v. mistletoe therapy is often offered with the aim to improve quality of life in a palliative setting, but is also used postoperatively across a range of tumour types to prevent recurrence and concurrent to chemotherapy to reduce ADRs [6, 19]. Our results are in agreement with the findings of Wiebelitz and Beer who concluded that "high dose intravenous mistletoe treatment is safe with few and manageable adverse events" [48]. Comparable to our results, the most common ADRs identified in a series of 17 patients with 107 i.v. applications of high dose mistletoe were temperature related (mild to moderate pyrexia) or skin related (generalised exanthema and pruritus) [48]. In contrast, s.c. applications are associated with frequent injection site reactions, along with pyrexia [25]. A retrospective study focusing on therapy with high doses of mistletoe found i.v. administration to be very well tolerated with only occasional light nausea and/or aching limbs with the very first infusion, while pyrexia was reported in <1% of patients [6]. Other reports on safety and tolerability of i.v. mistletoe applications have ranged from no ADRs at all to rare allergic incidents [18, 49, 50].

Limitations of this like most observational studies include the possibility of missing or erroneous data and assessment of a heterogeneous group of patients in terms of demographic, tumour entities, disease stage, varied doses, and frequencies of i.v. mistletoe applications and of concurrent therapies. On the other hand, this study attempted to provide an accurate picture of the clinical use of i.v. mistletoe therapy and of the safety of this use. There was no control group for this study, meaning that ADRs may have been overreported due to our inability to calculate the frequency of disease-related, other therapy-related, or spontaneous adverse events. Furthermore, since nearly half of the patients received intravenous mistletoe infusions alongside chemotherapy at least once, it is possible that some ADRs to chemotherapy might have been wrongly attributed to mistletoe, or vice versa.

5. Conclusions

I.v. infusion of mistletoe preparations by experienced clinicians appears to be safe, with an ADR frequency of almost half of that observed for s.c. mistletoe applications. Furthermore, only mild to moderate ADRs occurred and all patients recovered without sequelae. As a result, prospective studies assessing the efficacy of i.v. application of mistletoe preparations are recommended.

Conflict of Interests

The Network Oncology was funded by the Software AG Stiftung Darmstadt, Germany; Weleda AG Arlesheim, Switzerland; Abnoba GmbH Pforzheim, Germany; and Helixor GmbH Rosenfeld, Germany. By contract, researchers were independent from the funder. H. Matthes has been a member of Weleda AG directory board since March 2012. The other authors declare that they have no competing interests.

Acknowledgments

The authors would like to thank all medical documentation officers and all NO partners and participating institutions. MedDRA trademark is owned by IFPMA on behalf of ICH.

References

[1] M. A. Horneber, G. Bueschel, R. Huber, K. Linde, and M. Rostock, "Mistletoe therapy in oncology," *Cochrane Database of Systematic Reviews*, no. 2, Article ID CD003297, 2008.

[2] M. Mabed, L. El-Helw, and S. Shamaa, "Phase II study of viscum fraxini-2 in patients with advanced hepatocellular carcinoma," *British Journal of Cancer*, vol. 90, no. 1, pp. 65–69, 2004.

[3] M. Orange, M. Fonseca, A. Lace, H. B. von Laue, and S. Geider, "Durable tumour responses following primary high dose induction with mistletoe extracts: two case reports," *European Journal of Integrative Medicine*, vol. 2, no. 2, pp. 63–69, 2010.

[4] R. Huber, H. Barth, A. Schmitt-Gräff, and R. Klein, "Hypereosinophilia induced by high-dose intratumoral and peritumoral mistletoe application to a patient with pancreatic carcinoma," *Journal of Alternative and Complementary Medicine*, vol. 6, no. 4, pp. 305–310, 2000.

[5] F. Schad, J. Axtner, D. Buchwald et al., "Intratumoral mistletoe (*Viscum album* L) therapy in patients with unresectable pancreas carcinoma: a retrospective analysis," *Integrative Cancer Therapies*, 2013.

[6] M. Orange, *Mistletoe therapy for cancer patients [M.S. thesis]*, University of Birmingham, 2010, http://www.anthromed.org/.

[7] I. F. Pryme, S. Bardocz, A. Pusztai, and S. W. B. Ewen, "Suppression of growth of tumour cell lines in vitro and tumours in vivo by mistletoe lectins," *Histology and Histopathology*, vol. 21, no. 1–3, pp. 285–299, 2006.

[8] T. J. Yoon, Y. C. Yoo, O. B. Choi et al., "Inhibitory effect of Korean mistletoe (*Viscum album* coloratum) extract on tumour angiogenesis and metastasis of haematogenous and non-haematogenous tumour cells in mice," *Cancer Letters*, vol. 97, no. 1, pp. 83–91, 1995.

[9] K. S. Zaenker, H. Matthes, P. R. Bock, and J. Hanisch, "A specific mistletoe preparation (Iscador-Qu) in colorectal cancer (CRC) patients: more than just supportive care?" *Journal of Cancer Science & Therapy*, vol. 4, no. 9, pp. 264–270, 2012.

[10] S. Elluru, J.-P. D. van Huyen, B. Wootla et al., "Tumor regressive effects of *Viscum album* preparations. Exploration of immunomodulatory mechanisms," *Medicina*, vol. 67, supplement 2, pp. 85–89, 2007.

[11] J. Kuehn, "Different routes of application in mistletoe therapy—effect on bone marrow and blood profile. Clinical significance," *Phytomedicine*, vol. 18, supplement 1, p. S11, 2011.

[12] R. Zerm, M. Kröz, and H. Matthes, "Treatment of a patient suffering from advanced colorectal carcinoma with intravenous mistletoe application and Ardalan-chemotherapy," in *Fortschritte in der Misteltherapie: Aktueller Stand der Forschung und Klinische Anwendung*, R. Scheer, R. Bauer, H. Becker, V. Fintelmann, F. H. Kemper, and H. Schilcher, Eds., KVC, Essen, Germany, 2005.

[13] F. Schad, M. Kröz, M. Girke, H.-P. Lemmens, D. Brauer, and B. Matthes, "Intraläsionale und kombinierte subkutan-intravenöse Misteltherapie bei einem Patienten mit Kolonkarzinom," *Der Merkurstab*, vol. 52, no. 6, pp. 399–406, 1999.

[14] J. Pieper, "Der Einsatz von Plenosol bei inoperablen Tumoren," *EHK*, vol. 9, pp. 547–549, 1987.

[15] G. Spahn, V. Schindler, C. Heyder et al., "Integrative Behandlung des Pankreaskarzinoms unter Verwendung von Viscum-Präparaten—Ergebnisse aus dem klinischen Register der Klinik Öschelbronn 2007–2010," in *Die Mistel in der Tumortherapie 3—Aktueller Stand der Forschung und klinische Anwendung*, R. Scheer, S. Alban, H. Becker, and R. Stange, Eds., pp. 265–278, KVC, Essen, Germany, 2013.

[16] P. Wolf, N. Freudenberg, and M. Konitzer, "Analgetische und stimmungsaufhellende Wirkung bei Malignom-Patienten unter hochdosierter *Viscum album*-Infusionstherapie (Vysorel)," *Deutsche Zeitschrift für Onkologie*, vol. 26, pp. 52–54, 1994.

[17] M. Girke, *Innere Medizin: Grundlagen und therapeutische Konzepte der Anthroposophischen Medizin*, Salumed, Berlin, Germany, 2010.

[18] A. Büssing, U. Bückner, U. Enser-Weis et al., "Modulation of chemotherapy-associated immunosuppression by intravenous application of *Viscum album* L. extract, (Iscador): a randomised phase II study," *European Journal of Integrative Medicine*, vol. 1, supplement 1, pp. 2–3, 2008.

[19] A. Büssing, "Immune modulation using mistletoe (*Viscum album* L.) extracts Iscador: influencing cell function through subcutaneous and intravenous application," *Arzneimittel-Forschung*, vol. 56, no. 6A, pp. 508–515, 2006.

[20] M. Schink, W. Tröger, A. Dabidian et al., "Mistletoe extract reduces the surgical suppression of natural killer cell activity in cancer patients. A randomized phase III trial," *Forschende Komplementarmedizin*, vol. 14, no. 1, pp. 9–17, 2007.

[21] A. Büssing, M. Bischof, W. Hatzmann et al., "Beeinflussung der Granulozytenfunktion durch einmalige perioperative Mistelextrakt-Infusion," *Deutsche Zeitschrift für Onkologie*, vol. 36, no. 4, pp. 148–153, 2004.

[22] M. Cazacu, T. Oniu, C. Lungoci et al., "The influence of Isorel on the advanced colorectal cancer," *Cancer Biotherapy and Radiopharmaceuticals*, vol. 18, no. 1, pp. 27–34, 2003.

[23] J. Beuth, B. Schneider, and J. M. Schierholz, "Impact of complementary treatment of breast cancer patients with standardized mistletoe extract during aftercare: a controlled multicenter comparative epidemiological cohort study," *Anticancer Research*, vol. 28, no. 1B, pp. 523–527, 2008.

[24] J. Eisenbraun, R. Scheer, M. Kröz, F. Schad, and R. Huber, "Quality of life in breast cancer patients during chemotherapy and concurrent therapy with a mistletoe extract," *Phytomedicine*, vol. 18, no. 2-3, pp. 151–157, 2011.

[25] P. J. Mansky, D. B. Wallerstedt, T. S. Sannes et al., "NCCAM/NCI phase 1 study of mistletoe extract and Gemcitabine in patients with advanced solid tumors," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 964592, 11 pages, 2013.

[26] F. Schad, J. Axtner, A. Happe et al., "Network oncology (NO)—a clinical cancer register for health services research and the evaluation of integrative therapeutic interventions in anthroposophic medicine," *Forschende Komplementärmedizin*, vol. 20, no. 5, pp. 369–375, 2013.

[27] M. L. Steele, J. Axtner, A. Happe, M. Kröz, H. Matthes, and F. Schad, "Adverse drug reactions and expected effects to therapy with subcutaneous mistletoe extracts (*Viscum album* L.) in cancer patients," *Evidence-Based Complementary and Alternative Medicine*, vol. 2014, Article ID 724258, 11 pages, 2014.

[28] F. Schad, C. Teodoridis, U. Albrecht et al., "Network Oncology (NO)—a European approach to health service research on cancer treatment with focus on concurrent use of complementary therapies," *European Journal of Integrative Medicine*, vol. 1, no. 1, pp. 12–13, 2008.

[29] F. Schad, J. Pissarek, U. Albrecht, and H. Matthes, "QuaDoSta (quality assurance, documentation and statistics)—a flexible, platform-independent, open source database for oncological

documentation," *Cancer Research and Clinical Oncology*, vol. 130, supplement op, p. 785, 2004.

[30] J. M. M. Evans, D. McNaughton, P. T. Donnan, and T. M. MacDonald, "Pharmacoepidemiological research at the Medicines Monitoring Unit, Scotland: data protection and confidentiality," *Pharmacoepidemiology and Drug Safety*, vol. 10, no. 7, pp. 669–673, 2001.

[31] *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, R Core Team, Vienna, Austria, 2012.

[32] ICH, *E2A: Clinical safety data management: definitions and standards for expedited reporting*, http://www.ich.org/.

[33] UMC, Safety monitoring of medicinal products: guidelines for setting up and running a pharmacovigilance centre, http://www.who.int/medicinedocs.

[34] CTEP, Common Terminology Criteria for Adverse Events, http://ctep.cancer.gov/protocolDevelopment/.

[35] "Mistletoe products from Helixor," 2014, http://www.helixor.com/integrative-cancer-therapy/mistletoe-therapy/products/.

[36] Mistletoe therapy: patient information, 2014, http://www.abnoba.com/start.aspx.

[37] R. Wagner, *Praktische Prüfungsmethoden zur Beurteilung der Misteltherapie: ein Leitfaden für den niedergelassenen Arzt. Beiträge zur Krebstherapie*, Urachhaus, Stuttgart, Germany, 1994.

[38] "Iscador," March 2014, http://allgemein.mistel-therapie.de/index.php5?page=39&lang=1.

[39] Y. Zopf, C. Rabe, A. Neubert et al., "Women encounter ADRs more often than do men," *European Journal of Clinical Pharmacology*, vol. 64, no. 10, pp. 999–1004, 2008.

[40] N. Rouas-Freiss, P. Moreau, C. Menier, and E. D. Carosella, "HLA-G in cancer: a way to turn off the immune system," *Seminars in Cancer Biology*, vol. 13, no. 5, pp. 325–336, 2003.

[41] L. Rasmussen and A. Arvin, "Chemotherapy-induced immunosuppression," *Environmental Health Perspectives*, vol. 43, pp. 21–25, 1982.

[42] R. J. Wills, S. Dennis, and H. E. Spiegel, "Interferon kinetics and adverse reactions after intravenous, intramuscular, and subcutaneous injection," *Clinical Pharmacology and Therapeutics*, vol. 35, no. 5, pp. 722–727, 1984.

[43] M. McDonald and D. J. Sexton, *Drug Fever. UpToDate*, 2012, http://www.uptodate.com/contents/drug-fever#H11.

[44] P. Schöffski, S. Riggert, P. Fumoleau et al., "Phase I trial of intravenous aviscumine (rViscumin) in patients with solid tumors: a study of the European Organization for Research and Treatment of Cancer New Drug Development Group," *Annals of Oncology*, vol. 15, no. 12, pp. 1816–1824, 2004.

[45] R. Huber, J. Eisenbraun, B. Miletzki et al., "Pharmacokinetics of natural mistletoe lectins after subcutaneous injection," *European Journal of Clinical Pharmacology*, vol. 66, no. 9, pp. 889–897, 2010.

[46] C. A. Jacobs, D. H. Lynch, E. R. Roux et al., "Characterization and pharmacokinetic parameters of recombinant soluble interleukin-4 receptor," *Blood*, vol. 77, no. 11, pp. 2396–2403, 1991.

[47] N. Hayashi, H. Kinoshita, E. Yukawa, and S. Higuchi, "Pharmacokinetic analysis of subcutaneous erythropoietin administration with nonlinear mixed effect model including endogenous production," *British Journal of Clinical Pharmacology*, vol. 46, no. 1, pp. 11–19, 1998.

[48] K. R. Wiebelitz and A. M. Beer, "High dose intravenous mistletoe treatment—clinical results, laboratory findings and adverse events in a series of 17 patients with 107 intravenous applications," *Phytomedicine*, vol. 18, supplement 1, p. S11, 2011.

[49] P. Wolf, "Die Mistel-Infusionstherapie zur Behandlung von Tumorleiden," *Naturamed*, vol. 4, no. 12, pp. 720–724, 1989.

[50] G. S. Kienle, *Verträglichkeit, Nebenwirkungen, Überempfindlichkeitsreaktionen, Toxizität*, Wissenschaftliche Informationen, 2005, http://wissenschaft.mistel-therapie.de/index.php5?page=57.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 620974, 8 pages
http://dx.doi.org/10.1155/2014/620974

Research Article

Evaluation of Preclinical Assays to Investigate an Anthroposophic Pharmaceutical Process Applied to Mistletoe (*Viscum album* L.) Extracts

Stephan Baumgartner,[1,2,3] Heidi Flückiger,[1] Matthias Kunz,[1] Claudia Scherr,[1] and Konrad Urech[1]

[1] *Hiscia Institute, Society for Cancer Research, Kirschweg 9, 4144 Arlesheim, Switzerland*
[2] *Institute of Integrative Medicine, Gemeinschaftskrankenhaus, University of Witten-Herdecke, Gerhard-Kienle-Weg 4, 58313 Herdecke, Germany*
[3] *Institute of Complementary Medicine, Inselspital, University of Bern, Imhoof-Pavillon, 3010 Bern, Switzerland*

Correspondence should be addressed to Stephan Baumgartner; s.baumgartner@vfk.ch

Received 17 September 2013; Accepted 11 March 2014; Published 4 May 2014

Academic Editor: Steven Rosenzweig

Copyright © 2014 Stephan Baumgartner et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Extracts from European mistletoe (*Viscum album* L.) developed in anthroposophic medicine are based on specific pharmaceutical procedures to enhance remedy efficacy. One such anthroposophic pharmaceutical process was evaluated regarding effects on cancer cell toxicity *in vitro* and on colchicine tumor formation in *Lepidium sativum*. Anthroposophically processed *Viscum album* extract (APVAE) was produced by mixing winter and summer mistletoe extracts in the edge of a high-speed rotating disk and was compared with manually mixed *Viscum album* extract (VAE). The antiproliferative effect of VAE/APVAE was determined in five cell lines (NCI-H460, DU-145, HCC1143, MV3, and PA-TU-8902) by WST-1 assay *in vitro*; no difference was found between VAE and APVAE in any cell line tested ($P > 0.14$). Incidence of colchicine tumor formation was assessed by measurement of the root/shoot-ratio of seedlings of *Lepidium sativum* treated with colchicine as well as VAE, APVAE, or water. Colchicine tumor formation decreased after application of VAE (−5.4% compared to water, $P < 0.001$) and was even stronger by APVAE (−8.8% compared to water, $P < 0.001$). The high-speed mistletoe extract mixing process investigated thus did not influence toxicity against cancer cells but seemed to sustain morphostasis and to enhance resistance against external noxious influences leading to phenomenological malformations.

1. Introduction

Use of extracts from European mistletoe (*Viscum album* ssp. *album* L.) for cancer treatment is based on suggestions of Steiner [1], who founded anthroposophic medicine (AM) together with Wegman in the 1920s [2]. Subsequent preclinical research into mistletoe extracts revealed a multitude of highly interesting compounds (e.g., mistletoe lectins, viscotoxins, alkaloids, triterpenes, and oligo- and polysaccharides) with antitumoral (cytotoxic, antiangiogenic) as well as immunomodulating properties [3–5]. Clinical application increased overall survival time and improved quality of life of patients with various forms of cancer [6–8] and furthermore seemed to be safe [8, 9].

There is a variety of mistletoe extracts available, differing in mistletoe host trees and extraction methods as well as further pharmaceutical processing [10]. Mistletoe extracts used in anthroposophic medicine (Iscador, Helixor, abnobaVISCUM, Iscucin, and Isorel) rely on specific anthroposophic pharmaceutical procedures that were developed on the basis of suggestions of Steiner to enhance anticancer efficacy of mistletoe [11]. In general, extracts from mistletoe harvested in different seasons (summer and winter) are mixed together in a sophisticated, specifically designed apparatus.

From a pharmaceutical point of view, the question arises whether the anthroposophic pharmaceutical procedures applied indeed enhance anticancer efficacy as intended. This in turn leads to the following question: which methods

are suited and applicable to study any such increase in remedy efficacy? In general, mechanism studies performed on the cellular level are considered to be the scientifically and ethically appropriate first step prior proceeding to animal or human experimentation.

Correspondingly, determination of the toxicity of differently processed mistletoe extracts in cancer cell lines can be seen as first step to study the pharmaceutical process in question. This approach is based on the basic assumption that cancer is a cell-based disease, caused by multiple mutations of normal cells resulting in malignant cells, eventually supported by nonmalignant cells in the environment [12, 13].

A complementary view of cancer is that this disease is primarily a phenomenon at the level of multicellular organization. According to this view, tumors form if the organism as a whole is too weak to control individual cell growth [14]. Lately, the tissue organization field theory (TOFT) was put forward to challenge the somatic mutation theory (SMT) of cancer [15]. Anthroposophic medicine also presumes tumors to be failures of morphostasis and that cancer is primarily a coordination problem of the different super- and subordinated organizational levels within an organism [16, 17]. Correspondingly, anthroposophic tumor therapy complements conventional antitumor therapy with special measures to reestablish superordinate control and regulation [18, 19].

Methods and tools of preclinical research in cancer depend on the basic underlying paradigm, that is, the basic concept and understanding of cancer, its causes, and its formation (e.g., SMT or TOFT). Approaches based on the somatic mutation theory firstly center on cell-based bioassays and consequently on cell-tissue interactions. Preclinical approaches in cancer research based on the morphogenetic paradigm are much less developed [14]. For principal reasons, these primarily center on cancer in nonhuman biological models as a disorder phenomenon of the general morphological structure of entire organisms and subsequently also tissue-cell interactions, preferably in three-dimensional *in vitro* models.

The aim of the present study was to compare two complementary preclinical approaches to investigate the effects of one particular anthroposophic pharmaceutical process designed to enhance mistletoe efficacy. In this process, extracts from European mistletoe harvested in summer and winter are mixed together in the edge of a high-speed rotating disk. First, possible alterations in cell-based toxicity were tested in a panel of five cancer cell lines. Second, we investigated the potential of a newly developed morphological bioassay in which correspondingly processed mistletoe extracts were examined regarding their potential to protect plants from the damaging, shape changing effects of colchicine (colchicine tumor formation in *Lepidium sativum* L.).

2. Materials and Methods

2.1. Mistletoe Extracts and Pharmaceutical Processing. To produce the constituents for the investigated mistletoe extracts, mistletoe plants (*Viscum album* L. ssp. *album*) growing on either apple trees (*Malus domestica* Borkh.) or pine trees (*Pinus sylvestris* L.) were harvested shortly before midsummer and end of the year, respectively. The one- to two-year-old leaves, the stems, the generative organs, and in the winter harvest the ripened berries were mechanically opened in a roll mill and extracted by fermentation with mistletoe derived lactobacillus in distilled water. After three-day fermentation, the extract was obtained by pressing and sterile filtration. The basic winter and summer mistletoe extracts possess a drug-extract ratio of 1 : 5 (extract of 200 mg mistletoe fresh plant in 1 mL fluid).

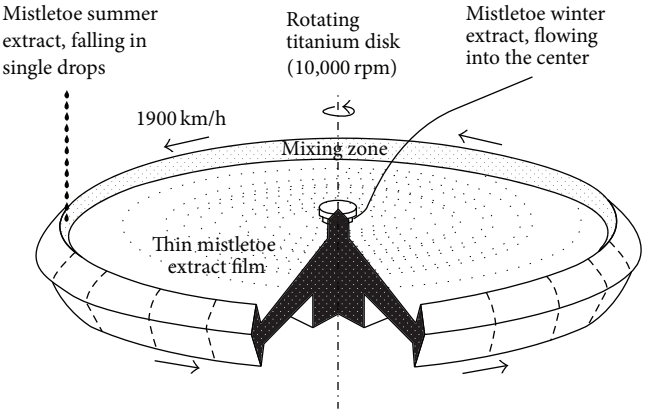


Figure 1: Schematic representation of the turning disc of the apparatus that has been used since autumn of 1996 at Hiscia Institute (Arlesheim, Switzerland) to produce anthroposophically processed *Viscum album* extract (APVAE). The winter mistletoe extract flows into the center of a 1 m diameter titanium disc rotating at 10,000 rpm and then spreads out horizontally. At the upturned disc edge ("mixing zone"), it combines with summer mistletoe extract dropping vertically from a height of 1 meter. Summer mistletoe extract enters the rotating disk not only at one position as schematically outlined above but also at 12 positions, equally spaced along the edge of the disk.

To produce anthroposophically processed *Viscum album* extract (APVAE), winter mistletoe extract is fed into the center of a 1 m diameter titanium disc rotating at 10,000 rpm. From here, it spreads out horizontally and combines subsequently with summer mistletoe extract dripping vertically from a height of 1 m into the upturned edge of the disc (Figure 1). The process runs continuously. After an average period of 20 seconds, the preparation leaves the disc edge. The disc is enclosed in an airtight stainless steel container floated with helium gas in order to avoid sonic boom, to reduce thermal friction loss, and to avoid oxidative reactions of the extract.

This specific blending process for winter and summer mistletoe extract was developed at the Hiscia Institute of the Society for Cancer Research (Arlesheim, Switzerland), with emphasis on realizing Steiner's original suggestions as precisely as possible [11, 20]. This process is being used for the production of Iscador.

As comparison sample relative to APVAE, we used a *Viscum album* extract (VAE) consisting of the same constituents

as the APVAE sample (winter and summer mistletoe extracts of the same batch as used for production of APVAE). For this purpose, a container was successively filled with winter and summer mistletoe extracts that were mixed together homogenously by upending the container 10 times.

For the experiments, APVAE and VAE were sterile filtered immediately after mixing and filled into 1 mL glass ampoules under aseptic conditions for storage at 4°C. Two sorts of mistletoe extracts were investigated: extracts from mistletoe plants growing on the host tree apple (APVAE/VAE Mali) and extracts from mistletoe plants growing on the host tree pine (APVAE/VAE Pini).

2.2. Cell Culture. The human carcinoma cell lines HCC1143 (breast carcinoma), PA-TU-8902 (pancreas adenocarcinoma), DU-145 (prostate carcinoma), and NCI-H460 (lung carcinoma) were obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Germany). MV3 cells (human metastatic melanoma) were kindly provided by Anka Dahl, University Hospital, Hamburg-Eppendorf (Germany). All cell lines except PA-TU-8902 were cultivated under standard cell culture conditions (37°C, 95% relative humidity, 5% CO_2) in RPMI-1640 medium (Sigma-Aldrich, Buchs, Switzerland) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich, Switzerland), 2 mM L-Glutamine (Sigma-Aldrich, Switzerland), and Penicillin-Streptomycin (Sigma-Aldrich, Switzerland; Penicillin 10,000 units/mL and 10 mg/mL Streptomycin). Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich, Switzerland) supplemented with the ingredients mentioned above was used to cultivate PA-TU-8902 cells. The cells were maintained in adherent, exponential growth in 25 cm^2 culture flasks (TPP, Faust Laborbedarf AG, Switzerland) and cells from subconfluent monolayer were harvested by brief exposure to trypsin-EDTA solution (Sigma-Aldrich, Switzerland).

2.3. Cell Growth Assay. Cells were seeded at a density of 2500 cells in 90 μL/well in a 96-well plate (TPP, Faust Laborbedarf AG, Switzerland) and incubated under standard conditions for 4 hours to attain adherence to the bottom. Thereafter, the cells were exposed during 48 hours to different concentrations of appropriate dilutions of *Viscum album* extract (VAE Mali, lot 1210/2338), anthroposophically processed *Viscum album* extract (APVAE Mali, lot 1210/2339), or blank medium (control) by adding a volume of 90 μL/well resulting in a total volume of 180 μL/well. After 2-day incubation, none of the cell lines had reached full confluence.

In this assay, only VAE/APVAE Mali was used due to the selective toxicity of the cancer cell lines used towards mistletoe lectins (VAE/APVAE Pini is almost void of mistletoe lectins). VAE Mali (lot 1210/2338) had a concentration of 10,400 ± 283 ng/mL mistletoe lectin, and APVAE Mali (lot 1210/2339) had a concentration of 10,350 ± 71 ng/mL. The mistletoe lectin content (ML I, ML II, and ML III) was determined by means of an ELISA with the aid of a combination of monoclonal antibodies specifically directed against the mistletoe lectins of mistletoes growing on deciduous trees [21].

Cell growth was assessed by using a colorimetric assay based on the cleavage of the tetrazolium salt WST-1 (Roche Diagnostica, Rotkreuz, Switzerland) to formazan by the mitochondrial dehydrogenase in viable cells [22]. For this purpose, after 48 h incubation of the cells, 20 μL of WST-1 reagent was added and allowed to react for 4 h at 37°C. Quantification of the produced formazan was done by a multiwell spectrophotometer (Labsystem Multiskan RC, BioConcept AG, Allschwil, Switzerland) and absorbance was measured against a background control (cell free) at a wavelength of 450 nm and a reference wavelength of 690 nm. All substances were tested in triplicates and each experiment was repeated 8 times. Note that in this assay cell growth reflects the total mitochondrial reducing ability of the cell populations, which itself is strongly influenced by the corresponding proliferation rate (in a positive way) and by the occurrence of cell death (in a negative way).

Possible effects depending on the position of the samples within the 96-well plate were eliminated by systematic exchange of the sample positions. All manipulations by the experimenter were performed with coded (blinded) VAE and APVAE samples. VAE and APVAE samples were diluted to obtain the following final concentrations of the extracts: 800, 600, 400, 200, 100, 50, 25, and 12.5 μg/mL. Furthermore, VAE/APVAE untreated cells were assessed. Each repetition of the experiments was done with freshly diluted extracts.

Viability of the cells was calculated by setting the OD values of the cell free background control to 0% and the OD values of the VAE/APVAE-untreated cells to 100%. ED50 was determined for each experiment by quadratic regression of viability versus concentration, including the concentrations with viability > 15% (12.5–200 μg/mL for NCI-H460, DU-145, HCC1143, and MV3 and 12.5–800 μg/mL for PA-TU-8902). Mean ED50 values ± standard deviation/error were calculated based on the eight ED50 values obtained in the eight independent experiments for each cell line. ED50 values for VAE and APVAE were compared using a t-test for independent samples. Calculations were carried out with Microsoft Excel for Mac 2011 (version 14.2.2) and Statistica 4 (Statsoft Inc., Tulsa, USA).

2.4. Morphological Colchicine Tumor Assay. Seedlings of *Lepidium sativum* L. (Ekkharthof, Lengwil, Switzerland) were cultivated in the dark at room temperature on chromatography paper (2043 A, Schleicher and Schuell, Dassel, Germany) in hanging LD-PE plastic bags (minigrip, Semadeni, Ostermundigen, Switzerland). Per bag, 16 sorted seeds were put on chromatography paper that had been soaked with 3 mL of fluid, namely, either (i) distilled water (Büchi, Fontavapor 250, Flawil, Switzerland), (ii) VAE Mali (lot 0404/4141) 2 mg/mL, (iii) APVAE Mali (lot 0404/4142) 2 mg/mL, (iv) VAE Pini (lot 0404/4143) 2 mg/mL, or (v) APVAE Pini (lot 0404/4144) 2 mg/mL. Mistletoe extracts were diluted 1 : 100 in distilled water (from 200 mg/mL to 2 mg/mL). Cultivation fluids were double coded and randomized. To all cultivation fluids, colchicine (Calbiochem, EMD Chemicals,

San Diego, USA) was added at a fixed concentration within one experiment (see below).

Growth status was documented by photocopying the seedlings after 93 ± 4 h. The photocopies of the seedlings were put on a 12 × 12 inch graphics tablet (Summasketch III, Summagraphics, GTCO CalComp Inc., Scottsdale, USA) connected to an Apple Macintosh G3/233 Desktop computer. The shape of each seedling was digitized using special software (Tracking 0.2.6, Fritschy-Informatik, Zürich, Switzerland). Seedlings either partially invisible (hidden behind another seedling), without visible shoot or root, or growing off the chromatographic paper were not digitized. Tracking every seedling with the cursor of the graphics tablet resulted in a series of coordinates in the graphics tablet resolution (0.127 mm). In addition, the software calculated the true curve length of the seedling. Each seedling's length was divided into shoot and root length by marking the beginning and end of every measurement phase for both shoot and root. Hourly measurements of a cardboard template allowed a drift control of the graphics tablet in both x- and y-directions. Length measurements were carried out with coded samples.

A total number of seven independent experiments were carried out. Within one experiment, each experimental group comprised 20 bags, with each containing 16 seeds, amounting to 320 seeds per parameter per experiment or 11,200 seeds in total. Concentration of colchicine in the cultivation fluid was 17 μg/mL for 1 experiment, 18 μg/mL for 4 experiments, and 20 μg/mL for 2 experiments.

Application of colchicine to *Lepidium sativum* led to dose-dependent shortening and thickening of the shoot (Figure 2) and to lengthening of the root. Hence, the ratio of root to shoot length increased due to colchicine. We therefore defined the ratio of root to shoot length as main outcome parameter for this morphological bioassay.

All data were analyzed with the statistics software Statistica 6 (Statsoft Inc., Tulsa, USA). Statistical evaluation was based on ANOVA (analysis of variance) procedures. Planned comparisons were evaluated with the LSD test only if the preceding global F-test was significant ($P < 0.05$) (protected Fisher's LSD). This procedure constitutes a good safeguard against type I as well as type II errors [23]. Statistical analysis was performed with data from $n = 10,612$ seedlings (data from 588 seedlings (5.25%) were missing, either due to failing germination or due to exclusion during length measurement according to the criteria defined above). Treatment parameters were decoded only after length measurement was accomplished.

3. Results

3.1. Antiproliferative Effects in Carcinoma Cell Lines. ED50 of *Viscum album* extract (VAE) Mali and anthroposophically processed *Viscum album* extract (APVAE) Mali was determined in five different cell lines (NCI-H460, DU-145, HCC1143, MV3, and PA-TU-8902), based on eight independent and coded (blinded) dose-response experiments each. ED50 of VAE Mali and APVAE Mali was statistically indistinguishable for all five cell lines (Table 1, Figure 3). In three

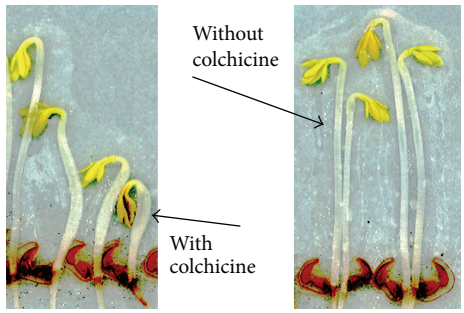


Figure 2: Colchicine (17 μg/mL) induces shortening and thickening of the shoots when applied to *Lepidium sativum*.

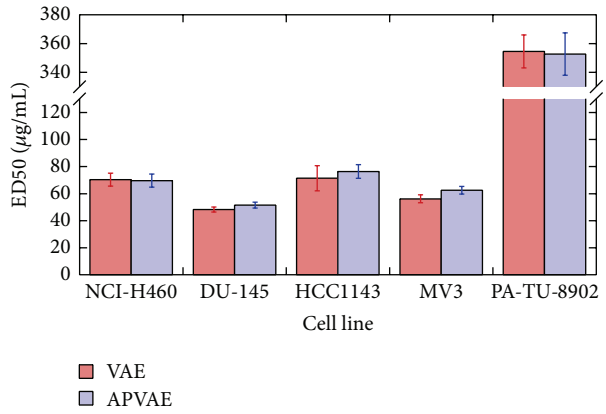


Figure 3: ED50 [μg/mL] of *Viscum album* extract (VAE) Mali and anthroposophically processed *Viscum album* extract (APVAE) Mali in five different cell lines. Mean ± SE based on 8 independent experiments each. No significant differences ($P < 0.05$) were observed for any pairwise comparison between VAE and APVAE.

cell lines (DU-145, HCC1143, and MV3), there was a trend for slightly elevated ED50 values for APVAE. In this assay, only VAE/APVAE Mali was investigated due to the selective toxicity of the cancer cell lines used towards mistletoe lectins (VAE/APVAE Pini is almost void of mistletoe lectins).

3.2. Morphological Bioassay. Experiments with colchicine-treated *Lepidium sativum* seedlings were analyzed with 2-way analysis of variance with the independent parameters treatment ($n = 5$: water control, VAE Mali, VAE Pini, APVAE Mali, and APVAE Pini) and experiment number ($n = 7$) and the dependent parameter root/shoot-ratio. All effects and interactions were highly significant ($P < 0.0001$, F-test; $n = 10,612$ in total).

The significant main effect for the independent parameter experiment number is due to the variations in absolute values of the outcome parameter root/shoot-ratio, which were most probably due to (i) the variations in colchicine concentration (17, 18, and 20 μg/mL) and (ii) differences in room temperature between the seven independent experiments.

The significant main effect for the independent parameter treatment reveals the impact of the mistletoe extracts on the root/shoot-ratio of *Lepidium sativum* seedlings (Figure 4(a));

Table 1: Comparison of ED50 [μg/mL] values of *Viscum album* extract (VAE) Mali and anthroposophically processed *Viscum album* extract (APVAE) Mali in five different cell lines. Mean ± SD based on 8 independent experiments each. 4th column: APVAE ED50 values expressed relative to VAE data set to 100%. 5th column: t-test for independent samples.

Cell line	ED50 VAE Mali [mean ± SD]	ED50 APVAE Mali [mean ± SD]	ED50 APVAE Mali [%]	P (t-test)
NCI-H460	70 ± 13 μg/mL	70 ± 14 μg/mL	99.0%	0.915
DU-145	48 ± 5 μg/mL	52 ± 6 μg/mL	106.7%	0.277
HCC1143	71 ± 26 μg/mL	76 ± 14 μg/mL	106.9%	0.647
MV3	56 ± 8 μg/mL	63 ± 8 μg/mL	111.3%	0.141
PA-TU-8902	355 ± 32 μg/mL	353 ± 42 μg/mL	99.5%	0.922

the LSD test differentiates the effects of the treatments water control, VAE, and APVAE ($P < 0.001$ for all pairwise comparisons). No significant difference in the root/shoot-ratio of *Lepidium sativum* seedlings was observed regarding mistletoe host tree (Malus versus Pinus, cf. Figure 4(a)).

Application of colchicine leads to the formation of colchicine tumors on the shoots, which decreases shoot length and increases root length and consequently increases the root/shoot-ratio in elongation. Both VAE and APVAE Mali/Pini counteracted the effect of colchicine on *Lepidium sativum*. APVAE Mali and Pini preparations both seemed to exhibit a stronger impact compared to the VAE samples. On the average, VAE reduced the root/shoot-ratio by 5.4%, whilst APVAE reduced it by 8.8%, compared to the water control. The increase in root/shoot-ratio reduction by APVAE is about 60% relative to VAE and is highly significant ($P < 0.001$).

In order to assess the effects of the variations in colchicine concentration, a 2-way analysis of variance with the independent parameters treatment ($n = 3$: water control, VAE Mali/Pini, and APVAE Mali/Pini) and colchicine concentration ($n = 3$: 17, 18, and 20 μg/mL) and the dependent parameter root/shoot-ratio was used. All effects and interactions were highly significant ($P < 0.0001$, F-test). The effects of VAE and APVAE were quite similar for the three colchicine concentrations, compared to the corresponding water control (Figure 4(b)). APVAE could be differentiated ($P < 0.05$) from the water control and VAE for all colchicine concentrations used; the effect of VAE was comparably weaker.

4. Discussion

The anthroposophic pharmaceutical process in question did not significantly alter the toxicity of *Viscum album* extracts in a panel of five carcinoma cell lines of different origin (pancreas adenocarcinoma, metastatic melanoma, and prostate, breast, and lung carcinoma). This result is in line with an earlier investigation of the same pharmaceutical process with two other cancer cell lines (Molt4 leukemia and Yoshida sarcoma cells) [24]. Viability reduction of carcinoma cell lines is most probably due to a specific compound found in aqueous mistletoe extracts, the mistletoe lectins [25]. In contrast, the Yoshida sarcoma cell line is specifically sensitive towards viscotoxins [26]. Thus, based on investigations in seven different cell lines, one may conclude that the anthroposophic pharmaceutical process studied does not seem to induce relevant changes in the biochemical properties of neither mistletoe lectins nor viscotoxins, though modifications of the complex molecules (60 kD and 5 kD, resp.) are conceivable due to the large sheering forces applied in the high-speed blending machine. In the present as well as an earlier investigation [24], concentration of lectins and viscotoxins was found unchanged after applying the anthroposophic pharmaceutical process in question.

In contrast, comparably large and highly significant differences between anthroposophically processed and unprocessed mistletoe extracts (APVAE versus VAE) were observed in the morphological bioassay with *Lepidium sativum*. The formation of the so-called colchicine tumors was reduced by application of VAE, and this reduction of tumor formation was enforced by APVAE, as depicted by the changes in the root/shoot-ratio being correlated to the formation of colchicine tumors. Furthermore, this decrease in tumor formation was comparable for extracts from mistletoe growing on the host trees apple and pine (VAE/APVAE Mali and Pini, resp.). VAE/APVAE Pini has only very low concentrations of mistletoe lectins, and concentration and composition of viscotoxins differ to a large extent between apple and pine mistletoe extracts [27]. Thus, neither mistletoe lectins nor viscotoxins can be the cause for the observed reduction in colchicine tumor formation.

In plants, colchicine inhibits microtubule assembly by binding to the dimeric subunit of the microtubule, tubulin [28]. This leads to reduction of cell polarity and—in dividing cells—also to polyploidy [29, 30], with the consequent formation of colchicine tumors, consisting of malformed cells or cell assemblies [31]. An interference of some compound(s) present in mistletoe extracts with the tubulin-colchicine reaction is in principle conceivable; we currently have no hypothesis which substance(s) might be responsible for any such interference, however. According to the results obtained and discussed above, mistletoe lectins and viscotoxins most probably have to be excluded as possible candidates for such interference.

The results of the present study are in line with an earlier investigation of colchicine tumor formation in *Triticum aestivum* shoots treated with VAE and APVAE Mali: an analogous reduction of tumor development through application of VAE Mali and an additional decrease of tumor incidence through application of APVAE Mali were observed [32]. Similarly, crown-gall-tumor formation in *Kalanchoe daigremontiana* induced by *Agrobacterium tumefaciens* was

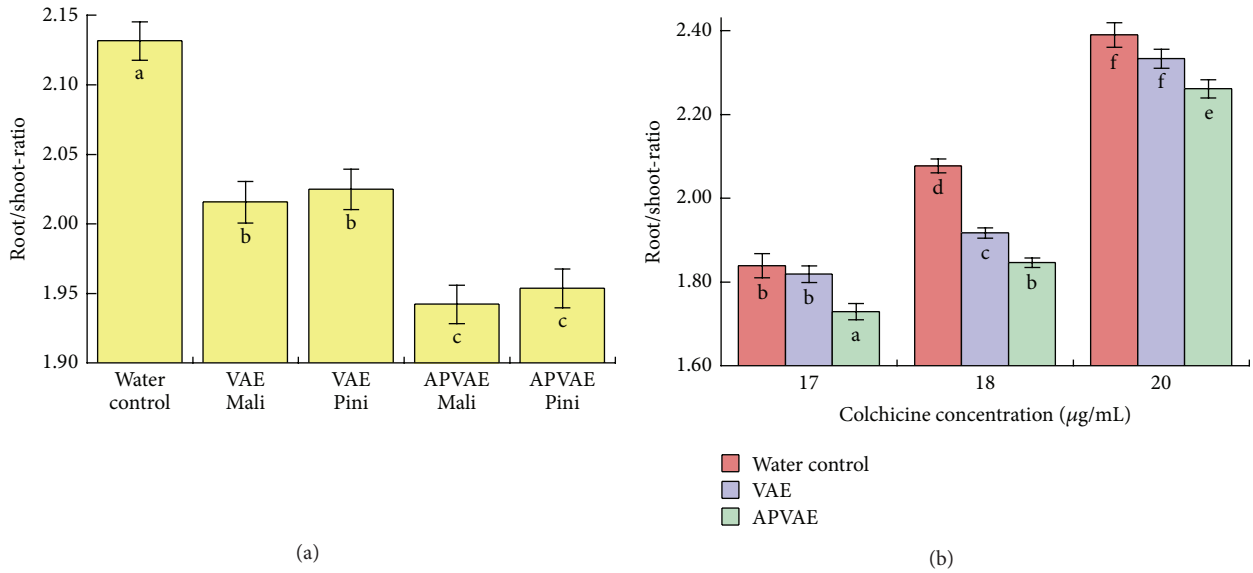


Figure 4: Root/shoot-elongation-ratio (mean ± SE) of colchicine-treated *Lepidium sativum*, with either water, *Viscum album* extract (VAE) Mali or Pini [2 mg/mL], or anthroposophically processed *Viscum album* extract (APVAE) Mali or Pini [2 mg/mL] added. (a) Average over all colchicine concentrations used. Parameters with different letters (a, b, and c) are statistically different ($P < 0.001$, LSD test); parameters with identical letters are statistically indistinguishable ($P > 0.53$, LSD test). (b) The same data as in (a) but differentiated according to the colchicine concentration used (17, 18, or 20 μg/mL). Data from VAE Mali and VAE Pini as well as APVAE Mali and APVAE Pini were pooled. Parameters with different letters (a, b, c, ...) are statistically different ($P < 0.05$, LSD test); parameters with identical letters are statistically indistinguishable ($P > 0.05$, LSD test).

reduced through application of VAE Mali; this decrease was further enforced after application of APVAE Mali [24]. Furthermore, damage induced by ultraviolet radiation (UV) on *Triticum aestivum* shoots as well as *Sinapis alba* shoots could be alleviated after application of VAE and APVAE Mali, but with stronger effects of APVAE compared to VAE [32].

Summarizing, effects of the anthroposophic pharmaceutical process in question were observed in five different whole system bioassays (colchicine tumor formation in *Lepidium sativum*, colchicine tumor formation in *Triticum aestivum*, crown-gall-tumor formation in *Kalanchoe daigremontiana*, UV damage in *Triticum aestivum*, and UV damage in *Sinapis alba*). In all five bioassays, anthroposophically processed *Viscum album* extracts (APVAE) induced a stronger morphostatic protection effect than unprocessed *Viscum album* extract (VAE). In contrast, no unambiguous effects of the investigated anthroposophic pharmaceutical process were observed regarding toxicity of *Viscum album* extracts in seven cancer cell lines (NCI-H460, DU-145, HCC1143, MV3, PA-TU-8902, Molt4, and Yoshida) of different origins (pancreas adenocarcinoma, metastatic melanoma, prostate, breast, and lung carcinoma, leukemia, and sarcoma).

This means that the high-speed blending process of summer and winter mistletoe extracts seems to induce some specific properties in *Viscum album* extracts that support morphostasis, that is, that help entire organisms to maintain their organization and form when threatened by noxious external factors. In contrast, no clear effect of the blending process was observed on lectin- and viscotoxin-based toxicity against cancer cell lines. Thus, it seems that preclinical methods derivable from the basic structure of the tissue organization field theory (TOFT) of cancer are more apt to investigate the anthroposophic pharmaceutical process in question, compared to preclinical methods derived from the somatic mutation theory (SMT) of cancer.

Anthroposophically processed *Viscum album* extracts from the host tree pine (APVAE Pini) led to a stabilization of the DNA of amniotic fluid cells *in vitro* [33]. Furthermore, APVAE Mali led to an improvement of DNA repair in gamma-ray or 4-hydroxycyclophosphamide damaged peripheral blood mononuclear cells (PBMC) *in vitro* [34]. A similar protection effect of APVAE Pini in PBMC poisoned with 4-hydroxycyclophosphamide was observed in a follow-up *in vitro* study, alongside the absence of any such protection effect in malignant Jurkat cells [35]. None of these three investigations was able to identify possible compounds of the mistletoe extracts that might be responsible for this DNA stabilizing effect.

In animal trials, anthroposophically processed *Viscum album* extracts exerted protective effects against carcinogenic compounds (N-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide, 20-methylcholanthrene) in trials with rats and mice [36, 37]. Furthermore, treatment with APVAE Mali resulted in a faster recovery from radiation- or cyclophosphamide-induced leukopenia in mice [38].

The results of the abovementioned preclinical investigations are in line with observations made in clinical trials. Kovacs et al. observed that DNA repair in lymphocytes of breast cancer patients could be substantially improved after subcutaneous injections of APVAE Mali [39]. Furthermore,

there are several clinical investigations that documented a reduction of side effects of conventional antitumor therapy by simultaneous APVAE therapy, without reducing the former's efficacy [40–43].

Taken together, these preclinical and clinical observations support the hypothesis that the application of APVAE may help organisms (cells, plants, animals, and humans) in their continuing quest for maintenance of organization and form, especially when endangered by external noxious influences or by endogenous tumor formation. In addition, based on the results of the present and earlier investigations [24, 32], we put forward the hypothesis that part of the effects of APVAE is due to the specific anthroposophic pharmaceutical processing applied.

5. Conclusions

Summarizing, preclinical and clinical observations seem to suggest that anthroposophically processed mistletoe extracts may support entire organisms in general (cells, plants, animals, and humans) in their maintenance of organization and form, when endangered by endogenous tumor formation or externally applied noxious influences. We raise the hypothesis that part of the observed morphostatic effects of anthroposophically processed *Viscum album* extracts is due to the specific anthroposophic pharmaceutical processing applied.

Furthermore, we argue that remedies and pharmaceutical procedures of anthroposophic medicine and homeopathy, as well as from other branches of CAM in general (e.g., European or Chinese phytotherapy), should be investigated by preclinical assays that are adapted to the theoretical context from which they were developed. Therefore, we see a need to further develop preclinical approaches in cancer research based on the morphogenetic paradigm, centering on cancer in nonhuman biological models as a disorder phenomenon of the general morphological structure of entire organisms and subsequently also tissue-cell interactions, preferably in three-dimensional *in vitro* models.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] R. Steiner, "Geisteswissenschaft und Medizin, 13th lecture, Dornach, 2. April 1920," in *Geisteswissenschaft und Medizin*, H. W. Zbinden, Ed., pp. 210–227, Rudolf-Steiner Nachlassverwaltung, Dornach, Switzerland, 4th edition, 1961.

[2] R. Steiner and I. Wegman, *Grundlegendes für eine Erweiterung der Heilkunst nach geisteswissenschaftlichen Erkenntnissen*, Rudolf-Steiner, Dornach, Switzerland, 1925.

[3] A. Büssing, "Biological and pharmacological properties of *Viscum album* L.," in *Mistletoe: The Genus Viscum*, A. Büssing, Ed., pp. 123–182, Harwood Academic, Amsterdam, The Netherlands, 2000.

[4] G. S. Kienle and H. Kiene, *Die Mistel in der Onkologie*, Schattauer, Stuttgart, Germany, 2003.

[5] J. Maldacker, "Preclinical investigations with mistletoe (*Viscum album* L.) extract Iscador," *Arzneimittel-Forschung*, vol. 56, no. 6, pp. 497–507, 2006.

[6] T. Ostermann, C. Raak, and A. Büssing, "Survival of cancer patients treated with mistletoe extract (Iscador): a systematic literature review," *BMC Cancer*, vol. 9, article 451, 2009.

[7] G. S. Kienle and H. Kiene, "Complementary cancer therapy: a systematic review of prospective clinical trials on anthroposophic mistletoe extracts," *European Journal of Medical Research*, vol. 12, no. 3, pp. 103–119, 2007.

[8] J. Melzer, F. Iten, K. Hostanska, and R. Saller, "Efficacy and safety of mistletoe preparations (*Viscum album*) for patients with cancer diseases," *Forschende Komplementärmedizin*, vol. 16, no. 4, pp. 217–226, 2009.

[9] G. S. Kienle, R. Grugel, and H. Kiene, "Safety of higher dosages of *Viscum album* L. in animals and humans—systematic review of immune changes and safety parameters," *BMC Complementary & Alternative Medicine*, vol. 11, article 72, 2011.

[10] A. Büssing, "Overview of *Viscum album* L. products," in *Mistletoe: The Genus Viscum*, A. Büssing, Ed., pp. 209–221, Harwood Academic, Amsterdam, The Netherlands, 2000.

[11] R. Leroi, "Die Mischung der Mistelsäfte—angaben Rudolf Steiners," *Beiträge zu einer Erweiterung der Heilkunst*, vol. 40, pp. 238–247, 1987.

[12] D. Hanahan and R. A. Weinberg, "The hallmarks of cancer," *Cell*, vol. 100, no. 1, pp. 57–70, 2000.

[13] D. Hanahan and R. A. Weinberg, "Hallmarks of cancer: the next generation," *Cell*, vol. 144, no. 5, pp. 646–674, 2011.

[14] M. Levin, "Morphogenetic fields in embryogenesis, regeneration, and cancer: non-local control of complex patterning," *Biosystems*, vol. 109, no. 3, pp. 243–261, 2012.

[15] A. M. Soto and C. Sonnenschein, "The tissue organization field theory of cancer: a testable replacement for the somatic mutation theory," *Bioessays*, vol. 33, no. 5, pp. 332–340, 2011.

[16] P. Heusser, "Führt die Molekularbiologie zu einem neuen Konzept des Organismus? Das Beispiel der Karzinogenese," *Forschende Komplementärmedizin und Klassische Naturheilkunde*, vol. 4, no. 2, pp. 106–111, 1997.

[17] G. S. Kienle and H. Kiene, "From reductionism to holism: systems-oriented approaches in cancer research," *Global Advances in Health and Medicine*, vol. 1, pp. 68–77, 2012.

[18] P. Heusser and G. S. Kienle, "Anthroposophic medicine, integrative oncology, and mistletoe therapy of cancer," in *Integrative Oncology*, D. Abrams and A. Weil, Eds., pp. 322–340, Oxford University Press, New York, NY, USA, 2009.

[19] J. Ritchie, J. Wilkinson, M. Gantley, G. Feder, Y. Carter, and J. Formby, *A Model of Integrated Primary Care: Anthroposophic Medicine*, Queen Mary, University of London, London, UK, 2001.

[20] G. Unger, "Kurze Geschichte der Heilmittelzentrifuge des Institutes Hiscia unter Einbeziehung der Hinweise Rudolf Steiners zur Erstellung der Mistelpräparate," in *Misteltherapie—Eine Antwort auf die Herausforderung Krebs*, R. Leroi, Ed., pp. 45–57, Freies Geistesleben, Stuttgart, Germany, 1987.

[21] H. Musielski and K. Rüger, "Verfahren zur quantitativen Bestimmung von Mistellektin I und Mistellektin II und/oder Mistellektin III in Mistelextrakten unter Verwendung monoklonaler Antikörper, die spezifisch mit Mistellektin reagieren," in *Grundlagen der Misteltherapie*, R. Scheer, H. Becker, and P. A. Berg, Eds., pp. 95–104, Hippokrates, Stuttgart, Germany, 1996.

[22] M. Ishiyama, H. Tominaga, M. Shiga, K. Sasamoto, Y. Ohkura, and K. Ueno, "A combined assay of cell viability and in vitro cytotoxicity with a highly water-soluble tetrazolium salt, neutral red and crystal violet," *Biological & Pharmaceutical Bulletin*, vol. 19, no. 11, pp. 1518–1520, 1996.

[23] S. G. Carmer and M. R. Swanson, "An evaluation of ten pairwise multiple comparison procedures by Monte Carlo methods," *Journal of the American Statistical Association*, vol. 68, pp. 66–74, 1973.

[24] S. Baumgartner, H. Flückiger, C. Jäggy, G. Schaller, D. S. Rossi, and K. Urech, "Untersuchung des Iscador-Maschinenprozesses in Modellsystemen der Zytotoxikologie und Phytopathologie," in *Fortschritte in der Misteltherapie—Aktueller Stand der Forschung und Klinische Anwendung*, R. Scheer, R. Bauer, H. Becker, V. Fintelmann, F. H. Kemper, and H. Schilcher, Eds., pp. 169–179, KVC, Essen, Germany, 2005.

[25] O. Janssen, A. Scheffler, and D. Kabelitz, "In vitro effects of mistletoe extracts and mistletoe lectins. Cytotoxicity towards tumor cells due to the induction of programmed cell death (apoptosis)," *Arzneimittel-Forschung*, vol. 43, no. 11, pp. 1221–1227, 1993.

[26] K. Urech, G. Schaller, P. Ziska, and M. Giannattasio, "Comparative study on the cytotoxic effect of viscotoxin and mistletoe lectin on tumour cells in culture," *Phytotherapy Research*, vol. 9, no. 1, pp. 49–55, 1995.

[27] K. Urech, G. Schaller, and C. Jäggy, "Viscotoxins, mistletoe lectins and their isoforms in mistletoe (*Viscum album* L.) extracts Iscador: analytical results on pharmaceutical processing of mistletoe," *Drug Research*, vol. 56, no. 6, pp. 428–434, 2006.

[28] R. L. Margolis and L. Wilson, "Addition of colchicine tubulin complex to microtubule ends: the mechanism of substoichiometric colchicine poisoning," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 74, no. 8, pp. 3466–3470, 1977.

[29] T. Butterfass, *Wachstums-und Entwicklungsphysiologie der Pflanze*, Quelle & Meyer, Heidelberg, Germany, 1970.

[30] A. F. Blakeslee and A. G. Avery, "Methods of inducing doubling of chromosomes in plants," *The Journal of Heredity*, vol. 28, pp. 393–412, 1937.

[31] A. Levan, "The effect of colchicine on root mitosis in Allium," *Hereditas*, vol. 24, pp. 471–486, 1938.

[32] H. Flückiger and S. Baumgartner, "Auswirkung des Iscador-Maschinenprozesses auf Mistelextrakte in botanischen Untersuchungssystemen," *Der Merkurstab*, vol. 56, pp. 114–121, 2003.

[33] A. Büssing, A. Lehnert, M. Schink, R. Mertens, and K. Schweizer, "Effect of *Viscum album* L. on rapidly proliferating amniotic fluid cells. Sister chromatid exchange frequency and proliferation index," *Arzneimittel-Forschung*, vol. 45, no. 1, pp. 81–83, 1995.

[34] E. Kovacs, "The in vitro effect of *Viscum album* (VA) extract on DNA repair of peripheral blood mononuclear cells (PBMC) in cancer patients," *Phytotherapy Research*, vol. 16, no. 2, pp. 143–147, 2002.

[35] J. Burkhart, C. Wälchli, P. Heusser, U. Weissenstein, S. Baumgartner, and A.-C. Andres, "In vitro investigation into the potential of a mistletoe extract to alleviate adverse effects of cyclophosphamide," *Alternative Therapies in Health and Medicine*, vol. 16, no. 3, pp. 40–48, 2010.

[36] R. Rentea, E. Lyon, and R. Hunter, *The Modulation of the Immune Response by* Viscum album *Proteins, Iscador*, Department of Pathology and Surgery, University of Chicago Hospitals and Clinics, Chicago, Ill, USA, 1976.

[37] G. Kuttan, L. G. Menon, and R. Kuttan, "Prevention of 20-methylcholanthrene-induced sarcoma by a mistletoe extract, Iscador," *Carcinogenesis*, vol. 17, no. 5, pp. 1107–1109, 1996.

[38] G. Kuttan and R. Kuttan, "Reduction of leukopenia in mice by "*Viscum album*" administration during radiation and chemotherapy," *Tumori*, vol. 79, no. 1, pp. 74–76, 1993.

[39] E. Kovacs, T. Hajto, and K. Hostanska, "Improvement of DNA repair in lymphocytes of breast cancer patients treated with *Viscum album* extract (Iscador)," *European Journal of Cancer*, vol. 27, no. 12, pp. 1672–1676, 1991.

[40] G. Bar-Sela, M. Wollner, L. Hammer, A. Agbarya, E. Dudnik, and N. Haim, "Mistletoe as complementary treatment in patients with advanced non-small-cell lung cancer treated with carboplatin-based combinations: a randomised phase II study," *European Journal of Cancer*, vol. 49, no. 5, pp. 1058–1064, 2013.

[41] H. Matthes, W. E. Friedel, P. R. Bock, and K. S. Zänker, "Molecular mistletoe therapy: friend or foe in established antitumor protocols? A multicenter, controlled, retrospective pharmaco-epidemiological study in pancreas cancer," *Current Molecular Medicine*, vol. 10, no. 4, pp. 430–439, 2010.

[42] W. E. Friedel, H. Matthes, P. R. Bock, and K. S. Zänker, "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, observational cohort study," *Journal of the Society for Integrative Oncology*, vol. 7, no. 4, pp. 137–145, 2009.

[43] P. R. Bock, W. E. Friedel, J. Hanisch, M. Karasmann, and B. Schneider, "Wirksamkeit und Sicherheit der komplementären Langzeitbehandlung mit einem standardisierten Extrakt aus Europäischer Mistel (*Viscum album* L.) zusätzlich zur konventionellen adjuvanten onkologischen Therapie bei primärem, nicht metastasiertem Mammakarzinom," *Drug Research*, vol. 54, pp. 456–466, 2004.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 210198, 9 pages
http://dx.doi.org/10.1155/2014/210198

Research Article

A Randomized Study on Postrelapse Disease-Free Survival with Adjuvant Mistletoe versus Oral Etoposide in Osteosarcoma Patients

Alessandra Longhi,[1] Marcus Reif,[2] Erminia Mariani,[3] and Stefano Ferrari[1]

[1] *Musculoskeletal Oncology Department, Istituto Ortopedico Rizzoli, Via Pupilli 1, 40136 Bologna, Italy*
[2] *Institute for Clinical Research, Hardenbergstraße 20, 10623 Berlin, Germany*
[3] *Immunorheumatology and Tissue Regeneration Laboratory, Istituto Ortopedico Rizzoli, Via Barbiano 1/10, 40136 Bologna, Italy*

Correspondence should be addressed to Alessandra Longhi; longhi.alessandra@libero.it

Received 20 November 2013; Accepted 2 March 2014; Published 31 March 2014

Academic Editor: Steven Rosenzweig

Copyright © 2014 Alessandra Longhi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Osteosarcoma is a highly malignant bone tumour. After the second relapse, the 12-month postrelapse disease-free survival (PRDFS) rate decreases below 20%. Oral Etoposide is often used in clinical practice after surgery as an "adjuvant" outside any protocol and with only limited evidence of improved survival. *Viscum album fermentatum Pini* (*Viscum*) is an extract of mistletoe plants grown on pine trees for subcutaneous (sc) injection with immunomodulatory activity. *Methods*. Encouraged by preliminary findings, we conducted a study where osteosarcoma patients free from disease after second metastatic relapse were randomly assigned to *Viscum* sc or Oral Etoposide. Our goal was to compare 12-month PRDFS rates with an equivalent historical control group. *Results*. Twenty patients have been enrolled, with a median age of 34 years (range 11–65) and a median follow-up time of 38.5 months (3–73). The median PRDSF is currently 4 months (1–47) in the Etoposide and 39 months (2–73) in the *Viscum* group. Patients getting *Viscum* reported a higher quality of life due to lower toxicity. *Conclusion*. *Viscum* shows promise as adjuvant treatment in prolonging PRDFS after second relapse in osteosarcoma patients. A larger study is required to conclusively determine efficacy and immunomodulatory mechanisms of *Viscum* therapy in osteosarcoma patients.

1. Introduction

Osteosarcoma is an aggressive malignant neoplasm, for which neoadjuvant chemotherapy with the four most effective drugs (Doxorubicin, Methotrexate, Cisplatin, and Ifosfamide) has improved 5-year disease-free survival (DFS) rate from 10% to 60%. Treatment for patients who relapse, either locally or with distant metastases mainly in lungs, is primarily surgical. The prognosis is poor, with long-term postrelapse survival <20%. Yet, most of the patients relapse a second time, mainly in the lung (40%; see [1]). Fagioli et al. [2] reported a 3-year DFS rate of 12% after the second relapse in patients who received surgery and chemotherapy, with 80% of patients rerelapsing within 1 year. In the study of Bacci et al. [3] on 235 osteosarcoma patients who relapsed after neoadjuvant chemotherapy, 120 (51%) patients had a second relapse with a median interval between second and third relapse of 11.8 months. Of these 120 patients only 14 (11.6%) achieved prolonged remission. The role of second-line chemotherapy for recurrent osteosarcoma is much less well defined than that of surgery, and there is no accepted standard regimen.

Besides surgery, different drugs have been employed (Ifosfamide, Cyclophosphamide, Etoposide, Methotrexate, Vinorelbine, and Gemcitabine plus Docetaxel) as "adjuvant" treatment postsurgery and eventually as monotherapy for unresectable disease, yet with scarce results [4]. A recent retrospective analysis (July 2013) on 110 relapsed osteosarcoma patients from St. Jude Hospital confirmed that surgery at relapse is essential for survival and chemotherapy may only slow disease progression in patients without second complete remission [5].

Etoposide is a topoisomerase II inhibitor mainly used intravenously in the treatment of several tumors (i.e., lymphomas, lung cancer, ovarian cancer, and Ewing's sarcoma), both in combination and in monotherapy. Some osteosarcoma protocols employ Etoposide iv in neoadjuvant schemes used for poor responders in postoperative treatment with intensification purpose (i.e., [6]). In the only osteosarcoma study on Oral Etoposide 50 mg/m^2/daily for 14 days as monotherapy, Kebudi et al. [7] reported a 15% response rate (RR) in relapsed pediatric patients. Sandri et al. [8] reported a successful use of Oral Etoposide at 50 mg/m^2 in children with recurrent ependymomas, showing a 40% RR. Oral Etoposide is well tolerated, with only mild adverse drug reactions like nausea, leucopenia, and alopecia. Yet, hematologic toxicity is one of the main limiting toxicities in second- or third-line chemotherapy in these heavily pretreated patients. Etoposide cannot be given for a longer period due to the risk of secondary hematologic malignancy.

Mistletoe therapy is widely used in cancer patients (>60% of tumor patients in Germany and Switzerland). It is derived from *Viscum album* L., a semiparasitic plant, which is classified into three subspecies (*deciduous tree mistletoe*, *pine mistletoe*, and *fir mistletoe*) each having its particular host tree (apple tree, oak, elm, pine, and fir). Its effects are similar to those of other biological response modifiers in targeting the immune system [9–15], (review in [16]). Main components of the whole plant extract are mistletoe lectins I, II, and III and six viscotoxins and polysaccharides. Lectins have shown cytostatic and immunomodulatory activity *in vitro* while viscotoxins have been reported to have cytoxic activity. Polysaccharides have shown immunomodulatory activities, that is, an increase in NK activity [17, 18]. The total plant extract is an immunostimulant (increase of NK cells, T-lymphocytes, and macrophages) and has apoptotic activity *in vitro* [19] and *in vivo* [20].

Viscum album fermentatum Pini (*Viscum*, equivalent to Iscador P, Weleda AG, Arlesheim, Switzerland) is one of several commercial *Viscum album* preparations and on the market since 1917. Administered as subcutaneous injection it is locally and systemically well tolerated. *Viscum* preparations have experimentally also been applied intravenously [21] but the subcutaneous injection is the only licensed form of application (exempt from the homeopathically potentiated mistletoe extracts ABNOBAviscum D6 to D30).

The study reported here examines the postrelapse disease-free survival (PRDFS) in patients at high risk for further relapse after surgery for a second relapse receiving either Etoposide or *Viscum*. Our aim is to compare the 12-month PRDFS rate of each of the two study arms with a historical cohort of patients. A preliminary report of the first ten patients of this study has already been published earlier [22]. Here, we present the clinical results for all patients.

2. Materials and Methods

This is a prospective, randomized, open-label study conducted according to the Declaration of Helsinki and approved by the ethics committee of the Istituto Ortopedico Rizzoli (IOR), Bologna, and by the Italian Competent Authorities. All patients provided written informed consent before study entry. The study is registered in the in the EU clinical trials register, EudraCT number 2006-002676-18.

2.1. Patients. Inclusion criteria comprised the histologically confirmed diagnosis of osteosarcoma or spindle cell sarcoma of the bone after a second relapse; absence of metastases and local relapse after surgery; age ≥10 years; ECOG ≤2; adequate bone marrow function (i.e., peripheral absolute neutrophils >1500, platelets >100.000); and further lab parameters restricted to bilirubin <2, creatinine < 1.5x normal, and a signed informed consent. Exclusion criteria were bone sarcomas of other histological type or any other malignancy prior to study; missing staging criteria; last antineoplastic treatment received within 30 days prior to study entry; treatment with Etoposide or *Viscum album* extract prior to study entry; concomitant treatment with drugs having either immunostimulatory or immunosuppressive properties; pregnancy.

2.2. Patient Assignment. Patients were randomized 1 : 1 to receive either Etoposide or *Viscum*. Randomization was requested by fax and performed centrally at the Institute for Clinical Research, Berlin, according to an unrestricted randomization list created by a statistician from the IOR uninvolved in any further aspect of the trial.

2.3. Study Medication and Treatment. The *Viscum album* extract applied in this study is an approved drug and has a marketing authorization under the name "Iscador P" (Weleda AG, Arlesheim, Switzerland) in Germany, Switzerland, and Austria. In Italy, it is registered as homeopathic remedy under the name *Viscum album fermentatum Pini*. According to the manufacturer, this mistletoe extract contained mistletoe lectins of up to 40 ng/mL in the 20 mg dose of Series II (personal communication). Immunomodulatory activity of this preparation has previously been demonstrated regarding an increase in TNF-α and IL-6 [23], natural killer (NK) cell cytotoxicity [24], activation of CD4$^+$ T-helper cells and monocytes [25], maturation of dendritic cells [26], and activation of macrophages [27]. In the actual study, immunological parameters including NK T lymphocytes, IL-2, IL-4, IL-12, IL-15, γ-IFN, and IP-10 were determined quarterly yet will be presented elsewhere.

Viscum album extract was injected subcutaneously (abdominal) 3 times/week. Starting dose was 2 boxes of Series 0 (0.01, 0.1, and 1 mg) with 14 vials all together, followed by 2 boxes of series I (0.1, 1, and 10 mg) with 14 vials; and subsequent treatment with series II (1, 10, and 20 mg) continuously until 12th month. Local reactions at the injection site (redness, slight swelling, and itching) with more than 5 cm diameter were followed by dose reduction, that is, injection of half an ampoule (discarding the rest).

Treatment with Oral Etoposide tablets was done at the dose of 50 mg/m^2 per day for 21 days, followed by one week rest. This schedule was repeated for 6 cycles. If G3/G4 hematological toxicity occurred, according to the study plan

Table 1: Schedule of events over the study.

Month	−0.5	0	1	2	3	6	9[a]	12[a]
Informed consent	X							
Inclusion/exclusion criteria	X							
Medical history/adverse events	X	X	X	X	X	X	X	X
Physical examination*	X	X	X	X	X	X	X	X
QoL (EORTC/POQOL)	X	X			X	X	X	X
CBC, biochemical profile**	X					X		X
Urine analysis	X							X
Pregnancy test***	X							
CT Lungs	X				X	X	X	X
Bone X-ray or CT					X	X	X	
Total bone scan	X							X
Ultrasound/CT abdomen	X					X		X
Medication dispense		X	X	X	X	X	X	
Return of unused medication			X	X	X	X	X	X
Immunological evaluation		X			X	X	X	X

*Full PE on month −0.5 and on exit visit; examination of disease-related findings only at other visits.
**Within two weeks prior to screening or within baseline period.
***For premenopausal women.
[a]Treatment duration for *Viscum*: 12 months; for Etoposide: 6 months.

the cycle was shortened to 14 days. If neutrophils were below 500/μL, G-CSF could be administrated until the count reached 1000/μL. If patients experienced G3/G4 toxicity over 2 cycles, total dose of Etoposide was reduced to 50%. Patients experiencing G3/G4 toxicity over the next cycle despite dose reduction were withdrawn from treatment.

The staging examinations performed at screening (month −0.5) and during the study at baseline (month 0) and 3, 6, 9, and 12 months after start of treatment are shown in Table 1.

2.4. Endpoints. Primary endpoint of the study was PRDFS after the second relapse in osteosarcoma patients, assessed at each visit by X-ray or computer tomography (CT) of the primary site of the tumor (bone) and CT of the lung and additionally by ultrasound examination or CT of the abdomen after 6 months of treatment. The primary efficacy parameter PRDFS rate was defined as the proportion of patients in a given treatment arm without any sign of relapse after 12 month of treatment with *Viscum* or Etoposide, respectively. As the PRDFS rate without treatment is known to be about 12% from retrospective studies [2, 3], the aim of this study was to assess whether any of the two treatments might have the potential to increase PRDFS rate to about 40% one year after surgery after the second relapse. Patients are followed up beyond the end of the trial and their PRDFS status is updated on an ongoing basis. Until July 2013 follow-up times up to 73 months have been documented.

Second endpoints were the quality of life (QoL) in both arms measured by the core questionnaire of the European Organization for Research and Treatment of Cancer (EORTC QOL-C30) in adults or by the Pediatric Quality of Life Cancer Module Acute Version 3.0 (PedsQL) in patients <18 years, respectively. The EORTC QLQ-C30 consists of 30 questions with four (28 questions) or seven (2 questions) response categories in the form of Likert scales. The questions are subsumed to five functioning scales (physical, role, emotional, cognitive, and social), three symptom scales (fatigue, nausea/vomiting, and pain), six single-item scales (dyspnea, sleep disturbance, appetite loss, constipation, diarrhea, and financial impact), and the global health/quality of life scale that can be regarded as overall QoL index. Regarding the PedsQL, there was only one Etoposide patient with postbaseline data, and therefore this questionnaire is disregarded here.

Safety endpoint of the study was the tolerability of Etoposide and *Viscum* treatments. For this, at each visit patients were asked for adverse events which were registered according to the Common Toxicity Criteria for Adverse Events (CTCAE) and assessed for their relationship to the study medications.

2.5. Sample Size Estimation. The sample size necessary to demonstrate a statistical superiority, based on the hypothesis that one or both drugs can improve the historically documented PRDFS rate of 12% up to approximately 35%, was estimated to require 18 patients per arm, assuming an alpha error level 5% and a power of 81%. Based on our experience, we anticipated no dropouts. Due to recruitment failure, the study was terminated early by protocol amendment after the inclusion of 20 patients (11 Etoposide, 9 *Viscum*).

2.6. Statistical Methods. Comparability between treatment arms was assessed using arithmetic mean, standard deviation, minimum, first, and third quartile, median, and maximum for continuous data, and contingence tables showing absolute and relative frequencies for categorical data. No tests for difference between treatment groups were performed for these baseline variables since P values smaller than 5% only

represent the expected 1 in 20 chance to find a difference where none exists in reality.

The analysis of the efficacy parameters followed the intention-to-treat approach; that is, all patients were included in the analysis as randomized. All efficacy analyses were done separately for each of the two treatment groups.

The evaluation of the primary endpoint PRDFS rate was performed as comparison of the 12-month PRDFS rate with the fixed value of 12% (i.e., the average PRDFS rate after second relapse in historical control groups) using an exact Binomial test [28].

A linear mixed model was used to analyze the QoL parameters of the EORTC QLQ-C30 as difference to baseline, including the respective baseline value of each QoL parameter, the treatment group and the visit as independent factors, and study patients as random factor. The dependency between successive visits within each patient was accounted for using a compound symmetry covariance matrix.

All tests were performed on an alpha error level of 5%; due to the exploratory character of this trial, no adjustment for multiple testing was applied. Together with the P values, 95% confidence intervals are reported.

3. Results

From June 2007 to July 2011, 20 patients had been enrolled. Eleven patients were randomly assigned to the Etoposide arm and nine to the *Viscum* arm. Histology confirmed osteosarcoma in all patients; all patients had undergone surgery for a second relapse of the disease in the lung, and two in the hip for local relapse of proximal femur primitive localization. A second chemotherapy had already been applied in 5 (55.5%) (*Viscum* arm) and 4 (36.4%) (Etoposide arm) patients after first relapse, respectively, the last one about three years before entry into the study.

Male to female ratio was 11 : 9; mean age was 33.9 years (range 11–65). Median DFS from first surgery to first relapse and from first to second relapse was 19.1 (2–40) and 21.1 (3–82) months in the *Viscum* arm and 26.9 (14–37) and 15.6 (3–40) months in the Etoposide arm, respectively. Other sociodemographics, disease, and treatment related baseline characteristics are shown in Tables 2 and 3.

After one year of treatment PRDFS rate in *Viscum* arm was 55.6%, compared to historical 12% rate: $P = 0.0041$; 95% CI (21.2%; 86.3%) (five out of nine patients) and it was 27.3%, $P = 0.2724$; 95% CI (6.0%; 61.0%) for Etoposide arm (three out of eleven patients) (see Figure 1).

Until July 2013 in the *Viscum* arm the median PRDFS (including censored dates) is 39 months (range 2–73 months). One out of 6 patients relapsed locally in the area of previous surgery (pelvis). In the Etoposide arm median PRDFS is 4 months (1–47 months) (see Figure 2). One patient enrolled in the Etoposide arm refused to accept Etoposide after randomization and withdrew from the trial and took *Viscum* instead; nevertheless, following intention-to-treat approach he was analyzed as assigned to Etoposide. Another patient relapsed after three months of Etoposide; after surgery for the third relapsed disease he crossed over to *Viscum* for 2 years. He is still free from disease from his 3rd relapse after 59 months. Two patients in the *Viscum* arm after one year of *Viscum* study treatment decided to continue *Viscum* therapy at least for another year spontaneously.

Regarding the quality of life assessment the trend was positive for *Viscum* treatment (Table 4); this can especially be seen in the global QoL scale, in the functional scales "physical functioning" and "social functioning," and for the symptom items "fatigue," "pain," "dyspnea," and "financial difficulties" of the EORTC QLQ-C30. A similar improvement

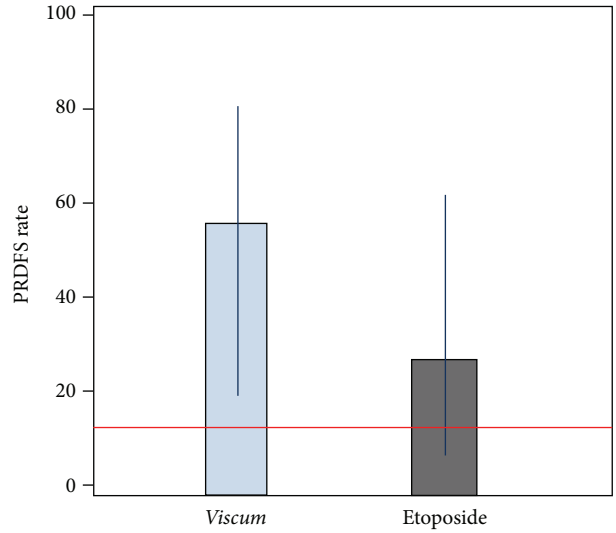


Figure 1: PRDFS rates and exact 95% confidence intervals after 12 months of treatment with *Viscum* or Etoposide, respectively. The horizontal line represents the 12% PRDFS rate derived from historical controls. By crossing the 12% line, the Etoposide confidence interval indicates that this treatment cannot statistically be distinguished from the historical rate, whereas for *Viscum* a significant difference can be deduced.

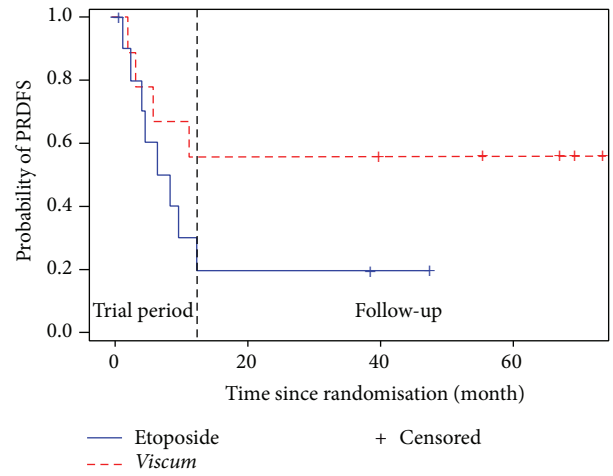


Figure 2: Kaplan-Meier graph of the course of PRDFS for *Viscum* and Etoposide patients, respectively, over the period of the trial and during follow-up. The vertical line indicates the end of the trial period. Last dates are from July 2013 and are updated on an ongoing basis.

Table 2: Sociodemographic and general health characteristics.

Patient characteristics	Frequency (percentage) or mean (range)	
	Viscum $n = 9$	Etoposide $n = 11$
Gender		
Male	4 (44.4)	7 (63.6)
Female	5 (55.6)	4 (39.4)
Age (years)	28 (18–48)	39 (11–66)
Ethnic group		
Caucasian	8 (88.9)	11 (100)
Asian	1 (11.1)	—
Family status		
Single/divorced	8 (88.9)	6 (54.5)
Married/in partnership	1 (11.1)	5 (45.5)
Highest education		
Vocational training	5 (55.6)	7 (63.6)
University graduate/student	4 (44.4)	4 (36.5)
ECOG		
0	3 (33.3)	6 (54.6)
1	6 (66.7)	5 (45.4)
≥2	—	—
Concomitant diseases	1 (11.1)	3 (27.3)
Paget syndrome	—	1 (11.1)
HCV	1 (11.1)	—
Primary hyperparathyroidism	—	1 (11.1)
Kidney tubulopathy	—	1 (11.1)
Current regular medication	5 (55.6)	6 (54.6)
Current signs and symptoms		
Pain	2 (22.2)	1 (9.1)
Weight loss	2 (22.2)	1 (9.1)
Cough	1 (11.1)	1 (9.1)
Dyspnea	1 (11.1)	—

Table 3: Disease and treatment specific baseline characteristics.

Tumor disease characteristics	Frequency (percentage) or mean (min–max)	
	Viscum $n = 9$	Etoposide $n = 11$
Time since primary diagnosis (years)	4.0 (1.5–10.5)	3.7 (1.4–7.2)
DFS 1° interval (months)	22.3 (2.9–43.3)	27.9 (14.5–39.4)
DFS 2° interval (months)	22.9 (3.0–82.1)	14.9 (1.8–47.4)
Time since 2° relapse (weeks)	13.9 (0.9–76.6)	7.6 (1.9–24.6)
Osteosarcoma		
Chondrosarcomatous	1 (11.1)	2 (18.2)
Osteoblastic	4 (44.4)	5 (45.5)
Spindle cell sarcoma	0	1 (9.1)
Not otherwise specified	4 (44.4)	3 (27.3)
Staging (Enneking)		
I (I B)	0	1 (9.1)
II (II A, II B)	6 (66.7)	8 (72.7)
III (III, III A, III B)	3 (33.3)	2 (18.2)
Grading		
2	8 (88.9)	11 (100)
3	1 (11.1)	—
4	—	—
Metastases present	9 (100)	11 (100)
2nd chemotherapy after 1st relapse	5 (55.6)	4 (36.4)
Time since last chemotherapy (years)	3.0 (0.6–10.5)	2.8 (0.4–7.2)
Radiotherapy	—	—
Frequency of surgeries		
3	5 (55.6)	9 (81.8)
4	3 (33.3)	1 (9.1)
5	1 (11.1)	1 (9.1)
Time since last surgery (months)	1.5 (0.7–2.0)	2.2 (1.2–5.9)

under Etoposide could only be seen for "social functioning." Here, on the other hand, deteriorations had to be observed for "nausea/vomiting" and "pain."

Regarding the safety of the patients, five Serious Adverse Events (SAE) occurred during the trial due to hospitalization of patients for surgery (2 Viscum, 1 Etoposide patient) and for pneumonia (2 Etoposide patients). Pneumonia was regarded as related to the Etoposide treatment; therefore, these SAEs constitute serious adverse drug reactions (SAR). Regarding further adverse drug reactions (ADR), no toxicity was reported under *Viscum* treatment except negligible local erythema after sc injection and hypotension in one patient. Under Etoposide, observed toxicity included G2, G3 hematologic toxicity (Table 5). G-CSF was necessary in three patients. Two patients needed dose reduction (14 instead of 21 days per cycle) due to hematologic toxicity, and one patient needed blood transfusion for G4 anemia (1 episode).

4. Discussion

The treatment of relapsed osteosarcoma patients is unsatisfactory especially after a second or further relapse because there is no effective adjuvant treatment besides surgery that can prolong PRDFS. In addition to this, heavily pretreated patients often do not want to receive another aggressive treatment with serious side effects.

The relationship between cancer and the immune system is well known [29]. Also, in osteosarcoma a relationship between infections as a favorable prognostic factor has been documented [30] and a new trend of immunotherapy as adjuvant treatment is emerging in the therapy of osteosarcoma. Interferon-Alpha (IFN) was used in osteosarcoma in the 1960ies at the Karolinska Institute before the chemotherapy era, and 10-year overall survival (OS) results are similar to those attained with chemotherapy alone [31]. Muramyl tripeptide (MTP) is a BCG derived drug with immunomodulating activity activating macrophage tested at Memorial

Table 4: Mean changes from baseline for the QoL scales of the EORTC QLQ-C30.

EORTC QLQ-C30 scale	Estimated changes*	95% CI	*P*-value
Physical functioning			
Viscum	7.30	[0.15; 14.44]	0.046
Etoposide	−2.45	[−8.93; 4.03]	0.430
Role functioning			
Viscum	3.80	[−7.94; 15.54]	0.827
Etoposide	−6.31	[−18.28; 5.65]	0.508
Emotional functioning			
Viscum	−5.98	[−10.58; −1.37]	0.014
Etoposide	−2.48	[−9.84; 4.87]	0.481
Cognitive functioning			
Viscum	−0.92	[−6.49; 4.65]	0.734
Etoposide	−5.94	[−12.19; 0.31]	0.061
Social functioning			
Viscum	11.76	[4.64; 18.88]	0.003
Etoposide	4.78	[0.51; 9.05]	0.031
Global health/QoL			
Viscum	11.17	[2.62; 19.72]	0.013
Etoposide	3.51	[−3.51; 10.54]	0.301
Fatigue			
Viscum	−9.85	[−16.31; −3.38]	0.005
Etoposide	1.13	[−5.72; 7.99]	0.73
Nausea/vomiting			
Viscum	0.43	[−2.70; 3.56]	0.779
Etoposide	5.47	[0.28; 10.66]	0.040
Pain			
Viscum	−10.71	[−18.83; −2.60]	0.012
Etoposide	10.54	[4.64; 16.45]	0.002
Dyspnoea			
Viscum	−12.63	[−16.94; −8.32]	<0.0001
Etoposide	5.82	[−1.04; 12.68]	0.090
Insomnia			
Viscum	−11.35	[−20.74; −1.96]	0.020
Etoposide	5.79	[−2.95; 14.53]	0.177
Appetite loss			
Viscum	−6.40	[−6.40; −6.40]	N.E.†
Etoposide	1.41	[−2.15; 4.96]	0.410
Constipation			
Viscum	−5.54	[−13.58; 2.50]	0.166
Etoposide	−0.62	[−9.65; 8.41]	0.884
Diarrhea			
Viscum	0.83	[−2.81; 4.47]	0.639
Etoposide	2.44	[−1.92; 6.80]	0.251
Financial problems			
Viscum	−11.46	[−16.21; −6.70]	<0.0001
Etoposide	−2.53	[−6.88; 1.83]	0.234

*Estimates resulting from a linear mixed model, including baseline score, treatment and visit as fixed factors, and patients as random factors.
†All postbaseline values in the *Viscum* group were 0; therefore no test statistic could be calculated.

Sloan Kettering Cancer Center together with chemotherapy with improved DFS and prolonged overall survival. A significant gain in OS from 70% to 78% could be observed after 6 years of follow-up [32].

IFN and MTP are quite expensive. MTP is reimbursed by the Italian health system only for the adjuvant treatment of high risk nonmetastatic osteosarcoma patients (<30 years old) together with chemotherapy, at a high cost (total treatment of 6 months is about € 100.000). *Viscum album fermentatum* has a long history being used for over 80 years; its toxicity is well known and its costs are much more affordable compared to the other two drugs.

Of course our study has major drawbacks. The interpretation of its results is limited by the low number of patients treated, and a larger study is needed for confirmative proof of these preliminary findings. Also, the use of *Viscum album fermentatum Pini* is based on the manufacturer's recommendations for the treatment of sarcomas, which do not include a rationale in this regard [33]. The chosen preparation may be remarkable since the pharmacological effects of mistletoe extracts have to a large extent been attributed to mistletoe lectins [34–36], and other mistletoe extracts of this manufacturer exceed *Viscum album fermentatum Pini* regarding their lectin contents by a factor ranging between 15 and 35. Yet, a pine mistletoe extract was shown to be more potent in enhancing the activity of lymphocytes compared to another extract (Iscador M) richer in mistletoe lectin content [37]. So either the efficacy of mistletoe extract is not (only) depending on the amount of mistletoe lectins or pharmacologically active principles other than mistletoe lectins contribute in a relevant way. Indeed, viscotoxins [36, 38, 39] have been acknowledged as pharmacologically active substances, and other constituents like Kuttan peptides [40], quercetin [41, 42], and polysaccharides [17, 18] have shown antitumor or immunomodulatory activities *in vitro*.

Regardless of these unresolved issues, so far the results indicate a positive trend in PRDFS for *Viscum* compared to historical control, and descriptively also compared to Etoposide. Moreover, *Viscum* patients tend to remain superior to Etoposide patients in several domains of their quality of life. Even if this study has an open-label design and the subjective assessment of QoL may be influenced by the patients knowing about their actual treatment, it seems doubtful that this knowledge unduly affects the patients' QoL assessments; rather, it is more likely that the lower quality of life in Etoposide patients is associated with the higher frequency and intensity of adverse drug reactions observed for this treatment.

5. Conclusions

Therapy with *Viscum* seems to be a promising adjuvant treatment in prolonging DFS of patients free from disease after their second relapse. Etoposide does not seem to prolong DFS. A larger study in this subgroup of patients might be of value, which might compare *Viscum* with other immunomodulators like IFN or MPT.

Table 5: Frequency and intensity of adverse events (AE) and adverse drug reactions (ADR).

AE characteristics	*Viscum* *N* [%]	Etoposide *N* [%]	Total *N* [%]
All AEs	16 [18.8]	69 [81.2]	85 [100.0]
Unfavorable AEs by			
Severity			
Severe	5 [5.88]	26 [30.59]	28 [36.47]
Outcome			
AE unchanged	4 [4.76]	4 [4.76]	8 [9.52]
AE exacerbated	—	2 [2.38]	2 [2.38]
Study medication			
Dose reduced	—	5 [5.88]	5 [5.88]
Use continued after interruption	1 [1.18]	18 [21.18]	19 [22.35]
Use discontinued	2 [2.35]	14 [16.47]	16 [18.82]
Adverse drug reactions (ADR)	2 [2.36]	47 [55.29]	49 [57.65]
Most frequent ADR			
Neutropenia	—	12 [25.53]	12 [24.49]
Anaemia	—	6 [12.77]	6 [12.24]
Leukopenia	—	6 [12.77]	6 [12.24]
Nausea	—	5 [10.64]	5 [10.20]
Alopecia	—	4 [8.51]	4 [8.16]

Conflict of Interests

All authors declare that there is no conflict of interests regarding the publication of this paper. All authors declare not to have employments, consultancies, stock ownerships, honoraria, paid expert testimonies, patent applications, travel grants, or other supports.

Acknowledgments

The authors thank all participants in the study, Dr. J. Johannes Kuehn for his help during the design of the study, Dr. Massimiliano Luppi for supervision of study medication storage, Dr. Silvia Cacciaguerra for monitoring, Susanne Schönberg and Silke Weippert for data management, and especially Dr. Silvana Becker for her ceaseless help on all problems upcoming during the trial. The Society for Cancer Research (Verein für Krebsforschung e.V.; VfK), Switzerland, supplied the *Viscum* medication and test kits for the measurements of some immunological lab parameters for free and gave financial support for monitoring costs. It was the only funding source. Marcus Reif is carrying out other studies for the VfK.

References

[1] A. Briccoli, M. Rocca, M. Salone et al., "Resection of recurrent pulmonary metastases in patients with osteosarcoma," *Cancer*, vol. 104, no. 8, pp. 1721–1725, 2005.

[2] F. Fagioli, M. Aglietta, A. Tienghi et al., "High-dose chemotherapy in the treatment of relapsed osteosarcoma: an Italian Sarcoma Group study," *Journal of Clinical Oncology*, vol. 20, no. 8, pp. 2150–2156, 2002.

[3] G. Bacci, A. Briccoli, A. Longhi et al., "Treatment and outcome of recurrent osteosarcoma: experience at Rizzoli in 235 patients initially treated with neoadjuvant chemotherapy," *Acta Oncologica*, vol. 44, no. 7, pp. 748–755, 2005.

[4] J. Ritter and S. S. Bielack, "Osteosarcoma," *Annals of Oncology*, vol. 21, supplement 7, pp. vii320–vii325, 2010.

[5] S. E. Leary, A. W. Wozniak, C. A. Billups et al., "Survival of pediatric patients after relapsed osteosarcoma: the St. Jude Children's Research Hospital experience," *Cancer*, vol. 119, no. 14, pp. 2645–2653, 2013.

[6] EURAMOS-1, "A randomized trial of the European and American Osteosarcoma Study Group to optimize treatment strategies for resectable osteosarcoma based on histological response to pre-operative chemotherapy," ISRCTN67613327, 2009, https://www.skion.nl/workspace/uploads/euramos_1_protocol_v2_1_2009_04_21.pdf.

[7] R. Kebudi, Ö. Görgün, and I. Ayan, "Oral etoposide for recurrent/progressive sarcomas of childhood," *Pediatric Blood and Cancer*, vol. 42, no. 4, pp. 320–324, 2004.

[8] A. Sandri, M. Massimino, L. Mastrodicasa et al., "Treatment with oral etoposide for childhood recurrent ependymomas," *Journal of Pediatric Hematology/Oncology*, vol. 27, no. 9, pp. 486–490, 2005.

[9] F. Hugo, T. Dittmar, E. K. Treutler, K. S. Zänker, and J. J. Kuehn, "The *Viscum album* extract Iscador P does not cause an autocrine interleukin-6 loop in B-Non-Hodgkin's lymphoma cell lines," *Onkologie*, vol. 28, no. 8-9, pp. 415–420, 2005.

[10] E. A. Mueller and F. A. Anderer, "A *Viscum album* oligosaccharide activating human natural cytotoxicity is an interferon γ inducer," *Cancer Immunology Immunotherapy*, vol. 32, no. 4, pp. 221–227, 1990.

[11] J. M. Braun, H. L. Ko, J. M. Schierholz, and J. Beuth, "Standardized mistletoe extract augments immune response and downregulates local and metastatic tumor growth in murine models," *Anticancer Research*, vol. 22, no. 6, pp. 4187–4190, 2002.

[12] B. Schaffrath, U. Mengs, T. Schwarz et al., "Anticancer activity of rViscumin (recombinant mistletoe lectin) in tumor colonization models with immunocompetent mice," *Anticancer Research*, vol. 21, no. 6, pp. 3981–3987, 2001.

[13] G. Kuttan, L. G. Menon, S. Antony, and R. Kuttan, "Anticarcinogenic and antimetastatic activity of Iscador," *Anti-Cancer Drugs*, vol. 8, no. 1, pp. S15–S16, 1997.

[14] T. Hajto, K. Hostanska, K. Frei, C. Rordorf, and H.-J. Gabius, "Increased secretion of tumor necrosis factor α, interleukin 1, and interleukin 6 by human mononuclear cells exposed to β-galactoside-specific lectin from clinically applied mistletoe extract," *Cancer Research*, vol. 50, no. 11, pp. 3322–3326, 1990.

[15] V. P. Chernyshov, P. Heusser, L. I. Omelchenko et al., "Immunomodulatory and clinical effects of *Viscum album* (Iscador M and Iscador P) in children with recurrent respiratory infections as a result of the chernobyl nuclear accident," *American Journal of Therapeutics*, vol. 7, no. 3, pp. 195–203, 2000.

[16] G. S. Kienle, F. Berrino, A. Büssing, E. Portalupi, S. Rosenzweig, and H. Kiene, "Mistletoe in cancer—a systematic review on controlled clinical trials," *European Journal of Medical Research*, vol. 8, no. 3, pp. 109–119, 2003.

[17] B. Möckel, T. Schwarz, H. Zinke, J. Eck, M. Langer, and H. Lentzen, "Effects of mistletoe lectin I on human blood cell lines and peripheral blood cells—cytotoxicity, apoptosis and induction of cytokines," *Arzneimittel-Forschung/Drug Research*, vol. 47, no. 10, pp. 1145–1151, 1997.

[18] J.-P. D. van Huyen, J. Bayry, S. Delignat et al., "Induction of apoptosis of endothelial cells by *Viscum album*: a role for antitumoral properties of mistletoe lectins," *Molecular Medicine*, vol. 8, no. 10, pp. 600–606, 2002.

[19] M. Harmsma, M. Ummelen, W. Dignef, K. J. Tusenius, and F. C. S. Ramaekers, "Effects of mistletoe (*Viscum album* L.) extracts Iscador on cell cycle and survival of tumor cells," *Arzneimittel-Forschung/Drug Research*, vol. 56, no. 6, pp. 474–482, 2006.

[20] T. Hajtò, T. Berki, L. Pàlinkàs, F. Boldizsàr, and P. Németh, "Investigation of the effect of mistletoe (*Viscum album* L.) extract Iscador on the proliferation and apoptosis of murine thymocytes," *Arzneimittel-Forschung/Drug Research*, vol. 56, no. 6, pp. 441–446, 2006.

[21] P. Schöffski, S. Riggert, P. Fumoleau et al., "Phase I trial of intravenous aviscumine (rViscumin) in patients with solid tumors: a study of the European Organization for research and treatment of cancer new drug development group," *Annals of Oncology*, vol. 15, no. 12, pp. 1816–1824, 2004.

[22] A. Longhi, E. Mariani, and J. J. Kuehn, "A randomized study with adjuvant mistletoe versus oral Etoposide on post relapse disease-free survival in osteosarcoma patients," *European Journal of Integrative Medicine*, vol. 1, no. 1, pp. 31–39, 2009.

[23] G. M. Stein, H. Meink, J. Durst, and P. A. Berg, "Release of cytokines by a fermented lectin-1 (ML-1) free mistletoe extract reflects differences in the reactivity of PBMC in healthy and allergic individuals and tumour patients," *European Journal of Clinical Pharmacology*, vol. 51, no. 3-4, pp. 247–252, 1996.

[24] E. A. Mueller, K. Hamprecht, and F. A. Anderer, "Biochemical characterization of a component in extracts of *Viscum album* enhancing human NK cytotoxicity," *Immunopharmacology*, vol. 17, no. 1, pp. 11–18, 1989.

[25] G. M. Stein and P. A. Berg, "Flow cytometric analyses of the specific activation of peripheral blood mononuclear cells from healthy donors after in vitro stimulation with a fermented mistletoe extract and mistletoe lectins," *European Journal of Cancer*, vol. 34, no. 7, pp. 1105–1110, 1998.

[26] G. M. Stein, A. Büssing, and M. Schietzel, "Stimulation of the maturation of dendritic cells in vitro by a fermented mistletoe extract," *Anticancer Research*, vol. 22, no. 6, pp. 4215–4219, 2002.

[27] M. D. Mossalayi, A. Alkharrat, and D. Malvy, "Nitric oxide involvement in the anti-tumor effect of mistletoe (*Viscum album* L.) extracts iscador on human macrophages: short communication," *Arzneimittel-Forschung/Drug Research*, vol. 56, no. 6, pp. 457–460, 2006.

[28] C. J. Clopper and E. S. Pearson, "The use of confidence or fiducial limits illustrated in the case of the binomial," *Biometrika*, vol. 26, no. 4, pp. 404–413, 1934.

[29] G. E. Blair and G. P. Cook, "Cancer and the immune system: an overview," *Oncogene*, vol. 27, no. 45, article 5868, 2008.

[30] L. M. Jeys, R. J. Grimer, S. R. Carter, R. M. Tillman, and A. Abudu, "Post operative infection and increased survival in osteosarcoma patients: are they associated?" *Annals of Surgical Oncology*, vol. 14, no. 10, pp. 2887–2895, 2007.

[31] C. R. Müller, S. Smeland, H. C. F. Bauer, G. Sæter, and H. Strander, "Interferon-α as the only adjuvant treatment in high-grade osteosarcoma: long term results of the Karolinska Hospital series," *Acta Oncologica*, vol. 44, no. 5, pp. 475–480, 2005.

[32] P. A. Meyers, C. L. Schwartz, M. D. Krailo et al., "Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival—a report from the children's oncology group," *Journal of Clinical Oncology*, vol. 26, no. 4, pp. 633–638, 2008.

[33] A. G. Weleda, *Iscador in Cancer Therapy. Recommendations for Treatment*, Weleda AG Medical Affairs, Schwäbisch-Gmünd, Germany, Society for Cancer Research, Arlesheim, Switzerland, 3rd edition, 2013.

[34] O. Janssen, A. Scheffler, and D. Kabelitz, "In vitro effects of mistletoe extracts and mistletoe lectins. Cytotoxicity towards tumor cells due to the induction of programmed cell death (apoptosis)," *Arzneimittel-Forschung/Drug Research*, vol. 43, no. 11, pp. 1221–1227, 1993.

[35] A. Büssing, K. Suzart, J. Bergmann, U. Pfüller, M. Schietzel, and K. Schweizer, "Induction of apoptosis in human lymphocytes treated with *Viscum album* L. is mediated by the mistletoe lectins," *Cancer Letters*, vol. 99, no. 1, pp. 59–72, 1996.

[36] A. Büssing, W. Vervecken, M. Wagner, B. Wagner, U. Pfüller, and M. Schietzel, "Expression of mitochondrial Apo2.7 molecules and Caspase-3 activation in human lymphocytes treated with the ribosome-inhibiting mistletoe lectins and the cell membrane permeabilizing viscotoxins," *Cytometry*, vol. 37, no. 2, pp. 133–139, 1999.

[37] G. M. Stein and P. A. Berg, "Immunmodulation durch Mistelextrakte: ergebnisse von in vitro und ex vivo Studien (abstract)," *Der Merkurstab*, vol. 50, no. 2, p. 35, 1997.

[38] A. Büssing, G. Schaller, and U. Pfüller, "Generation of reactive oxygen intermediates (ROI) by the thionins from *Viscum album* L.," *Anticancer Research*, vol. 18, no. 6, pp. 4291–4296, 1998.

[39] J. Tabiasco, F. Pont, J.-J. Fournié, and A. Vercellone, "Mistletoe viscotoxins increase natural killer cell-mediated cytotoxicity," *European Journal of Biochemistry*, vol. 269, no. 10, pp. 2591–2600, 2002.

[40] G. Kuttan, D. M. Vasudevan, and R. Kuttan, "Isolation and identification of a tumour reducing component from mistletoe extract (Iscador)," *Cancer Letters*, vol. 41, no. 3, pp. 307–314, 1988.

[41] H. Becker and J. Exner, "Vergleichende Untersuchungen von Misteln verschiedener Wirtsbäume an Hand der Flavonoide and Phenolcarbonsäuren," *Zeitschrift für Pflanzenphysiologie*, vol. 97, no. 5, pp. 417–428, 1980.

[42] Z. A. Sekeroğlu and V. Sekeroğlu, "Effects of *Viscum album* L. extract and quercetin on methotrexate-induced cyto-genotoxicity in mouse bone-marrow cells," *Mutation Research*, vol. 746, no. 1, pp. 56–59, 2012.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 430518, 9 pages
http://dx.doi.org/10.1155/2014/430518

Research Article

Additional Therapy with a Mistletoe Product during Adjuvant Chemotherapy of Breast Cancer Patients Improves Quality of Life: An Open Randomized Clinical Pilot Trial

Wilfried Tröger,[1] Zdravko Ždrale,[2] Nevena Tišma,[2] and Miodrag Matijašević[2]

[1] *Clinical Research Dr. Tröger, Zechenweg 6, 79111 Freiburg, Germany*
[2] *Institute of Oncology and Radiology of Serbia, Pasterova 14, 11000 Belgrade, Serbia*

Correspondence should be addressed to Wilfried Tröger; troeger@crdt.de

Received 10 October 2013; Revised 24 December 2013; Accepted 13 January 2014; Published 20 February 2014

Academic Editor: Matthias Kröz

Copyright © 2014 Wilfried Tröger et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Breast cancer patients receiving adjuvant chemotherapy often experience a loss of quality of life. Moreover chemotherapy may induce neutropenia. Patients report a better quality of life when additionally treated with mistletoe products during chemotherapy. *Methods*. In this prospective randomized open-label pilot study 95 patients were randomized into three groups. All patients were treated with an adjuvant chemotherapy. The primary objective of the study was quality of life, the secondary objective was neutropenia. Here we report the comparison of HxA ($n = 34$) versus untreated control ($n = 31$). *Results*. In the explorative analysis ten of 15 scores of the EORTC QLQ-C30 showed a better quality of life in the HxA group compared to the control group ($P < 0.001$ to $P = 0.038$ in Dunnett-T3 test). The difference was clinically relevant (difference of at least 5 points, range 5.4–12.2) in eight of the ten scores. Neutropenia occurred in 7/34 HxA patients and in 8/31 control patients ($P = 0.628$). *Conclusions*. This pilot study showed an improvement of quality of life by treating breast cancer patients with HxA additionally to CAF. Although the open design may be a limitation, the findings show the feasibility of a confirmatory study using the methods described here.

1. Introduction

Quality of life of cancer patients is frequently reduced during and after chemotherapy [1]. But physicians have reported better quality of life in breast cancer patients additionally treated with mistletoe products during chemotherapy, compared to patients receiving chemotherapy alone or together with a placebo [2–5]. A systematic review of clinical trials in breast cancer patients [6] identified nine studies in which mistletoe products were given additionally to conventional chemotherapy, thereof three retrolective studies [7–9] and six randomized clinical trials (three are open-label [2, 3, 10] and three double-blind [4, 5, 11]). In one study, data on natural killer-cell activity and quality of life assessed by EORTC QLQ-C30 was collected. In all other studies disease- or therapy-related symptoms or quality of life, assessed by different questionnaires, were documented and showed an improvement favouring the additional therapy with mistletoe.

Because 70% of cancer patients use mistletoe products in Germany, randomized clinical trials are difficult to conduct: low recruitment rates and noncompliance because of therapy preferences are the consequences [11–14]. Therefore, we conducted this trial in Serbia, a country where mistletoe therapy was unknown.

This randomized clinical pilot trial was carried out to assess the effects of two different mistletoe products on quality of life and on neutrophil count when administered during CAF chemotherapy for breast cancer. The aim was to identify promising outcome measures and methods for future studies in Serbia and to use the results for sample size calculation for a following confirmative study. Here we present the results with the mistletoe product Helixor A (HxA).

2. Methods

2.1. Objectives. The objectives of this pilot study were to determine the clinical response (primary: quality of life, and secondary: neutropenia) of breast cancer patients to an additional mistletoe therapy during CAF. Our primary hypotheses were: breast cancer patients receiving mistletoe products during six cycles of consecutive treatment with CAF will show a better quality of life and less neutropenia compared to patients receiving CAF alone.

2.2. Design. We conducted a prospective randomized open-label pilot study with equal-size randomization into three groups. All three groups received six cycles of CAF. In addition, one group received Helixor A (HxA), another group received Iscador M, and a control group had no additional therapy. The study was not placebo-controlled because currently no active placebo is known that can imitate the typical and time-dependent reactions following subcutaneous injections of mistletoe products (reactions at the injection site, increased body temperature, and flu-like symptoms). Here we report the comparison of HxA versus control. The other part of this combined pilot study has been published elsewhere [10].

2.3. Participants. At the Institute of Oncology and Radiology, National Cancer Research Centre of Serbia in Belgrade (IORS), breast cancer patients in the stages $T_{1-3}N_{0-2}M_0$ scheduled to receive six consecutive cycles of CAF after surgery were assessed for eligibility.

Additional inclusion criteria were female gender, age ≥ 18 years, Karnofsky-Index ≥ 60, leukocytes ≥ 3,000/mm^3, thrombocytes ≥ 100,000/mm^3, serum creatinine ≤ 2 mg%, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) ≤ 2.5 × the upper institutional limits.

Exclusion criteria were pregnancy or lactation, distant metastases, planned radiation or hormone therapy during the CAF treatment period, use of immunostimulant or immunosuppressive agents (e.g., corticosteroids) except for nausea and emesis, current use of other investigational agents, clinically relevant physical or mental illness such as serious infections, hepatic, renal or other organ dysfunction, or major depression, alcohol abuse, alcoholism, oral or parenteral drug abuse, and methadone treatment.

2.4. Randomization. The chance to be allocated to any of the three groups (HxA, other mistletoe product, and control) was 1 : 1 : 1, for randomization variable block sizes were used. No stratification took place prior to randomization. The randomization sequence was generated by Clinical Research Dr. Tröger (CRDT), using SPSS (SPSS 14.0.1, SPSS Inc., Chicago, Ill, USA). Allocation concealment was implemented by using sealed envelopes, prepared by CRDT. Patients were enrolled by investigators at the Outpatient Clinic of the IORS, while the sealed randomization envelopes were stored in the Department of Study Coordination of the IORS and released consecutively for each enrolled patient.

2.5. Interventions. CAF was administered in six cycles with a three-week interval between each cycle. The scheduled dosage was 500 mg cyclophosphamide, 50 mg adriamycin, and 500 mg 5 FU per 1 m^2 skin surface administered at one day. All patients received antiemetic therapy with a single dose of ondansetron chloride 8 mg, dexamethasone 8 mg, and ranitidine 50 mg, respectively, administered prior to each CAF cycle.

No other antineoplastic or immunomodulatory therapies were permitted during the study. Patient compliance was examined by account of the questionnaires and patient diaries and at each visit.

Patients randomly allocated to the first group with additional therapy received Helixor A (HxA; aqueous extract of *Viscum album* from fir tree (*Abies alba*), fresh plant material). HxA was manufactured and provided by Helixor Heilmittel GmbH & Co. KG, Rosenfeld, Germany, and prepared in 1 mL ampoules for injection, each ampoule containing aqueous extract of 1, 5, 10, 20, 30, or 50 mg of fresh mistletoe herb, respectively, in isotonic saline solution. HxA was administered by subcutaneous injection of 1 mL HxA into the upper abdominal region three times per week. The patients were instructed to inject HxA themselves. The dosage of HxA followed the guidelines of the manufacturer with a stepwise increase: 3×1 mg, 3×5 mg, 3×10 mg, 3×20 mg, 3×30 mg, and remaining doses 50 mg. Dose-dependent inflammatory reactions at the injection site (redness and swelling, sometimes accompanied by itching) were monitored. If such reactions exceeded 5 cm in diameter, the dosage was decreased or the therapy was paused until the reactions had ceased. Depending on the beginning of the injections in the first week, 52–54 injections of HxA were planned. An average of 52.3±2.8 injections with altogether 2,813 mg (min = 1 mg; max = 6,818 mg of HxA per patient) were administered in the HxA group.

2.6. Outcomes. The primary objective of the study was quality of life and the secondary objective neutropenia. Quality of life was documented with the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) in the official Serbian translation [15]. The EORTC QLQ-C30 has 30 questions and is analysed in 15 scores: six scores for functioning and nine symptom scores. Before each of the six CAF cycles and at least three weeks after the last CAF cycle, EORTC QLQ-C30 was filled in by the patients and the absolute number of neutrophils in the peripheral blood was determined. Neutropenia was defined as neutrophil count <1,000/μL.

2.7. Assessment of Adverse Events. Adverse events (AE) were assessed by interviewing the patients and by analysing laboratory data at each visit. The Common Terminology Criteria of Adverse Events (v3.0) were used for grading. Local reactions to HxA less than 5 cm in diameter were expected reactions and therefore not classified as adverse events.

Neutropenia was one of the study objectives and was not classified as AE.

2.8. Statistical Methods. Statistical analysis (SPSS 14.0) was performed on the intention-to-treat population, a population consisting of all patients randomised into one of the three groups. Missing data were not replaced. Because of the pilot character of this study, all results of the analysis are explorative and do not have a confirmatory character. Therefore no sample size calculation was performed. A sample size of 90 patients (30 per group) was considered to be sufficient. For calculation of the EORTC QLQ-C30 scores, missing data within one scale were replaced according to the manual [16].

Quality of life (EORTC QLQ-C30) was analysed as follows: for each EORTC QLQ-C30, the mean change from baseline during follow-up in each group was compared among the HxA group, the group with the other mistletoe product, and the control group, using nonparametric marginal models according to Brunner and Langer [17] with therapy as whole-plot factor and time as subplot factor and a possible interaction between these two factors (results not shown). As a sensitivity analysis, a parametric covariance pattern model was also applied and found to qualitatively concur with the nonparametric results. For a more comprehensible presentation, the estimates of this parametric model will be shown: post-hoc analyses of differences between the HxA group and the control group were performed using the Dunnett-T3 test. Clinical relevance of between-group differences of EORTC QLQ-C30 scores was classified according to Osoba et al. [18] as small (5–10 points), moderate (11–20 points), and large (>20 points).

For the incidence of neutropenia, the difference between the HxA and control groups was analysed by chi-square test according to the sequential rejective Holm procedure.

2.9. Adherence to Regulations and Guidelines. The study was approved by the Ethics Committee of the National Cancer Research Centre of Serbia without modifications (date: 3 October 2005) and by the Serbian Drug Agency (date: 01 November 2005). Due to its pilot character, this study was not registered in a public study registry. The study was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice guidelines, and national laws. A patient insurance was provided for all participants. All patients provided signed informed consent prior to inclusion. CRDT was responsible for planning, conduct, monitoring, and analysis of the study. Two audits at the CRDT office and one at the study site were performed by the two sponsors during the study; no violation of Good Clinical Practice was detected.

This publication followed the CONSORT-PRO statement for reporting of randomized trials [19], therefore "standardized mean differences" (effect sizes) are calculated [20].

3. Results

3.1. Recruitment, Participant Flow, Assessment, and Numbers Analysed. From 14 December 2005 to 15 February 2007 a total of 123 breast cancer patients were scheduled for treatment with CAF and assessed for eligibility at the IORS study centre. 28 patients did not fulfil the eligibility criteria (reasons: see Figure 1), whereas 95 patients were included and randomized into the 3 therapy groups: CAF and HxA ($n = 34$), CAF and another mistletoe product ($n = 30$), and CAF without additional therapy ($n = 31$). One patient in the control group was withdrawn from further CAF therapy after three cycles of CAF because of heart disease (Figure 1).

In the HxA group, 5 patients dropped out; reasons were withdrawal of informed consent ($n = 4$, in one of these patients travel distance to study centre was the reason) and rhinoconjunctivitis with putative relationship to mistletoe therapy ($n = 1$). These dropout patients were replaced to achieve at least 30 in both groups. All other patients in the HxA group and all patients in the control group received the six scheduled CAF cycles.

The EORTC QLQ-C30 was evaluable for 86.9% (207 of 238) of planned visits in the HxA group, and for 97.2% (211 of 217) of planned visits in the control group. None of the expected questionnaires were missing in the follow-up phase of the study (Table 1). The neutrophil count was determined at 88.6 % (211 of 238) and 98.2 % (213 of 217) of planned visits in the HxA group and control group, respectively.

3.2. Baseline Data of the Patient Groups. The HxA group and the control group did not differ significantly regarding age, tumour stage, body mass index, physical status, vital signs, previous diseases, EORTC QLQ-C30 scores, and neutrophil counts (Table 2).

3.3. Quality of Life during Chemotherapy. During chemotherapy with CAF, a deterioration of the quality of life occurred in 14 of 15 EORTC QLQ-C30 mean scores in the control group of which 4 were clinically relevant; whereas a deterioration occurred in 5 of 15 scores in the HxA group, of which 2 were clinically relevant.

In the adjusted analyses, mean differences from baseline were compared between the two groups for each EORTC QLQ-C30 score: 14 of 15 comparisons favoured the HxA group and one comparison (financial difficulties) favoured the control group. Ten of 14 comparisons favouring the HxA group showed significant differences (Figures 2 and 3). Eight of these 10 significant between-group differences of the EORTC QLQ-C30 scores were clinically relevant (>5 points), of which two (Pain −10.8 points, Role function +10.5 points) were moderate and the remaining six were small (5–10, Figures 2 and 3).

In explorative analyses, the differences from baseline for each of the 15 scores at each of the six follow-up assessments were compared between the two groups. 80 out of 90 differences favoured the HxA group, while 10 differences favoured the control group (data not shown). Furthermore, moderate differences of at least 10 points were found once in nine scores (role function, emotional function, social function, and global health: pain, insomnia, nausea/emesis, appetite loss, and diarrhoea), small differences of 5–10 points were found in three scores (cognitive function, fatigue, and constipation), and differences <5 points were found in the remaining three scores.

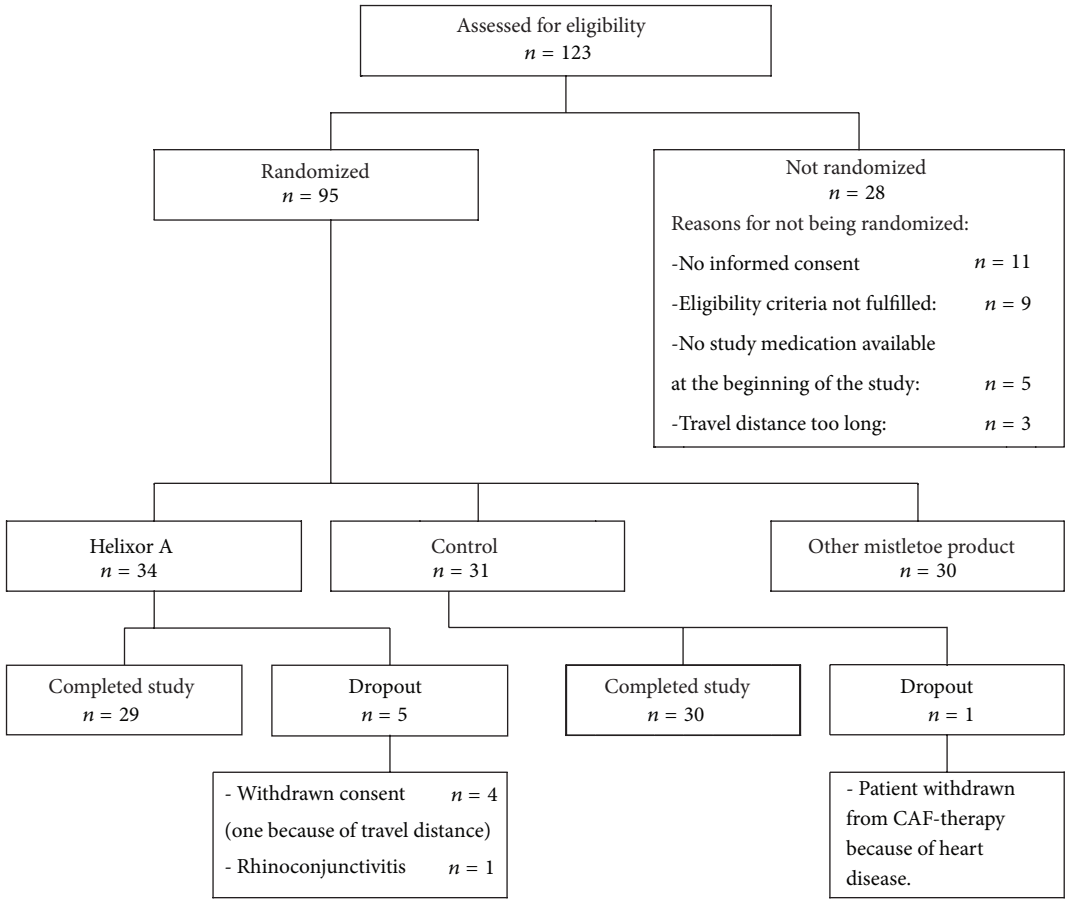


Figure 1: Detailed flow chart of the patient disposition.

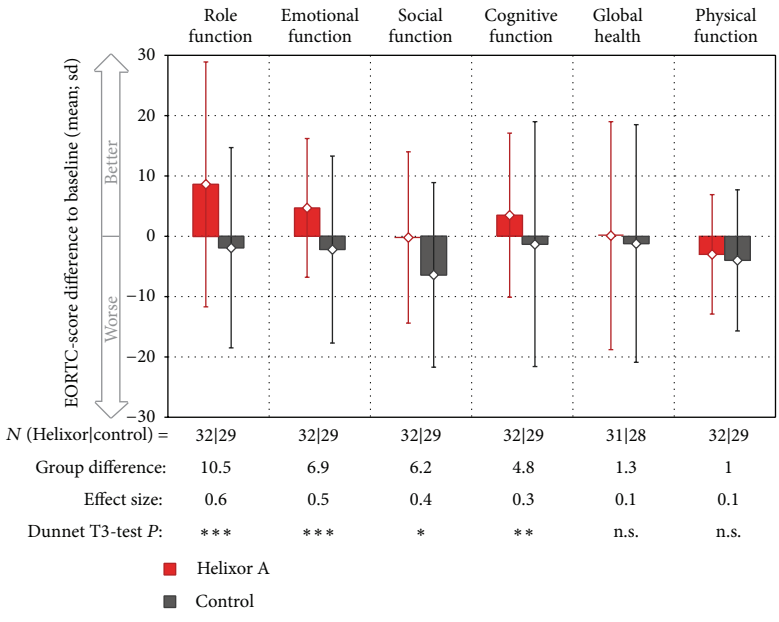


Figure 2: Differences of the mean of six follow-up values to baseline values of the EORTC QLQ-C30 function scores (mean ± sd), sorted by between-group group differences. All patients with at least one follow-up visit are displayed. P values are corrected using Bonferroni's method regarding 15 EORTC scores tested and defined as follows: $^{***}P < 0.001$ (extremely significant); $^{**}P < 0.01$ (highly significant); $^{*}P < 0.05$ (significant); n.s.: not significant.

Table 1: Baseline status.

	Group		P values
	HxA (n = 34)	Control (n = 31)	
Age in years (mean ± SD; t-test)	50.4 ± 6.9	50.8 ± 8.0	0.228
Tumor status (n; Chi square test)			
Tumor classification			0.619
T1	9	9	
T2	24	19	
T3	1	2	
Tx	0	1	
Pos. lymph nodes			0.849
N0	16	16	
N1	16	14	
N2	2	1	
Tumor grade			0.474
G1	3	1	
G2	28	24	
G3	3	6	
Menopause status (n; Kruskal-Wallis test)			0.475
Premenopausal	18	13	
Perimenopausal	2	1	
Postmenopausal	13	17	
Unknown	1	0	
Receptor status estrogen			0.507
Positive	21	17	
Negative	9	12	
unknown	4	2	
Receptor status progesterone			0.620
Positive	22	19	
Negative	8	10	
Unknown	4	2	
Body Mass Index (mean ± SD; t-test)	26.1 ± 4.3	25.6 ± 4.7	0.709
Karnofsky-Index (mean ± SD)	100 ± 0.0	100 ± 0.0	—
Abnormal findings on physical examination (n)	0	0	—
Vital signs (mean ± SD; t-test)			
Blood pressure systolic (mmHg)	126.1 ± 12.9	132.2 ± 19.0	0.139
Blood pressure diastolic (mmHg)	79.9 ± 9.2	83.6 ± 13.6	0.209
Pulse (/min)	77.3 ± 13.0	77.1 ± 10.1	0.945
Temperature (°C)	36.6 ± 0.1	36.6 ± 0.1	1.000
Primary outcomes			
EORTC QLQ-C30 (mean ± SD; Mann-Whitney U-test)			
Global health status	66.9 ± 21.5	68.5 ± 18.3	0.713
Physical functioning	85.6 ± 14.6	86.0 ± 12.4	0.872
Role functioning	68.6 ± 23.5	73.0 ± 16.9	0.443
Emotional functioning	69.4 ± 17.9	74.1 ± 18.1	0.274
Cognitive functioning	82.8 ± 17.2	79.3 ± 23.4	0.23
Social functioning	73.5 ± 22.5	80.5 ± 18.9	0.226
Fatigue	25.5 ± 18.3	25.7 ± 19.5	0.894
Nausea and vomiting	7.4 ± 16.0	2.9 ± 7.8	0.285
Pain	21.1 ± 20.2	16.1 ± 20.6	0.273
Dyspnea	2.9 ± 9.6	3.4 ± 10.3	0.839
Insomnia	20.6 ± 26.0	23.0 ± 31.0	0.902
Appetite loss	14.7 ± 27.5	10.3 ± 20.1	0.704
Constipation	8.8 ± 18.9	6.9 ± 13.7	0.914
Diarrhoea	4.9 ± 14.5	2.3 ± 8.6	0.498
Financial difficulties	20.6 ± 23.2	20.7 ± 30.1	0.661
Secondary outcomes			
Incidence of neutropenia	0	0	—

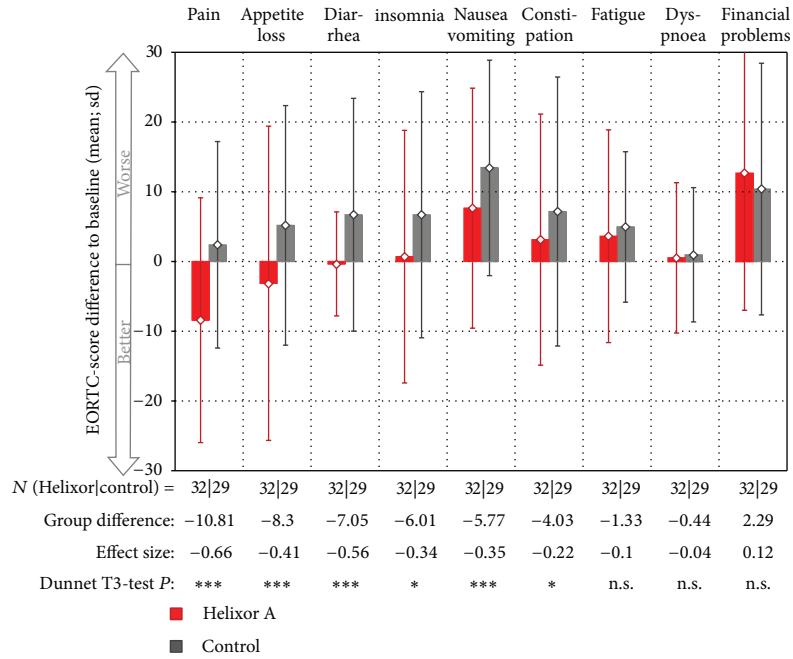


FIGURE 3: Differences of the mean of six follow-up values to baseline of the EORTC QLQ-C30 symptom scores (mean ± sd) sorted by group differences. All patients with at least one follow-up visit are displayed. P values are corrected using Bonferroni's method regarding 15 EORTC scores tested and defined as follows: $^{***}P < 0.001$ (extremely significant); $^{**}P < 0.01$ (highly significant); $^{*}P < 0.05$ (significant); n.s.: not significant.

TABLE 2: Number of patients and compliance in the completion of QoL forms by visit and groups.

Group Visit	Time schedule (days)	Time window (days)	Dropout	Forms expected	Forms received
Control					
1	0	0-0	0	31	29 (94%)
2	21	21–25	0	31	31 (100%)
3	42	42–49	0	31	31 (100%)
4	63	63–74	1	30	30 (100%)
5	84	84–98	1	30	30 (100%)
6	105	105–122	1	30	30 (100%)
7	$182 \geq x \geq 26$	126–143	1	30	30 (100%)
Helixor:					
1	0	0-0	0	34	34 (100%)
2	21	18–28	2	32	32 (100%)
3	42	39–51	5	29	29 (100%)
4	63	63–75	5	29	29 (100%)
5	84	84–96	5	29	29 (100%)
6	105	105–117	5	29	29 (100%)
7	$182 \geq x \geq 126$	126–155	5	29	29 (100%)

3.4. Neutropenia during Chemotherapy. Neutropenia (neutrophil count < 1,000/μL) was detected seven times in seven different patients of the HxA group and nine times in eight different patients of the control group (Table 3) ($P = 0.628$).

3.5. Adverse Events. Altogether 1,527 injections of HxA were administered. Twenty of 34 patients (59%) of the HxA group reported altogether 45 adverse events (AE). The 45 AE were: local inflammatory reaction at the injection site > 5 cm, with definite causal relation to HxA ($n = 42$, representing 2.7% of 1,527 injections); rhinoconjunctivitis at one eye, documented by the investigator to be probably caused by HxA—the patient withdrew informed consent and gave no further information ($n = 1$); and AEs without any causal relationship to the administration of HxA ($n = 2$).

In the control group, 12 AEs and one serious adverse event (SAE: acute hospitalization because of dehydration upon severe emesis; see Table 4) occurred.

Table 3: List of patients experiencing a neutropenia.

Group	ID	Visit number	Date (Visit)	Age	Stage	T	N	M	G	Leuco-cytes/nL	Neutro-phils/nL
HxA	18	2	26.04.2006	43	2	2	1	0	2	10.2	0.6
	61	2	18.09.2006	50	2	2	0	0	2	2.6	0.9
	42	7	03.11.2006	47	2	2	1	0	2	1.4	0.5
	53	7	06.12.2006	44	2	2	1	0	2	1.6	0.4
	57	7	22.12.2006	64	2	2	1	0	3	1.5	0.3
	58	7	22.12.2006	37	2	2	0	0	3	2.5	0.7
	75	7	07.03.2007	55	2	2	0	0	2	3.2	0.9
Control	13	3	26.04.2006	32	2	2	1	0	2	2.8	0.9
	33	5	29.08.2006	60	2	2	0	0	2	2.4	0.9
	87	6	26.03.2007	45	2	2	1	0	2	3.3	0.3
	90	6	29.03.2007	52	2	1	0	0	3	2.6	0.8
	51	7	08.12.2006	66	2	2	1	0	2	1.3	0.3
	56	7	21.12.2006	53	2	2	1	0	2	3.5	0.9
	62	7	31.01.2007	62	2	1	1	0	2	2.5	0.8
	66	7	23.01.2007	44	1	1	0	0	2	2.4	0.8
	90	7	19.04.2007	52	2	1	0	0	3	2.7	0.9

Seven patients of the HxA group and eight patients of the control group experienced a neutropenia ($P = 0.628$; 2-sided Chi square test) at day 21 of the respective cycle/visit.

Table 4: List adverse events.

Group AE	Code*	Number	Grade**	number	Relation	Duration	Outcome
Helixor A						Adverse events	
Localized skin reaction at the injection site	10022096	42	Mild Moderate Severe	1 13 27	Definite	Median 4 days (lq 2–uq 6,5)	Resolved without sequelae
Conjunctivitis	10010741	1	Moderate	1	Probable	Unknown	Unknown
Febrile temperature	10021113	1	Severe	1	Unrelated	2 days	Resolved without sequelae
Sting	—	1	Severe	1	Unrelated	Unknown	Unknown
Control						Adverse events	
Urticaria (localized)	10046749	1	Mild	1	Unrelated	3 days	Resolved without sequelae
Wound infection	10048038	1	Severe	1	Unrelated	6 days	Resolved without sequelae
Nausea/emesis	10028813	3	Severe	3	Unrelated	2 days	Resolved without sequelae
Control						Serious adverse events:	
Nausea/emesis***	10028813	1	Severe	1	Unrelated	4 days	Resolved without sequelae

*Code according to the Medical Dictionary for Regulatory Activities.
**Grading according to the Common Terminology Criteria of Adverse Events (v3.0).
***Patient was hospitalized to treat dehydration. According to the ICH 2A Guidelines hospitalization is defined as "serious adverse event".
Abbreviations: lq: lower quartile; uq: upper quartile; ICH: International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use.

4. Discussion

In this randomized pilot study quality of life (EORTC QLQ-C30) and incidence of neutropenia were investigated in breast cancer patients undergoing adjuvant CAF chemotherapy. Patients receiving HxA in addition to CAF had significantly better quality of life, compared to patients receiving CAF alone. HxA therapy was well tolerated.

Strengths of this study include a high recruitment rate, detailed assessments of therapy implementation, high therapy compliance, and very low dropout rates.

Due to the open-label design, the study cannot distinguish between direct drug effects on quality of life and possible indirect effects from therapy expectations, therapy administration, and so forth, in the HxA group.

Generally, medication trials are blinded to separate pharmacological effects from placebo effects. However, the lack of blinding may not necessarily have had relevant effects on the results of this study: an updated Cochrane review of randomized trials comparing placebo to no treatment found no significant placebo effects on eight out of ten evaluable indications, small effects on self-reported pain, and

moderate effects on phobia. Even these effects might have been confounded by biases [21–23].

This study was designed as a pilot study, and the limited sample size of 30 patients per group does not allow for hypothesis confirmation. Moreover dropout patients or patients with missing data were more frequent in the HxA-group. As the results were calculated using data from all included patients (ITT), data of two patients in each group were missing. The remaining differences are additional three patients (ITT) in the HxA-group, which may lead to a biased result. Nevertheless, significant differences in 10 of 15 EORTC QLQ-C30 scores favouring the HxA group were found. All of these scores showed a clinically relevant difference of at least 5 points. The latter scores include role function, emotional function, pain, nausea, emesis, appetite loss, and diarrhoea, which are relevant symptoms in patients during chemotherapy with CAF.

These results are consistent with findings from another randomized phase III trial, wherein the administration of HxA to patients undergoing chemotherapy for breast cancer, lung cancer, or ovarian cancer was associated with significant beneficial effects on quality of life [3]. Notably, in the latter study, different methods for assessment of quality of life were used ("Functional Living Index-Cancer" questionnaire filled in by the patients, and Karnofsky performance index classified by the attending physician).

On the other hand, in a trial of early breast cancer patients receiving radiochemotherapy (CMF schedule), no influence of adjunctive therapy with HxA on any of the EORTC QLQ-C30 scores was found [11]. This discrepancy to the present study may be attributed to the fact that in the latter trial, patients did not experience any significant deterioration of the EORTC QLQ-C30 scores during radiochemotherapy. Consequently, a favourable effect of the additive mistletoe therapy could not be achieved.

On the whole, there is some evidence that mistletoe extracts may have an impact on quality of life and reduction of side effects of chemotherapy especially in case of breast cancer patients as concluded in two recent comprehensive systematic reviews [24, 25].

In this pilot study, the effects of HxA on quality of life increased over time with the largest effects observed at the last follow-up visit. A longer follow-up period might show additional long- term benefits of HxA therapy.

5. Conclusions

In this randomized pilot study of patients receiving adjuvant CAF chemotherapy for breast cancer, additional treatment with mistletoe therapy (Helixor A) was associated with significant and clinically relevant improvements of quality of life. Although the open design may be a limitation, the findings show the feasibility and justification of a phase III study using the methods described here.

Abbreviations

5-FU:	5-Fluorouracil
AE:	Adverse event
CAF:	Cyclophosphamide, adriamycine, and 5-fluoro-uracil
CRDT:	Clinical Research of Dr. Tröger
EORTC QLQ-C30:	European Organization for Research and Treatment of Cancer Quality of Life Questionnaire
HxA:	Helixor A
IORS:	Institute of Oncology and Radiology of Serbia
ITT:	Intention to treat
SAE:	Serious adverse event.

Conflict of Interests

The sponsor, Helixor Heilmittel GmbH & Co. KG, had no influence on study design, planning, conduct, or analysis. The authors declare that they have no conflict of interests.

Authors' Contribution

Miodrag Matijašević was the responsible principal investigator. Zdravko Ždrale was the coinvestigator. Nevena Tišma carried out the blood analysis. Wilfried Tröger was principal author of the paper, wrote the study protocol, coordinated the study, had full access to all data, and is guarantor. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to thank Petra Siemers, Anja Glockmann, and Claudia Bihl for monitoring and data management, Biostatistik Fischer for statistical analyses, and Dusanka Jelecanin for the coordination of patients in the centre. Special thanks go to the study nurses Milijana Sokol and Zorica Randelović and the participating patients. The part of the study that is reported here was financially supported by Helixor Heilmittel GmbH & Co. KG, Rosenfeld, Germany.

References

[1] P. A. Ganz, L. Kwan, A. L. Stanton et al., "Quality of life at the end of primary treatment of breast cancer: first results from the moving beyond cancer randomized trial," *Journal of the National Cancer Institute*, vol. 96, no. 5, pp. 376–387, 2004.

[2] B.-M. Heiny, "Additive therapie mit standardisiertem Mistelextrakt reduziert die Leukopenie und verbessert die Lebensqualität von Patientinnen mit fortgeschrittenem Mammakarzinom unter palliativer Chemotherapie (VEC-Schema)," *Krebsmedizin*, vol. 12, pp. 1–14, 1991.

[3] B. K. Piao, Y. X. Wang, G. R. Xie et al., "Impact of complementary mistletoe extract treatment on quality of life in breast, ovarian and non-small cell lung cancer patients. A prospective randomized controlled clinical trial," *Anticancer Research*, vol. 24, no. 1, pp. 303–309, 2004.

[4] V. F. Semiglasov, V. V. Stepula, A. Dudov, W. Lehmacher, and U. Mengs, "The standardised mistletoe extract PS76A2 improves QoL in patients with breast cancer receiving adjuvant CMF chemotherapy: a randomised, placebo-controlled,

double-blind, multicentre clinical trial," *Anticancer Research*, vol. 24, no. 2, pp. 1293–1302, 2004.

[5] V. F. Semiglazov, V. V. Stepula, A. Dudov, J. Schnitker, and U. Mengs, "Quality of life is improved in breast cancer patients by standardised mistletoe extract PS76A2 during chemotherapy and follow-up: a randomised, placebo-controlled, double-blind, multicentre clinical trial," *Anticancer Research*, vol. 26, no. 2, pp. 1519–1529, 2006.

[6] G. S. Kienle, A. Glockmann, M. Schink, and H. Kiene, "Viscum album L. extracts in breast and gynaecological cancers: a systematic review of clinical and preclinical research," *Journal of Experimental and Clinical Cancer Research*, vol. 28, no. 1, article 79, 2009.

[7] J. Beuth, B. Schneider, and J. M. Schierholz, "Impact of complementary treatment of breast cancer patients with standardized mistletoe extract during aftercare: a controlled multicenter comparative Epidemiological Cohort Study," *Anticancer Research*, vol. 28, no. 1, pp. 523–527, 2008.

[8] P. R. Bock, W. E. Friedel, J. Hanisch, M. Karasmann, and B. Schneider, "Efficacy and safety of long-term complementary treatment with standardized european mistletoe extract (Viscum album L.) in addition to the conventional adjuvant oncologic therapy in patients with primary non-metastasized mammary carcinoma / results of a multicenter, comparative, epidemiological cohort study in Germany and Switzerland," *Drug Research*, vol. 54, no. 8, pp. 456–466, 2004.

[9] K. Schumacher, B. Schneider, G. Reich et al., "Influence of postoperative complementary treatment with lectin-standardized Mistletoe extract on breast cancer patients. a controlled epidemiological multicentric retrolective cohort study," *Anticancer Research*, vol. 23, no. 6, pp. 5081–5087, 2003.

[10] W. Tröger, S. Jezdić, Z. Ždrale, N. Tišma, H. J. Hamre, and M. Matijašević, "Quality of life and neutropenia in patients with early stage breast cancer: a randomized pilot study comparing additional treatment with mistletoe extract to chemotherapy alone," *Breast Cancer*, vol. 3, no. 1, pp. 35–45, 2009.

[11] L. Auerbach, V. Dostal, I. Václavik-Fleck et al., "Signifikant höherer Anteil aktivierter NK-Zellen durch additive Misteltherapie bei chemotherapierten Mamma-CA-Patientinnen in einer prospektiven randomisierten doppelblinden Studie," in *Fortschritte in der Misteltherapie. Aktueller Stand der Forschung Und Klinischen Anwendung*, R. Scheer, R. Bauer, H. Becker, V. Fintelmann, F. H. Kemper, and H. Schilcher, Eds., pp. 543–554, KCV, Essen, Germany, 2005.

[12] I. Gerhard, "Problems of randomised treatment studies in complementary medicine. Forsch Komplementämed," *Klass Naturheilkd*, vol. 7, pp. 34–35, 2000.

[13] M. Rostock and R. Huber, "Randomized and double-blind studies—demands and reality as demonstrated by two examples of mistletoe research," *Forschende Komplementarmedizin und Klassische Naturheilkunde*, vol. 11, no. 1, pp. 18–22, 2004.

[14] R. Ziegler, "Mistletoe preparation iscador: are there methodological concerns with respect to controlled clinical trials?" *Evidence-Based Complementary and Alternative Medicine*, vol. 6, no. 1, pp. 19–30, 2009.

[15] N. K. Aaronson, S. Ahmedzai, B. Bergman et al., "The european organization for research and treatment of cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology," *Journal of the National Cancer Institute*, vol. 85, no. 5, pp. 365–376, 1993.

[16] P. M. Fayers, N. K. Aaronson, K. Bjordal, M. Groenvold, D. Curran, and A. Bottomley, *The EORTC QLQ-C30 Scoring Manual*, EORTC Quality of Life Group, European Organization for Research and Treatment of Cancer, Brussels, Belgium, 3rd edition, 2001.

[17] E. Brunner and F. Langer, *Nichtparametrische Analyse Longitudinaler Daten*, Oldenbourg, München, Germany, 1999.

[18] D. Osoba, A. Bezjak, M. Brundage, B. Zee, D. Tu, and J. Pater, "Analysis and interpretation of health-related quality-of-life data from clinical trials: basic approach of the National Cancer Institute of Canada Clinical Trials Group," *European Journal of Cancer*, vol. 41, no. 2, pp. 280–287, 2005.

[19] M. Calvert, J. Blazeby, D. G. Altman, D. A. Revicki, D. Moher, and M. D. Brundage, "Reporting of patient-reported outcomes in randomized trials: the CONSORT PRO extension," *The Journal of the American Medical Association*, vol. 309, no. 8, pp. 814–822, 2013.

[20] J. Cohen, *Statistical Power Analysis for the Behavioral Sciences*, Routledge Academic, New York, NY, USA, 1988.

[21] A. Hróbjartsson and P. C. Gøtzsche, "Is the placebo powerless? An analysis of clinical trials comparing placebo with no treatment," *The New England Journal of Medicine*, vol. 344, no. 21, pp. 1594–1602, 2001.

[22] A. Hróbjartsson and P. C. Gøtzsche, "Is the placebo powerless? Update of a systematic review with 52 new randomized trials comparing placebo with no treatment," *Journal of Internal Medicine*, vol. 256, no. 2, pp. 91–100, 2004.

[23] A. Hróbjartsson and P. C. Gøtzsche, "Placebo interventions for all clinical conditions," *Cochrane Database of Systematic Reviews*, no. 3, 2004.

[24] M. A. Horneber, G. Bueschel, R. Huber, K. Linde, and M. Rostock, "Mistletoe therapy in oncology," *Cochrane Database of Systematic Reviews*, no. 2, p. CD003297, 2008.

[25] G. S. Kienle and H. Kiene, "Influence of Viscum album L (European Mistletoe) extracts on quality of life in cancer patients: a systematic review of controlled clinical studies," *Integrative Cancer Therapies*, vol. 9, no. 2, pp. 142–157, 2010.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 606348, 2 pages
http://dx.doi.org/10.1155/2014/606348

Erratum

Erratum to "NCCAM/NCI Phase 1 Study of Mistletoe Extract and Gemcitabine in Patients with Advanced Solid Tumors"

Patrick J. Mansky,[1,2] Dawn B. Wallerstedt,[3] Timothy S. Sannes,[4] Jamie Stagl,[5] Laura Lee Johnson,[2] Marc R. Blackman,[6] Jean L. Grem,[7] Sandra M. Swain,[8] and Brian P. Monahan[9]

[1] *The Cancer Team at Bellin Health, 1580 Commanche Avenue, Green Bay, WI 54313, USA*
[2] *National Center for Complementary and Alternative Medicine, NIH, Bethesda, MD 20892, USA*
[3] *Samueli Institute, Alexandria, VA 22314, USA*
[4] *Department of Clinical and Health Psychology, University of Florida, Gainesville, FL 32611, USA*
[5] *University of Miami, Miami, FL 33124, USA*
[6] *Research Service (151), Veterans Affairs Medical Center and Department of Medicine, Georgetown University School of Medicine, Washington, DC 20422, USA*
[7] *University of Nebraska Medical Center, Omaha, NE 68198, USA*
[8] *Washington Cancer Institute, Washington Hospital Center, Washington, DC 20010, USA*
[9] *Department of Medicine, Hematology and Medical Oncology Division, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA*

Correspondence should be addressed to Patrick J. Mansky; manpaj@bellin.org

Received 22 November 2013; Accepted 18 December 2013; Published 12 February 2014

Copyright © 2014 Patrick J. Mansky et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

There were several modifications in the paper. They are shown as follows.

Page 1, Abstract: the sentence in the abstract "Dose limiting toxicities (DLT) were grade (G) 3 nonhematologic and G4 hematologic events" should read "... and G4 hematologic events *related to platelets and granulocytes only*."

"GEM 1380 mg/m^2 and mistletoe 250 mg combined were the MTD" should read "GEM 1300 mg/m^2 and mistletoe 250 mg combined were the MTD."

Page 2, Introduction: "While ML antibodies were absent in patients without adverse effects, ..." should read "While ML *antibodies type IgE* were absent in patients ...."

Page 2, Materials and Methods: "A whole plant mistletoe extract (HELIXOR Apis (A), growing on fir trees)" should read "A whole plant mistletoe extract (Helixor A (*Abietis*), growing on fir trees)."

Page 2, Materials and Methods: "Helixor, GmbH, Rosenfeld" should read "Helixor Heilmittel *GmbH & Co. KG,* Rosenfeld."

Page 3, Study Design and Outcomes: the sentence "... was administered with gemcitabine in 20% dose increments per dose level (900, 1080, 1380, and 1560 mg/m^2 ...)" should read "... (900, 1080, 1300, and 1560 mg/m^2 ...)."

Page 4, Results: Maximum Tolerated Dose and Dose Limiting Toxicities. The last sentence in this paragraph should read as follows: Thus, we achieved the MTD at dose level 8 (*gemcitabine* 1300 mg/m^2 and mistletoe 250 mg).

Paragraph 3.4. "Three study participants experienced individual DLTs at dose level 9 (gemcitabine 1560 mg/m^2 with 250 mg daily of mistletoe). These included grade 3 cellulitis at the mistletoe injection site, grade 4 acute renal failure, and grade 4 neutropenia" should read "... and grade 4 *thrombocytopenia.*"

Page 8, Discussion: the sentence "The MTD for the gemcitabine/mistletoe combination in this study was gemcitabine 1380 mg/m^2 ..." should read "The MTD for the gemcitabine/mistletoe combination in this study was gemcitabine 1300 mg/m^2 ...."

Table 4: Dose limiting toxicities by dose level.

Stage I (fixed GEM dose of 750 mg/m^2)			Stage II (fixed mistletoe dose, established in stage 1)			
Level	*N*	DLT	Level/dosage	*N*	DLT	Action taken per protocol
(1) (escalating daily mistletoe injections, reaching: 20 mg/day)	3	None	(6) (250 mg/day mistletoe; **900** mg/m^2 GEM on day 1/8 of 3-week cycle)	7	Grade 4 neutropenia	Dose reduced; enrolled 3 more patients at this dose level.
(2) (escalating daily mistletoe injections, reaching: 50 mg/day)	3	None	(7) (250 mg/day mistletoe; **1080** mg/m^2 GEM on day 1/8 of 3-week cycle)	7	Grade 4 thrombocytopenia	Dose reduced; enrolled 3 more patients at this dose level.
(3) (escalating daily mistletoe injections, reaching: 100 mg/day)	3	None	(8)* (250 mg/day mistletoe; **1300** mg/m^2 GEM on day 1/8 of 3-week cycle)	6	None	N/A
(4) (escalating daily mistletoe injections, reaching: 200 mg/day)	6	None	(9) (250 mg/day mistletoe; **1560** mg/m^2 GEM on day 1/8 of 3-week cycle)	4	Grade 3 cellulitis[a]; grade 4 acute renal failure[b]; grade 4: thrombocytopenia[c]	[a]mistletoe therapy withheld; patient rechallenged and developed hypersensitivity reaction. Mistletoe discontinued; [b]Pt. treated for renal failure and subsequently withdrawn from study; [c]Pt. hospitalized d/t other AE; *Maximum tolerated dose reached per protocol.*
(5) (escalating daily mistletoe injections, reaching: 250 mg/day)	5	None				

* Per study protocol, this level represents the maximum tolerated dose, as 3 DLT's were observed in the subsequent dose level.

Page 8, Table 4: Dose limiting toxicities by dose level. "Level/dosage 8 (250 mg/day mistletoe; 1380 mg/m^2 GEM on day 1/8 of 3-week cycle)" should read "Level/dosage 8 (250 mg/day mistletoe; 1300 mg/m^2 GEM on day 1/8 of 3-week cycle)."

Page 9, Discussion: the sentence "None of the study patients developed febrile neutropenia even at the highest gemcitabine dose of 1650 mg/m^2" should read "None of the study patients developed febrile neutropenia even at the highest gemcitabine dose of 1560 mg/m^2."

Page 9, Conclusion: the sentence "the MTD was gemcitabine 1380 mg/m^2 weekly …" should read "the MTD was gemcitabine 1300 mg/m^2 weekly …."

Page 10, Acknowledgments: the second sentence should read: They greatly appreciate … the support of Drs. *Dietrich* Schlodder and *Jörg* Schierholz.

The sentence "… as well as Sabine Rieger from Helixor GmbH" should read "… as well as Sabine Rieger from *Helixor Heilmittel GmbH & Co. KG*."

The sentence "Mistletoe extract (Helixor A) was provided by Helixor GmbH, Rosenfeld, …" should read "Mistletoe extract (Helixor A) was provided by *Helixor Heilmittel GmbH & Co. KG, Rosenfeld, …*."

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 724258, 11 pages
http://dx.doi.org/10.1155/2014/724258

Research Article

Adverse Drug Reactions and Expected Effects to Therapy with Subcutaneous Mistletoe Extracts (*Viscum album* L.) in Cancer Patients

Megan L. Steele,[1] Jan Axtner,[1] Antje Happe,[1] Matthias Kröz,[1,2,3] Harald Matthes,[1,2] and Friedemann Schad[1,2]

[1] *Research Institute Havelhoehe, 14089 Berlin, Germany*
[2] *Hospital Havelhoehe, 14089 Berlin, Germany*
[3] *Institute for Social Medicine, Epidemiology and Health Economics, Charité-University Medical Center, 10117 Berlin, Germany*

Correspondence should be addressed to Friedemann Schad; fschad@havelhoehe.de

Received 23 September 2013; Accepted 16 December 2013; Published 19 January 2014

Academic Editor: Gene Feder

Copyright © 2014 Megan L. Steele et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. In Europe, mistletoe extracts are widely used as a complementary cancer therapy. We assessed the safety of subcutaneous mistletoe as a conjunctive therapy in cancer patients within an anthroposophic medicine setting in Germany. *Methods.* A multicentre, observational study was performed within the Network Oncology. Suspected mistletoe adverse drug reactions (ADRs) were described by frequency, causality, severity, and seriousness. Potential risk factors, dose relationships and drug-drug interactions were investigated. *Results.* Of 1923 cancer patients treated with subcutaneous mistletoe extracts, 283 patients (14.7%) reported 427 expected effects (local reactions <5 cm and increased body temperature <38°C). ADRs were documented in 162 (8.4%) patients who reported a total of 264 events. ADRs were mild (50.8%), moderate (45.1%), or severe (4.2%). All were nonserious. Logistic regression analysis revealed that expected effects were more common in females, while immunoreactivity decreased with increasing age and tumour stage. No risk factors were identified for ADRs. ADR frequency increased as mistletoe dose increased, while fewer ADRs occurred during mistletoe therapy received concurrent with conventional therapies. *Conclusion.* The results of this study indicate that mistletoe therapy is safe. ADRs were mostly mild to moderate in intensity and appear to be dose-related and explained by the immune-stimulating, pharmacological activity of mistletoe.

1. Introduction

Effective treatment of cancer remains one of the biggest challenges to modern medicine. Due to conventional therapies such as chemotherapy and radiation often falling short of their goals, and to patient dissatisfaction concerning adverse effects associated with these treatments, complementary and alternative medicines (CAM) are becoming increasingly popular [1]. Anthroposophic medicine (AM), founded in the 1920s by Rudolf Steiner and Ita Wegman, is a person-centred medical approach which combines conventional medicine with the use of CAM remedies and specialised therapies, such as physical and artistic therapies [2]. AM uses an integrative approach to treat cancer, focusing not only on elimination of pathological entities (conventional therapies), but also activating salutogenetic resources by using European mistletoe extracts (*Viscum album* L.), and other therapies, with the aim of improving health related functions or preventing further disease [2]. Mistletoe therapy is amongst the most frequently used complementary treatments by cancer patients in Europe [3]. In 2003 more than 18 million defined daily doses were prescribed in Germany [4].

Mistletoe extracts have been shown to kill cancer cells in vitro and can stimulate immune system cells both in vitro and in vivo [5–7]. Two components of mistletoe, namely, viscotoxins and lectins, have been shown to be largely responsible for these effects [7–9]. Reports on the clinical efficacy of mistletoe therapy have been conflicting and systematic

literature reviews often criticise studies for poor design [10, 11]. Although an effect of mistletoe on tumour shrinkage in vivo or overall survival might remain to be proven more thoroughly, a growing number of studies indicate beneficial effects on quality of life of cancer patients and reduction of adverse drug reactions (ADRs) associated with conventional cancer treatments [12–17]. Until recently, however, the safety of mistletoe therapy itself and CAM medications in general has been largely overlooked. Reporting of CAM-related ADRs was neglected in the past due to the belief that CAM are "natural" and therefore safe [18]. Furthermore the possibility of harmful interactions between CAM and conventional medications has been discussed [19, 20]. A number of recent controlled clinical trials, mostly using low doses of one type of mistletoe, have focused on tolerability and safety of mistletoe therapy [16, 21, 22]. The conclusions of these studies ranged from "no safety concerns" to "excellent tolerability" [16, 22]. In clinical practice, however, patients generally receive a wide range of doses and mistletoe products, often in conjunction with conventional therapies. Therefore, the aim of this study was to assess the safety of subcutaneous application of mistletoe extracts as a conjunctive therapy in cancer patients, under standard clinical practice within an AM setting in Germany. This involved detection and classification of ADRs according to internationally accepted guidelines to describe the frequency, causality, severity, and seriousness of ADRs attributed to mistletoe therapy. Additionally, potential risk factors, dose relationships, and drug interactions were investigated in order to identify populations with increased susceptibility to experiencing an ADR to mistletoe.

2. Methods

2.1. Study Design and Data Sources. The present study was designed as a multicentre, observational study within the Network Oncology (NO), a conjoint clinical registry of German hospitals and outpatient practitioners specialised in AM [23, 24]. The NO was established with the aim of developing a database to allow evaluation of integrative therapeutic interventions in AM. Qualified medical documentation officers systematically extract basic patient information, cancer diagnoses, therapies, adverse events, and disease progress from patient files, and record data using the QuaDoSta (Quality management, Documentation, and Statistics) software that was developed at Havelhoehe Research Institute [25]. Along with conventional therapies, such as chemotherapy, radiation, and surgery, the NO documents detailed information about mistletoe therapies received, including dosage data, therapy start and end dates, and related adverse events. Data are entered through a web-based interface comprising checklists and drop down menus, which are linked to regularly updated catalogues (e.g., International Classification of Diseases-10: ICD-10) to ensure uniform data [26]. Additionally, regular training and a telephone support are provided to maintain and enhance data quality. All analyses were conducted and figures created with R version 2.15.1 [27].

2.2. Selection of Patient Data. Patient data recorded in the NO database between June 2009 and June 2012 were analysed by trained investigators. Data was checked for completeness and plausibility, corrected if necessary, and subjected to inclusion/exclusion criteria. Only patients with a valid identification number, birth date, gender, cancer diagnosis date, ICD-10 code, and at least a start or end date for mistletoe therapy were included in the final analyses. Descriptive statistics were used to describe patient demographics and the Wilcoxon rank sum (W) was used to test for differences between groups. The disease stage of patients at diagnosis was described according to Union for International Cancer Control (UICC) staging. The relationship between age and UICC stage at diagnosis was assessed by a one-way analysis of variance. Types of mistletoe and conventional therapies received are summarised.

2.3. Assessment and Classification of Expected Effects and Adverse Drug Reactions. All mistletoe-related expected effects and adverse events reported by physicians were assessed by the study centre. Expected effects were local reactions <5 cm and increased body temperature <38°C. Local reactions and increased temperatures beyond these desired levels, and all other adverse events, were assessed as suspected mistletoe ADRs if a causal relationship between mistletoe and an event was described by physicians as at least a reasonable possibility. Causality of suspected ADRs was then assessed by the investigators according to the World Health Organization Uppsala Monitoring Centre (WHO-UMC) causality assessment system [28]. Expected effects and ADRs with "possible," "probable," or "certain" causality were classified as MedDRA 15.0 preferred terms (developed under the auspices of the International Conference on Harmonization: ICH) and grouped by organ manifestation (WHO SOC-code) [28, 29]. ADRs were evaluated in terms of severity (grades 1 to 5) according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 [30] and designated as serious or nonserious according to ICH guidelines [29]. The numbers of expected effects and ADRs experienced per person were determined and the management and outcome of ADRs are summarised.

2.4. Predictive Factors for Experiencing at Least One Expected Effect or Adverse Drug Reaction. A logistic regression model was developed to evaluate odds ratios (OR) and their 95% confidence intervals (CI) to predict expected effects or ADRs based on potential influencing factors. The regression model included age, gender, and UICC stage. The incidences of ADRs in response to very low, low, moderate, and high doses of mistletoe were compared in order to determine whether experiencing an ADR was dose-dependent. Additionally, the incidences of ADRs during mistletoe therapy with concurrent conventional therapies (chemotherapy, targeted therapies, hormone therapy, bisphosphates, radiation and surgery) were determined in order to see whether receiving mistletoe and a conventional therapy concurrently increased the risk of experiencing an ADR.

3. Results

3.1. Patient Characteristics and Summary of Conventional Treatments Received. The medical records of 2,131 cancer patients from three hospitals, one rehabilitation clinic, and seven outpatient oncologists in Germany that received mistletoe extract therapy were analysed. The data of 208 patients were excluded due to not meeting the inclusion criteria (patients only received mistletoe extracts by application types other than subcutaneous: $n = 191$; no start or end date for mistletoe therapy recorded: $n = 17$). The final analysis was performed on 1923 cancer patients made up of 1,325 (68.9%) females and 598 (31.1%) males treated with mistletoe extracts by subcutaneous application between July 1, 1999 and June 30, 2012. The age of female patients at first diagnosis ranged from 25 to 92 years and for male patients ranged from 27 to 91 years. The median age of male patients (65 years) was six years older than that of female patients (59 years) ($W = 298932$, $P < 0.001$). The age distribution of patients, with respect to gender, is shown in Figure 1. Breast cancer (582 patients) was by far the most common cancer entity, followed by colorectal, lung, and pancreas (313, 264, and 233 patients, resp.). The relative frequencies of the most common cancer entities in the treated population, with respect to gender, are shown in Figure 2. At the time of diagnosis, 23 patients (1.2%) had a UICC stage of 0, 292 (15.2%) were stage I, 431 (22.4%) were stage II, 369 (19.2%) were stage III, and 491 (25.5%) were stage IV. The UICC stage at diagnosis was not known for 317 (16.5%) patients. There was a highly significant relationship between increasing UICC stage and increasing age at diagnosis ($P < 0.001$). In addition to mistletoe therapy, chemotherapy was used in 55.6% of patients, hormonal therapy in 17.0%, targeted therapies (i.e., monoclonal antibodies, proteasome inhibitors, signal transduction inhibitors) in 16.6%, and bisphosphonates in 5.8%. Radiation therapy was used in 35.6% of patients and 82.4% of patients had at least one surgery.

3.2. Mistletoe Extract Therapy. The median length of time between first diagnosis and the start of mistletoe therapy was 3.6 months (CI = 2.9–4.0). Total periods of time for which patients received mistletoe therapy ranged from one day to 11 years. The median length of therapy was 4.6 months (CI = 4.1–5.3) and the mean length was 1.1 years (standard deviation of 1.6 years). Generally, patients received mistletoe injections three times per week. Mistletoe extracts from Abnoba were the most frequently used (1315 patients), followed by Iscador (444 patients), Helixor (323 patients) and Iscucin (67 patients). Rarely used mistletoe extracts were from Lektinol (12 patients), Isorel (5 patients), and Eurixor (1 patient). The most common mistletoe host trees were ash (fraxini = 789 patients), apple (mali = 561 patients), oak (quercus = 357 patients), pine (pini = 268 patients), and spruce (abietis = 195 patients). Other host trees were maple (aceris), birch (betulae), elm (ulmi), willow (salicis), almond (amygdali), poplar (populi), hawthorn (cratagi), and linden (tiliae) (146 patients). Although the dose ranges of different mistletoe products vary markedly, doses of mistletoe extracts received by patients can be summarised by classifying doses as very low, low, moderate, or high depending on the product, as shown in Table 1. The numbers of patients that received very low, low, moderate, or high doses of different types of mistletoe extracts are shown in Figure 3. As per the summary of product characteristics (SPC) for each of the products, mistletoe extract therapy mostly started with a very low or low dose of mistletoe extract, increasing to a moderate or high dose over time [31–33].

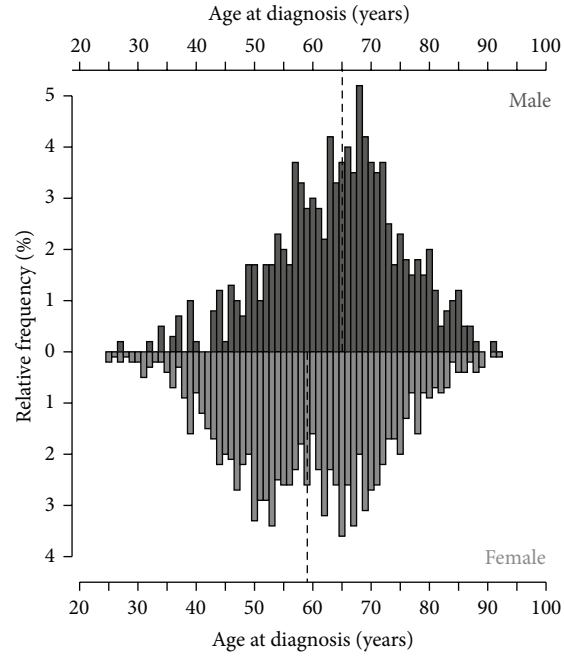


Figure 1: Age of cancer patients treated with mistletoe extracts with respect to gender. The dashed lines denote the respectable median age of both genders.

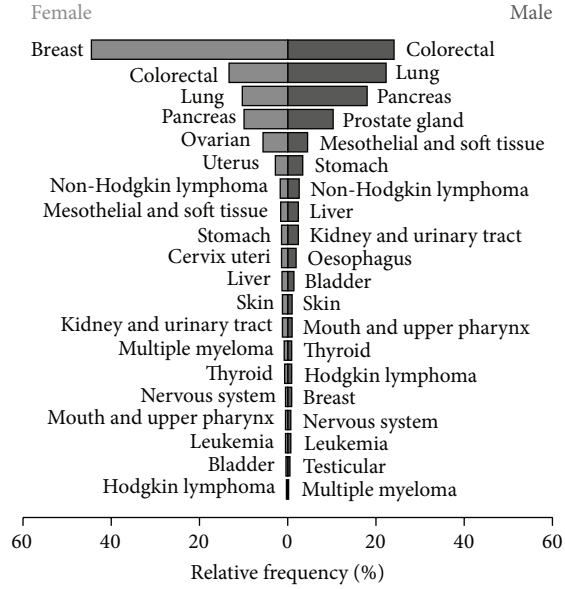


Figure 2: Relative frequency of the most common cancer types in patients treated with mistletoe extracts with respect to gender.

Table 1: Dose levels for different mistletoe products.

	Very low	Low	Moderate	High
Abnoba	≤0.02 mg/mL	>0.02–0.2 mg/mL	>0.2–2.0 mg/mL	>2.0 mg/mL
Helixor	<1 mg/mL	1–<10 mg/mL	10–<30 mg/mL	≥30 mg/mL
Iscador	≤0.01 mg/mL	>0.01–<1 mg/mL	1–<10 mg/mL	≥10 mg/mL
Iscucin	Strengths A and B	Strengths C and D	Strengths E and F	Strengths G and H
Isorel	Strength 1	Strengths 6 and 12	Strengths 24 and 32	Strength 60

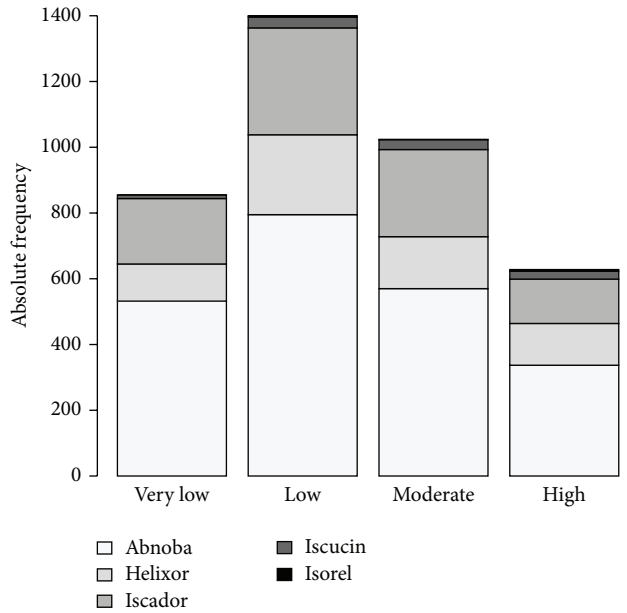


Figure 3: Number of patients that received subcutaneous applications of mistletoe extracts at different dose levels. Dose ranges for each level are shown in Table 1.

3.3. Expected Effects and Adverse Drug Reactions Attributed to Mistletoe Extract Therapy. Of the 1923 patients treated with mistletoe extracts, 414 (21.5%) patients experienced either an expected effect or an ADR. Of 691 events in total, 666 (21.2% of total patients) were local or temperature related reactions and 25 (0.9% of total patients) were systemic reactions (blood glucose/pressure changes, chills, diarrhoea, fatigue, headache, malaise, nausea, vomiting, syncope, rash, urticaria, and pruritus). Expected effects made up the majority of reactions, with 283 patients (68.4% of patients experiencing a reaction and 14.7% of total patients) reporting 427 expected effects (61.8% of all events). These consisted of local reactions <5 cm (erythema = 44.7%, induration = 5.6%, burning sensation = 4.7%, lymphadenopathy = 1.6%, eosinophilia = 0.2%) and increased body temperature <38°C (43.1%).

A total of 264 ADRs were documented in 162 (8.4%) cancer patients treated with mistletoe extracts (Table 2). ADRs were classified as having possible (42.1%), probable (53.4%), or certain (4.5%) causality according to the WHO-UMC causality assessment. The majority (92.4%) of ADRs fell under the system organ class of general disorders and administration site conditions. These consisted of local reactions >5 cm (injection site erythema, swelling, and urticaria) and increased body temperature >38°C, along with chills, fatigue and malaise. The remaining 7.6% of ADRs were made up by diarrhoea, nausea, vomiting, headache, increased blood glucose, decreased blood pressure, rash, syncope and generalised pruritus, and urticaria.

Of the 264 ADRs, nearly all were rated as being of mild/stage I (50.8%) or moderate/stage II (45.1%) intensity, according to the CTCAE. Increased body temperature between 39°C and 40°C made up 87.4% of the ADRs rated as having moderate intensity, with local reactions >5 cm making up 7.6% and other reactions (chills, diarrhoea, vomiting, pruritus, and urticaria) making up the remaining 5.0%. Only 11 ADRs (4.2% of ADRs), experienced by 11 patients (0.6% of total patients), were judged to be severe/stage III according to CTCAE. These were eight patients with increased body temperature >40°C (less than 24 h), one patient with severe injection site swelling, one patient with generalised urticaria, and one patient with syncope. Therapy was immediately stopped for these patients and all of the patients made full recoveries. There were no life threatening/stage IV ADRs or deaths/stage V ADRs related to mistletoe therapy. Although three ADRs in two patients were reported as serious by physicians, on closer inspection by the investigators it was determined that one of the ADRs (injection site swelling) was severe but not serious according to the ICH E2A guideline. Furthermore, after consulting the original medical records of one patient who experienced two serious ADRs (generalised pruritus, and urticaria), it was discovered that the ADRs were most likely caused by penicillin and not mistletoe. Therefore no serious ADRs were attributed to mistletoe therapy.

The number of expected effects per patient ranged from zero to seven. Detailed numbers are presented in Figure 4, along with the number of ADRs per patient which showed a similar pattern ranging from zero to five. More than half of the patients experiencing an expected effect or an ADR had only one.

3.4. Management and Outcomes of Adverse Drug Reactions. Most ADRs (71.6%) did not require any type of treatment or intervention. For some ADRs, the mistletoe dose was reduced (7.6%) or the product was changed (6.8%). Therapy was stopped in response to only three ADRs (diarrhoea, injection site swelling, local reaction) and two local reactions required dressings. Seven ADRs were treated with AM remedies. These were calcium quercus for injection site urticaria, Combudoron gel for two local reactions, Gelsemium for two cases of pyrexia (one intravenous and one subcutaneous application), Nux vomica for vomiting, and Thrombocutan gel for injection site erythema (initiated

Table 2: Adverse drug reactions recorded in cancer patients treated with mistletoe extracts.

System organ class (SOC)	Preferred term (PT)	Total patients	Total events	Incidence (%)
Gastrointestinal disorders	Diarrhoea	1	2	0.05
	Nausea	2	2	0.10
	Vomiting	2	2	0.10
Gastrointestinal disorders total		5	6	0.26
General disorders and administration site conditions	Chills	4	4	0.21
	Fatigue	1	2	0.05
	Injection site erythema (>5 cm)	22	24	1.14
	Injection site swelling (>5 cm)	4	4	0.21
	Injection site urticaria (>5 cm)	2	2	0.10
	Local reaction (>5 cm)	31	39	1.61
	Malaise	1	1	0.05
	Pyrexia	124	171	6.45
General disorders and administration site conditions total		157*	244	8.16
Investigations	Blood glucose increased	1	1	0.05
	Blood pressure decreased	1	1	0.05
Investigations total		2	2	0.10
Nervous system disorders	Headache	2	2	0.10
Nervous system disorders total		2	2	0.10
Skin and subcutaneous tissue disorders	Pruritus generalised	3	3	0.16
	Rash	2	2	0.10
	Urticaria	4	4	0.21
Skin and subcutaneous tissue disorders total		7*	9	0.36
Vascular disorders	Syncope	1	1	0.05
Vascular disorders total		1	1	0.05
Total		162*	264	8.42

*This value is not equal to the sum of patients listed for each adverse drug reaction since some patients experienced multiple reactions.

Table 3: Odds ratios of predictive factors for experiencing at least one expected effect or adverse drug reaction during mistletoe extract therapy.

	Expected effects		Adverse drug reactions	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Female	1.61 (1.11, 2.39)	0.015*	0.95 (0.64, 1.45)	0.821
Age (years)	0.97 (0.96, 0.99)	$2.28e-05$***	0.99 (0.97, 1.00)	0.110
UICC stage				
0	Reference			
I	0.39 (0.16, 0.97)	0.038*	2.10 (0.41, 38.41)	0.479
II	0.39 (0.16, 0.95)	0.033*	2.00 (0.40, 36.45)	0.505
III	0.26 (0.11, 0.65)	0.003**	2.60 (0.52, 47.43)	0.357
IV	0.15 (0.06, 0.39)	$6.11e-05$***	2.23 (0.44, 40.72)	0.441

OR: odds ratio; CI: confidence interval; UICC: International Union Against Cancer.
Significance levels: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

by the patient). Only one patient with urticaria required treatment with a conventional medication (intravenous H2 receptor antagonist). Intervention data was missing for 24 (9.1%) patients. Outcome data revealed that 237 (89.8%) patients had "completely recovered," four (1.5%) patients had "not yet recovered" but were expected to make a full recovery, and data was missing for 23 (8.7%) patients.

3.5. Logistic Regression Analysis of Expected Effects and Adverse Drug Reactions. Logistic regression analyses were performed in order to determine potential influencing factors for experiencing an expected effect or an ADR while receiving mistletoe extract therapy. Gender, age, and UICC stage were assessed as variables for predicting expected effects (local reactions <5 cm and increased body temperature <38°C) or ADRs. The results from both analyses are presented in Table 3. It was not possible to include mistletoe dosage in these analyses due to the complexity of the dosage data (i.e., patients often received many different doses and types of mistletoe during their therapy and may have reacted

Table 4: Incidences of adverse drug reactions experienced during mistletoe therapy with concurrent conventional therapies.

Conventional therapies	Number of patients			Incidence (%)
	Included in analysis*	Mistletoe and conventional therapy crossover	ADR during therapy crossover	
Chemotherapy	832	415	10	2.41
Targeted therapies	226	120	1	0.83
Hormone therapy	185	113	1	0.88
Bisphosphates	42	20	0	0.00
Radiation therapy	543	207	5	2.42
Surgery	1371	270	3	1.11

*Only patients that had both start and end dates for mistletoe extract therapy and the appropriate conventional therapy were included in each analysis. ADR: adverse drug reaction.

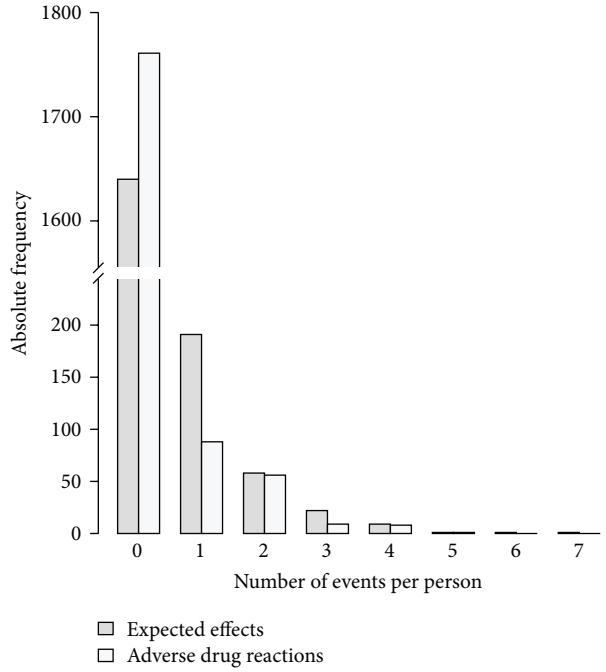


Figure 4: Number of expected effects or adverse drug reactions to subcutaneous mistletoe therapy per patient. Dark grey = expected effects; light grey = adverse drug reactions.

to a certain dose on one occasion, but not on another). The number of patients analysed by logistic regression was reduced from 1923 to 1606 due to the UICC stage at diagnosis being unknown or not applicable for 317 patients. When expected effects were considered (238 patients out of 1606 patients), females were more likely to experience a reaction (OR = 1.61, CI = 1.11–2.39, $P = 0.015$), while older age (OR = 0.97 per year, CI = 0.96–0.99, $P < 0.001$) and increasing UICC stage (UICC I: OR = 0.39, CI = 0.16–0.97, $P = 0.038$; UICC II: OR = 0.39, CI = 0.16–0.95, $P = 0.033$; UICC III: OR = 0.26, CI = 0.11–0.65, $P = 0.003$; UICC IV: OR = 0.15, CI = 0.06–0.39, $P < 0.001$) were associated with less reactions. No risk factors were identified for ADRs (133 patients out of 1606 patients). Based on the rule of ten events per variable [34–36], there were too few events to accurately analyse potential risk factors for more specific types of ADRs (e.g., severe or systemic ADRs).

3.6. Relationship between Mistletoe Dose and Incidence of Adverse Drug Reactions. In order to investigate the relationship between increasing doses of mistletoe extracts and incidence of ADRs, doses were divided into 4 levels: very low, low, moderate, and high. The number of ADRs that occurred at doses within each level was divided by the total number of patients that received mistletoe extracts within each dose level. There was a highly significant relationship between dose level and the incidence of ADRs (Figure 5). Only 0.5%, 1.0% and 1.6% of patients who received very low, low, and moderate doses of mistletoe extracts experienced an ADR at those dose levels. On the other hand, 20.4% of patients who received high doses of mistletoe extracts reported an ADR. This relationship was also seen when only pyrexia and injection site ADRs were considered (very low = 0.1%, low = 0.9%, moderate = 1.3%, high = 20.0%). There was no significant dose-dependent relationship observed for other ADRs however (diarrhoea, nausea, vomiting, chills, fatigue, malaise, headache, increased blood glucose, decreased blood pressure, pruritus, rash, urticaria, and syncope; very low = 0.5%, low = 0.4%, moderate = 1.5%, high = 1.3%). The odds ratios and 95% confidence intervals for the effect of mistletoe dosage on relative frequencies of all ADRs, pyrexia and injection site ADRs only, and other ADRs are shown in Figure 5. Of the 11 severe ADRs (pyrexia x 8, injection site swelling, generalised urticaria, syncope), all occurred in response to high doses (10–20 mg) of mistletoe extracts, apart from the injection site swelling which occurred in response to a moderate dose (0.2 mg).

3.7. Incidences of ADRs during Mistletoe Therapy with Concurrent Conventional Therapies. To see whether the frequency of ADRs increased during concurrent therapy with mistletoe extracts and a conventional therapy, the numbers of ADRs that occurred during different therapy crossovers were determined. The results presented in Table 4 show lower incidences of ADRs during therapy crossovers (chemotherapy: 2.41%, targeted therapies: 0.83%, hormone therapy: 0.88%, bisphosphates: 0%, radiation therapy: 2.42%, and surgery:

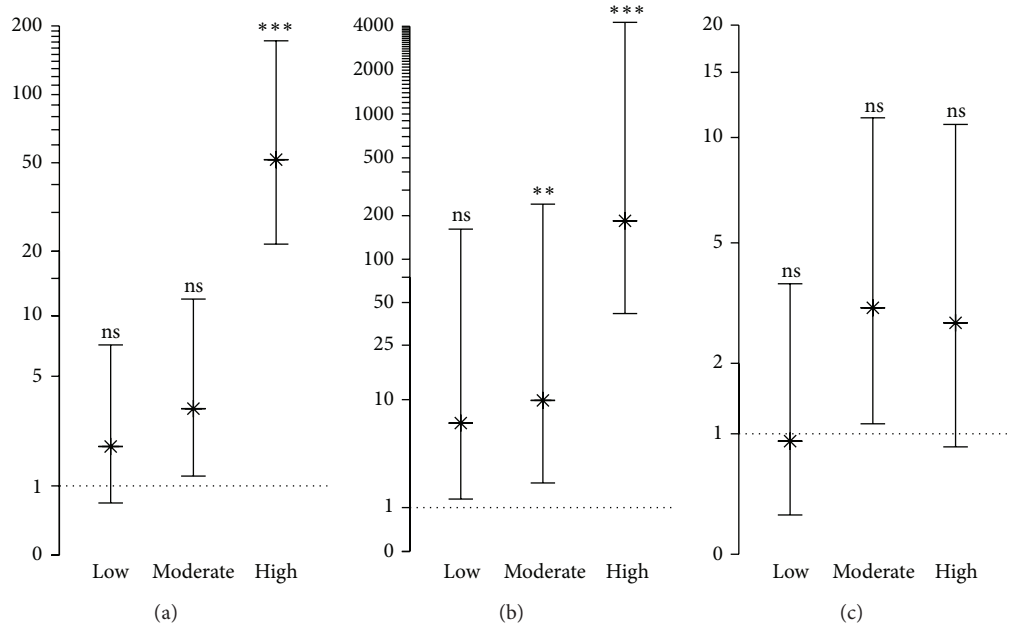


Figure 5: Odds ratios with 95% confidence intervals for the effect of mistletoe dosage on relative frequencies of (a) all ADRs, (b) pyrexia and injection site ADRs only, and (c) other ADRs (diarrhoea, nausea, vomiting, chills, fatigue, malaise, headache, increased blood glucose, decreased blood pressure, pruritus, rash, urticaria, and syncope). The relative frequencies of ADRs in response to low, moderate and high doses of mistletoe were compared to the relative frequency of ADRs that occurred in response to very low dose mistletoe. ns = not significantly different ($P > 0.05/3$), significance levels: $^{**}P < 0.01/3$, $^{***}P < 0.001/3$. Note that normally accepted significance levels are divided by three in accordance with the Bonferroni correction for multiple comparisons.

1.11%) compared to the overall incidence of ADRs (8.42%) recorded in Table 2.

4. Discussion

In the present study we assessed expected effects and ADRs in 1923 cancer patients that received subcutaneous mistletoe applications. Expected effects, classified as local reactions <5 cm and increased temperature <38°C, were reported in 14.7% of patients and ADRs were reported in 8.4% of patients. Our results are closely comparable with the published literature, both in terms of incidence and types of ADRs [13, 37, 38]. A retrolective, pharmacoepidemiological cohort study by Bock et al. [37] found that 0.8% of breast cancer patients treated with mistletoe extracts (Iscador) had a systemic reaction and 17.3% experienced local reactions at the injection site, sometimes with mild fever. Our finding that 0.9% of patients experienced a systemic reaction is almost identical. It is not clear whether local reactions <5 cm and increased temperature <38°C were included as ADRs in the analysis by Bock et al. Since expected effects were not discussed in the publication, however, we can assume that there was no cutoff point and that all observed local and fever reactions were recorded as ADRs. In the current study, if we include expected effects along with ADRs, 21.2% of patients experienced local (plus pyrexia) reactions compared to 17.3% as reported by Bock et al. This difference might be explained by the fact that our study specifically concentrated on recording mild reactions (expected effects) such as redness and slight increases in body temperature, in addition to more obvious ADRs. These results are also similar to the results of a systematic review, which included 18 clinical trials examining mistletoe preparations, and reported incidences of 1.6% and 15.9% for systemic and local reactions, respectively [13].

While 8.4% of patients experienced a suspected ADR, which is described as "common" according to the Council for International Organizations of Medical Sciences guidelines [39], only 0.6% of patients had a severe (grade III) suspected ADR, making severe reactions "uncommon." Severe reactions were mostly pyrexia >40°C which lasted less than 24 h. There were no life threatening (stage IV) ADRs and all reactions were nonserious. ADRs were mostly general disorders and administration site conditions, with other reactions (diarrhoea, nausea, vomiting, headache, increased blood glucose, decreased blood pressure, rash, syncope and generalised pruritus and urticaria) making up only 7.4% of ADRs. Of the recorded ADRs, all have been reported previously by the Federal Institute for Drugs and Medical Devices of Germany (Bundesinstitut für Arzneimittel und Medizinprodukte, www.bfarm.de) and all except for decreased blood pressure, syncope, and increased blood glucose are listed in the SPCs of the products [31–33]. Each of these three suspected ADRs occurred once only and all were described as having "possible" causality. Mistletoe extracts have been shown previously to possess hypotensive activity [40]. In Wistar rats injected with mistletoe extracts, arterial blood pressure was significantly decreased and hexocycline, a selective antagonist of muscarinic receptors, blocked this effect. As with any hypotensive drugs it is possible that an

excessive decrease in blood pressure could lead to fainting (syncope). Only one case of possible mistletoe therapy related syncope was found in the literature in a female patient who received high dose mistletoe at six o'clock in the evening, with the intention of inducing pyrexia [41]. The patient collapsed upon getting out of bed in the early hours of the morning (between two and three o'clock) with the result of bruises and a head laceration. It is not clear however whether mistletoe was responsible for the collapse, or if it was a disease-related or spontaneous event [41]. The only studies that were found indicating mistletoe induced changes in blood glucose levels, demonstrated hypoglycaemic effects [42, 43], making it a high possibility of this suspected ADR not actually being related to mistletoe.

Attribution of causality of ADRs is a complex task, often because patients can be receiving multiple therapies at once and might also experience disease-related symptoms that could be confused for ADRs. We classified the recorded ADRs as having "possible," "probable," or "certain" causality according to the WHO-UMC causality assessment. Our findings of 42.1% possible, 53.4% probable, and 4.5% certain ADRs correspond with other published findings, which range from 44.4% to 53.2% for possible, 34.0% to 49.8% for probable and 3.3% to 17.7% for certain ADRs [44–46].

A somewhat controversial topic concerning classification of ADRs is the concept of what constitutes an "adverse" event, and what is actually an expected, or even desired effect. Mistletoe therapy is thought to benefit cancer patients through the modulation of cellular and humoral immune responses [47, 48]. Mild local reactions at injection sites and increases in body temperature (<24 h) are frequent observations following applications, and according to product information, are an important part of dose finding strategies during mistletoe therapy [31–33]. These reactions are not considered by physicians as ADRs, but rather as positive indicators of expected pharmacological activity and immune system stimulation. Furthermore, the effect of increasing body temperature and the associated "feeling of warmth" is thought to benefit cancer patients, who often feel cold due to a loss of thermoregulation and reduced temperature amplitudes, especially after chemotherapy and in breast cancer patients [49–51]. Interestingly, significant survival benefits have been achieved in cancer patients treated with hyperthermia in combination with radiation and/or chemotherapy [52–56]. A review by Peer et al. [57] summarized the recent literature identifying complex effects of temperature on immune cells and potential cellular mechanisms by which increased temperature may enhance immune surveillance and tumour control. Although further research is required to better understand the role of body temperature regulation during tumour development and treatment, the temperature boosting property of mistletoe might indeed be a beneficial effect. As a result of differences in the interpretation of what constitutes an ADR, it is not surprising that data regarding incidences of mistletoe-induced ADRs ranges widely. One systematic review on adverse events during mistletoe therapy revealed that incidences ranged from 0.9% to 43% for local reactions and from 0.8% to 4% for systemic reactions [58].

Based on logistic regression results, young to middle-aged females with early-stage cancer were the most likely to experience an expected effect. An identical outcome, in terms of young to middle-aged females reporting the highest incidence of events, was found in a study which assessed the age and gender distribution of suspected ADRs to a variety of newly marketed drugs in general practice in England [59]. A prospective multicentre study based on intensive pharmacovigilance involving 2,371 patients also found a higher risk of ADRs among female subjects compared to males [60]. Although several explanations such as gender-specific pharmacokinetic and pharmacodynamic behaviour of drugs were investigated by Zopf et al. [60], a reason for the gender difference remains unclear but is possibly related to a higher rate of autonomic dysregulation in females [61]. As expected, increasing age at diagnosis was highly correlated with increasing cancer stage ($P < 0.001$). It is well known that cancer and conventional cancer treatments (chemotherapy and radiation) can suppress the immune system [62, 63]. Therefore, it makes sense that patients with late-stage cancer (who were also generally older and had received more chemotherapies/radiation) experienced less expected effects, which are related to stimulation of the immune system, than patients with early-stage cancer. Interestingly, there were no risk factors identified for experiencing an ADR.

The results of our investigation into the relationship between increasing doses of mistletoe extracts and incidence of ADRs suggest that ADRs that are related to the expected pharmacological action of mistletoe, such as local inflammatory reactions and pyrexia, were dose-related. The relationship between dose and other reactions (e.g., diarrhoea, nausea, and vomiting), which are not obviously related to the expected pharmacological action of mistletoe, was less clear. It is important to note however that the low frequency of these reactions makes it difficult to draw any conclusions. Whether specific mistletoe products or host trees were associated with an increased risk of experiencing an ADR was outside the scope of this study but will be investigated in the future.

Finally, the incidence of suspected ADRs to mistletoe injections decreased rather than increased when mistletoe therapy was combined with conventional therapies such as chemotherapy, hormone therapy, targeted therapies, bisphosphates, radiation therapy, and surgery. Importantly, studies have shown that the pharmacokinetics of gemcitabine, an antimetabolite chemotherapeutic agent, are not affected when combined with mistletoe therapy [64, 65]. The reduction in mistletoe ADRs during cotherapy with conventional drugs was probably due to the immunosuppressing nature of many of these therapies [62, 63], providing further evidence that mistletoe extracts are safe for use in cancer patients. Additionally, it has been reported previously that not only is conjunctive therapy with mistletoe extracts safe, it can also reduce the frequency and severity of chemotherapy ADRs [16, 66].

The present study assessed a heterogeneous group of patients in terms of demographic, tumour entities, disease stage, and varied doses, types, and frequencies of subcutaneous mistletoe applications and concurrent therapies. Though potentially limited in terms of too many factors

being involved that could have influenced the outcome, our study provides an accurate picture of the clinical use of subcutaneous mistletoe therapy within the NO and its overall safety. A weakness of observational studies is missing or erroneous information for some patients. This problem was addressed by only including patients with complete and valid core data (birth and diagnosis dates, gender, an ICD-10 code, either a start or end date for mistletoe therapy and the product name). For the 1923 eligible patients, all ADRs were reported as long as a valid date was listed. Other limitations of this study include the possibility of underreporting by physicians, especially of expected effects and mild ADRs, and less frequent reporting of ADRs by ambulatory patients due to reduced contact with physicians. On the other hand, it is possible that ADRs were overreported since there was no untreated patient group for which disease-related or spontaneous events could be controlled for. A study by Reidenberg and Lowenthal [67] revealed that even when healthy, young, non-medicated subjects were asked to document everyday symptoms for three days, 41% of patients reported fatigue, 15% a headache, and 10% muscle pain, among others, indicating a high probability of false positive findings in our clinical findings. Furthermore, only conventional or AM interventions related to cancer treatment were recorded for patients in the present study, meaning it is possible that reactions to external interventions (e.g., self-medication, herbal remedies) could have influenced our results.

5. Conclusions

The results of this study indicate that mistletoe therapy is safe. While expected effects and mild local or temperature related ADRs were common, severe ADRs (which were mostly temperature related) were uncommon, no serious ADRs occurred, and no ADRs were associated with long-term injury or disability. Observed ADRs were mostly dose-dependent and believed to be related to the immune-stimulating, pharmacological activity of mistletoe. Future research, involving larger numbers of patients, might be important for identifying risk factors for ADRs to mistletoe, especially severe ADRs. Furthermore, continuing research is required to draw conclusions on clinical efficacy of mistletoe therapy.

Conflict of Interests

The Network Oncology was funded by the Software AG Stiftung Darmstadt, Germany, Weleda AG Arlesheim, Switzerland, Abnoba GmbH Pforzheim, Germany, and Helixor GmbH Rosenfels, Germany. By contract, researchers were independent from the funder. H. Matthes has been a member of Weleda AG directory board since March 2012. The other authors declare that they have no conflict of interests.

Acknowledgments

The authors would like to thank all medical documentation officers, all NO partners, and participating institutions. MedDRA trademark is owned by IFPMA on behalf of ICH.

References

[1] M. Horneber, G. Bueschel, G. Dennert, D. Less, and M. Zwahlen, "How many cancer patients use complementary and alternative medicine: a systematic review and metaanalysis," *Integrative Cancer Therapies*, vol. 11, no. 3, pp. 187–203, 2012.

[2] G. S. Kienle, H. Kiene, and H. U. Albonico, "Anthroposophic medicine: health technology assessment report—short version," *Forschende Komplementarmedizin*, vol. 13, supplement 2, pp. 7–18, 2006.

[3] M. A. Horneber, G. Bueschel, R. Huber, K. Linde, and M. Rostock, "Mistletoe therapy in oncology," *Cochrane Database of Systematic Reviews*, no. 2, Article ID CD003297, 2008.

[4] J. W. Zeller, "Immuntherapeutika und zytostatika," in *Arzneimittelverordnungs-Report 2004*, U. Schwabe and D. Paffrath, Eds., pp. 609–624, Springer, Berlin, Germany, 2004.

[5] G. Seifert, P. Jesse, A. Laengler et al., "Molecular mechanisms of mistletoe plant extract-induced apoptosis in acute lymphoblastic leukemia in vivo and in vitro," *Cancer Letters*, vol. 264, no. 2, pp. 218–228, 2008.

[6] N. E. Gardin, "Immunological response to mistletoe (*Viscum album* L.) in cancer patients: a four-case series," *Phytotherapy Research*, vol. 23, no. 3, pp. 407–411, 2009.

[7] K. Hostanska, T. Hajto, G. C. Spagnoli, J. Fischer, H. Lentzen, and R. Herrman, "A plant lectin derived from *Viscum album* induces cytokine gene expression and protein production in cultures of human peripheral blood mononuclear cells," *Natural Immunity*, vol. 14, no. 5-6, pp. 295–304, 1995.

[8] R. Huber, K. Claßen, M. Werner, and R. Klein, "In vitro immunoreactivity towards lectin-rich or viscotoxin-rich mistletoe (*Viscum album* L.) extracts iscador applied to healthy individuals: a randomised double-blind placebo controlled study," *Arzneimittel-Forschung*, vol. 56, no. 6, pp. 447–456, 2006.

[9] C. Jaggy, H. Musielski, K. Urech, and G. Schaller, "Quantitative determination of lectins in mistletoe preparations," *Arzneimittel-Forschung*, vol. 45, no. 8, pp. 905–909, 1995.

[10] T. Ostermann, C. Raak, and A. Büssing, "Survival of cancer patients treated with mistletoe extract (*Iscador*): a systematic literature review," *BMC Cancer*, vol. 9, article 451, 2009.

[11] G. S. Kienle, A. Glockmann, M. Schink, and H. Kiene, "*Viscum album* L. extracts in breast and gynaecological cancers: a systematic review of clinical and preclinical research," *Journal of Experimental and Clinical Cancer Research*, vol. 28, no. 1, article no. 79, 2009.

[12] J. Beuth, B. Schneider, and J. M. Schierholz, "Impact of complementary treatment of breast cancer patients with standardized mistletoe extract during aftercare: a controlled multicenter comparative Epidemiological Cohort Study," *Anticancer Research*, vol. 28, no. 1, pp. 523–527, 2008.

[13] J. Melzer, F. Iten, K. Hostanska, and R. Saller, "Efficacy and safety of mistletoe preparations (*Viscum album*) for patients with cancer diseases," *Forschende Komplementarmedizin*, vol. 16, no. 4, pp. 217–226, 2009.

[14] A. Büssing, C. Raak, and T. Ostermann, "Quality of life and related dimensions in cancer patients treated with mistletoe

extract (*Iscador*): a meta-analysis," *Evidence-based Complementary and Alternative Medicine*, vol. 2012, Article ID 219402, 8 pages, 2012.

[15] H. Matthes, W. E. Friedel, P. R. Bock, and K. S. Zänker, "Molecular mistletoe therapy: friend or foe in established antitumor protocols? a multicenter, controlled, retrospective pharmaco-epidemiological study in pancreas cancer," *Current Molecular Medicine*, vol. 10, no. 4, pp. 430–439, 2010.

[16] J. Eisenbraun, R. Scheer, M. Kröz, F. Schad, and R. Huber, "Quality of life in breast cancer patients during chemotherapy and concurrent therapy with a mistletoe extract," *Phytomedicine*, vol. 18, no. 2-3, pp. 151–157, 2011.

[17] P. Heusser, S. B. Braun, M. Bertschy et al., "Palliative in-patient cancer treatment in an anthroposophic hospital: II. Quality of life during and after stationary treatment, and subjective treatment benefits," *Forschende Komplementarmedizin*, vol. 13, no. 3, pp. 156–166, 2006.

[18] S. P. Myers and P. A. Cheras, "The other side of the coin: safety of complementary and alternative medicine," *Medical Journal of Australia*, vol. 181, no. 4, pp. 222–225, 2004.

[19] M. Tascilar, F. A. de Jong, J. Verweij, and R. H. J. Mathijssen, "Complementary and alternative medicine during cancer treatment: beyond innocence," *Oncologist*, vol. 11, no. 7, pp. 732–741, 2006.

[20] B. R. Cassileth and G. Deng, "Complementary and alternative therapies for cancer," *Oncologist*, vol. 9, no. 1, pp. 80–89, 2004.

[21] K. C. Kim, J. H. Yook, J. Eisenbraun, B. S. Kim, and R. Huber, "Quality of life, immunomodulation and safety of adjuvant mistletoe treatment in patients with gastric carcinoma—a randomized, controlled pilot study," *BMC Complementary and Alternative Medicine*, vol. 12, article 172, 2012.

[22] R. Huber, H. Lüdtke, J. Wieber, and C. Beckmann, "Safety and effects of two mistletoe preparations on production of Interleukin-6 and other immune parameters—a placebo controlled clinical trial in healthy subjects," *BMC Complementary and Alternative Medicine*, vol. 11, article 116, 2011.

[23] F. Schad, C. Teodoridis, U. Albrecht et al., "Network oncology (NO)—a European approach to health service research on cancer treatment with focus on concurrent use of complementary therapies," *European Journal of Integrative Medicine*, vol. 1, supplement 1, pp. 12–13, 2008.

[24] F. Schad, J. Axtner, A. Happe et al., "Network oncology (NO)—a clinical cancer register for health services research and the evaluation of integrative therapeutic interventions in anthroposophic medicine," *Research in Complementary Medicine*, vol. 20, pp. 353–360, 2013.

[25] F. Schad, J. Pissarek, U. Albrecht, and H. Matthes, "QuaDoSta (quality assurance, documentation and statistics)—a flexible, platform-independent, open source database for oncological documentation," *Cancer Research and Clinical Oncology*, vol. 130, supplement op, p. 785, 2004.

[26] E. Jeschke, F. Schad, J. Pissarek, B. Matthes, U. Albrecht, and H. Matthes, "QuaDoSta—a freely configurable system which facilitates multi-centric data collection for healthcare and medical research," *GMS Medizinische Informatik, Biometrie und Epidemiologie*, vol. 3, no. 2, 2007.

[27] R Core Team, "R: A language and environment for statistical computing," in R Foundation for Statistical Computing, 2012.

[28] UMC, "Safety monitoring of medicinal products: guidelines for setting up and running a pharmacovigilance centre," http://www.who.int/medicinedocs.

[29] ICH, "E2A: clinical safety data management: definitions and standards for expedited reporting," http://www.ich.org.

[30] CTEP, "Common terminology criteria for adverse events," http://ctep.cancer.gov/protocolDevelopment.

[31] "Arzneimittelinformation AbnobaVISCUM," 2007, http://www.abnoba.de.

[32] "Iscador: Anthroposophisches Arzneimittel. Fachinformation des Arzneimittel-Kompendium der Schweiz," 2007, http://www.weleda.de.

[33] "Helixor: Anthroposophische Arzneimittel. Fachinformation des Arzneimittel-Kompendium der Schweiz," 2007, http://www.helixor.de.

[34] J. Concato, P. Peduzzi, T. R. Holford, and A. R. Feinstein, "Importance of events per independent variable in proportional hazards analysis I. Background, goals, and general strategy," *Journal of Clinical Epidemiology*, vol. 48, no. 12, pp. 1495–1501, 1995.

[35] P. Peduzzi, J. Concato, A. R. Feinstein, and T. R. Holford, "Importance of events per independent variable in proportional hazards regression analysis II. Accuracy and precision of regression estimates," *Journal of Clinical Epidemiology*, vol. 48, no. 12, pp. 1503–1510, 1995.

[36] P. Peduzzi, J. Concato, E. Kemper, T. R. Holford, and A. R. Feinstem, "A simulation study of the number of events per variable in logistic regression analysis," *Journal of Clinical Epidemiology*, vol. 49, no. 12, pp. 1373–1379, 1996.

[37] P. R. Bock, W. E. Friedel, J. Hanisch, M. Karasmann, and B. Schneider, "Efficacy and safety of long-term complementary treatment with standardized european mistletoe extract (*Viscum album* L.) in addition to the conventional adjuvant oncologic therapy in patients with primary non-metastasized mammary carcinoma / results of a multicenter, comparative, epidemiological Cohort study in Germany and Switzerland," *Arzneimittel-Forschung*, vol. 54, no. 8, pp. 456–466, 2004.

[38] G. S. Kienle, R. Grugel, and H. Kiene, "Safety of higher dosages of *Viscum album* L. in animals and humans—systematic review of immune changes and safety parameters," *BMC Complementary and Alternative Medicine*, vol. 11, article 72, 2011.

[39] CIOMS, *Guidelines for Preparing Core Clinical-Safety InFormation on Drugs*, Council for International Organizations of Medical Sciences, Geneva, Switzerland, 2nd edition, 1999.

[40] M. Radenkovic, V. Ivetic, M. Popovic, S. Brankovic, and L. Gvozdenovic, "Effects of mistletoe (*Viscum album* L., *Loranthaceae*) extracts on arterial blood pressure in rats treated with atropine sulfate and hexocycline," *Clinical and Experimental Hypertension*, vol. 31, no. 1, pp. 11–19, 2009.

[41] R. Penter, "Methodische aspekte zu einer goetheanistisch-anthroposophischen klinischen forschung am beispiel der entwicklung der mistelfiebertherapie," *Der Merkurstab*, vol. 5, pp. 350–368, 2002.

[42] O. Adaramoye, M. Amanlou, M. Habibi-Rezaei, P. Pasalar, and M. Ali, "Methanolic extract of African mistletoe (*Viscum album*) improves carbohydrate metabolism and hyperlipidemia in streptozotocin-induced diabetic rats," *Asian Pacific Journal of Tropical Medicine*, vol. 5, no. 6, pp. 427–433, 2012.

[43] E. Frederick, A. Kayode, and U. Slyvester, "Evaluation of the methanolic extract of mistletoe (*Tapinanthus bangwensis*) leaves grown on orange trees for the phytochemical properties and its physiological effects on streptozotocin induced diabetes mellitus in laboratory animals," *World Applied Sciences Journal*, vol. 9, no. 9, pp. 975–979, 2010.

[44] M. Tabali, E. Jeschke, A. Bockelbrink et al., "Educational intervention to improve physician reporting of adverse drug reactions (ADRs) in a primary care setting in complementary and alternative medicine," *BMC Public Health*, vol. 9, article 274, 2009.

[45] M. Tabali, T. Ostermann, E. Jeschke, C. M. Witt, and H. Matthes, "Adverse drug reactions for CAM and conventional drugs detected in a network of physicians certified to prescribe CAM drugs," *Journal of Managed Care Pharmacy*, vol. 18, no. 6, pp. 427–438, 2012.

[46] M. Süsskind, P. A. Thürmann, C. Lüke et al., "Adverse drug reactions in a complementary medicine hospital: a prospective, intensified surveillance study," *Evidence-based Complementary and Alternative Medicine*, vol. 2012, Article ID 320760, 8 pages, 2012.

[47] S. Fischer, A. Scheffler, and D. Kabelitz, "Stimulation of the specific immune system by mistletoe extracts," *Anti-Cancer Drugs*, vol. 8, supplement 1, pp. S33–S37, 1997.

[48] G. Stein, W. Henn, H. von Laue, and P. Berg, "Modulation of the cellular and humoral immune responses of tumor patients by mistletoe therapy," *European Journal of Medical Research*, vol. 3, no. 4, pp. 194–202, 1998.

[49] H. B. von Laue and W. Henn, "Zeitphänomene der krebskrankheit," *Deutsche Zeitschrift für Onkologie*, vol. 23, pp. 64–73, 1991.

[50] K. M. Kokolus, C. Hong, and E. A. Repasky, "Feeling too hot or cold after breast cancer: is it just a nuisance or a potentially important prognostic factor?" *International Journal of Hyperthermia*, vol. 26, no. 7, pp. 662–680, 2010.

[51] E. M. Bessell, C. Selby, and I. O. Ellis, "Severe hypoglycaemia caused by raised insulin-like growth factor II in disseminated breast cancer," *Journal of Clinical Pathology*, vol. 52, no. 10, pp. 780–781, 1999.

[52] R. Valdagni and M. Amichetti, "Report of long-term follow-up in a randomized trial comparing radiation therapy and radiation therapy plus hyperthermia to metastatic lymphnodes in stage IV head and neck patients," *International Journal of Radiation Oncology Biology Physics*, vol. 28, no. 1, pp. 163–169, 1994.

[53] E. L. Jones, J. R. Oleson, L. R. Prosnitz et al., "Randomized trial of hyperthermia and radiation for superficial tumors," *Journal of Clinical Oncology*, vol. 23, no. 13, pp. 3079–3085, 2005.

[54] J. van der Zee, D. González González, G. C. van Rhoon, J. D. P. van Dijk, W. L. J. van Putten, and A. A. M. Hart, "Comparison of radiotherapy alone with radiotherapy plus hyperthermia in locally advanced pelvic tumours: a prospective, randomised, multicentre trial," *The Lancet*, vol. 355, no. 9210, pp. 1119–1125, 2000.

[55] K. Sugimachi, H. Kuwano, H. Ide, T. Toge, M. Saku, and Y. Oshiumi, "Chemotherapy combined with or without hyperthermia for patients with oesophageal carcinoma: a prospective randomized trial," *International Journal of Hyperthermia*, vol. 10, no. 4, pp. 485–493, 1994.

[56] K. Sugimachi, K. Kitamura, K. Baba et al., "Hyperthermia combined with chemotherapy and irradiation for patients with carcinoma of the oesophagus—a prospective randomized trial," *International Journal of Hyperthermia*, vol. 8, no. 3, pp. 289–295, 1992.

[57] A. J. Peer, M. J. Grimm, E. R. Zynda, and E. A. Repasky, "Diverse immune mechanisms may contribute to the survival benefit seen in cancer patients receiving hyperthermia," *Immunologic Research*, vol. 46, no. 1–3, pp. 137–154, 2010.

[58] R. Saller, S. Kramer, F. Iten, and J. Melzer, "Unerwünschte wirkungen der misteltherapie bei tumorpatienten—eine systematische Übersicht," in *Fortschritte in der Misteltherapie: Aktueller Stand der Forschung und klinische Anwendung*, R. Scheer, H. Bauer, V. Becker et al., Eds., pp. 367–403, KVC, Essen, Germany, 2005.

[59] R. M. Martin, P. N. Biswas, S. N. Freemantle, G. L. Pearce, and R. D. Mann, "Age and sex distribution of suspected adverse drug reactions to newly marketed drugs in general practice in England: Analysis of 48 Cohort studies," *British Journal of Clinical Pharmacology*, vol. 46, no. 5, pp. 505–511, 1998.

[60] Y. Zopf, C. Rabe, A. Neubert et al., "Women encounter ADRs more often than do men," *European Journal of Clinical Pharmacology*, vol. 64, no. 10, pp. 999–1004, 2008.

[61] Y. S. Ali, N. Daamen, J. Giris et al., "Orthostatic intolerance: a disorder of young women," *Obstetrical and Gynecological Survey*, vol. 55, no. 4, pp. 251–259, 2000.

[62] L. Rasmussen and A. Arvin, "Chemotherapy-induced immunosuppression," *Environmental Health Perspectives*, vol. 43, pp. 21–25, 1982.

[63] N. Rouas-Freiss, P. Moreau, C. Menier, and E. D. Carosella, "HLA-G in cancer: a way to turn off the immune system," *Seminars in Cancer Biology*, vol. 13, no. 5, pp. 325–336, 2003.

[64] P. J. Mansky, J. Grem, D. B. Wallerstedt, B. P. Monahan, and M. R. Blackman, "Mistletoe and gemcitabine in patients with advanced cancer: a model for the phase I study of botanicals and botanical-drug interactions in cancer therapy," *Integrative Cancer Therapies*, vol. 2, no. 4, pp. 345–352, 2003.

[65] P. J. Mansky, D. B. Wallerstedt, T. Sannes et al., "NCCAM/NCI phase I study of mistletoe extract and gemcitabine in patients with advanced solid tumors," *Journal of Clinical Oncology*, vol. 28, supplement 2559, no. 15, 2010.

[66] B. K. Piao, Y. X. Wang, G. R. Xie et al., "Impact of complementary mistletoe extract treatment on quality of life in breast, ovarian and non-small cell lung cancer patients. A prospective randomized controlled clinical trial," *Anticancer Research*, vol. 24, no. 1, pp. 303–309, 2004.

[67] M. M. Reidenberg and D. T. Lowenthal, "Adverse nondrug reactions," *The New England Journal of Medicine*, vol. 279, no. 13, pp. 678–679, 1968.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2013, Article ID 718105, 7 pages
http://dx.doi.org/10.1155/2013/718105

Research Article

Effects of Lipophilic Extract of *Viscum album* L. and Oleanolic Acid on Migratory Activity of NIH/3T3 Fibroblasts and on HaCat Keratinocytes

R. Kuonen,[1] U. Weissenstein,[1] K. Urech,[1] M. Kunz,[1] K. Hostanska,[2] M. Estko,[3] P. Heusser,[4] and S. Baumgartner[1,4,5]

[1] *Society for Cancer Research, Hiscia Institute, 4144 Arlesheim, Switzerland*
[2] *Institute for Complementary Medicine, University Hospital Zurich, 8091 Zurich, Switzerland*
[3] *Witten/Herdecke University, 58448 Witten, Germany*
[4] *Institute of Integrative Medicine, Witten/Herdecke University, 58448 Witten, Germany*
[5] *Institute of Complementary Medicine KIKOM, University of Bern, 3010 Bern, Switzerland*

Correspondence should be addressed to U. Weissenstein; u.weissenstein@vfk.ch

Received 16 July 2013; Revised 25 September 2013; Accepted 27 September 2013

Academic Editor: Srini Kaveri

Copyright © 2013 R. Kuonen et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Viscum album L. lipophilic extract (VALE) contains pharmacologically active pentacyclic triterpenes that are known to exhibit immunomodulatory, antitumor, and wound healing activity. Preliminary clinical observations indicate that VALE was able to influence cutaneous wound healing *in vivo*. The objective of this study was to investigate wound closure related properties of VALE *in vitro*. As measured in a wound healing assay, VALE and its predominant triterpene oleanolic acid (OA) significantly and dose dependently promoted the migration of NIH/3T3 fibroblasts *in vitro*, thereby leading to an enhanced wound closure. Compared to the negative control, maximal stimulation by 26.1% and 26.2%, respectively, was attained with 10 μg/mL VALE and 1 μg/mL OA. Stimulation of proliferation in NIH/3T3 fibroblasts by VALE and OA could be excluded. At higher concentrations both substances affected proliferation and viability of NIH/3T3 fibroblasts and HaCat keratinocytes. In the toxic range of concentrations of VALE and OA, migration of NIH/3T3 fibroblasts was suppressed. The extent of the stimulatory effect on cell migration of VALE quite closely corresponded to the effect expected by the concentrations of OA contained in the crude extract VALE. These data support the casual observation that *Viscum album* L. lipophilic extract might modulate wound healing related processes *in vivo*.

1. Introduction

Pentacyclic triterpenes like OA, betulinic acid, and ursolic acid are lipophilic compounds of mistletoe with promising pharmacological activities [1–3]. Several properties are described for OA and betulinic acid including antitumor [4–6], anti-inflammatory [7], anti-HIV [8, 9], and immunomodulatory activities [10]. Additionally they exert remarkable wound healing properties [11] and it has been shown that plant extracts containing pentacyclic triterpenes are effective in the topical treatment of actinic keratosis [12]. In a prospective case series study, Kunz et al. [13] used an ointment containing *Viscum album* lipophilic extract (VALE unguentum) for the topical treatment of basal cell carcinoma (BCC). Their clinical findings include promotion of wound healing and antitumoral properties of the lipophilic mistletoe extract. A primary hemostatic effect of the treatment followed by wound closure and partial or complete remission of BCC was observed.

Cutaneous wound healing is a complex self-limiting process characterized by a well coordinated, progressive sequence of events and is structured with regard to both time and space [14]. The acute wound healing process proceeds in three partly overlapping phases: hemostasis and inflammation, granulation tissue formation and reepithelialization, and wound remodeling, and many different cell types are

involved, for example, fibroblasts and keratinocytes [15, 16]. As fibroblasts are responsible for initiating angiogenesis, epithelialization, collagen formation, and synthesis of extracellular matrix proteins [15, 17] an important step of the second phase consists in activation of fibroblast migration into the wounded area.

In our study we used a well-established *in vitro* assay to mimic the migration of mouse NIH/3T3 fibroblasts to an artificial wound with the objective to evaluate the pharmacological effects of VALE and OA on wound healing and we tested their effects on viability and proliferation of fibroblasts and keratinocytes.

2. Material and Methods

2.1. Cell Lines and Cell Culture Conditions. NIH/3T3 mouse fibroblasts (ATCC, Rockville, MD, USA) were cultured in RPMI-1640 medium supplemented with 5% heat-inactivated fetal calf serum (FCS), 2 mM L-Glutamine, and 1% penicillin-streptomycin. HaCat human adult keratinocytes (Institute of Pathology, University Hospital Basel, Switzerland) were cultured in Dulbecco's Modified Eagle's medium (DMEM low glucose) supplemented with 10% heat-inactivated FCS, 2 mM L-Glutamine, and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. For the experiments, cells were collected from subconfluent monolayers using trypsin/EDTA and further cultured in medium containing 1% FCS. All cell culture reagents were obtained from Sigma (Buchs, Switzerland).

2.2. Reagents and Extracts. Oleanolic acid (OA, purity > 98%) and dimethyl sulfoxide (DMSO) were purchased from MP Biomedicals Europe and from Sigma (Buchs, Switzerland), respectively. *Viscum album* L. lipophilic extract (VALE), containing 10% of OA, was obtained by ultracritical CO_2 extraction and was kindly provided by Hiscia Institute, Verein für Krebsforschung Arlesheim, Switzerland. OA and VALE were dissolved in DMSO. Final concentrations of DMSO in all cell culture assays never surpassed the concentration of 1%.

2.3. WST-1 Cell Viability Assay. The viability of NIH/3T3 and HaCat cells was measured by the use of a colorimetric WST-1 assay based on the cleavage of the tetrazolium salt WST-1 by viable cells, according to the supplier's instructions (Roche Diagnostica, Rotkreuz, Switzerland). In brief, cells were seeded into 96-well microtiter plates at a density of 2.5–5 $\times$ 10^3 cells per well. NIH/3T3 cells were treated with either VALE (25–2000 μg/mL) or OA (6.13–100 μg/mL) for 24 h and 48 h and HaCat cells were treated with either VALE (100–1600 μg/mL) or OA (1.25–80 μg/mL) for 24 h and 48 h, followed by incubation with WST-1 for 4 h. Absorbance at 450 nm and 650 nm against a background control was measured in a multi-well plate reader (Labsystems Multiscan RC, Helsinki, Finland). The effect of vehicle DMSO on the cell viability was tested in parallel.

Three independent experiments performed in triplicates were evaluated. Cell growth inhibition was calculated as follows: inhibition in % = 100 − (absorbance of treated cells/absorbance of untreated control $*$ 100).

2.4. In Vitro Wound Healing Assay. The effect of VALE and OA on wound closure was investigated using a CytoSelect 24-well wound healing assay (Cell Biolabs, Inc., San Diego, USA).

3×10^4 NIH/3T3 fibroblasts in DMEM (1% FCS) were seeded into the inserts of a CytoSelect 24-well plate and cultured overnight to allow adhering and reaching a 60–80% confluence. The inserts were carefully removed, leaving a defined and precise 0.9 mm wound field. The medium was aspirated and cells were further incubated for 24 h with either VALE (0.1–2000 μg/mL) or oleanolic acid (0.1–50 μg/mL) in DMEM medium supplemented with 1% FCS. For the determination of cell migration to the wound field, cells were stained according to manufacturer's instructions.

To define confluent area (100%), NIH/3T3 cells were seeded into wells without created wounded area, allowed adhering and reaching confluence for 24 h, and subsequently stained.

DMEM with 5% FCS was used as positive control and medium with 1% FCS as negative control.

For visualization microscopic images focused on the center of the wound were photographed and analyzed using CellD imaging software [18]. The wound healing assay was repeated 7 times.

For the statistical analysis, all data were standardized to the density of confluent cells (100%) and expressed as percentage of cells in wounded area (%) = (test compound/confluent area) $\times$ 100.

2.5. BrdU Proliferation Assay. Cell proliferation was evaluated by 5-bromo-2-deoxyuridine (BrdU) incorporation using a colorimetric BrdU Cell Proliferation ELISA (Roche Applied Science, Indianapolis, IN, USA) according to manufacturer's instructions. Briefly, NIH/3T3 cells were seeded at a density of 5×10^3/well into a 96-well microtiter plate and allowed to adhere for 4–6 h in DMEM medium supplemented with 1% FCS. Then cells were cultured in DMEM in the presence of VALE (0.01–100 μg/mL) or OA (0.001–10 μg/mL) or in the absence of tested compounds for 24 h and 48 h. During the final 24 h of culture, 10 μM BrdU was added. After DNA denaturation and incubation with anti-BrdU-POD antibody the absorbance at 450/690 nm was measured using a multiwell plate reader. Wells with unlabelled cells were set as background control and wells with culture medium alone were set as blank. Three independent experiments performed in triplicates were evaluated.

2.6. Statistics. Viability data from the WST-1 test were assessed with descriptive statistics (calculation of mean and standard error (SE)). Data of the wound healing and proliferation assays were analyzed with analysis of variance (ANOVA) using the software Statistica 4.0 (Statsoft, Tulsa, USA). Global significance was assessed with the F-test. Only if the preceding F-test was significant pairwise comparisons

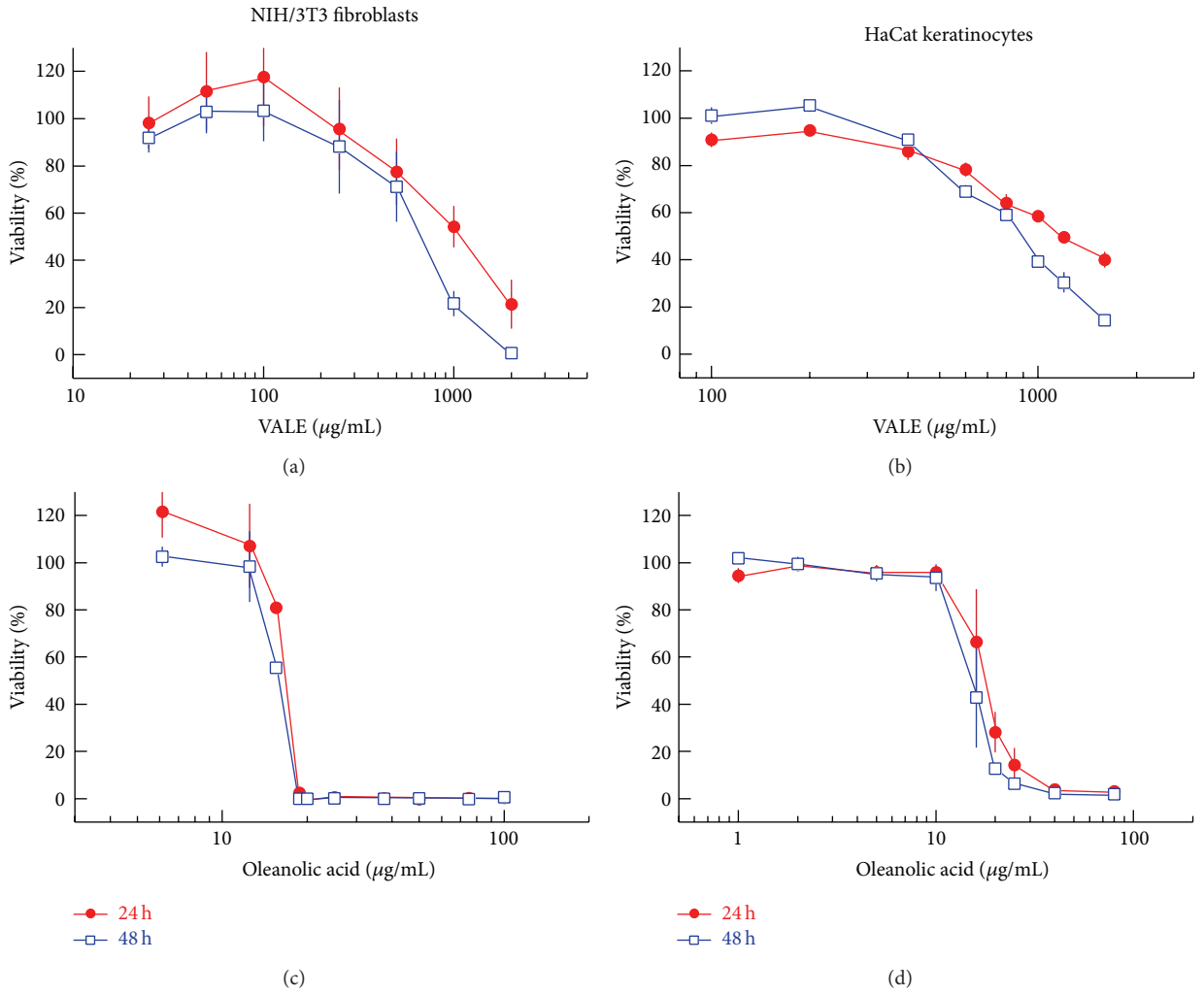


Figure 1: Dose-dependent cytotoxic effect of VALE on (a) NIH/3T3 and (b) HaCat cells and of OA on (c) NIH/3T3 and (d) HaCat cells after 24 h and 48 h of incubation. Mean values ± SE of three experiments are expressed in percentage of cell viability compared to the untreated control.

with the LSD-test was carried out (protected Fisher's LSD-test). P values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Effects of VALE and OA on Cell Viability. As cytotoxic effects can influence results of the functional wound healing assay, the effect of VALE and OA on cell viability after 24 h and 48 h treatment was measured by determining metabolic activity using the WST-1 assay.

VALE investigated at concentrations from 25 to 2000 μg/mL in NIH/3T3 cells and from 100 to 1600 μg/mL in HaCat cells decreased the viability of both cell lines in a dose-dependent manner (Figures 1(a) and 1(b)). NIH/3T3 cells were slightly more sensitive to VALE than HaCat cells.

OA displayed a steep dose-dependent cytotoxic effect on both NIH/3T3 and HaCat cells within a small dose range (Figures 1(c) and 1(d)). Both cell lines exhibited an almost complete abrogation of cell viability by concentrations between 18 and 100 μg/mL OA after 48 h of treatment. Concentrations of VALE < 100 μg/mL and OA < 12.5 μg/mL were nontoxic for NIH/3T3 fibroblasts, and VALE < 200 μg/mL and OA < 10 μg/mL were nontoxic for HaCat keratinocytes (cell viability > 90%).

The amount of DMSO contained in the tested samples was used as control to determine the effect of DMSO on untreated cells. No toxic effect was observed below 1% DMSO (data not shown).

3.2. Effect of VALE and OA on Migratory Activity and Cell Growth of NIH/3T3 Fibroblasts. The effect of VALE and OA on the migratory activity of NIH/3T3 fibroblasts was evaluated using a wound healing assay. Results are expressed as percentage of cells in wounded area (Figure 2(a)). 36.9% of NIH/3T3 positive control cells (5% FCS) migrated to the wounded area in contrast with 13.5% in the untreated negative control (1% FCS). Compared to the negative control,

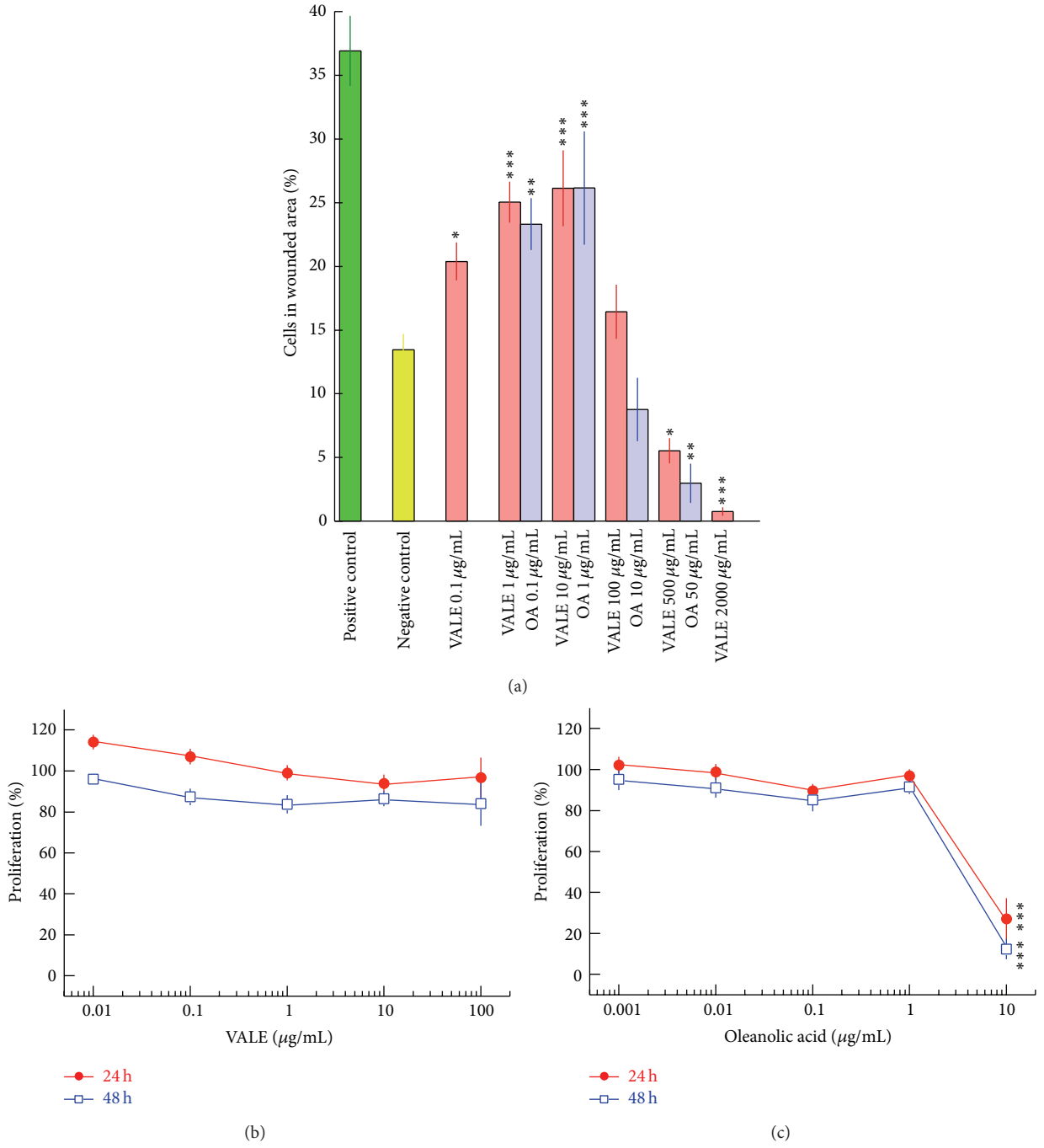


Figure 2: (a) Effect of VALE and OA on wound closure of NIH/3T3 fibroblasts expressed in % of cells migrated to the wounded area. As positive control DMEM with 5% FCS and as negative control untreated samples (1% FCS) were used. Density of confluent cells without created wound was set as 100% wound closure. (b), (c) Effect of VALE and OA on the proliferation of NIH/3T3 fibroblasts was expressed as proliferation of cells compared to the untreated control. Results are expressed as means ± SE of 7 independent wound healing or 3 proliferation experiments, respectively. Significance levels are given compared to the negative control ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, LSD-test).

VALE at concentrations between 0.1 and 10 μg/mL and OA at concentrations of 0.1 μg/mL and 1.0 μg/mL significantly promoted the migration of NIH/3T3 cells thereby leading to an enhanced wound closure. VALE at concentrations from 500 to 2000 μg/mL and 50 μg/mL of OA significantly inhibited the wound closure (Figure 2(a)). Maximal stimulation of NIH/3T3 cell migration by VALE and OA was found at concentrations of 10 μg/mL (26.1%, $P < 0.001$) and 1 μg/mL (26.2%, $P < 0.001$), respectively. The dose-dependent wound healing effect of VALE and OA is illustrated in Figure 3 showing different extents of wound closure in the original microphotographs of one representative experiment.

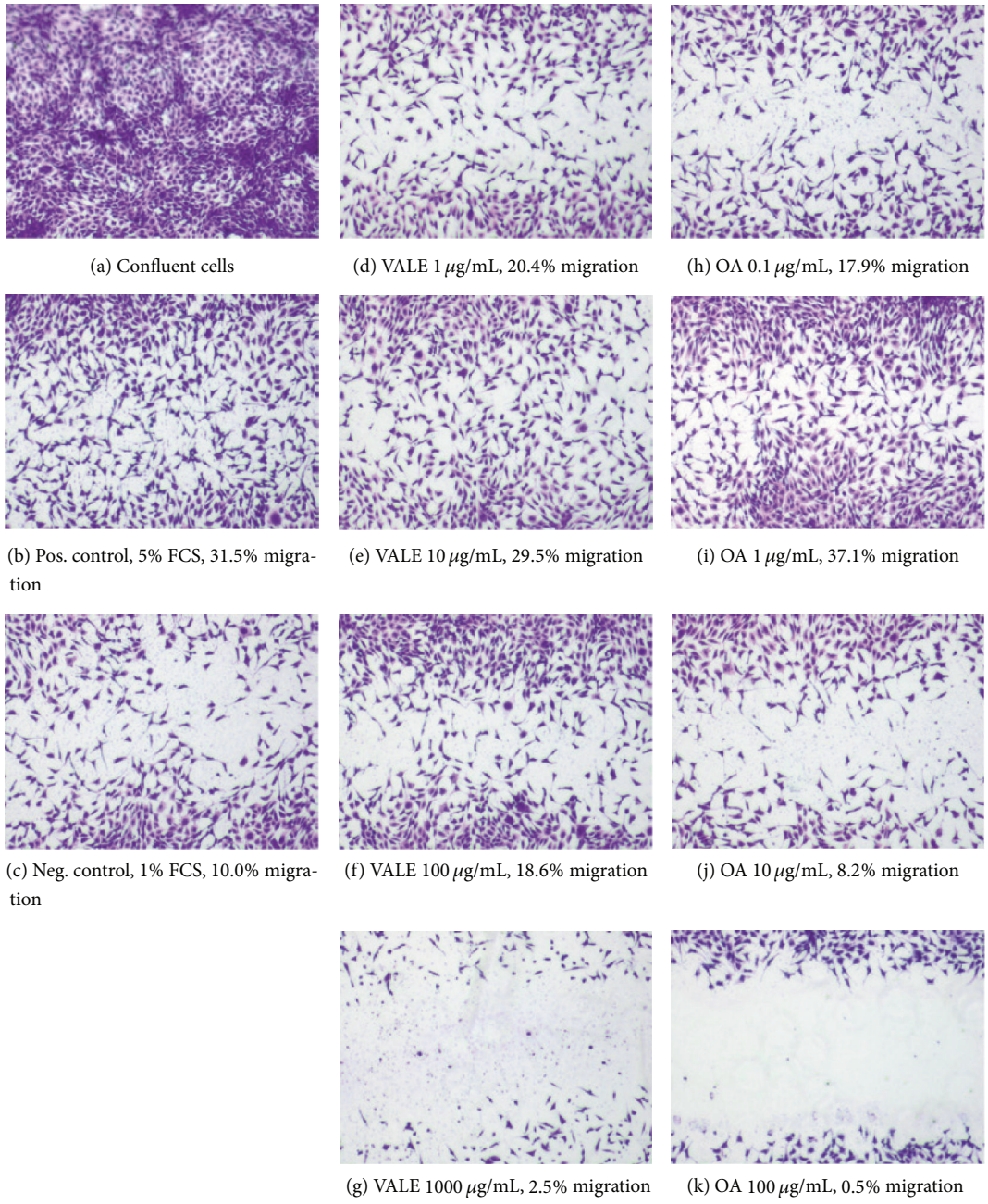


Figure 3: Representative microphotographs of one experiment, illustrating the dose-dependent effect of VALE (d)–(g) and OA (h)–(k) on NIH/3T3 fibroblast migration 24 h after monolayer wounding. Data were standardized to the density of confluent cells (100%) (a) and expressed as percentage of cells in wounded area (% migration) = (test compound/confluent area) × 100. Samples treated with 5% FCS were set as positive control (b) and untreated samples (1% FCS) were set as negative control (c).

The proliferative response of NIH/3T3 cells on VALE and OA was assessed by quantitative analysis of the percentage of cells staining positive for BrdU incorporation. The levels of proliferation found in response to VALE and OA were compared to those of the untreated control (1% FCS).

No proliferation stimulating effect could be found with both substances for the cells at any concentration (Figures 2(b) and 2(c)). A strong and statistically significant inhibition of proliferation by about 80% was found with OA at a concentration of 10 μg/mL.

4. Discussion

An important finding of our *in vitro* study was the stimulatory effect of VALE and OA on the migratory activity of NIH/3T3 fibroblasts leading to an enhanced wound closure. This effect

resulted from stimulation of fibroblasts motility rather than from their mitosis.

Several *in vivo* studies show wound healing activities of triterpenes. Metelmann et al. reported remarkable advantage in promoting wound healing in superficial wounds treated with triterpenes compared to a moist wound dressing [11]. Severe necrotizing herpes zoster of an immunosuppressed patient was successfully treated with an emulsion of the pentacyclic triterpene betulin without causing any side effects [19]. Oleanolic acid isolated from *Anredera diffusa* induced an enhanced wound healing activity in mice [20]. In experimental animal models the activity of the triterpene glycoside asiaticoside, isolated from *Centella asiatica*, improved and facilitated normal as well as delayed-type wound healing and related properties like increase in hydroxyproline, tensile strength, and collagen content and promoted epithelialization and angiogenesis [21]. Kunz et al. [13] observed in a prospective case series study wound healing promoting and anti-tumoral effects by topical treatment of BCC with VALE unguentum and more specifically an achievement of hemostasis in bleeding tumor wounds and after a prolonged treatment period a wound reepithelialization with a thin epithelial layer (personal communication).

In the present study we investigated the effect of lipophilic mistletoe extract on cutaneous wound healing *in vitro*. NIH/3T3 fibroblast cell cultures have been widely used as a model for testing wound healing activity *in vitro* [22, 23] because cell migration, proliferation, and collagen production of fibroblasts play an essential role in cutaneous wound healing. Since growth inhibiting and apoptosis-inducing properties of lipophilic extract from *Viscum album* L. and of its predominant triterpene oleanolic acid have been demonstrated [2], initially the determination of the nontoxic range of VALE and OA was crucial to choose appropriate concentrations when performing the functional wound healing assay. Cell survival was estimated after 24 h and 48 h of treatment using the WST-1 assay. Cells with a viability > 90% of the untreated control were considered to be unaffected. Our results demonstrated that concentrations of VALE < 100 μg/mL and of OA < 12.5 μg/mL were nontoxic for NIH/3T3 fibroblasts. HaCat keratinocytes were slightly less sensitive and concentrations of VALE < 200 μg/mL and of OA < 10 μg/mL were revealed to be nontoxic for this cell type. We thus observed a comparable effect of VALE and OA on the viability of fibroblasts and keratinocytes, cell types whose proliferation, migration, and complex interactions play a primary role in wound healing [24]. Parallel experiments revealed similar dose response curves for NIH/3T3 cell viability after treatment with betulinic acid with nontoxic concentrations <2.5–5.0 μg/mL (data not shown).

Fibroblast migration is one of the essential steps during the proliferative phase of wound healing and during wound closure. The effect of VALE and OA on the migration of NIH/3T3 fibroblasts was investigated using the CytoSelect 24-well wound healing assay. Former experiments performed under similar cell culture and assay conditions illustrate the reliability of the assay to study the influence of wound healing compounds like Bepanthen Plus (Bayer Schweiz AG) on NIH/3T3 fibroblast migration (see supplementary material available online at http://dx.doi.org/10.1155/2013/718105). We observed a dose-dependent stimulation of migration at nontoxic doses of VALE (0.1–10 μg/mL) and of OA (0.1–1 μg/mL) and an inhibition of migration at higher doses of VALE (500–2000 μg/mL) and of OA (50 μg/mL). This inhibitory effect on fibroblast migration corresponds to our results of the cytotoxic properties of VALE and OA in NIH/3T3 fibroblasts. With a proportion of 10%, OA is the most prominent triterpene found in VALE. The strongest stimulation of migration was found with 10 μg/mL of VALE and its corresponding concentration of 1 μg/mL of OA. For this reason we hypothesize that the main active component in VALE responsible for the stimulatory effect on NIH/3T3 fibroblast migration was OA.

Cell migration is not the only component which contributes to wound closure. Cell proliferation has also to be taken into account. We investigated a possible induction of NIH/3T3 fibroblast proliferation by VALE and OA as the wound healing assay used does not allow a clear distinction between migration and proliferation. The results of the BrdU incorporation assay revealed no mitogenic effect of VALE and OA on NIH/3T3 fibroblasts under the experimental conditions used. Enhanced NIH/3T3 cell numbers in an artificial wound by triterpene containing birch bark extract as well as isolated triterpenes in a concentration-dependent manner were also reported by Naumann et al. [25]. In their study, fibroblast proliferation as well as cell migration was influenced. Additionally, HaCat cells treated with the birch bark extract revealed a time-dependent increase of COX-2 mRNA, the key enzyme for the prostaglandin E2 production. Cells use different signaling pathways for migration from those for proliferation. For example, migratory effects of members of heparin-binding fibroblast growth factor family (FGFs) are clearly discriminated from proliferative effects [26]. The mechanisms and signaling pathways responsible for the migratory effect of VALE and OA on NIH/3T3 fibroblasts are unclear at present and need further investigation.

5. Conclusions

Clinical observations indicate a relevant contribution of *Viscum album* lipophilic extract (VALE) to wound healing *in vivo*, thus highlighting the therapeutic potential of the lipophilic fraction of mistletoe. Our *in vitro* results demonstrate that VALE and oleanolic acid stimulated the migration of NIH/3T3 fibroblasts *in vitro*, which led to an enhanced wound closure. These findings are a first step in the process of investigating the underlying mechanisms of the effects of VALE on wound healing.

Conflict of Interests

The authors declare that they have no conflict of interests.

References

[1] T. Krzaczek, "Pharmacobotanical research of the sub-species *Viscum album* L. IV. Acids and amines," *Annales Universitatis Mariae Curie-Sklodowska*, vol. 32, pp. 281–291, 1977.

[2] K. Urech, J. M. Scher, K. Hostanska, and H. Becker, "Apoptosis inducing activity of viscin, a lipophilic extract from *Viscum album* L," *Journal of Pharmacy and Pharmacology*, vol. 57, no. 1, pp. 101–109, 2005.

[3] S. Jäger, H. Trojan, T. Kopp, M. N. Laszczyk, and A. Scheffler, "Pentacyclic triterpene distribution in various plants—rich sources for a new group of multi-potent plant extracts," *Molecules*, vol. 14, no. 6, pp. 2016–2031, 2009.

[4] D. A. Eiznhamer and Z.-Q. Xu, "Betulinic acid: a promising anticancer candidate," *The Investigational Drugs Journal*, vol. 7, no. 4, pp. 359–373, 2004.

[5] K. A. Lúcio, G. G. da Rocha, L. C. Monção-Ribeiro, J. Fernandes, C. M. Takiya, and C. R. Gattass, "Oleanolic acid initiates apoptosis in non-small cell lung cancer cell lines and reduces metastasis of a B16F10 melanoma model in vivo," *PLoS ONE*, vol. 6, no. 12, Article ID e28596, 2011.

[6] Z. Ovesná, A. Vachálková, K. Horváthová, and D. Tóthová, "Pentacyclic triterpenoic acids: new chemoprotective compounds. Minireview," *Neoplasma*, vol. 51, no. 5, pp. 327–333, 2004.

[7] H. Safayhi and E.-R. Sailer, "Anti-inflammatory actions of pentacyclic triterpenes," *Planta Medica*, vol. 63, no. 6, pp. 487–493, 1997.

[8] F. Mengoni, M. Lichtner, L. Battinelli et al., "In vitro anti-HIV activity of oleanolic acid on infected human mononuclear cells," *Planta Medica*, vol. 68, no. 2, pp. 111–114, 2002.

[9] C. Aiken and C. H. Chen, "Betulinic acid derivatives as HIV-1 antivirals," *Trends in Molecular Medicine*, vol. 11, no. 1, pp. 31–36, 2005.

[10] U. Weissenstein, U. D. Toffol-Schmidt, S. Baumgartner, and K. Urech, "Effect of *Viscum album* lipohilic extract on human immunocompetent cells in vitro," *Phytomedicine*, vol. 18, pp. S22–S23, 2011.

[11] H. R. Metelmann, J. Brandner, H. Schumann, F. Bross, M. Hoffmann, and F. Podmelle, "Accelerating the aesthetic benefit of wound healing by triterpene," *Journal of Cranio-Maxillofacial Surgery*, vol. 40, no. 5, pp. e150–e154, 2012.

[12] C. Huyke, J. Reuter, M. Rödig et al., "Treatment of actinic keratoses with a novel betulin-based oleogel. A prospective, randomized, comparative pilot study," *Journal der Deutschen Dermatologischen Gesellschaft*, vol. 7, no. 2, pp. 128–134, 2009.

[13] C. Kunz, A. Hintze, H. Heiligtag, and K. Urech, "Treatment of basal cell carcinoma with *Viscum album* lipophilic extract—a case series study," *Phytomedicine*, vol. 18, supplement 8, p. S25, 2011.

[14] S. A. Eming, T. Krieg, and J. M. Davidson, "Inflammation in wound repair: molecular and cellular mechanisms," *The Journal of Investigative Dermatology*, vol. 127, no. 3, pp. 514–525, 2007.

[15] A. J. Singer and R. A. F. Clark, "Cutaneous wound healing," *The New England Journal of Medicine*, vol. 341, no. 10, pp. 738–746, 1999.

[16] G. Broughton II, J. E. Janis, and C. E. Attinger, "The basic science of wound healing," *Plastic and Reconstructive Surgery*, vol. 117, no. 7, supplement, pp. 12S–34S, 2006.

[17] G. C. Gurtner, S. Werner, Y. Barrandon, and M. T. Longaker, "Wound repair and regeneration," *Nature*, vol. 453, no. 7193, pp. 314–321, 2008.

[18] J. Selinummi, J. Seppälä, O. Yli-Harja, and J. A. Puhakka, "Software for quantification of labeled bacteria from digital microscope images by automated image analysis," *BioTechniques*, vol. 39, no. 6, pp. 859–862, 2005.

[19] S. Weckesser, M. N. Laszczyk, M. L. Müller, C. M. Schempp, and H. Schumann, "Topical treatment of necrotising herpes zoster with betulin from birch bark," *Forschende Komplementarmedizin*, vol. 17, no. 5, pp. 271–273, 2010.

[20] G. Moura-Letts, L. F. Villegas, A. Marçalo, A. J. Vaisberg, and G. B. Hammond, "In vivo wound-healing activity of oleanolic acid derived from the acid hydrolysis of Anredera diffusa," *Journal of Natural Products*, vol. 69, no. 6, pp. 978–979, 2006.

[21] A. Shukla, A. M. Rasik, G. K. Jain, R. Shankar, D. K. Kulshrestha, and B. N. Dhawan, "In vitro and in vivo wound healing activity of asiaticoside isolated from Centella asiatica," *Journal of Ethnopharmacology*, vol. 65, no. 1, pp. 1–11, 1999.

[22] E. Ranzato, M. Patrone, M. Pedrazzi, and B. Burlando, "Hmgb1 promotes wound healing of 3T3 mouse fibroblasts via RAGE-dependent ERK1/2 activation," *Cell Biochemistry and Biophysics*, vol. 57, no. 1, pp. 9–17, 2010.

[23] K. Hostanska, M. Rostock, J. Melzer, S. Baumgartner, and R. Saller, "A homeopathic remedy from arnica, marigold, St. John's wort and comfrey accelerates in vitro wound scratch closure of NIH 3T3 fibroblasts," *BMC Complementary and Alternative Medicine*, vol. 12, article 100, 2012.

[24] S. Werner, T. Krieg, and H. Smola, "Keratinocyte-fibroblast interactions in wound healing," *Journal of Investigative Dermatology*, vol. 127, no. 5, pp. 998–1008, 2007.

[25] K. Naumann, S. Ebeling, M. Laszczyk, A. Scheffler, and I. Merfort, "Triterpenes from birch bark extract beneficially affect wound healing," *Planta Medica*, vol. 76, p. P575, 2010.

[26] B. Boilly, A. S. Vercoutter-Edouart, H. Hondermarck, V. Nurcombe, and X. Le Bourhis, "FGF signals for cell proliferation and migration through different pathways," *Cytokine and Growth Factor Reviews*, vol. 11, no. 4, pp. 295–302, 2000.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2013, Article ID 964592, 11 pages
http://dx.doi.org/10.1155/2013/964592

Research Article

NCCAM/NCI Phase 1 Study of Mistletoe Extract and Gemcitabine in Patients with Advanced Solid Tumors

Patrick J. Mansky,[1,2] Dawn B. Wallerstedt,[3] Timothy S. Sannes,[4] Jamie Stagl,[5] Laura Lee Johnson,[2] Marc R. Blackman,[6] Jean L. Grem,[7] Sandra M. Swain,[8] and Brian P. Monahan[9]

[1] *The Cancer Team at Bellin Health, 1580 Commanche Avenue, Green Bay, WI 54313, USA*
[2] *National Center for Complementary and Alternative Medicine, NIH, Bethesda, MD, USA*
[3] *Samuelli Institute, Alexandria, VA, USA*
[4] *Department of Clinical and Health Psychology, University of Florida, Gainesville, FL, USA*
[5] *University of Miami, Miami, FL, USA*
[6] *Research Service (151), Veterans Affairs Medical Center, and Department of Medicine, Georgetown University School of Medicine, Washington, DC, USA*
[7] *University of Nebraska Medical Center, Omaha, NE, USA*
[8] *Washington Cancer Institute, Washington Hospital Center, Washington, DC, USA*
[9] *Department of Medicine, Hematology and Medical Oncology Division, Uniformed Services University of the Health Sciences, Bethesda, MD, USA*

Correspondence should be addressed to Patrick J. Mansky; manpaj@bellin.org

Received 14 April 2013; Revised 23 July 2013; Accepted 17 September 2013

Academic Editor: Gunver Kienle

Copyright © 2013 Patrick J. Mansky et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Purpose. European Mistletoe (*Viscum album* L.) extracts (mistletoe) are commonly used for cancer treatment in Europe. This phase I study of gemcitabine (GEM) and mistletoe in advanced solid cancers (ASC) evaluated: (1) safety, toxicity, and maximum tolerated dose (MTD), (2) absolute neutrophil count (ANC) recovery, (3) formation of mistletoe lectin antibodies (ML ab), (4) cytokine plasma concentrations, (5) clinical response, and (6) pharmacokinetics of GEM. *Methods.* Design: increasing mistletoe and fixed GEM dose in stage I and increasing doses of GEM with a fixed dose of mistletoe in stage II. Dose limiting toxicities (DLT) were grade (G) 3 nonhematologic and G4 hematologic events; MTD was reached with 2 DLTs in one dosage level. Response in stage IV ASC was assessed with descriptive statistics. Statistical analyses examined clinical response/survival and ANC recovery. *Results.* DLTs were G4 neutropenia, G4 thrombocytopenia, G4 acute renal failure, and G3 cellulitis, attributed to mistletoe. GEM 1380 mg/m^2 and mistletoe 250 mg combined were the MTD. Of 44 patients, 24 developed nonneutropenic fever and flu-like syndrome. GEM pharmacokinetics were unaffected by mistletoe. All patients developed ML3 IgG antibodies. ANC showed a trend to increase between baseline and cycle 2 in stage I dose escalation. 6% of patients showed partial response, 42% stable disease. Median survival was 200 days. Compliance with mistletoe injections was high. *Conclusion.* GEM plus mistletoe is well tolerated. No botanical/drug interactions were observed. Clinical response is similar to GEM alone.

1. Introduction

European mistletoe (*Viscum album* L.), a semiparasitic plant growing on various trees [1], has been used in folklore and as a medicinal plant for several thousand years. In the modern era, it was first introduced as a plant extract preparation for the treatment of malignant diseases by Steiner [2]. A number of studies have reported immunostimulatory effects of mistletoe extracts, on mononuclear cells [3], lymphocytes [4–6], macrophages [7], and NK cells [8, 9]. Mistletoe extracts contain a number of biologically active components, including mistletoe lectins (reviewed in [10–16]) and viscotoxins [17, 18]. Mistletoe extracts may also have antiangiogenic properties [1]. Mistletoe lectins stimulate secretion of

a number of cytokines including IL-6, IL-12, IL-1, and TNF-α [19–21], may enhance cytotoxic NK-cell activity, and may induced apoptosis [22] and induction of FAS ligand [23]. Some of these findings have been supported by microarray gene expression profiling [24]. Mistletoe extract reduces leukopenia in chemotherapy-treated mice and stimulates neutropoiesis in mice after cyclophosphamide chemotherapy [25]. In a dose-dependent fashion, ML-1 may upregulate protein synthesis in neutrophils at low doses, while high doses resulted in neutrophil apoptosis via a caspase-dependent mechanism [26]. Mixed findings have been reported on mistletoe antibody formation. *In vivo* antibody formation has a protective effect against the toxicity of mistletoe lectins to normal somatic cells [27]. While ML antibodies were absent in patients without adverse effects [28], a potential role of ML-antibodies in the neutralization of mistletoe lectin activity *in vivo* has been debated [27, 29]. Collectively, the mounting preclinical data with mistletoe therapy suggests that rigorous clinical trials are needed.

In a phase I study in HIV-positive patients treated with *Viscum album* Quercus Frischsaft (QuFrF) [30], limited toxicities included flu-like symptoms, gingivitis, eosinophilia, and a slight rise in serum urea nitrogen and creatinine. Natural mistletoe lectins were detected in normal volunteers 2 weeks after single dose injection. Fever and flu-like symptoms were observed [31]. Thus, mistletoe preparations appear to be well tolerated, and antibody response appears to be robust. However, the clinical efficacy of mistletoe in oncology settings remains unclear. Notably, in a large retrospective study of mistletoe therapy in nonmetastatic breast cancer patients, fewer adverse events and longer survival were observed in the mistletoe therapy group compared to conventional therapy alone [32]. In a comparable retrospective study design in pancreatic cancer patients, similar clinical outcomes were observed with fewer adverse events and improved survival in patients treated with mistletoe [33]. Despite compelling preclinical data and these isolated reports of clinical benefit of mistletoe preparations, recent reviews of clinical trials still note methodological weaknesses of current published studies on mistletoe [34] as well as conflicting results on tumor response and survival prolongation on treatment using various mistletoe preparations [35–38]. A number of recently published studies confirm this mixed picture [39–42].

With converging preclinical evidence suggesting immunostimulatory and antiangiogenic properties of mistletoe, in addition to a dearth of well-designed clinical trials testing the safety and efficacy of mistletoe, the present study sought to administer mistletoe to advanced stage cancer patients, in combination with a standard, well-known chemotherapy regimen (GEM), hypothesizing that gemcitabine and mistletoe can be administered safely in combination to patients with advanced cancer. The study aims were to evaluate the following: (1) safety, toxicity, and maximum tolerated dose (MTD) of the mistletoe/GEM combination in patients with advanced solid cancers (ASC), (2) neutrophil count recovery, (3) formation of mistletoe lectin antibodies (ML ab), (4) cytokine plasma concentrations, (5) clinical response, and (6) GEM pharmacokinetics as an indicator of possible interactions of the mistletoe/GEM combination regimen.

2. Materials and Methods

2.1. Mistletoe Extract Quality and Content Verification. A whole plant mistletoe extract (HELIXOR Apis (A), growing on fir trees), Lot 021224 and Lot 0406, was used and supplied by Helixor, GmbH, Rosenfeld, Germany. Study agent content analyses was performed by the manufacturer. Product content verification analyses were conducted by Lawrence Livermore National Laboratories, Livermore, CA, USA. Verification analyses were consistent with the manufacturer's analyses and showed no evidence for product contamination with pesticides, heavy metals, or the prescription drugs listed. The Helixor mistletoe extract was assayed for approximately 60 elemental species by inductively-coupled-plasma/mass spectrometry (ICP-MS). In addition, Helixor A mistletoe extract was assayed for a variety of pesticides and street drugs including stimulants, narcotics, and tranquilizers using gas chromatography-mass spectroscopy GC-MS.

Liquid Chromatography-Mass Spectrometry (LC-MS and LC-MS/MS) analysis of Helixor mistletoe formulation for commonly used oncology drugs was negative. Helixor A mistletoe extract was assayed by thin-layer chromatography (TLC) via a QA/QC protocol supplied by Helixor. TLC assay results of the submitted Helixor solution were very similar to those expected from the Helixor QA/QC protocol.

Both lots were tested in parallel up to this final assay performed on 14 February 2006. Lot 021224 contained 3.4 (±0.2) ng/mL ML-I and 178 (±4) ng/mL ML-III. Lot 040686 contained 9.2 (±0.9) ng/mL ML-I and 293 (±12) ng/mL ML-III.

For the study, two lots of mistletoe were manufactured, as the period of study enrollment spanned more than 2 years. The initial mistletoe lot was tested for ML-I and ML-III stability at the beginning of the study, and at 6, 15, and 18 months. ML-I and ML-III concentrations remained stable for 2 years.

2.2. Patient Recruitment and Screening Statistics. The protocol, informed consent, and patient recruitment materials were reviewed and approved by the National Cancer Institute's Institutional Review Board (IRB) on July 15, 2002 and by the National Naval Medical Center's IRB on December 12, 2002 (study number 02-AT-0260). A total of 704 persons expressed interest in this study and were contacted over this five year study. Forty-four persons (6%) were enrolled on-study after meeting all study eligibility criteria and signing written informed consent.

2.3. Study Eligibility Criteria. Patients with histologically confirmed treated or untreated, advanced pancreatic or non-small cell lung cancer (NSCLC), or recurrent metastatic colorectal or breast cancer were eligible for study participation. Additionally, study participants needed to be able and willing to administer daily subcutaneous injections of mistletoe by themselves or with assistance.

2.4. Study Design and Outcomes. The objective of this two stage, dose escalation phase I clinical trial was to observe

the safety of the combination of gemcitabine and subcutaneously injected mistletoe extract in a population with advanced solid cancers and limited treatment options.

The study design, and rationale for this two agent, dose escalation paradigm, is published elsewhere [43]. In brief, in stage I, a fixed dose of gemcitabine (750 mg/m^2) was administered intravenously on day 1 and day 8 of a 3-week cycle with an escalating mistletoe dose (1 mg, 5 mg, 10 mg, 20 mg, 50 mg, 100 mg, 200 mg, and 250 mg/day subcutaneously). As the manufacturer recommends mistletoe dosing from 50 to 200 mg, this dosing covered a range from 20% to 125% of the manufacturer recommended dose, which was considered a reasonable dosing range in a drug where there is precedence for clinical use and there are no prospective dosing data in combination with GEM. In stage II, a fixed mistletoe daily dose (as determined in stage I) was administered with gemcitabine in 20% dose increments per dose level (900, 1080, 1380, and 1560 mg/m^2, resp., with the maximum dose being more than 50%, higher than the manufacturer recommended dose of 1000 mg/m^2) [43]. This stage of the study examined whether participants' ability to tolerate gemcitabine would be differentially affected by concurrently administered mistletoe injections. Enrollment of 3 patients per dose level was planned. Grade 3 nonhematologic and grade 4 hematologic events were considered dose limiting toxicities (DLT). If three patients enrolled in a dose level successfully completed three cycles of the gemcitabine-mistletoe regimen with no DLT, then subsequently enrolled patients were assigned to the next higher dosage level. However, if 1 DLT occurred, an additional 1–3 patients were added to the cohort at that dose level for a maximum of 6 patients per dose level. The occurrence of 2 DLTs in one dosage level was considered to represent the maximum tolerated dose (MTD).

Primary study outcomes were the MTD and DLT of the combination regimen and the plasma gemcitabine pharmacokinetics alone and in combination with mistletoe extract. Secondary study outcomes were neutrophil count recovery, the stimulation of selected plasma cytokine levels (IL-6, IL-12, IFNγ, and TNF-α), the time to production and the circulating plasma concentrations of mistletoe lectin-1 (ML-1) and mistletoe lectin-3 (ML-3) antibodies, measured as IgG1-4 subclasses, and tumor response.

2.5. Participants and Data Collection Procedures. Enrolled participants were evaluated and treated in the hematology-oncology clinic at the National Naval Medical Center by the study investigators and NCI fellows. Prior to signing informed consent, the study investigators informed the participants about the purpose and methods of the study and explained where the study was in terms of stage and dose escalation. Once enrolled on-study, one of the study investigators instructed the study participant and family members on how to administer the daily subcutaneous mistletoe injections, with special attention to rotating the sites of injection and avoiding reinjection in the same area. Each participant (or family member) demonstrated successful subcutaneous administration of the mistletoe extract and was supplied with study supplies (i.e., sterile syringes, alcohol swabs, and sharps containers). Participants were informed that localized skin reactions, including discomfort at the injection site, redness, and itching, were commonly reported and were advised to inform the study staff if any skin reactions or other adverse events occurred.

Laboratory values were monitored twice a week, and clinical on-study evaluations were performed every cycle. CT scans were performed at baseline, and every 3 cycles. Adverse events were monitored weekly by the study investigators using ToxGrade, a software program designed for this study using the Common Terminology Criteria for Adverse Events (CTCAEv3) guidelines. Study data were tracked in a database monitored by the EMMES Corporation (Rockville, MD, USA). Independent study monitoring was provided by EMMES and KAI (both in Rockville, MD, USA).

2.6. Analytic Plan. The primary aim of this phase I study was to investigate the safety and toxicity of the mistletoe/GEM treatment regimen. As such, adverse events (any clinical event while on-study, considered related to mistletoe or gemcitabine based on published effects of the respective agents [44] rated as not related, possibly related, likely related, or definitely related), number of dose limiting toxicities, and clinical response (defined as progressive/stable disease or partial response at the time patients as assessed every 3 cycles and/or when patients were taken off of the study at disease progression, using RECIST criteria) are reported with corresponding descriptive statistics for the 44 study participants.

Secondary analyses included used Kaplan Meier [45] to assess time from study enrollment to death. Progression free survival and time to progression were initially considered but were difficult quantities to assess due to the lack of precise measurement of progression and assessment bias even when a rigorous definition is used (U.S. Food and Drug Administration CDER and CBER. Guidance for Industry Clinical Trial Endpoints for the Approval of Cancer Drugs and Biologics http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm071590.pdf May 2007), and as such, time to disease progression analyses are not included in this paper.

An exploratory aim of the study was to examine potential trends of mistletoe and gemcitabine escalation on immune functioning. The *a priori* hypothesis was that ANC values would increase over the course of treatment; however, each group to be assessed had a small sample size. The Jonckheere-Terpstra trend test [46] was used to examine absolute neutrophil count (ANC) trends across time and across varying levels of gemcitabine and mistletoe treatments. This nonparametric statistical approach is similar to a Kruskal-Wallis test and has more power than the Kruskal-Wallis when there is *a priori* ordering of the populations from which the samples are drawn.

Pharmokinetics analyses used area under the curve analyses and plasma concentrations (CP, nmol/mL) from 20 minutes to 25 minutes following the infusion, comparing between cycle 1 (gemcitabine alone) and cycle 3 (gemcitabine plus mistletoe) using a Wilcoxon signed rank test.

3. Results

A total of 44 study participants were enrolled on this study; twenty patients were treated in stage I (mistletoe dose escalation phase) and 24 in stage II (gemcitabine dose escalation phase). The study population's demographic information is presented in Table 1(a). All patients had stage IV disease; the majority had received previous chemo-, hormonal, immunological, or radiation therapy, and 23% were chemotherapy-naïve. Patients' disease characteristics are listed in Table 1(b).

3.1. Adverse Events. A total of 706 discrete hematologic adverse events (AEs) were documented, occurring in 95% of study participants (Table 2(a)). The most common were low lymphocyte counts (for example, lymphopenia) (n = 200 events), anemia (n = 158), leukopenia (e.g., total WBC count) (n = 149), thrombocytopenia (n = 100), and neutropenia (e.g., low granulocyte or absolute neutrophil count) (n = 99). The majority (85%) of observed hematologic AEs were grade 1-2, 104 grade 3 (15%) and five grade 4 toxicities were observed. The grade 4 AEs included 1 neutropenia event (defined as an ANC < 500), 2 thrombocytopenia events (defined as platelet count < 25,000), and 2 lymphopenia events. A total of 570 nonhematologic AEs were recorded. The most common were hyperglycemia and hypoalbuminemia, followed by almost equal numbers of nausea and fatigue (Table 2(b)).

Nonneutropenic fever and flu-like syndrome, which have been previously described with mistletoe treatment and are also known AEs associated with gemcitabine, were observed in 24 of 44 (55%) patients. More patients experienced these symptoms in stage II of the study (15/24) than in stage I (9/20). Only one grade 3 febrile event was observed during stage II, all other events were grade 2 or less.

3.2. Mistletoe-Related Adverse Events. Mistletoe-related nonhematologic adverse events are represented in Table 3. A total of 112 adverse events were attributed to mistletoe treatment. The most common AEs attributed to mistletoe treatment were injection site reactions (42 events), localized induration (20 events), grade 1-2 nonneutropenic fever (22 events), and grade 1-2 flu-like symptoms (10 events). All of these AEs were expected as they had been documented as known mistletoe-related AEs in the Investigators' Brochure. Seventy-five events were grade 1, thirty-five were grade 2, and two events were grade 3. The two grade 3 events were cellulitis at the mistletoe injection site.

3.3. Gemcitabine-Related Adverse Events. A total of 473 hematologic AEs at least possibly related to gemcitabine were documented. Most commonly occurring number of events were leukopenia, thrombocytopenia, neutropenia, and anemia, which is consistent with the previously published data. Thirty patients developed a low WBC, and 28 patients developed thrombocytopenia. 30% of the low WBC events and close to 10% of the thrombocytopenic events were grade 3.

A total of 249 nonhematologic events were attributed at least possibly to gemcitabine. The most common were nausea (n = 47) and vomiting (n = 31), followed by liver enzyme elevation (elevated AST n = 25; elevated ALT n = 20), nonneutropenic fever (n = 21), and fatigue (n = 19). Thirteen grade 3 events were recorded, most commonly vomiting (n = 3) and fatigue (n = 2).

3.4. Maximum Tolerated Dose and Dose Limiting Toxicities. Five dose limiting toxicities (DLTs) were observed (Table 4). One study participant experienced grade 4 neutropenia at dose level 6 (mistletoe 250 mg/gemcitabine 900 mg/m^2). An additional three participants enrolled onto this dose level did not subsequently experience a DLT. One study participant experienced grade 4 thrombocytopenia at dose level 7 (mistletoe 250 mg/gemcitabine 1180 mg/m^2); three subsequent participants enrolled at this dose level did not experience a DLT. Three study participants experienced individual DLTs at dose level 9 (gemcitabine 1560 mg/m^2 with 250 mg daily of mistletoe). These included grade 3 cellulitis at the mistletoe injection site, grade 4 acute renal failure, and grade 4 neutropenia. As per the protocol's study design, one dose level below the dose level at which 3 DLTs were reached was defined as the maximum tolerated dose. Thus, we achieved the MTD at dose level 8 (gemcitabine 1380 mg/m^2 and mistletoe 250 mg).

3.5. Pharmacokinetics of Gemcitabine. Plasma concentrations of gemcitabine from patients treated during stage I were measured in nmol/mL. Fifteen of 20 patients treated in stage I had plasma samples obtained for analysis. Twelve patients had paired samples obtained during cycle 1 (without mistletoe) and cycle 3 (with mistletoe).

The addition of mistletoe did not affect gemcitabine pharmacokinetics as measured during cycle 3 (cycle 3, day 8 of gemcitabine/mistletoe combination) compared to cycle 1 of treatment (gemcitabine alone on day 1 of treatment before mistletoe was added on day 8, P values ranging from 0.47 to 0.97; Table 5).

3.6. Best Clinical Response to Treatment. Figure 1(a) shows the best overall response. Of the 44 enrolled study patients, 33, completed at least 3 cycles of therapy. Of these 33, six percent (n = 2) had a partial response, 42% (n = 14) had stable disease, and 43% (n = 14) progressed on treatment. Nine percent (n = 3) were not evaluable for response.

3.7. Best Clinical Response according to Diagnosis. Both partial responses were observed in patients with pancreatic cancer. Three of 4 evaluable patients with NSCLC had stable disease, and 5/11 patients with breast cancer had stable disease (Figure 1(b)). Only 1 out of 8 patients with colorectal cancer had stable disease.

3.8. Survival Analyses. Of the 44 study participants, three participants died on study, 10 participants requested to

Table 1: (a) Study population demographics. (b) Prior treatment by disease type*.

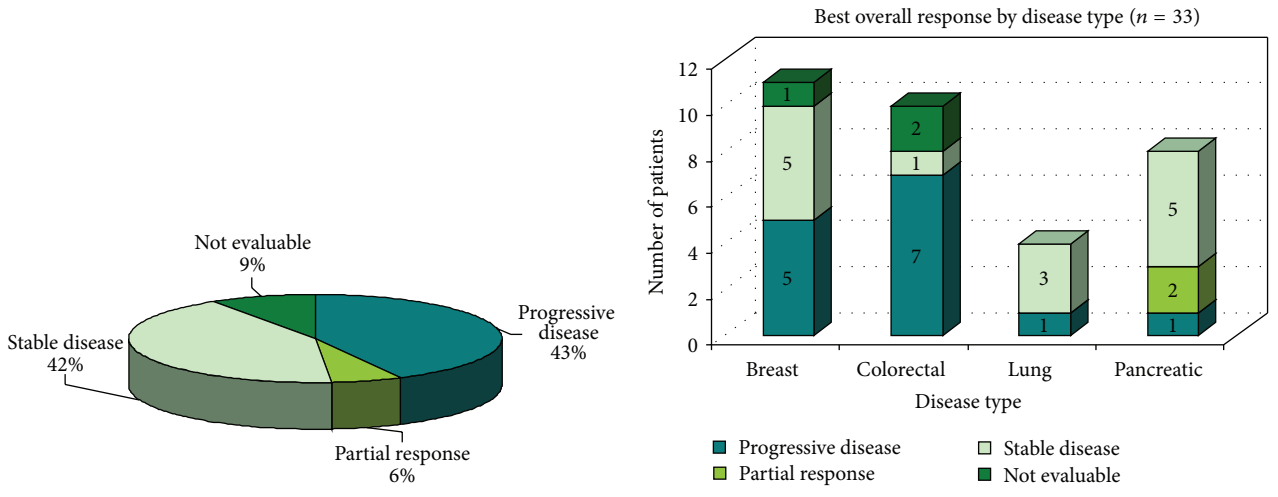
(a)

	Stage I (*n*)	Stage II (*n*)	Total (*n*)	Total (%)
Number enrolled	20	24	44	—
Age (years)				
Mean	55.0	55.1	55.1	—
Range	29–81	29–76	29–81	—
Gender				
Male	10	13	23	52%
Female	10	11	21	48%
Race				
White	17	19	36	82%
Black	2	2	4	9%
Asian	0	3	3	7%
Ethnicity				
Not Hispanic	17	24	41	93%
Hispanic	3	0	3	7%
Cancer diagnosis				
Colorectal	4	13	17	39%
Breast	6	6	12	27%
Pancreatic	6	4	10	23%
Lung	4	1	5	11%
Disease stage				
IV	20	24	44	100%

(b)

Disease type	No prior treatment	Chemotherapy only	Radiation only	Chemotherapy and radiation	Chemotherapy and surgery	Chemotherapy, surgery, and radiation	Total
Colorectal	0	4	0	1	8	4	17
Breast	0	1	0	0	1	10	12
Pancreatic	9	0	0	1	0	0	10
Lung	1	1	1	2	0	0	5
Total (*n*/%)	10 (22.7%)	6 (13.6%)	1 (2.3%)	4 (9.1%)	9 (20.5%)	14 (31.8%)	44

*No study participants were treated solely with surgery or with surgery plus radiation only.



(a) (b)

Figure 1: (a) Best clinical response and (b) best overall response by diagnosis.

Table 2: (a) Overall hematologic CTCAE adverse events ($n = 44$). (b) Most common nonhematologic CTCAE adverse events ($n = 44$).

(a)

Hematologic adverse events	Number of events (possibly multiple events from same participant)					Number of participants experiencing (multiple) events	Number of participants (with most severe event if there are multiple)			
	Grade 1	Grade 2	Grade 3	Grade 4	Total		Grade 1	Grade 2	Grade 3	Grade 4
Lymphopenia	42	105	51	2	200	34		15	17	2
Anemia	85	61	12		158	41	13	22	6	
Leukopenia (Total WBC)	83	50	16		149	30	8	12	10	
Thrombocytopenia	78	13	7	2	100	29	20	4	3	2
Neutropenia (ANC/AGC)	41	39	18	1	99	26	4	11	10	1
Total hematologic AEs	329	268	104	5	706					

(b)

Nonhematologic adverse events	Number of events (possibly multiple events from same participant)					Number of participants experiencing (multiple) events	Number of participants (with most severe event if there are multiple)			
	Grade 1	Grade 2	Grade 3	Grade 4	Total		Grade 1	Grade 2	Grade 3	Grade 4
Hyperglycemia	81	35	8		124	34	17	12	5	
Hypoalbuminemia	53	38	2		93	29	11	16	2	
Hypocalcemia	59	19	1		79	33	20	12	1	
Hyponatremia	50	0	9	1	60	29	22	0	6	1
Elevated AST, SGOT	42	13	2		57	30	20	8	2	
Elevated ALP, ALKP	29	22	5		56	25	10	10	5	
Nausea	27	23	1		51	24	9	14	1	
Fatigue	20	25	5		50	29	7	17	5	
Total: most commonly occurring nonheme AEs	361	175	33	1	570		116	89	27	1
Total: nonheme AEs (overall)	751	398	85	6	1243					

terminate the study, 23 participants progressed while on study, one terminated the study due to a dose limiting toxicity, 6 left due to complicating disease issues which may be tied to progression, and one voluntarily withdrew. An attempt was made to follow study subjects once they terminated study treatment until death. At the study's last attempt to contact former participants, three were still alive and five others were lost to followup. A Kaplan Meier curve was used to illustrate time to death in Figure 2. The median time to death of any cause was approximately 200 days.

3.9. Jonckheere-Terpstra Trend Test Results for ANC Values across Increasing Dose Levels. We prospectively followed ANC nadir and ANC maximum as one of the study outcomes, hypothesizing that the ANC may be influenced by mistletoe exposure. ANC values showed a trend for increase between baseline and cycle 2 in stage I ($P = 0.06$). When ANC maximum was measured, there was a significant trend ($P = 0.034$) for the maximum ANC level achieved in stage II during cycle 1. However, if patients were eliminated based on dexamethasone exposure, the trend for stage I diminished ($P = 0.092$) but was maintained for cycle 1 ANC maximum during stage II ($P = 0.017$).

3.10. Development of Mistletoe Lectin 3 IgG Antibodies and Cytokine Release. Helixor A extract is low in ML-1 and high in ML-3 content. Therefore, ML-3 content was followed. All study patients eventually developed mistletoe lectin 3 IgG antibodies. The formation of antibodies was higher at increasing doses of mistletoe.

For stage II, when all patients were exposed to the same mistletoe regimen with increasing doses of gemcitabine,

Table 3: Nonhematologic CTCAE adverse events at least possibly related to mistletoe ($n = 44$).

Nonhematologic adverse events	Number of events (possibly multiple same events from participant)				Number of participants experiencing (multiple) events	Number of participants (with most severe event if there are multiple)		
	Grade 1	Grade 2	Grade 3	Total		Grade 1	Grade 2	Grade 3
Injection site reaction	30	12		42	26	14	12	
Fever (in the absence of neutropenia)	18	4		22	14	11	3	
Induration/fibrosis skin and subcutaneous tissue	13	7		20	15	8	7	
Flu-like syndrome	6	4		10	9	5	4	
Pruritus	3	1		4	3	2	1	
Cellulitis (with normal ANC or grade 1 or 2 ANC)		1	1	2	2		1	1
Allergic reaction/hypersensitivity		1		1	1		1	
Dermatology skin reaction-NOS		1		1	1		1	
Cellulitis with unknown ANC			1	1	1			1
Lymphatics-NOS	1			1	1	1		
Lymphedema		1		1	1		1	
Myalgia NOS	1			1	1	1		
Pain-joint		1		1	1		1	
Pain-skin	1			1	1	1		
Phlebitis		1		1	1		1	
Rash: erythema multiforme		1		1	1		1	
Rigors/chills	1			1	1	1		
Total nonheme AEs	75	35	2	112				

NOS: not otherwise specified.

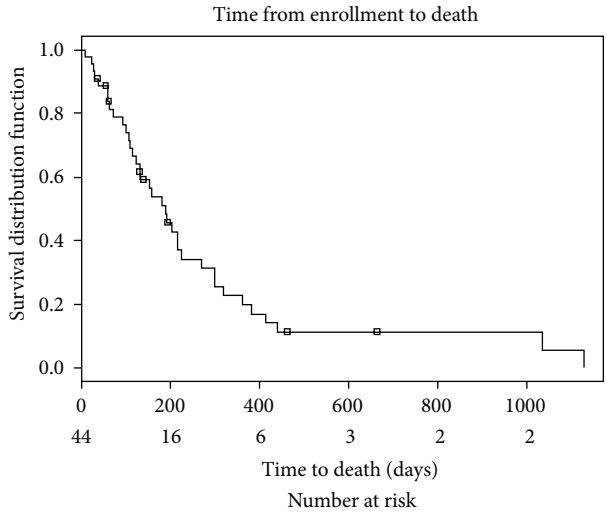


Figure 2: Time from enrollment to death.

only IgG3 antibody levels increased with increasing doses of gemcitabine (data not shown). Cytokines were minimally affected by this combination regimen.

4. Discussion

To our knowledge, this is the first reported dose escalation study of a whole mistletoe extract combined with single agent gemcitabine in patients with advanced solid cancers. As per the manufacturer's information [44], nonfebrile neutropenia as a function of dose in gemcitabine was observed in 63% (19% grade 3, 6% grade 4). Thrombocytopenia occurred in 24% (4% grade 3, 1% grade 4). Gemzar as a single agent was administered at doses between 800 mg/m^2 and 1250 mg/m^2 over 30 minutes intravenously, once weekly, in 979 patients with a variety of malignancies.

We observed 30% grade 3 neutropenia and 10% grade 3 thrombocytopenia, while single agent gemcitabine testing has resulted in 19% grade 3 nonfebrile neutropenia and 4% grade 3 thrombocytopenia [44]. Thirty-seven percent of patients experienced nonneutropenic fever, while single agent gemcitabine was associated with 41% fever. There was no documented incidence of febrile neutropenia for the combination regimen. Flu-like symptoms occurred in 18% of patients.

The hematologic toxicity profile of the mistletoe/gemcitabine combination and febrile reactions in this study were similar to single agent gemcitabine [44]. The addition of

Table 4: Dose limiting toxicities by dose level.

Stage I (fixed GEM dose of 750 mg/m^2)			Stage II (fixed mistletoe dose, established in stage 1)			
Level	*n*	DLT	Level/dosage	*n*	DLT	Action taken per protocol
(1) (escalating daily mistletoe injections, reaching: 20 mg/day)	3	None	(6) (250 mg/day mistletoe; **900** mg/m^2 GEM on day 1/8 of 3-week cycle)	7	Grade 4 neutropenia	Dose reduced; enrolled 3 more patients at this dose level
(2) (escalating daily mistletoe injections, reaching: 50 mg/day)	3	None	(7) (250 mg/day mistletoe; **1080** mg/m^2 GEM on day 1/8 of 3-week cycle)	7	Grade 4 thrombocytopenia	Dose reduced; enrolled 3 more patients at this dose level
(3) (escalating daily mistletoe injections, reaching: 100 mg/day)	3	None	(8)* (250 mg/day mistletoe; **1380** mg/m^2 GEM on day 1/8 of 3-week cycle)	6	None	N/A
(4) (escalating daily mistletoe injections, reaching: 200 mg/day)	6	None	(9) (250 mg/day mistletoe; **1560** mg/m^2 GEM on day 1/8 of 3-week cycle)	4	Grade 3 cellulitis[a]; grade 4 acute renal failure[b]; grade 4: thrombocytopenia[c]	[a]Mistletoe therapy withheld; patient rechallenged and developed hypersensitivity reaction. Mistletoe discontinued; [b]Pt. treated for renal failure and subsequently withdrawn from study; [c]Pt. hospitalized d/t other AE; *Maximum tolerated dose reached per protocol*
(5) (escalating daily mistletoe injections, reaching: 250 mg/day)	5	None				

* Per study protocol, this level represents the maximum tolerated dose, as 3 DLT's were observed in the subsequent dose level.

Table 5: Pharmacokinetics of gemcitabine (cycle 1) and gemcitabine plus mistletoe (cycle 3).

	Cycle 1 Median (25th%, 75th%)	Cycle 3 Median (25th%, 75th%)	*P* value Signed rank test
Gemcitabine AUC (min ∗ nmol/mL)	664 (514, 870)	670 (625, 851)	0.97
Gemcitabine Average Cp (nmol/mL)	47.7 (42.7, 64.6)	49.7 (45.7, 53.8)	0.85

mistletoe did not exacerbate hematologic gemcitabine toxicity. Interestingly, there was a trend ($P = 0.06$) towards increased ANC nadir during the first 3 weeks of initiation of mistletoe and of ANC maximum during the first 6 weeks as a function of mistletoe dose ($P = 0.034$). Others have claimed that mistletoe may boost chemotherapy tolerance, but published data on dosing and mistletoe schedule are lacking, while these were collected in detail in this study.

Flu-like symptoms may be more common when mistletoe is added to gemcitabine. We observed febrile and flu-like reactions attributable to mistletoe across the entire mistletoe dosing spectrum that did not seem to be dose dependent.

The addition of mistletoe did not affect the pharmacokinetics of gemcitabine at any of the mistletoe dose levels tested, suggesting that mistletoe can be added to gemcitabine without concern about adversely affecting gemcitabine's pharmacokinetic profile. The MTD for the gemcitabine/mistletoe combination in this study was gemcitabine 1380 mg/m^2 given weekly on day one and eight of a three-week cycle with

mistletoe 250 mg s.c. daily. As per the manufacturer recommendations, gemcitabine is commonly dosed at 1000 mg i.v. weekly for three weeks on a 28-day cycle. In our study a higher dose was tolerated.

Stimulatory effects of mistletoe on neutrophils and lymphocytes have been reported *in vitro* as well as in patients. We observed a mistletoe dose-dependent trend towards increased absolute neutrophil count ANC nadir during cycle 1 and ANC maximum during cycle 2. None of the study patients developed febrile neutropenia even at the highest gemcitabine dose of 1650 mg/m^2. As this study employed a dose escalation scheme in a diverse group of patients with advanced cancer, many of whom were heavily pretreated, this observation would have to be verified and confirmed in a setting of increased homogeneity of patient population and treatment regimen with a larger sample size.

There is a sizable body of literature on the effects of mistletoe on cytokine production [21]. We selected testing for IL-6, IL-12, IFN gamma, and TNF alpha based on their previously described role in tumor development and proliferation as well as existing publications of possible effects of mistletoe on the production of these cytokines. The production of these cytokines in patients with cancer however has not been studied in detail when chemotherapy was combined with mistletoe. We did not detect any consistent pattern of increased or decreased production of any of the cytokines tested.

Mistletoe lectin (ML)-3 antibody formation of the IgG type was detected in all patients by cycle 3 of therapy or 9 weeks and thus was independent of the actual mistletoe dose administered. The physiologic effect of the formation of ML antibodies is not well understood. While we did not compare participants' injections site reactions to this immunological data, we did observe injection site reactions early in treatment of all study participants. Others have reported local reactions in 87% [31]. In most studies, mistletoe is injected three times per week, while our patients injected mistletoe daily. It is thus not surprising that our study would find a higher rate of local injection site reactions. Skin reactions decreased over the course of therapy. This phenomenon may have resulted from the increasing formation of ML antibodies over time mitigating the mistletoe related injection site reactions. Febrile reactions occurred in more than one-third of the patients. It is not clear from our data that there was any relationship between the appearance of ML antibodies and febrile reactions or other toxicities. The study was not designed to yield reliable data on clinical response to the study regimen. Therefore, we are unable to determine associations between clinical response and the formation of ML antibodies or febrile reactions. Future studies may add the understanding of the physiological reactions to mistletoe therapy by connecting immunological data to changes in symptom presentation.

The finding of a partial response rate of 6% is comparable to what would be expected from single agent gemcitabine in this population of patients with advanced, mostly heavily pretreated carcinomas. The median survival from study enrollment of about 200 days is within the range of what would be expected from single agent gemcitabine. Compliance with the mistletoe regimen was high, and no episode of febrile neutropenia was observed in any of the 44 patients. The lack of episodes of febrile neutropenia in a set of heavily pretreated patients of whom almost 50% received gemcitabine doses of 1100 mg/m^2 or higher is noteworthy, but would have to be confirmed in a larger, more homogenous cancer population.

The above results should be interpreted in light of several study limitations. First, the study sample included 4 different types of solid tumors, each of which may respond differently to GEM or mistletoe/GEM therapy. As such, the results presented herein may not extend to more homogenous groups of cancer patients. Second, the overall sample size was small ($n = 44$). As a result, the study may have been underpowered to detect significant trends for the study outcomes. Despite the majority of study outcomes remaining descriptive in nature, the statistical tests applied (e.g., survival analyses and Jonckheere-Terpstra trend test) should be interpreted in light of the small sample sizes used in each respective test. The overall small sample size precluded subgroup analyses (e.g., treatment naïve participants' response), which may be a worthwhile area for future studies to pursue. Finally, the study was not designed to examine the effectiveness of a mistletoe/GEM combination. Future work should apply double blind, randomized controlled study designs to examine.

5. Conclusion

The combination of mistletoe and gemcitabine was well tolerated and treatment compliance was high. The MTD was gemcitabine 1380 mg/m^2 weekly on day one and eight of a 3-week cycle combined with mistletoe 250 mg daily. Gemcitabine pharmacokinetics were not affected by mistletoe. The lack of febrile neutropenia even at higher gemcitabine doses is noteworthy. The formation of ML antibodies is common. A consistent effect of the study regimen on the serum levels of selected cytokines could not be demonstrated. Clinical response of the combination appeared to be similar to single agent gemcitabine reported previously.

Conflict of Interests

All authors were contacted, and the possibility of conflict of interests was explored. None of the authors listed reported any conflict of interests regarding the content of this paper or investigational agents used for the research reported therein.

Authors' Contribution

Dawn B. Wallerstedt, Timothy S. Sannes, and Jamie Stagl contributed to data collection and interpretation and paper writing. All other authors also contributed to the design and funding of the study.

Acknowledgments

The authors would like to acknowledge the help and advice of Drs. Agnes Nguyenpho, FDA; Maria Merino, Laboratory of Pathology, NCI; Alex Ling, Diagnostic Imaging Department,

Clinical Center, NIH; and Julia T. Arnold, NCCAM. They greatly appreciate the technical assistance of Howard Baldwin, NCCAM, and the support of Drs. Dieter Schlodder and Juergen Schierholz as well as Sabine Rieger from Helixor GmbH. The authors also thank the NIH and NNMC fellows, nurses, pharmacy staff, and all the patients who made it possible to conduct this study. This research was supported, in part, by the Intramural Research Programs of the NIH, NCCAM, and NCI and was done at the Intramural Research Program of the National Center for Complementary and Alternative Medicine, National Institutes of Health. Conclusions drawn and recommendations for future directions made are the opinions of the authors and do not represent the view of the National Center for Complementary and Alternative Medicine, The Department of the Navy, Department of Defense, or the National Cancer Institute. Mistletoe extract (Helixor A) was provided by Helixor GmbH, Rosenfeld, Germany, which also supported the mistletoe lectin analyses.

References

[1] S. Elluru, J. D. van Huyen, S. Delignat et al., "Molecular mechanisms underlying the immunomodulatory effects of mistletoe (*Viscum album* L.) extracts Iscador," *Arzneimittel-Forschung*, vol. 56, no. 6, pp. 461–466, 2006.

[2] R. Steiner, *Geisteswissenschaft und Medizin*, Rudolf Steiner, Dornach, Switzerland, 1985.

[3] K. Hostanska, T. Hajto, G. C. Spagnoli, J. Fischer, H. Lentzen, and R. Herrman, "A plant lectin derived from *Viscum album* induces cytokine gene expression and protein production in cultures of human peripheral blood mononuclear cells," *Natural Immunity*, vol. 14, no. 5-6, pp. 295–304, 1995.

[4] G. Stein and P. A. Berg, "Non-lectin component in a fermented extract from *Viscum album* L, grown on pines induces proliferation of lymphocytes from healthy and allergic individuals *in vitro*," *European Journal of Clinical Pharmacology*, vol. 47, no. 1, pp. 33–38, 1994.

[5] R. Rentea, E. Lyon, and R. Hunter, "Biologic properties of Iscador: a *Viscum album* preparation. I. Hyperplasia of the thymic cortex and accelerated regeneration of hematopoietic cells following X-irradiation," *Laboratory Investigation*, vol. 44, no. 1, pp. 43–48, 1981.

[6] J. Beuth, H. L. Ko, L. Tunggal et al., "Thymocyte proliferation and maturation in response to galactoside-specific mistletoe lectin-1," *In Vivo*, vol. 7, no. 5, pp. 407–410, 1993.

[7] G. Kuttan, "Tumoricidal activity of mouse peritoneal macrophages treated with *Viscum album* extract," *Immunological Investigations*, vol. 22, no. 6-7, pp. 431–440, 1993.

[8] K. Hamprecht, R. Handgretinger, W. Voetsch, and F. A. Anderer, "Mediation of human NK-activity by components in extracts of *Viscum album*," *International Journal of Immunopharmacology*, vol. 9, no. 2, pp. 199–209, 1987.

[9] T. Hajto and C. Lanzrein, "Natural killer and antibody-dependent cell-mediated cytotoxicity activities and large granular lymphocyte frequencies in *Viscum album*-treated breast cancer patients," *Oncology*, vol. 43, no. 2, pp. 93–97, 1986.

[10] A. Bussing, "Mistletoe therapy and immunological research," *Anti-Cancer Drugs*, vol. 8, no. 1, p. S65, 1997.

[11] G. M. Stein and P. A. Berg, "Mistletoe extract-induced effects on immunocompetent cells: *in vitro* studies," *Anti-Cancer Drugs*, vol. 8, no. 1, pp. S39–S42, 1997.

[12] H. J. Gabius, S. André, H. Kaltner, H. C. Siebert, C. W. von der Lieth, and S. Gabius, "The mistletoe myth: claims, reality and provable perspectives," *Zeitschrift fur Arztliche Fortbildung*, vol. 90, no. 2, pp. 103–110, 1996.

[13] H. Franz, "Mistletoe lectins and their A and B chains," *Oncology*, vol. 43, no. 1, pp. 23–34, 1986.

[14] U. Elsasser-Beile, M. Voss, R. Schuhle, and U. Wetterauer, "Biological effects of natural and recombinant mistletoe lectin and an aqueous mistletoe extract on human monocytes and lymphocytes *in vitro*," *Journal of Clinical Laboratory Analysis*, vol. 14, no. 6, pp. 255–259, 2000.

[15] U. Elsässer-Beile, S. Lusebrink, T. Grussenmeyer, U. Wetterauer, and W. Schultze-Seemann, "Comparison of the effects of various clinically applied mistletoe preparations on peripheral blood leukocytes," *Arzneimittel-Forschung*, vol. 48, no. 12, pp. 1185–1189, 1998.

[16] T. Hajto, T. Berki, L. Palinkas, F. Boldizsar, and P. Nemeth, "Investigation of the effect of mistletoe (*Viscum album* L.) extract Iscador on the proliferation and apoptosis of murine thymocytes," *Arzneimittelforschung*, vol. 56, no. 6, pp. 441–446, 2006.

[17] R. Samtleben, T. Hajto, and K. Hostanska, "Mistletoe lectins as immunostimulants (chemistry, pharmacology and clinic)," in *Immunomodulatory Agents from Plants*, H. Wagner, Ed., pp. 223–241, Birkhäauser, Basel, Switzerland, 1999.

[18] R. T. Lee, H. J. Gabius, and Y. C. Lee, "Ligand binding characteristics of the major mistletoe lectin," *The Journal of Biological Chemistry*, vol. 267, no. 33, pp. 23722–23727, 1992.

[19] G. Ribéreau-Gayon, S. Dumont, C. Muller, M. L. Jung, P. Poindron, and R. Anton, "Mistletoe lectins I, II and III induce the production of cytokines by cultured human monocytes," *Cancer Letters*, vol. 109, no. 1-2, pp. 33–38, 1996.

[20] T. Hajto, K. Hostanska, K. Weber et al., "Effect of a recombinant lectin, *Viscum album* agglutinin on the secretion of interleukin-12 in cultured human peripheral blood mononuclear cells and on NK-cell-mediated cytotoxicity of rat splenocytes *in vitro* and *in vivo*," *Natural Immunity*, vol. 16, no. 1, pp. 34–46, 1998.

[21] T. Hajto, K. Hostanska, K. Frei, C. Rordorf, and H. J. Gabius, "Increased secretion of tumor necrosis factor α, interleukin 1, and interleukin 6 by human mononuclear cells exposed to β-galactoside-specific lectin from clinically applied mistletoe extract," *Cancer Research*, vol. 50, no. 11, pp. 3322–3326, 1990.

[22] H. O. Pae, W. G. Seo, G. S. Oh et al., "Potentiation of tumor necrosis factor-α-induced apoptosis by mistletoe lectin," *Immunopharmacology and Immunotoxicology*, vol. 22, no. 4, pp. 697–709, 2000.

[23] A. Büssing, G. M. Stein, U. Pfüller, and M. Schietzel, "Induction of Fas ligand (CD95L) by the toxic mistletoe lectins in human lymphocytes," *Anticancer Research*, vol. 19, no. 3, pp. 1785–1790, 1999.

[24] J. Eggenschwiler, A. Patrignani, U. Wagner et al., "Gene expression profiles of different breast cancer cells compared with their responsiveness to fermented mistletoe (*Viscum album* L.) extracts Iscador from oak (Quercus), pine (Pinus), white fir (Abies) and apple tree (Malus) *in vitro*," *Arzneimittel-Forschung*, vol. 56, no. 6, pp. 483–496, 2006.

[25] K. Weber, U. Mengs, T. Schwarz, H. Becker, and H. Lentzen, "Stimulation of neutropoiesis by a special standardized mistletoe preparation after cyclophosphamide chemotherapy in mice," *Arzneimittel-Forschung*, vol. 46, no. 12, pp. 1174–1178, 1996.

[26] A. Savoie, V. Lavastre, M. Pelletier, T. Hajto, K. Hostanska, and D. Girard, "Activation of human neutrophils by the plant lectin *Viscum album* agglutinin-I: modulation of de novo protein synthesis and evidence that caspases are involved in induction of apoptosis," *Journal of Leukocyte Biology*, vol. 68, no. 6, pp. 845–853, 2000.

[27] A. Stettin, J. L. Schultze, E. Stechemesser, and P. A. Berg, "Anti-mistletoe lectin antibodies are produced in patients during therapy with an aqueous mistletoe extract derived from *Viscum album* L. and neutralize lectin-induced cytotoxicity *in vitro*," *Klinische Wochenschrift*, vol. 68, no. 18, pp. 896–900, 1990.

[28] G. M. Stein and P. A. Berg, "Characterisation of immunological reactivity of patients with adverse effects during therapy with an aqueous mistletoe extract," *European Journal of Medical Research*, vol. 4, no. 5, pp. 169–177, 1999.

[29] G. M. Stein, U. Pfüller, and P. A. Berg, "Recognition of different antigens of mistletoe extracts by anti-mistletoe lectin antibodies," *Cancer Letters*, vol. 135, no. 2, pp. 165–170, 1999.

[30] R. W. Gorter, M. van Wely, M. Reif, and M. Stoss, "Tolerability of an extract of European mistletoe among immunocompromised and healthy individuals," *Alternative Therapies in Health and Medicine*, vol. 5, no. 6, pp. 37–48, 1999.

[31] R. Huber, J. Eisenbraun, B. Miletzki et al., "Pharmacokinetics of natural mistletoe lectins after subcutaneous injection," *European Journal of Clinical Pharmacology*, vol. 66, no. 9, pp. 889–897, 2010.

[32] P. R. Bock, W. E. Friedel, J. Hanisch, M. Karasmann, and B. Schneider, "Efficacy and safety of long-term complementary treatment with standardized european mistletoe extract (*Viscum album* L.) in addition to the conventional adjuvant oncologic therapy in patients with primary non-metastasized mammary carcinoma/results of a multicenter, comparative, epidemiological cohort study in Germany and Switzerland," *Arzneimittel-Forschung*, vol. 54, no. 8, pp. 456–466, 2004.

[33] H. Matthes, W. E. Friedel, P. R. Bock, and K. S. Zanker, "Molecular mistletoe therapy: friend or foe in established anti-tumor protocols? A multicenter, controlled, retrospective pharmacoepidemiological study in pancreas cancer," *Current Molecular Medicine*, vol. 10, no. 4, pp. 430–439, 2010.

[34] T. Ostermann and A. Bussing, "Retrolective studies on the survival of cancer patients treated with mistletoe extracts: a meta-analysis," *Explore*, vol. 8, no. 5, pp. 277–281, 2012.

[35] G. S. Kienle, A. Glockmann, M. Schink, and H. Kiene, "*Viscum album* L. extracts in breast and gynaecological cancers: a systematic review of clinical and preclinical research," *Journal of Experimental and Clinical Cancer Research*, vol. 28, article 79, 2009.

[36] T. Ostermann, C. Raak, and A. Bussing, "Survival of cancer patients treated with mistletoe extract (Iscador): a systematic literature review," *BMC Cancer*, vol. 9, article 451, 2009.

[37] M. A. Horneber, G. Bueschel, R. Huber, K. Linde, and M. Rostock, "Mistletoe therapy in oncology," *Cochrane Database of Systematic Reviews*, no. 2, Article ID CD003297, 2008.

[38] G. S. Kienle and H. Kiene, "Complementary cancer therapy: a systematic review of prospective clinical trials on anthroposophic mistletoe extracts," *European Journal of Medical Research*, vol. 12, no. 3, pp. 103–119, 2007.

[39] R. Grossarth-Maticek, H. Kiene, S. M. Baumgartner, and R. Ziegler, "Use of iscador, an extract of European mistletoe (*Viscum album*), in cancer treatment: prospective nonrandomized and randomized matched-pair studies nested within a cohort study," *Alternative Therapies in Health and Medicine*, vol. 7, no. 3, pp. 57–76, 2001.

[40] D. Lenartz, U. Dott, J. Menzel, J. M. Schierholz, and J. Beuth, "Survival of glioma patients after complementary treatment with galactoside-specific lectin from mistletoe," *Anticancer Research*, vol. 20, no. 3, pp. 2073–2076, 2000.

[41] M. K. Steuer-Vogt, V. Bonkowsky, P. Ambrosch et al., "The effect of an adjuvant mistletoe treatment programme in resected head and neck cancer patients: a randomised controlled clinical trial," *European Journal of Cancer*, vol. 37, no. 1, pp. 23–31, 2001.

[42] G. Bar-Sela, M. Wollner, L. Hammer, A. Agbarya, E. Dudnik, and N. Haim, "Mistletoe as complementary treatment in patients with advanced non-small-cell lung cancer treated with carboplatin-based combinations: a randomised phase II study," *European Journal of Cancer*, vol. 49, no. 5, pp. 1058–1064, 2013.

[43] P. J. Mansky, J. Grem, D. B. Wallerstedt, B. P. Monahan, and M. R. Blackman, "Mistletoe and gemcitabine in patients with advanced cancer: a model for the phase I study of botanicals and botanical-drug interactions in cancer therapy," *Integrative Cancer Therapies*, vol. 2, no. 4, pp. 345–352, 2003.

[44] http://pi.lilly.com/us/gemzar.pdf.

[45] E. L. Kaplan and P. Meier, "Nonparametric estimation from incomplete observations," *Journal of the American Statistical Association*, vol. 53, no. 282, pp. 457–481, 1958.

[46] A. R. Jonckheere, "A distribution-free k-sample test against ordered alternatives," *Biometrika*, vol. 41, no. 1-2, pp. 133–145, 1954.