



ISCADOR® in Cancer Therapy

Scientific Information and Study Results

ISCADOR  AG

Solutions for Integrative Oncology

This literature is intended for professionals.

ISCADOR® in Cancer Therapy

Scientific Information and Study Results



Contents

1	Introduction	6
2	Mistletoe botany	7
2.1	Occurrence and dispersal	7
2.2	Morphology	9
2.3	Mistletoe flower and fruit	10
3	From mistletoe to ISCADOR	12
3.1	Mistletoe harvest	12
3.2	Processing of the fresh plant and fermentation	12
3.3	Mixing of summer and winter extracts	13
3.4	Manufacture of the end product	14
3.5	Quality control	15
4	Mistletoe constituents and their effects	16
4.1	Mistletoe lectins	16
4.2	Viscotoxins	19
4.3	Kuttan's peptides	20
4.4	Oligosaccharides, polysaccharides	21
4.5	Flavonoids	21
4.6	Thiols	21
4.7	Triterpenes	21
5	Immunological properties of mistletoe extracts	23
5.1	Principles of immunological tumour defence	23
5.1.1	Non-specific (natural) immune system	24
5.1.2	Specific (adaptive) immune system	24
5.1.3	Interactions between the non-specific and specific immune system	25
5.2	Effects of mistletoe extracts on cells of the non-specific immune system	26
5.2.1	Influence of mistletoe extracts on monocytes and macrophages	26
5.2.2	Influence of mistletoe extracts on neutrophil granulocytes	27
5.2.3	Influence of mistletoe extracts on natural killer cells	27

5.3	Effects of mistletoe extracts on cells of the specific immune system	28	6.3.6	Osteosarcoma	57
5.4	Effects on tumour development	29		- ISCADOR versus oral etoposide as adjuvant therapy in osteosarcoma patients after a second relapse	58
5.4.1	Influence of mistletoe extracts on tumour cell proliferation	29	6.4	ISCADOR in cancer-related fatigue syndrome	59
5.4.2	Influence of mistletoe extracts on apoptosis	29	6.5	ISCADOR therapy and immune checkpoint inhibitors	60
5.4.3	Cytokine induction	29	6.5.1	Principles	60
6	Clinical efficacy of ISCADOR	31	6.5.2	Practical use exemplified in the treatment of non-small cell lung cancer - First clinical experiences with the combination of immune checkpoint inhibitors and ISCADOR	61
6.1	Study designs	31	7	Therapy with ISCADOR	62
6.1.1	Randomised controlled trials (RCT)	32	7.1	General points	62
6.1.2	Controlled, pharmaco-epidemiological, retrospective cohort studies	33	7.2	Initial phase	63
6.2	Clinical effects of ISCADOR in detail	34	7.3	Optimum dose response	63
6.3	ISCADOR in the treatment of different tumour entities	35	7.4	Maintenance phase	64
6.3.1	Breast cancer	35	7.5	Indications	65
	- Efficacy and safety of ISCADOR in patients with primary, non-metastasised breast cancer	35	7.6	Side effects	65
	- Adjuvant mistletoe treatment and chemotherapy in patients with breast cancer	37	7.7	Combination with standard therapies	66
	- ISCADOR and chemotherapy in patients with breast cancer	38	7.7.1	Chemotherapy	66
	- ISCADOR and chemotherapy in patients with breast cancer: five-year follow-up	40		- Interactions between ISCADOR and chemotherapeutics	67
6.3.2	Lung cancer	42	7.7.2	Radiotherapy	68
	- Mistletoe therapy in patients with advanced non-small cell lung cancer	42	7.7.3	Hormone/antihormone therapy	68
6.3.3	Colorectal cancer	43	7.8	Interactions with other drugs	69
	- Postoperative ISCADOR treatment in patients with primary, non-metastasised colorectal cancer	44	7.9	Contraindications	69
	- ISCADOR reduces cancer-related fatigue syndrome in patients with primary, non-metastasised colorectal cancer	47	8	References	70
6.3.4	Pancreatic cancer	48	9	Drug information	77
	- Improvement of supportive therapy using ISCADOR in patients with pancreatic cancer	48	10	Imprint and contact	78
	- Mistletoe therapy in locally advanced or metastasised pancreatic cancer	51			
6.3.5	Malignant melanoma	55			
	- Efficacy and safety of ISCADOR in long-term treatment of patients with primary malignant melanoma	55			

1 Introduction

There is a long tradition, going back to the fourth century BC, of using mistletoe as a remedy. This evergreen plant has been used over the ages for menstruation complaints, epilepsy, ulcers, cardiac insufficiency, hypertension and oedema (Ramm 2015).

Based on research by Dr. Rudolf Steiner (1861–1925), the founder of anthroposophy, the white-berried mistletoe (*Viscum album* L.) was first specifically used in cancer therapy at the beginning of the 20th century. At the end of 1916, he suggested to Dr. Ita Wegman, a physician closely collaborating with him, that mistletoe has specific potential for cancer treatment. Working with an experienced pharmacist, she developed an injection preparation (Iscar), and first treated cancer patients with mistletoe in the summer of 1917 (Selg 2016). The preparation was further developed in the following years and registered under the name ISCADOR in 1926. Today, after almost a century of clinical and practical experience, ISCADOR is the most frequently prescribed mistletoe preparation in complementary tumour therapy, with the longest and most thorough research history.

ISCADOR, which is manufactured from the leaves, stems and berries of the plant, is a fermented aqueous extract of the white-berried mistletoe (*Viscum album* L.) originating from the host trees of apple (ISCADOR M = Mali), oak (ISCADOR Qu = Quercus), pine (ISCADOR P = Pini) and elm (ISCADOR U = Ulmi). In Switzerland, fir mistletoe ISCADOR is also available (ISCADOR A = Abietis).

This brochure gives a full account of the constituents of mistletoe, their effects and immunological properties, and current ISCADOR study findings. ISCADOR treatment is also described here in more detail.

Readers who would like more information on ISCADOR studies can download the current version of «Documentation on published clinical studies with ISCADOR» as a PDF from <http://studien.vfk.ch>. This covers all publications on subjects including immunology, DNA repair, quality of life/pain, tumour remissions, survival periods, safety, and systematic reviews. Summaries of numerous selected studies are used to give an overview of results on the efficacy and safety of ISCADOR.

2 Mistletoe botany

Mistletoe grows as a hemiparasite on trees. Globally, approximately 1,500 plants are identified as mistletoes in the widest sense. However, only the white-berried mistletoe (*Viscum album* L.) – named after its white fruits – is used in complementary cancer therapy. It is divided into three botanical subspecies: the deciduous mistletoe (*Viscum album* ssp. *album*, Fig. 1) grows frequently on poplars and apple trees. It can also be found on maple, birch, lime, robinia, willow, hawthorn, and almond. On oak and elm, mistletoe is found very rarely in nature, but is today cultivated in natural habitats for pharmaceutical purposes. Inside the fruits, the pip is connected to the pericarp by threads. In contrast, the pips of both conifer mistletoe subspecies are not attached with threads to the pericarp but lie freely in the fruit tissue. These subspecies are named pine mistletoe (*Viscum album* ssp. *austriacum*, Fig. 2) and fir mistletoe (*Viscum album* ssp. *abietis*, Fig. 3), after the respective conifer species on which they grow (Becker 1986, Ramm et al. 2016).



Fig. 1: Deciduous mistletoe
ISCADOR M, Qu, U



Fig. 2: Pine mistletoe
ISCADOR P



Fig. 3: Fir mistletoe
ISCADOR A

2.1 Occurrence and dispersal

The mistletoe is native to many parts of Europe and is therefore called European mistletoe. However, it rarely grows in the North and far East of Europe, as it cannot survive extreme frost below minus 20 degrees Celsius. In the South, its occurrence is limited by strong sunshine and aridity. The mistletoe is most strongly established in France, and the natural occurrence of very rare oak mistletoe is almost exclusively limited to that country (Ramm et al. 2000, Ramm 2016).

Three bird species specialise on mistletoe berries as food source, and are responsible for the natural dispersal of mistletoe.



Fig. 4: Mistle thrush

The **mistle thrush** (*Turdus viscivorus*) feeds almost exclusively on ripe mistletoe fruits from October to March and excretes the undigested fruit skin and pips after ingestion. These sticky excretions often remain stuck to twigs, where mistletoe embryos can germinate in spring (Ramm 2017).



Fig. 5: Blackcap

The **blackcap** (*Sylvia atricapilla*), a migratory bird that returns from the South to Central Europe in March, picks single berries left by mistle thrushes on mistletoe bushes, and eats the fruit flesh and fruit skin. In doing so, it sloughs off the sticky mistletoe pip on a host twig, where mistletoe embryos can germinate close to the mother plant (Ramm 2017).



Fig. 6: Waxwing

The third mistletoe distributor is the **waxwing** (*Bombycilla garrulus*), which is native to the tundra of Northern Europe. When these birds cannot find enough food in this location after a humid and cold summer, they move to the South in large flocks and raid mistletoe berry stocks in Central and Southern Europe. Like mistle thrushes, waxwings also swallow several mistletoe berries at once and excrete the pips undigested, often back onto the same trees, sometimes leading to strong proliferation of mistletoe plants (Ramm 2017).

2.2 Morphology

Mistletoe can only grow on its subspecies-specific host tree. Thus pine mistletoe only grows on pines, and fir mistletoe on firs. The deciduous mistletoe grows on several broad-leaved tree species and can move across a whole range of deciduous species (Ramm 2016).

Mistletoe pips remain in their dried, sticky shell on the twigs for the whole winter before the enclosed embryos start to germinate in April. As they do so, a germinating stem (hypocotyl) develops. In contact with the bark, the tip of the hypocotyl expands into a kind of adhesive disc attached to the bark by excretion of a sticky substance (Fig. 7). From the centre of this adhesive disc, the haustorial stem actively grows through the bark until it reaches the cambium, the thin layer of vital and meristematic cells located between bark and wood. From there a so-called sinker develops, enclosed by the continuously growing young wood of the host tree (Fig. 8). In this young host wood, newly formed water-conducting vessels are redirected towards the sinker. The host tree therefore provides the mistletoe plant with water and dissolved minerals, and also with organic substances such as carbohydrates and amino acids (Becker 1986, Ramm 2016).

When the mistletoe seedling has connected with the host tree's water-conducting tissue, the first small leaves, which look like cotyledons, develop in the following spring (Fig. 9). Subsequently, another one-year dormant phase follows. The next spring, a new stem emerges, again with only one pair of leaves. One year later, the first branching occurs through shoots emerging from axillary buds: two lateral shoots accompany the central shoot, but again each new mistletoe twig consists of only one stem with a terminal pair of leaves. In contrast to common leaves, mistletoe leaves lack a polarised morphology, their upper- and under-surfaces being identically structured (Becker 1987, Ramm 2016).



Fig. 7: A hypocotyl emerging from the mistletoe seedling



Fig. 8: Mistletoe sinker in the young wood of an oak

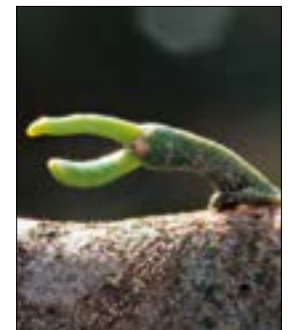


Fig. 9: A new seedling with two new leaves



Fig. 10: Female mistletoe flower



Fig. 11: Male mistletoe flower

2.3 Mistletoe flower and fruit

The white-berried mistletoe is a dioecious plant. Thus, there are mistletoe bushes with only female flowers (Fig. 10), which later bear the fruit, and mistletoe bushes with only male flowers (Fig. 11), which release pollen. In nature, female mistletoe plants are up to four times more common than male ones. Generally, three inflorescences develop from generative buds, which have formed between the terminal leaf primordia in July of the previous year. The three flower buds of the male plants are enclosed by only one upper leaf (bract). In the female flower, one bract encloses the two lateral flowers and a second bract encloses the terminal flower. Male flowers consist of four simple perigon leaves. Sepals, petals and stamens therefore grow together and form a deep funnel. Male flowers are distinctly larger than female flowers, which consist mainly of a spherical fruit primordium, on which four small, yellowish perigon leaves open to present the stigma (Ramm 2016).

Depending on geographical location and weather conditions, the main flowering period of *Viscum album* is in February and March; thus insects active in winter, such as flies (Fig. 12, 13), ants, hoverflies, bumblebees, and midges, pollinate the mistletoe flower. After successful pollination, the fruit begins to develop in April. In the first weeks, nutritive tissue accumulates in the pip, before the organs of the embryos are formed from July onward. At the end of September, the embryo is fully developed within the still green-coloured fruit, and viable. Depending on the degree of cold, the fruits begin to ripen at the end of October. Fruit skin (exocarp) and fruit pulp (mesocarp) lose their green colour and become translucent. Tiny lipid droplets, embedded in the fruit pulp, reflect the light so that the whole fruit appears white (Ramm et al. 2000, Ramm 2016). The endosperm inside the fruit, and the embryos inside the pip remain green throughout the winter, indicating that the young mistletoe germ is continually supplied with light and thus can stay alive (Ramm et al. 2016).

Thus, the white-berried mistletoe differs in growth, development and reproduction in many ways from other plant species:

- It grows on trees and shrubs, not directly in mineral soil.
- It has no roots, but forms a so-called sinker with which it anchors itself into the wood of its host tree. The sinker does not grow actively into the wood, but expands outwards synchronously with the expanding young wood.
- Although the mistletoe plant contains chlorophyll and can produce organic nutrients by means of photosynthesis, in addition to water and minerals it receives 20 to 40 percent of its organic substances from the host tree via its sinker.
- At the end of May, the mistletoe starts to perform unusual motions (nutations), which are typical of this plant. The young leaves and stems orientate themselves each day in a different direction for four weeks, until the end of June when all young branches are directed towards the centre of the mistletoe bush. The mistletoe has its own centre, and in June emancipates itself from the positive (roots) and negative (leaves) geotropic orientation typical of common plants. Form reduction and nutational motions are the basis of its typical spherical form, which is not found in any other plants.
- The mistletoe plant grows very slowly, not forming its first typical leaves before the second year. In subsequent years, too, only one stem with two opposite leaves grows from each leaf axillary bud. At the age of five to seven years, the mistletoe plant starts to flower and is able to reproduce from then on.
- Mistletoe leaves are evergreen, biannual, and maintain their growth potential, expanding in length, width and thickness in the spring of the second year. In late summer, the two-year-old green mistletoe leaves fall off without any previous sign of wilting.
- Mistletoe embryos, which are embedded in the endosperm of the green pips inside the white berries, need constant light during the winter to maintain their capacity to germinate in spring.



Fig. 12: A fly on the male mistletoe flower



Fig. 13: A fly on the female mistletoe flower

3 From mistletoe to ISCADOR

3.1 Mistletoe harvest

Mistletoe harvesting, strictly separated according to host tree, takes place twice a year: in summer when the plant is at the peak of its vegetative development, and in winter when the buds and fruits have fully developed. In this way, harvesting takes optimum account of mistletoe's various physiological stages (Urech et al. 2009). One- and two-year-old leaves, stems and buds are harvested (Fig. 14). At winter harvest, additionally, ripened berries are gathered.

The major sources of apple tree and pine mistletoe are the extensive, wild stocks in France, though apple tree mistletoe is also cultivated and harvested from the own natural habitats. Fir mistletoe is harvested in Switzerland. Very rare oak mistletoe is harvested from wild stocks in France, and from the own natural habitats, the latter also supplying all the elm mistletoe required.

During harvest, the plant parts to be processed are carefully picked from the mistletoe bushes and then taken to the place of manufacture as soon as possible for sorting (Fig. 15).



Fig. 14: Harvest of the mistletoe branches



Fig. 15: Sorting the plants

3.2 Processing of the fresh plant and fermentation

After sorting, the plant parts are mechanically macerated using a rolling mill for subsequent fermentation (Fig. 16). Macerated plant parts are mixed in equal amounts with water in quality for pharmaceutical use and starter cultures from the mistletoe's own microflora (lactobacilli) are added (Fig. 17). Within three (plant) or four days (berries), an extraction equilibrium between mistletoe and fluid has developed and is stabilised by lactic acid, so that the solid, insoluble plant residues can be separated from the extract by pressing (Fig. 18).



Fig. 16: Shredding the plant



Fig. 17: Adding starter cultures to the mistletoe mash



Fig. 18: Pressing of mistletoe extract

3.3 Mixing of summer and winter extracts

The respective extracts of summer and winter mistletoe are mixed together in a 1:1 ratio on a special machine with a titanium disc one meter in diameter that rotates at 10,000 revolutions per minute. In the mixing process, drops of the summer extract fall to the edge of the rotating disc through twelve droppers from a height of around one metre. The winter extract is continuously fed into the centre of the disc, and spreads out horizontally. Both juices combine at the edge of the disc to form the ISCADOR concentrate (Fig. 19).



Fig. 19: ISCADOR manufacturing machine

In an intensive mixing process, summer extracts are dropped into winter extracts, resulting in a preparation constituted of well-balanced substances. At the same time, «non-material information» is incorporated into the preparation through this special process, which enhances the efficacy of mistletoe extracts in their use as ISCADOR therapy.

According to Rudolf Steiner's spiritual-scientific research, the forces at work in the mistletoe growth process must be transformed through mixing into a new and different type of «composite process» to enhance the efficacy of the mistletoe extract (Steiner 1924).

To test this postulated increase in efficacy through the ISCADOR machine process, studies were undertaken on the cell viability of Molt4 and Yoshida tumour cells, and also on the development of crown gall tumours in *Kalanchoe daigremontiana*. These tests showed that the machine process had no relevant effect either on amounts of lectins or viscotoxins in the extracts, nor on their biological activity in the Molt4- and the Yoshida-cell culture test. By contrast, in the phyto-pathological tumour model with *Kalanchoe* a significant increase in the efficacy of mistletoe extracts mixed by the ISCADOR machine process was demonstrated. Similar results gained with other botanical control systems support the conclusion that processing mistletoe extracts with the ISCADOR manufacturing machine significantly enhances protective effects against external noxious influences and thus increases their effectiveness. This protective effect only extends to physically, chemically and biologically noxious effects, but not to the toxicity of lectins and viscotoxins. This phenomenon corresponds to results of other botanical test systems with external contaminants. Thus the resistance of mustard and wheat plants to external noxious substances such as UV radiation and colchicine, was significantly higher with ISCADOR manufactured using the mixing machine, compared with a Viscum control product not mixed by the machine (Flückiger and Baumgartner 2003, Baumgartner et al. 2005).

3.4 Manufacture of the end product

ISCADOR concentrate obtained from the mixing process (Fig. 20) is then processed into a finished medicinal product under GMP conditions. At first the mistletoe extract is diluted to the final ampoule concentration with isotonic saline solution (Fig. 21). Due to the heat sensitivity of constituents, such as mistletoe lectins and viscotoxins, heat sterilisation is not used. Instead, the solution is sterile filtered and subsequently filled in ampoules under aseptic conditions (Fig. 22). Finally, the ampoules are packaged (Fig. 23) and ready for medical use (Fig. 24).



Fig. 20: ISCADOR extract



Fig. 21: Diluting the ISCADOR concentrate



Fig. 22: Production of the injection ampoules



Fig. 23: Packaging of ISCADOR



Fig. 24: Finished medicinal product

3.5 Quality control

To ensure consistent quality, mistletoe extracts are subject to extensive tests and controls. Thus, for example, quantitative and qualitative analyses of mistletoe lectins and viscotoxins are carried out during the production process. And throughout the medicine's shelf life, stability tests document its specification-compliance.

4 Mistletoe constituents and their effects

Mistletoe extracts contain various constituents with multiple antitumoural effects (Kovacs et al. 2006, Maldacker 2006, Urech et al. 2006). The spectrum of biologically active substances ranges from glycoproteins, including mistletoe lectins I, II and III (ML I, ML II, ML III), polypeptides (e.g. viscotoxins), peptides, amino acids and amines, oligosaccharides and polysaccharides (arabinogalactanes), numerous enzymes, sulphurous compounds, fats, plant acids, phyto-sterols and sterols, flavonoids, phenylpropanes, lignans, minerals, trace elements, to various other proteins and in some extent triterpenes (Büssing 2000, Kienle and Kiene 2003, Saller et al. 2004, 2005, Urech et al. 2005, 2006, Urech and Baumgartner 2015, Nazaruk and Orlikowski 2016). Two chief mechanisms are considered in the anti-carcinogenic action of mistletoe extracts: firstly, an immunomodulation effect leading to the body's own immune system better recognising and combating tumour cells. Secondly, direct and indirect inhibitory effects on tumour growth (Saller et al. 2004, 2005). The composition of the different constituents varies depending on season, stage of development of the plant, habitat and host tree (Urech et al. 2009), and account is taken of these factors at harvesting and during the pharmaceutical processing of the medicinal product ISCADOR.

4.1 Mistletoe lectins

Currently, mistletoe lectins, and particularly mistletoe lectin I, are the most thoroughly investigated mistletoe constituents in terms of their structure, profile and mode of action. Mistletoe lectins are glycoproteins with a carbohydrate content of 4 – 12% depending on the host tree and time of harvest. Lectins agglutinate cells as well as specifically recognise and bind certain sugars. As monomers they have a molecular weight between 50 and 63 kDa, and ML I, especially, can also be combined to form a dimer or trimer. Mistletoe lectins consist of a toxic A-chain (29 kDa, 254 amino acids) with enzymatic properties and a carbohydrate-binding B-chain (34 kDa, 264 amino acids), which are connected by a disulphide bridge (Fig. 25). Based on the sugar specificity and its molecular weight, three various mistletoe lectins ML I, ML II and ML III can be differentiated into more than 20 different isoforms. ML I specifically binds to D-galactose, ML III to N-acetyl-galactosamine, and ML II to both.

While passing the membrane of a cell, a reduction of the disulphide bridge connecting A- and B-subunits occurs. Thus A- and B-chains dissociate. The free A-subunit of the mistletoe lectin acts

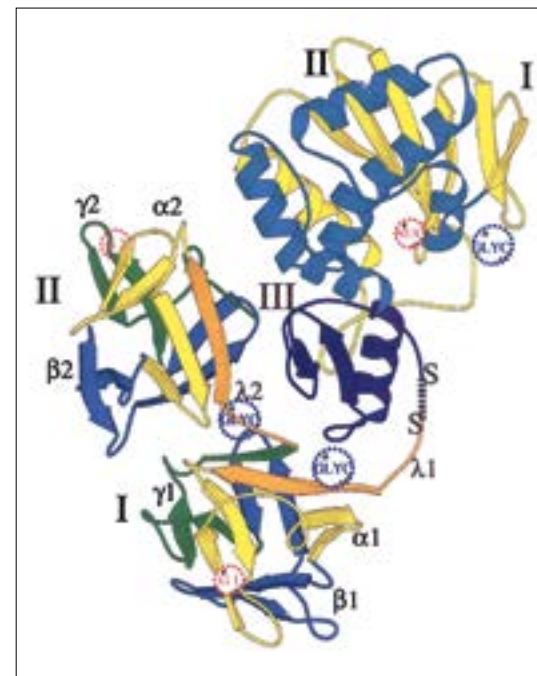


Fig. 25: Structure of ML I (Modified according to Krauspenhaar et al. 1999) The three domains of the A-chain (I, II and III) are illustrated in yellow, blue and dark blue. The homologous subdomains of the two domains (I and II) of the B-chain ($\alpha 1, 2$, $\beta 1, 2$ and $\gamma 1, 2$) are yellow, blue and green. The linking regions $\lambda 1, 2$ are orange, the disulphide bridge between the A- and B-chain is represented as a dark blue, dotted line. The red dotted circle represents the nucleotide-binding site of the A-chain (NUC) or the low and highly affine galactose-binding sites of the B-chain (G1 and G2). The glycosylation sites (GLYC) are marked as blue circles with a dotted line. Spiral: α -helix, arrow: β -sheet.

as a potent ribosome-inactivating protein in the cytosol so that protein biosynthesis is irreversibly inhibited and consequently apoptosis is induced. Mistletoe lectins I, II and III therefore belong to type 2 ribosome-inhibiting proteins (RIP) (Lee et al. 1994, Pfüller 2000, Franz 2003, Kienle and Kiene 2003, Fischer 2006, Sander 2008, Kreis 2009).

It also proved possible to isolate other lectins from the mistletoe plant, for example VisalbcBA (chitin-binding mistletoe lectin), which is completely different from other mistletoe lectins. VisalbcBA is a sugar-free dimer with two identical subunits and a much lower molecular weight of 10.8 kDa per subunit (Pfüller 2000, Franz 2003, Kreis 2009).

Mistletoe lectins only represent around 1% of mistletoe proteins. The lectin content is subject to a wide range of variations and is particularly dependent on host tree and season. Pine mistletoes show the lowest concentration of lectins and predominantly contain ML III and hardly any ML I. Oak, poplar and apple tree mistletoes are particularly rich in lectin, with ML I clearly predominating. The mistletoe plant contains significantly higher concentrations of lectin in winter than in summer, concentration increasing towards the centre of the mistletoe bush, especially in old stems and the sinker. Mistletoe lectins are very similar to the lectin from the castor-oil plant in their structure (Büssing and Schietzel 1999, Becker and Scher 2005, Urech et al. 2009).

Tab. 1: Properties of mistletoe lectins (according to Pfüller 2000)

Lectin	Molecular weight (Da)	Sugar specificity	Number of chains
ML I	115.000	D-galactose	4 (dimer)
ML II	63.900	D-galactose, N-acetyl-galactosamine	2
ML III	61.600	N-acetyl-galactosamine	2
VisalbcBA	21.600	N-acetyl-glucosamine and oligomers	2

Although mistletoe extracts contain lectins in very small concentrations, their pharmacological activity is an important aspect of the efficacy of mistletoe preparations. The antitumoural effects of mistletoe lectins have already been assessed and analysed through a multitude of preclinical experiments since the seventies of the last century. From the experiments it was evident that mistletoe lectins act, on the one hand, by direct antiproliferative effects on tumour cells, and on the other indirectly, by stimulating immunological processes. Direct cytotoxicity is primarily based on inhibition of protein synthesis and induction of programmed cell death, apoptosis (Fig. 27). Among other things, activation of the immune system is shown by an increase in the number and activity of natural killer cells (NK cells) and T helper cells, the release of β -endorphins, the reduction of tumour progression and the alleviation of side effects of conventional cancer therapies. An increased state of activity of the lymphatic cells, a significant increase in the cytokines IL-1, IL-6, IL-10, IL-12, IFN- γ and TNF- α in the serum, increased phagocytosis activity and an increase in respiratory burst have been reported in treatment with mistletoe lectins. Protection of cellular DNA against methylation processes has also been observed (Franz 2003, Kienle and Kiene 2003, Fischer 2006).

It is suspected that the cytotoxic effect of lectins is most important at the beginning of mistletoe therapy. Later their immunomodulatory effect is more significant, as the toxic effect decreases after around six weeks due to formation of antibodies against mistletoe lectins (Kienle and Kiene 2003).

4.2 Viscotoxins

Besides the lectins, viscotoxins belong to the pharmacologically important and typical mistletoe constituents. These are low-molecular, heat-resistant, strongly alkaline polypeptides, consisting of 46 amino acids. They have a molecular weight of around 5 kDa. Three disulphide bridges are responsible for the high stability of viscotoxins. Due to their high cysteine content they are categorised as thionines. Various isoforms are known: viscotoxin A1 (Fig. 26), A2, A3, B and 1-PS. In their chemical structure, viscotoxins resemble cardiotoxins from snakes, especially those contained in high concentrations in cobra poison (Ribereau-Gayon et al. 1986, Büssing 2000, Pfüller 2000, Debreczeni et al. 2003, Becker and Scher 2005, Fischer 2006, Pal 2008, Kreis 2009).

Similar to lectins, the viscotoxin content is dependent on the time of harvest and host tree. Thus, mistletoe plants of various host trees contain different amounts of viscotoxins, which are concentrated in the periphery of the plant, mainly in young leaves, stems and short, blossom-bearing shoots. The sinker does not contain viscotoxins. Their content is highest in June. The viscotoxins therefore behave in exactly the opposite way from mistletoe lectins (Urech et al. 2009).

The action of viscotoxins has not been investigated as thoroughly as that of lectins. It is known that, like lectins, they have immunogenic effects, and that they induce anti-viscotoxin antibodies after repeated exposure. A cytotoxic effect has also been demonstrated, due, in contrast to the

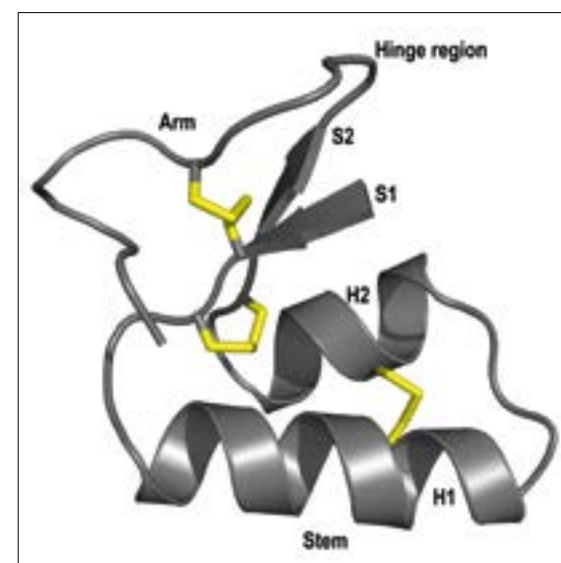


Fig. 26: Structure of viscotoxin A1 (Pal 2008) Each molecule of viscotoxin A1 consists of two helices (H1 and H2) and two antiparallel β -strands (S1 and S2) which are stabilised by three disulphide bridges.

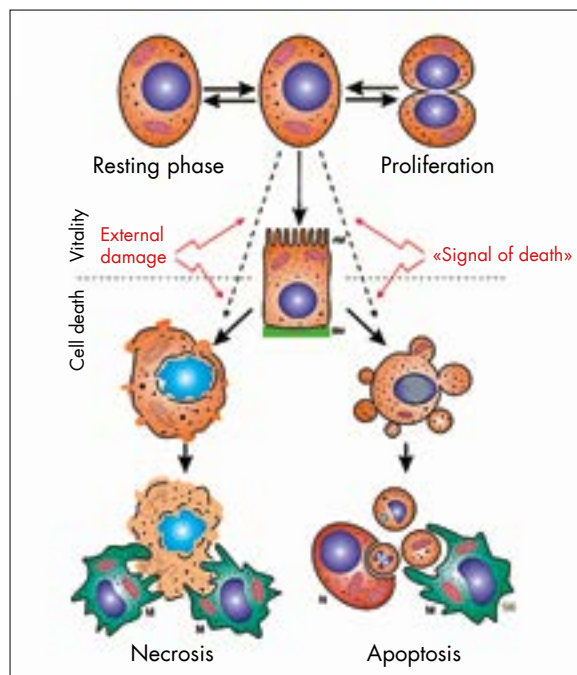


Fig. 27: Diagram of apoptosis and necrosis

lectins, to necrosis via rapid lysis of the cell membrane (Büssing 2000, Becker and Scher 2005, Fig. 27). Presumably their toxic effects are caused by the linking of thionines to membrane phospholipids and subsequent pore formation and cell-wall damage. The various viscotoxins have significantly different cytotoxicity. The viscotoxins A3 and 1-PS show the highest cytotoxicity, in contrast to viscotoxin B, which only has around 1/15 of the cytotoxic effect of viscotoxins A3 and 1-PS. Viscotoxins also increase the activity of the cytotoxic T cells and granulocytes (respiratory burst, phagocytosis) so that bacteria and presumably tumour cells can be more effectively destroyed. They inhibit RNA, DNA and protein synthesis, increase NK cell-mediated cytotoxicity against tumour cells, and cause a release of IL-6 (Büssing 2000, Kienle and Kiene 2003, Giudici et al. 2005, Fischer 2006).

4.3 Kuttan's peptides

Kuttan's peptides are low-molecular, heat-resistant peptides with a molecular weight of around 5 kDa, which have some of the properties of viscotoxins. The molecule shows cytotoxic and immunostimulatory effects. In animal experiments, an antitumoural action was observed from local application (Kuttan 1988).

4.4 Oligosaccharides, polysaccharides

The mistletoe plant also contains oligosaccharides and polysaccharides, whose content is likewise subject to seasonal fluctuations. Polysaccharides are high-molecular sugar polymers formed from monosaccharides, to which proteins can also be bound. Both poly- and oligosaccharides are substances with immunomodulating and antitumoural effects. In preclinical experiments, mistletoe polysaccharides protect against radiation damage, and the survival time of gamma-irradiated mice was significantly increased. It is presumed that the binding of polysaccharides to mistletoe lectins modulates their pharmacological effect, and that this may stabilise lectins in the mistletoe extract (Fischer 1996, Kienle and Kiene 2003, Becker and Scher 2005, Fischer 2006).

4.5 Flavonoids

Numerous different flavonoid derivatives can also be identified in mistletoe, in particular quercetin and quercetin methyl ester, which are predominantly present in glycosylated form. These induce apoptosis in various cell culture models and show scavenger properties (Fischer 1996, Kienle and Kiene 2003, Becker and Scher 2005, Fischer 2006).

4.6 Thiols

Mistletoe extracts also contain high concentrations of thiols, for example, glutathione, which exhibit good scavenger properties. Furthermore, the activity of poly-ADP-ribose-polymerase (PARP), the key enzyme in DNA repair processes, is significantly influenced by the thiol content. It is possible that the antioxidative potential of these substances contributes to mistletoe's cumulative efficacy (Kreis 2009).

4.7 Triterpenes

Although triterpenes were discovered very early as mistletoe constituents, it was only around the turn of the century that their antitumoural potential became a greater focus of attention (Urech 2003). Amongst seven identified triterpenes in the mistletoe plant, oleanolic acid is the main agent with concentrations of up to 3% of the dry weight. Oleanolic acid and additional pentacyclic triterpenes such as betulinic acid and ursolic acid isolated from the mistletoe plant have been shown to induce antiproliferative effects and apoptosis in tumour cells (Urech et al. 2005). However, aqueous mistletoe extracts contain only small amounts, or no detectable triterpenes.

Tab. 2: Mistletoe constituents and their effects (modified by Kienle and Kiene 2003)

Structural types	Substance classes	Effects on tumour cells	Effects on immune cells
Glycoproteins	Mistletoe lectins I, II, III	<ul style="list-style-type: none"> • Cytotoxicity due to inhibition of ribosomal protein synthesis • Induction of apoptosis 	<ul style="list-style-type: none"> • Release of TNF-α, IL-1, IL-2, IL-6 • Activation of NK cells • Increase in activity of phagocytosis
	VisalbCBA	<ul style="list-style-type: none"> • Low cytotoxicity 	<ul style="list-style-type: none"> • Stimulation of lymphocytes
Polypeptides	Viscotoxins A1–3, B, 1-PS	<ul style="list-style-type: none"> • Cytotoxicity due to lysis of cell membranes • Inhibition of RNA, DNA and protein synthesis 	<ul style="list-style-type: none"> • Activation of macrophages • Activation of granulocytes (respiratory burst, phagocytosis) • Release of cytokines (IL-6) • Increase of NK cell mediated toxicity against tumour cells
Peptides	Peptide 5 kDa (Kuttan et al.)	<ul style="list-style-type: none"> • Increase of cytotoxic activity • Tumour inhibition in animal experiments 	<ul style="list-style-type: none"> • Stimulation of macrophages • Activation of NK cells
Oligo- and polysaccharides	Arabinogalactans, galacturonans	<ul style="list-style-type: none"> • Tumour inhibition in animal experiments • Protection against radiation damage 	<ul style="list-style-type: none"> • Stimulation of T helper cells (TH-1 \uparrow, INFγ \uparrow, IL-6, TNF-α) and phagocytes • Increase of NK cell activity • Release of interferone-γ
Flavonoide	Quercetin derivatives	<ul style="list-style-type: none"> • Induction of apoptosis • Tumour inhibition in animal experiments 	<ul style="list-style-type: none"> • Antioxidative effects • Protective effects
Thiols	Glutathion	–	<ul style="list-style-type: none"> • Antioxidative effects
Triterpenes	Oleanolic acid, betulinic acid, ursolic, lupeol, β -amyrinacetate, lupeolacetate	<ul style="list-style-type: none"> • Induction of apoptosis • Tumour inhibition in animal experiments 	<ul style="list-style-type: none"> • Anti- and pro-inflammatory effects • Activation of macrophages and T-helper cells

In mistletoe extracts such as ISCADOR, numerous different components with diverse action profiles can be identified. The complex and system-wide mode of action of an extract with agonistic or antagonistic and synergistic or co-stimulatory effects therefore, cannot be reduced to single constituents. Only the total extract can display its full effect.

5 Immunological properties of mistletoe extracts

The action of mistletoe extracts on immunocompetent cells of both the non-specific and specific immune system such as natural killer cells (NK cells), monocytes, macrophages, antigen-presenting cells, T lymphocytes (T cells) including T helper cells, neutrophil and eosinophil granulocytes and a number of cytokines, has now been clearly proven. At times, mistletoe lectin I (ML I) was thought responsible for the various immunological reactions. Nowadays it is known that other lectins such as ML II, ML III, the chitin-binding ML and other components such as viscotoxins, oligosaccharides and polysaccharides also have an immunomodulating effect (Kienle and Kiene 2003, Saller et al. 2004, 2005, Klein 2005, 2009).

5.1 Principles of immunological tumour defence

It is widely believed that tumours develop when the homeostasis between proliferative and antiproliferative or apoptosis-inducing physiological factors is disturbed at the cellular level. As a result of this, and additional factors, tumour cells can escape immune surveillance. The selection of the tumour cells leads to their low antigenicity, to antigen masking or even to antigen loss. The immune system cannot recognise them anymore. In addition, tumour-induced secondary immunosuppression may occur through cytokines such as tumour growth factor β (TGF- β), interleukin 10 (IL-10), soluble immune complexes or receptors such as the interleukin 2 receptor (IL-2R), soluble adhesion molecules, prostaglandins or acidic proteins. This aspect of immunological escape phenomena is firstly associated with impaired genetic growth control (oncogenes, tumour suppressor genes and apoptosis genes) and secondly with inadequate immunological growth control at a humoral, cellular or cytokine level (Berg and Stein 2001, Schleyerbach 2004).

Cytotoxic T cells, NK cells, macrophages and eosinophil granulocytes play a role in tumour defence and in the complex interaction of the specific and non-specific immune systems. The immune reaction depends on the density and type of the presented antigens, the mutual influence of the cytokine milieu and T helper cell population, and co-stimulatory signals (Berg and Stein 2001, Schleyerbach 2004).

Since the injection of mistletoe extracts may provoke a reaction by the immune system, the general principles of immune defence will now be explained in more detail.

5.1.1 Non-specific (natural) immune system

The term non-specific immune system covers innate defence measures, which are mostly activated independently of a specific antigen. Since no individual cell clones are required for them to manifest, they are also called non-clonal defence mechanisms. This includes the intact skin, the mucous membranes, the protective acid mantle of the skin, substances similar to pectin, the complement system, the anti-microbial enzyme system and non-specific mediators such as interferon or interleukin (Unger and Hildenbrand 2004).

At cellular level, phagocytic blood cells can be found. These are granulocytes, monocytes, and macrophages, which also represent a significant component of the effector arm of the specific immune system. Inflammatory reaction is therefore of particular importance in defending against pathogens. It is characterised by a high amount of defence strength at the focus of inflammation, and by a complex interaction of soluble and cellular components.

Subsequently, mediators are released which widen the blood vessels and assure a higher permeability of the capillary walls. The granulocytes and macrophages then migrate to the focus and form the first line of defence, in which a large part of the pathogens are destroyed (Unger and Hildenbrand 2004, Klein 2005).

5.1.2 Specific (adaptive) immune system

The specific immune system, which was previously called the acquired immune system, develops in addition and as complement to the innate immune system, and features the following characteristics (according to Unger and Hildenbrand 2004):

- It specifically reacts with proteins, glycoproteins, lipids and carbohydrates functioning as antigens. The lymphocytes have suitable surface structures (receptors) to specifically detect these antigens.
- A distinction is made between humoral and cellular immune defence, whereby the antibodies producing B lymphocytes represent the humoral immune defence and the antigen-specific T lymphocytes represent cellular immune defence.
- The antibodies of the B lymphocytes and the antigen receptors of the T lymphocytes are able to detect different alien structures.
- The specific immune system is subject to various control mechanisms so that B and T lymphocytes only react after specific activation, and self-activated B and T lymphocytes are either eliminated in the maturation phase (central tolerance) or inactivated later on (peripheral tolerance).

- Antigen-presenting cells (APC), B lymphocytes, T lymphocytes and NK cells are particularly important in the activation of the antigen-specific effector mechanism, since they also communicate with each other by direct cell contact and with the help of adhesion molecules or using messenger substances (cytokines), which form a complex information system.

5.1.3 Interactions between the non-specific and specific immune system

The non-specific and specific immune systems are closely related. On the first contact with antigens, the cells of the non-specific immune systems such as NK cells, eosinophils, mast cells or antigen-presenting cells (APC) are activated as an initial step. They then activate further cell populations of the non-specific and finally the specific immune system (Berg and Stein 2001, Klein 2005).

The invasive agent or the infected cell is killed and eliminated by activated cells from the non-specific immune system and by the release of various factors. If the antigens are not fully eliminated in this phase, the specific immune response is activated via the APC, for example dendritic cells (DC), monocytes, B cells and endothelial cells. Antigens are processed in the APC, therefore crushed, and the resulting peptide is then expressed on the surface together with the major histocompatibility complex (MHC) of classes II (exogenous antigens) and I (endogenous antigens). Native T cells are stimulated with a suitable T cell receptor using this MHC peptide complex for proliferation and differentiation. The expression of MHC I peptide complexes leads to the activation of CD8⁺ cytotoxic T cells and the expression of MHC II peptide complexes to the stimulation of CD4⁺ T helper cells (Fig. 28). It is important for the activation of the T cells that the APCs – in addition to the MHC peptide complex – also express co-stimulating molecules such as for example CD80 or CD86, without allowing the T cell response to be initiated (Unger and Hildenbrand 2004, Klein 2005).

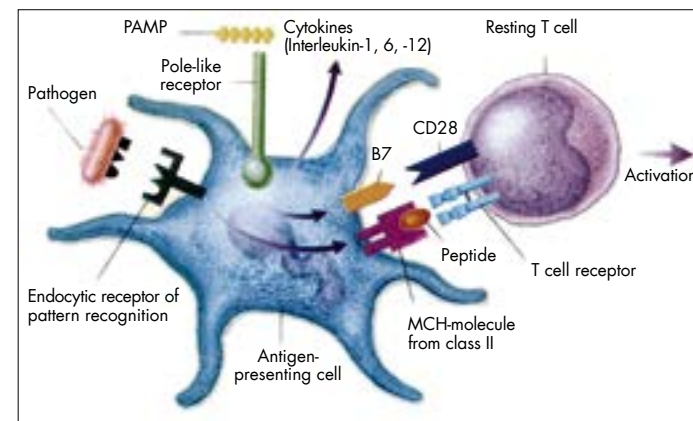


Fig. 28: Antigen uptake, activation by PAMP (pathogen-associated molecular pattern) and antigen-presentation through class II MHC molecules using antigen-presenting cells (according to Unger and Hildenbrand 2004)

5.2 Effects of mistletoe extracts on cells of the non-specific immune system

ISCADOR may activate cells from the innate immune system such as NK cells, macrophages, granulocytes and eosinophils. This has been well documented in numerous *in vitro* and *in vivo* studies. An increase in inflammatory cytokines, such as tumour necrosis factor α (TNF- α), IL-1 or IL-6 was observed under exposure to mistletoe extracts *in vitro* and in tumour patients and healthy subjects. This applied to lectin-rich as well as lectin-poor or viscotoxin-rich extracts (Heinzerling et al. 2006, Huber et al. 2006, Hajt6 et al. 2009, Klein 2009). In a randomised, double blind, placebo-controlled study on 47 healthy subjects, they received ISCADOR Qu special (ML-rich), ISCADOR P (ML-low) or a placebo (physiological saline solution) for 12 weeks. It was shown that the production of granulocyte/macrophage-colony-stimulating factor (GM-CSF) was significantly increased through the mononuclear cells in the peripheral blood (PBMC) by the administration of ML-rich extracts (Huber et al. 2006). GM-CSF is responsible for the maturation and recruitment of precursor cells of granulocytes and monocytes from the bone marrow, and is an important factor in the release of eosinophils from the bone marrow (Wong et al. 2002). The increased release of GM-CSF could therefore explain the activation of cells from the non-specific immune system and the increase in the leukocytes observed in therapy – particularly neutrophils and eosinophils (Klein 2005, 2009). There is also evidence that administration of mistletoe extracts counteracts post-surgical suppression of granulocytes and NK cells (Schink et al. 2007, 2009).

5.2.1 Influence of mistletoe extracts on monocytes and macrophages

Many constituents of mistletoe can directly and indirectly activate monocytes and macrophages (isolated mistletoe lectin, Kuttan's peptides, oligosaccharides, polysaccharides or total extracts). Antigen-presenting functions are stimulated, co-stimulating molecules are expressed, and phagocytosis activity and cytotoxicity are increased. Finally, cytokines are induced and the antitumoural efficacy of the macrophages/monocytes is improved (Kienle and Kiene 2003).

Numerous investigations showed that mistletoe lectin prefers to bind to monocytes and macrophages. The binding increases the intracellular calcium and stimulates the synthesis and release of cytokines (TNF- α , IL-1 α , IL-1 β , IL-6) (Elsässer-Beile et al. 2000). Mistletoe lectin caused a dose-related increase in phagocyte capacity on macrophages (Fischer 2006).

The antitumoural effects of mistletoe extracts were often identified in connection with an increase in monocytes and macrophages. The administration of mistletoe-activated macrophages alone showed an antitumoural effect in animal experiments (Kienle and Kiene 2003).

Additionally, the survival time of mice suffering from tumours was doubled by transferring *in vivo*-activated macrophages from healthy mice to animals with tumours. This effect could not be achieved with non-activated macrophages (Fischer 2006). It is also presumed that mistletoe extracts inhibit neoangiogenesis by release of TNF- α from macrophages. (Kienle and Kiene 2003).

5.2.2 Influence of mistletoe extracts on neutrophil granulocytes

Mistletoe extracts can directly activate granulocytes *in vitro*. Viscotoxin has shown itself to be particularly effective here: it significantly increased phagocytosis and oxidative burst of human granulocytes. All available mistletoe extracts, all known viscotoxins and also viscotoxin-free mistletoe extracts are able to do this, but not isolated mistletoe lectins. If granulocytes were incubated with mistletoe extracts and viscotoxins, there was an additional strengthening of granulocyte activation. Thus two different routes are possible for activating granulocytes: firstly, granulocytes seem to be activated by the mistletoe extract independently of viscotoxins or mistletoe lectin. Secondly, the total mistletoe extract and the viscotoxins appear involved in the activation route (Stein et al. 1999, 2001, Kienle and Kiene 2003, Büssing et al. 2005, Fischer 2006).

5.2.3 Influence of mistletoe extracts on natural killer cells

The immunomodulating effect of mistletoe constituents (polysaccharides, lectins, Kuttan's peptides, viscotoxins or total extracts) is also characterised by an increase in the number and activity of NK cells. NK cells can kill their target cells, such as tumour cells, virus-infected cells or microorganisms, by releasing cytotoxic substances and inducing apoptosis. Mistletoe extracts increase the cell formation rate in bone marrow and the cytotoxicity of NK cells. Mistletoe-activated NK cells also showed an antitumoural effect in animal experiments (Kienle and Kiene 2003, Fischer 2006, Elluru et al. 2007, Braedel-Ruoff 2010). An experiment with mice injected with specific melanoma cells demonstrated significant decrease in metastasis when, four hours later, they received injections of *in vivo*-activated splenic cells from healthy mice treated with mistletoe. Their survival time was also significantly increased. In contrast, administration of normal, non mistletoe-activated splenic cells did not have the same effect. It can be concluded from this that NK cells are responsible for this effect (Antony et al. 1999, 2000).

Some constituents of the mistletoe extract also have an influence on the NK cells *in vitro*. Thus the polysaccharide rhamnogalacturonan, isolated from the mistletoe plant, led, via formation of a bridge between the effector cells and tumour cells, to an increase in cytotoxicity of NK cells. Oligosaccharides also indirectly increased the cytotoxicity of CD56⁺ NK cells by releasing lymphokines (Fischer 2006, Braedel-Ruoff 2010). Isolated viscotoxins likewise led to significant

increases in the cytotoxic activity of NK cells on various tumour cell lines, a mechanism it has not yet been possible to explain (Tabiasco et al. 2002). In contrast to other mistletoe constituents, isolated mistletoe lectins exert a cytotoxic action predominantly on individual lymphocyte sub-populations. After the application of 1 ng/ml mistletoe lectin, a selective decline of the portion of NK cells in the lymphocyte subsets was detected after 72 hours. However, there were no significant changes within the T cell subset. The ratio of T and B cells also remains the same. It was possible to fully suppress the preferential killing of NK cells with anti-mistletoe lectin serum. Among the lymphoid cells, the NK cells therefore seem to be a population particularly sensitive to mistletoe lectin (Schink 2001).

5.3 Effects of mistletoe extracts on cells of the specific immune system

As early on as the 1980s, it was shown that subjects who received a subcutaneous injection of a mistletoe extract formed antibodies to mistletoe lectin I (ML I) (Stettin et al. 1990). It was also shown that therapy with mistletoe extracts leads to the specific proliferation of lymphocytes, and thus the humoral and cellular immune system are activated. This activation of a specific immune response was initially attributed chiefly to ML I. It is now known, however, that lymphocyte proliferation and the formation of antibodies can be stimulated by ML II, ML III or viscotoxins (Stein et al. 1997, Klein et al. 2002, Klein 2005). These antibodies occur in tumour patients receiving mistletoe therapy and also in healthy subjects treated with mistletoe extracts over a longer period. The antibodies are predominantly of an IgG isotype (IgG1 and IgG3). In rare cases the anti-ML I and anti-ML III antibodies may also be of the IgE type (Stein et al. 1997, Klein 2005, Huber et al. 2006, Klein 2009).

An additional chitin-binding mistletoe lectin (cbML or VisalbcBA) has also been identified in the last few years. In tumour patients and healthy subjects it induces antibodies belonging only to the IgG class. This lectin (cbML) increases the proliferation of lymphocytes *in vitro*. Interestingly, antibodies to cbML were also identified in people who had never had contact with mistletoe extracts. Maybe a «natural immunity» to cbML can be postulated (Klein et al. 2004, Klein 2005, 2009). This is in contrast to antibody formation in response to ML I, ML III or viscotoxins, where a previous contact with mistletoe extracts is essential.

5.4 Effects on tumour development

5.4.1 Influence of mistletoe extracts on tumour cell proliferation

Although a proliferation-inducing potential of many plant lectins on lymphoid cells has been discussed, this has not yet been identified for mistletoe lectins. On the contrary, there are numerous studies on different tumour cell lines, which confirm that mistletoe preparations do not have any proliferative effects on tumour cells. Büssing et al. (2005) examined the metabolic activity of cells with different concentrations of isolated ML I and total mistletoe extract in 14 tumour cell lines. They did not identify any proliferative effects from ML I or the total mistletoe extract on the examined cell lines. Additionally, Kelter and Fiebig (2006) examined the three ISCADOR preparations ISCADOR M special, ISCADOR Qu special and ISCADOR P for their growth-stimulating properties in 26 human cell lines, in a low dose range. The results clearly showed that there was no growth stimulation from the three tested mistletoe extracts in the cell lines.

5.4.2 Influence of mistletoe extracts on apoptosis

Mistletoe extracts and ML I induce apoptosis in tumour cells, leukaemia cells, lymphocytes, monocytes and granulocytes. The total extract and ML I significantly suppress the proliferation of cells, depending on the dose (Kienle and Kiene 2003, Elluru et al. 2006, 2007). The details of the cellular mechanisms induced by the mistletoe constituents leading to apoptosis have been investigated, but no final explanation has been found. It is known that mistletoe lectin plays a significant role in apoptosis induction. Even low concentrations trigger the cells' «suicide program» (Büssing 2001, Kienle and Kiene 2003, Fischer 2006). Studies show that apoptosis can also be induced, independently of conventional death receptors, by mitochondrial, receptor-independent signal paths (cytochrome c/Apaf-1). This involves the caspase cascade being activated as a significant component of the signal path. An interaction of the mistletoe lectin with DNA is discussed as a cause of apoptosis induction (Barbieri et al. 1997, Elluru et al. 2006, 2007). Mistletoe lectins can also indirectly induce apoptosis by increasing the expression of fas ligands in lymphocytes, which then become able to initiate programmed cell death in Fas⁺ target cells (Büssing 2001).

5.4.3 Cytokine induction

There are numerous studies on cytokine induction by individual mistletoe lectins and by isolated A and B chains. It was shown that incubating mononuclear cells (PBMC) with ML I or isolated B-chains leads to the release of TNF- α , IL-6, IL-1 β , but not IL-1 α . These results suggest substantial involvement of monocytes, since ML I favours binding to these cells (Fischer 2006, Elluru et al. 2008). There are numerous studies on cytokine induction by total mistletoe extract. A study with ISCADOR P in various concentrations on the *in vitro* reactivity of mononuclear cells showed that release of IL-6 and TNF- α is induced, sometimes to an intense degree. IL-2, IL-4,

IL-5 and IFN- γ are induced much less frequently (Stein and Berg 1997). In a study by van Huyen et al. (2006), the tumour inhibition due to ISCADOR was related to the release of IL-12, this mechanism being influenced by the increase in T cell and NK cell activities.

Mistletoe-induced cytokine production *in vivo* has mostly been investigated in tumour patients. The studies showed highly individual variability and differences in the induced cytokine patterns between individual lectins and depending on the composition of the different mistletoe preparations (host trees). Despite this high variability, almost all studies showed a significant increase in the release of IL-6 and TNF- α . However, mistletoe therapy did not always lead to an increase in cytokine expression or release. This suggests that mistletoe therapy can have a regulating effect on the cytokine network, which is part of the immune system (Fischer 2006). This was confirmed in preclinical studies on B-cell lines: it was shown that, using ISCADOR P, an autocrine loop can be fully blocked in relation to IL-6 production and stimulation of B cell lines with IL-6 (Kuehn 2009).

6 Clinical efficacy of ISCADOR

There are 139 clinical studies on the use of anthroposophic mistletoe preparations for treating different tumour entities. In terms of methodology, they comprise 33 prospective randomised clinical studies, 18 non-randomised prospective comparative studies, 44 retrospective comparative studies and 44 larger cohort studies and smaller case series (Kienle 2014). Of the 139 studies on mistletoe therapy, 87 were performed with ISCADOR. The ISCADOR studies comprise 23 prospective randomised clinical studies, 17 non-randomised prospective comparative studies, 32 retrospective comparative studies and 15 larger cohort studies and smaller case series. ISCADOR is therefore the best and most comprehensively investigated medicinal product in complementary cancer therapy.

Of the 139 studies, 136 showed a benefit of mistletoe therapy. Mistletoe extracts such as ISCADOR increase the efficacy of conventional tumour therapies, reduce side effects and stimulate defence mechanisms against cancer cells, as well as triggering non-specific immune responses. They can inhibit the growth of malignant cells and prolong the survival time of patients. They show significant results in terms of limiting and stopping tumour growth, and occasionally in tumour regression; they reduce rates of recurrence and metastasis, and improve quality of life. Temperature increase may also occur as the desired expression of metabolic process activation.

Furthermore, patients report an improvement in their general condition: they regain their appetite, feel more energy, and often gain some body weight (Büssing 2000, Kienle and Kiene 2003, Kienle et al. 2003, Saller et al. 2004, 2005, Kienle 2009, 2014, Ostermann et al. 2009, Tröger et al. 2013, 2014). Detailed information can be found at <http://studien.vfk.ch> in «Documentation of published clinical studies with ISCADOR».

6.1 Study designs

The efficacy of a drug for a specific indication is considered to be proven when more patients can expect recovery or improvement of their disorders than without the drug. This must be demonstrated with reasonable reliability (Decision of the German Federal Administrative Court (BVerwG 3.). Senate, Reference: 3 C 21/91). The standard method for such proof is the randomised controlled clinical trial (RCT).

In 2000, a comparison of numerous epidemiological and randomised clinical studies showed that carefully planned and evaluated epidemiological studies yielded results broadly comparable to those of randomised clinical studies (Kjell Benson and Hartz 2000, Concato et al 2000). In fact, the results of the epidemiological cohort studies deviated markedly less from each other, thus were in more accord, than the results of the randomised studies, strongly supporting their evidential value. An additional strength of epidemiological studies is that they much better reflect patients' actual treatment reality, and therefore have greater external validity, or in other words more general application.

6.1.1 Randomised controlled trials (RCT)

Randomised controlled trials (RCT) are studies in which patients are allocated randomly to either a treatment or a control group. Neither physician nor patient have any influence on group selection. With a sufficient number of patients, randomisation guarantees comparability of these two groups in relation to known and unknown risk factors (Kabisch et al. 2011).

Double blinding, in addition to randomisation, is used to demonstrate the efficacy of a drug in a group of patients by minimising the influence and the expectations of patients or physicians. In a double-blinded, often placebo-controlled study, neither the investigator nor the patient knows whether the latter has been assigned to the treatment or control group (Kabisch et al. 2011).

It is believed that double-blinded, randomised controlled studies show the pure efficacy of a drug. Such studies therefore tend to underestimate the efficacy of the drug in question compared to clinical practice situations. Such trials are also often far removed from daily clinical practice since patients are carefully selected, and all concomitant therapies have to be excluded. Randomised controlled studies have further ethical disadvantages, since the doctor-patient relationship has to be eliminated. The individual situation of the patient cannot be taken into account and those receiving a placebo are deprived of a potentially better therapy (Willich 2006).

Last but not least, medical and technical problems often impede the implementation of double-blind studies. During therapy with ISCADOR, typical reactions may include local red-dening, raised temperature, and subjective feelings of wellbeing (IsCADOR AG 2015) which cannot be imitated by a placebo, so that the patient is then often «unblinded».

6.1.2 Controlled, pharmaco-epidemiological, retrospective cohort studies

For drugs which have been on the market for longer, the results of pharmaco-epidemiological studies can, in accordance with the EU Directive 2001/83/EC of the European Union Commission, be used as valid proof of efficacy and safety. According to the «Levels of Evidence» of the Oxford Centre for Evidence-Based Medicine, controlled pharmaco-epidemiological cohort studies correspond to the second-highest level of evidence (Level II). The medical records of patients treated for the indicated diseases or complaints within a specific period are selected from representative institutions (practices, clinics). All relevant data is taken from the files – including the baseline condition, the treatments used, and the findings and results observed from this – and transferred to a standardised case report form (CRF). The selection of institutions and patient records, the scope and type of findings to be collected, and the measures to check the completeness and accuracy of data (monitoring, audit) is established in a detailed study protocol before the study begins. To allow comparison of the different therapies, patients who are treated with the tested product and those who are not – the control group – are recorded as parallel groups. This means that the control group is not a historic cohort.

Since controlled pharmaco-epidemiological retrospective cohort studies cannot be randomised, it must be assumed that specific factors relating to both physician and patient could influence the result of treatment. The most important task of the evaluation, therefore, is to determine the influence of parameters distinct from that of the tested product, and to compensate for them. For this purpose, there exist proven statistical procedures, which can mathematically calculate the degree to which the treatment result depends on various influencing parameters, and modify the result accordingly. Epidemiological cohort studies thus also feature a largely undistorted comparison of therapies, and can be used to evaluate the evidence for a drug's efficacy.

6.2 Clinical effects of ISCADOR in detail

- **Improvement of quality of life** (2, 4, 5, 6, 13, 16, 17, 20, 23)
 - increase in appetite and weight gain
 - normalisation of sleep, temperature sensitivity and performance
 - improvement in mood and initiative
- **Reduction of adverse drug reactions of conventional therapies such as chemotherapy and radiotherapy** (2, 5, 13, 16, 17, 20, 21)
- **Reduction of symptoms related to the disease or therapy** (2, 5, 15, 16, 17, 20, 21), such as:
 - nausea
 - vomiting
 - diarrhoea
 - immunodepression
- **Alleviation of cancer-related fatigue syndrome** (2, 5, 17, 20, 23, 25)
- **Alleviation of cancer-related pain** (20, 23, 24)
- **Reduction of hospitalisation period** (5, 16, 17)
- **Improvement of immunological responsiveness** (3, 10, 11, 12, 14, 19, 24)
 - increase of the body's own defences
 - reduction of susceptibility to infection
- **Prevention of recurrence and metastases** (1, 5, 7, 8, 9)
- **Prolongation of survival** (1, 2, 5, 6, 7, 16, 17, 18, 22, 26)

- | | |
|---------------------------------------|--|
| 1) Augustin et al. 2005 | 14) Kuehn und Fornalski 1997 |
| 2) Bock et al 2004 | 15) Loewe-Mesch et al. 2008 |
| 3) Büssing et al. 2005 | 16) Matthes et al. 2009 |
| 4) Carlsson et al. 2006 | 17) Matthes et al. 2010 |
| 5) Friedel et al. 2009 | 18) Ostermann et al. 2009 |
| 6) Grossarth-Maticek et al. 2001 | 19) Schink et al. 2007 |
| 7) Grossarth-Maticek und Ziegler 2006 | 20) Tröger et al. 2009 |
| 8) Grossarth-Maticek und Ziegler 2007 | 21) Tröger et al. 2012 |
| 9) Grossarth-Maticek und Ziegler 2008 | 22) Tröger et al. 2013 |
| 10) Huber et al. 2001 | 23) Tröger et al. 2014 |
| 11) Huber et al. 2005 | 24) Wagner 2007 |
| 12) Huber et al. 2006 | 25) Wode et al. 2009 |
| 13) Kienle und Kiene 2010 | 26) Ziegler und Grossarth-Maticek 2008 |

6.3 ISCADOR in the treatment of different tumour entities

6.3.1 Breast cancer

Breast cancer is by far the most common cancer in women in Germany, with around 70,000 new diagnoses per year. The mortality rate is around 18,000. Breast cancer occurs significantly earlier than most other types of cancer. Thirty percent of affected women are younger than 55 at first diagnosis. Despite rising numbers, nowadays fewer women die from breast cancer than 20 years ago (Katalinic et al. 2015). Treatment usually requires a multimodal therapy comprising surgery, radiotherapy, chemotherapy and/or antihormone therapy. Due to the common side effects of these therapies, chemotherapy in particular may have a severe impact on quality of life. As a result, mistletoe therapy is becoming increasingly important. Doctors report that patients receiving mistletoe therapy in addition to chemotherapy have a better quality of life and less neutropenia. Mistletoe preparations have immunomodulating properties, and a protective effect on healthy cells during chemotherapy can also be assumed.

Efficacy and safety of ISCADOR in patients with primary, non-metastasised breast cancer (Bock et al. 2004)

Patients and methods: A multicentre, controlled, pharmaco-epidemiological, retrospective cohort study was performed in accordance with GEP guidelines with 1,431 patients in a parallel group design from randomly selected centres in Germany and Switzerland. Patients with primary, non-metastasised breast cancer of UICC stages I to III were included. The study investigated the therapeutic efficacy and safety of long-term complementary therapy with ISCADOR (in most cases ISCADOR M) in addition to conventional adjuvant oncological therapies. The patients in the control group were exclusively treated with the conventional therapy.

Study objectives: The frequency of adverse drug reactions (ADR) of the conventional therapy was assessed as a primary outcome parameter of efficacy. The secondary outcome parameters were the assessment of the disease-related symptoms and the overall survival time (OS). The safety of the therapy was evaluated according to the frequency and degree of severity of the ADRs.

Results: Baseline analysis showed a disadvantage for the ISCADOR group. ISCADOR patients were in a more advanced stage and had more unfavourable prognostic factors. Nevertheless, after median duration of ISCADOR therapy of 52 months, patients in the ISCADOR group had fewer side effects compared to patients in the control group receiving conventional therapy only (16.3% vs 54.1%; adjusted Odds Ratio 95% confidence interval): OR = 0.47 (0.32–0.67), $p < 0.0001$, (Fig. 29).

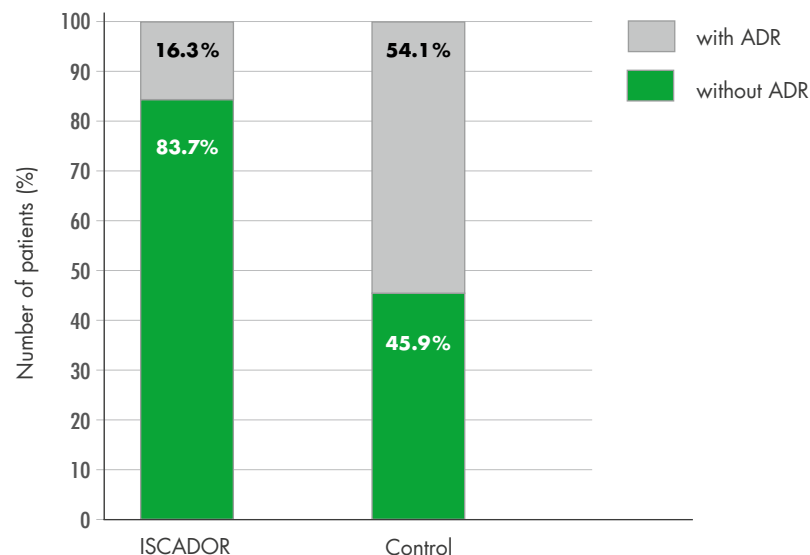


Fig. 29: Proportion of breast cancer patients with adverse drug reactions (ADR) after conventional treatment, $p < 0.0001$ (only patients who received chemo- or radiotherapy were evaluated)

In the ISCADOR group, most disease-related symptoms such as nausea, vomiting, appetite loss, headaches, tiredness/exhaustion improved with significantly greater frequency than in the control group. The Karnofsky index improved more frequently, body weight increased more significantly and the adjusted relative hazard rate for mortality (adjusted hazard ratio, HR) was significantly lower (Cox regression (95% confidence interval): HR = 0.46 (0.23–0.96), $p = 0.038$), which represents a reduction in the relative mortality risk of 54% (Fig. 30).

ISCADOR-related systemic side effects such as weakness, tiredness/exhaustion or gastrointestinal complaints developed in only 6 of 631 patients (0.8%). Small local inflammatory skin reactions at injection sites, such as erythema, induration, oedemas, and itching, developed in 123 patients (17.3%). All of these side effects were slight to moderate (WHO/CTC grade 1–2). No severe side effects were observed.

Conclusions: The results of this cohort study confirm that complementary long-term treatment with ISCADOR in patients with primary, non-metastasising breast cancer is generally well tolerated and can be considered safe. In comparison to a parallel control group, significantly fewer side effects of conventional therapy, fewer disease-related symptoms and a longer survival time were observed in the ISCADOR group.

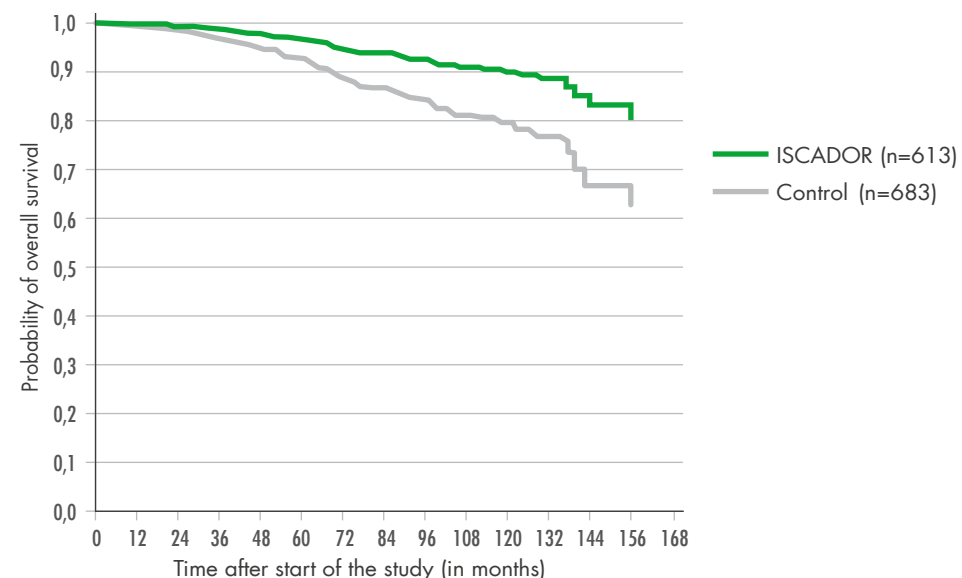


Fig. 30: Overall survival (OS) for breast cancer, Cox proportional hazard regression, HR = 0.46 (0.22–0.96), $p = 0.038$

Adjuvant mistletoe treatment and chemotherapy in patients with breast cancer (Loewe-Mesch et al. 2008)

Patients and methods: In a prospective, open, two-arm, non-randomised study, 33 patients with primary breast cancer received ISCADOR M during adjuvant chemotherapy (CMF or EC). The control group ($n = 33$) did not receive mistletoe therapy.

Study objectives: The influence of adjuvant, simultaneous mistletoe treatment/chemotherapy before, and 14 days after the mistletoe treatment/chemotherapy on blood count, differential blood count, lymphocyte subpopulations, lymphocyte activity, quality of life (EORTC QLQ-C30, BR23) and the therapeutic tolerability of chemotherapy were investigated.

Results: The patients in the ISCADOR group took fewer oral glucocorticoids ($p=0.006$) and reported a better quality of life than the patients in the control group. The counts of the lymphocyte subpopulations of these patients did not decrease so rapidly. However, there was no difference between the groups in their chemotherapy-related immunosuppression. Finally, nausea and vomiting (EORTC QLQ-C30, $p = 0.02$) and other chemotherapy-related side effects (EORTC QLQ-BR23, $p = 0.02$) decreased significantly in the ISCADOR group. Good therapeutic tolerability of the simultaneous mistletoe treatment/chemotherapy was observed. There were no side effects of the mistletoe therapy.

Conclusions: The quality of life of the patients in the ISCADOR group was less impaired than in the control group. However, the laboratory parameters did not illustrate advantages or disadvantages of ISCADOR therapy under the study conditions.

ISCADOR and chemotherapy in patients with breast cancer (Tröger et al. 2009)

Patients and methods: This randomised clinical study examined whether therapy with ISCADOR M in addition to chemotherapy improves quality of life and reduces neutropenia. The additional therapy with ISCADOR was restricted to the duration of the chemotherapy. A five-year follow-up should show whether the additional ISCADOR therapy has had an impact on the therapeutic effects of chemotherapy, antihormone treatment or radiotherapy. 95 patients with breast cancer of up to stage $T_{1-3}N_{0-2}M_0$ receiving adjuvant chemotherapy (Cyclophosphamide, Adriamycin, 5-FU = CAF) were randomly assigned to one of three groups. One group received ISCADOR, a second group received another mistletoe preparation and the third group received no treatment other than chemotherapy. Therapy with ISCADOR ceased with the end of the chemotherapy. Quality of life, including cancer-related fatigue, was assessed by the EORTC QLQ-C30 questionnaire (European Organisation for Research and Treatment of Cancer). Quality of life and the neutrophil granulocytes were determined at the start of the study and one day before the next chemotherapy cycle.

Results: The ISCADOR and control group did not differ at baseline in terms of age, tumour stage, body mass index, physical well-being, vital signs and previous diseases. In the basic examination (1st visit), no neutropenia was found in any of the patients. After evaluation of the EORTC QLQ-C30 throughout the visits, a better quality of life was detected in the ISCADOR group, compared to the control group. Twelve of 15 scores showed a significant difference ($p < 0.02$), and nine of the scores were also clinically relevant (Fig. 31 and 32 show a selection of the scores).

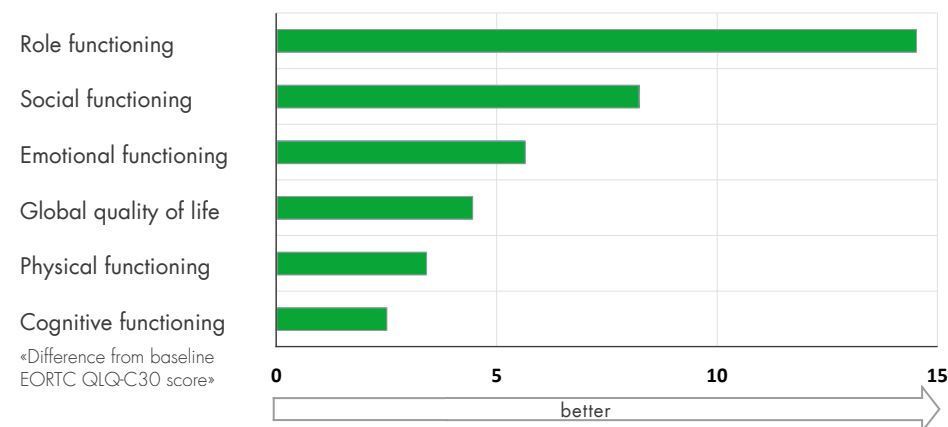


Fig. 31: Quality of life (mean changes in **functions** in accordance with EORTC QLQ-C30). A difference of more than 5 points is clinically relevant.

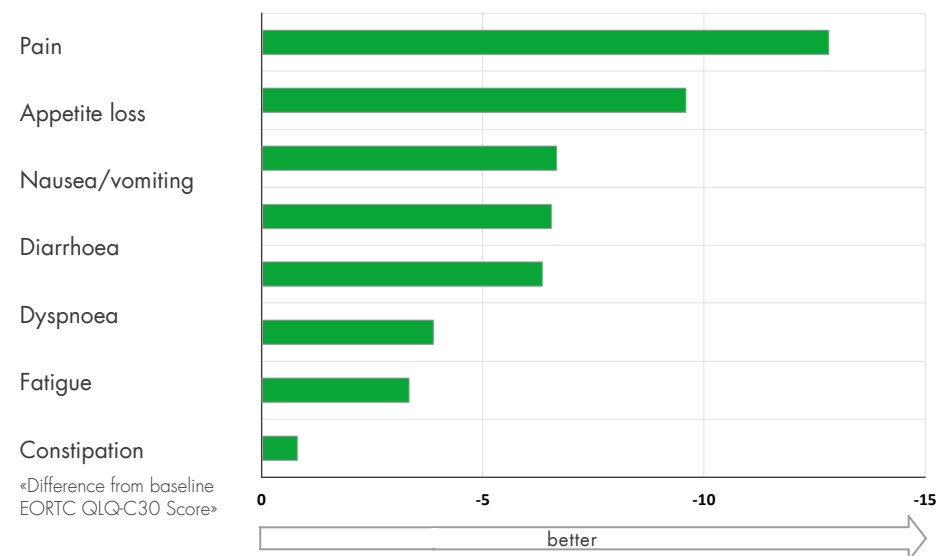


Fig. 32: Quality of life (mean changes in **symptoms** in accordance with EORTC QLQ-C30). A difference of more than 5 points is clinically relevant.

Neutropenia occurred during the course of treatment in 3 of 30 ISCADOR patients and 8 of the 31 control patients, but this was not significant due to the low number of cases ($p = 0.182$). Therapy with ISCADOR was well tolerated.

Conclusions: In this randomised study, the additional therapy with ISCADOR M improved the quality of life of breast cancer patients during chemotherapy with CAF. A trend to reduced frequency of chemotherapy-related neutropenia was shown in the ISCADOR group. Verification of these promising results is currently in progress.

ISCADOR and chemotherapy in patients with breast cancer: five-year follow-up

(Tröger et al. 2012)

Patients and methods: The patients described above were subsequently followed up for five years. None of these patients received ISCADOR therapy after the end of the chemotherapy but some of the patients in both groups received antihormone therapy or radiotherapy.

The aim of this prospective non-interventional five-year follow-up study was to examine whether ISCADOR M treatment additional to chemotherapy had an influence on median disease-free survival (DFS), frequency of recurrence and metastasis. The following inclusion criteria needed to be met for participation in this five-year follow-up study:

- Patients received the prescribed chemotherapy cycles every six weeks
- Patients had no metastasis before the start of chemotherapy
- The patients had given consent for participation in the follow-up study

Before the start of chemotherapy, two patients in the ISCADOR group had an unknown metastasis status ($M = x$). In the control group, one patient was excluded because she had only 2 cycles of chemotherapy due to heart disease. A further patient did not agree to participate in the follow-up, and thus 28 of 30 patients from the ISCADOR group and 29 of 31 patients from the control group were included in the study. The follow-up lasted from June 2006 to May 2012. Incidents of recurrence and/or metastases were documented annually over this five-year period as part of yearly routine visits to the study centre. A deviation of ± 2 months was tolerated for the visits. However, follow-up for individual patients ended if they experienced a relapse or developed metastasis.

Results: After chemotherapy and mistletoe therapy ended, the patients received further therapies which may have influenced their disease-free survival. Therapies in both groups were therefore recorded in the documentation forms. The most commonly forms of therapy used in

both groups were radiotherapy ($n = 37$) and antihormone therapies (tamoxifen; $n=32$) so that these therapies could be assessed as separate subgroups.

Trastuzumab ($n = 4$), goserelin ($n = 2$), docetaxel ($n = 1$) and letrozole ($n = 1$) were used as subsequent treatments. Even though they were used equally in both groups, the numbers were too low to be able to form separate subgroups.

The median disease-free survival period could not be determined, since the greatest probability of recurrence or metastasis developing in the five years was only 28%. Six of the 28 patients in the ISCADOR group and eight of the 29 patients in the control group had recurrences or metastasis after five years (Fig. 33). This difference was not statistically significant ($p = 0.551$; Cox-Mantel log-rank test).

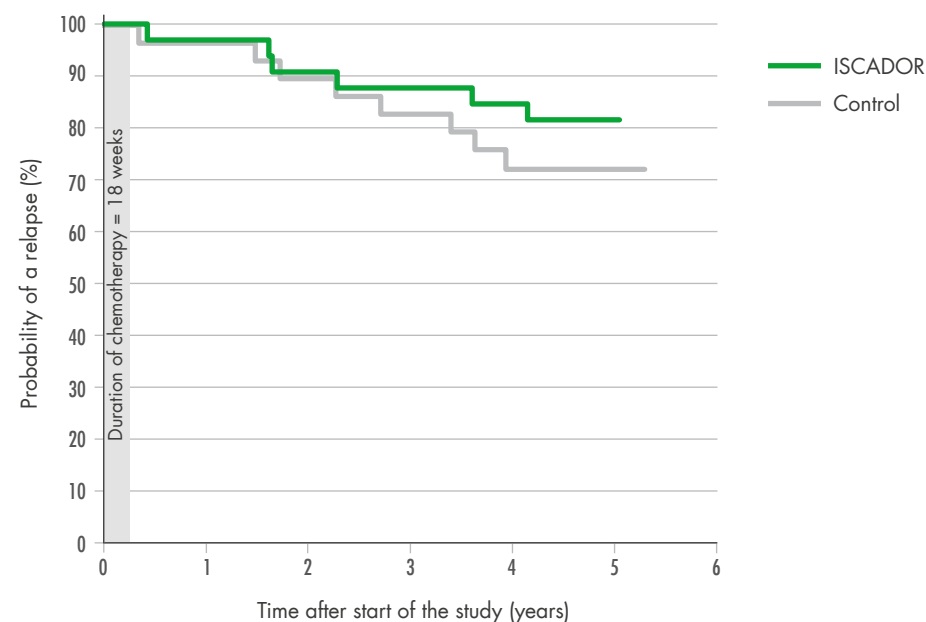


Fig. 33: Disease-free survival of both therapy groups of patients with breast cancer
(the results did not show a difference from the control group)

In a subgroup of patients who received radiotherapy, four of the 19 patients in the ISCADOR group and three of the 18 patients in the control group developed relapses or metastases after five years. In the subgroup of patients treated with antihormone therapy, this was four out of 18 in the ISCADOR group and four out of 14 patients in the control group. Differences ascertained within both subgroups were not statistically significant (Fisher's Exact Test $p = 0.792$ and $p = 0.659$).

Conclusions: ISCADOR therapy in addition to chemotherapy improves quality of life in patients with breast cancer in the early stages and may prevent neutropenia. It was also shown that additional ISCADOR therapy during chemotherapy can prevent patients from interrupting or postponing the chemotherapy cycles since the chemotherapy is better tolerated. The results of the subsequent five-year follow-up study show that addition of ISCADOR to chemotherapy had no influence on five-year disease-free survival. Consequently, there were no indications that ISCADOR therapy administered during chemotherapy had any negative effects on the chemotherapy. On the other hand, the duration of mistletoe therapy (18 weeks) is probably too short to positively influence the incidence of a recurrence or metastases over 5 years.

6.3.2 Lung cancer

With around 52,500 new cases in 2012, lung cancer is one of the most common types of cancer in Germany. Due to the unfavourable prognosis, it is also the most common cause of cancer death in men (25%) and the third most common in women (15%). The main risk factor for lung cancer is smoking. Around 90% of men and at least 60% of women who develop lung cancer have actively smoked. Passive smoking also increases the risk. Lung cancer is one of the tumours with the least favourable prognosis: the relative five-year survival period in Germany is 21% for women and 16% for men. The survival chances vary greatly according to the stage of the disease, as for all types of cancer. Since lung cancer often does not cause any complaints in the early stage, the disease is often discovered late on and by chance (Katalinic et al. 2015). Almost 60% of patients with lung cancer in the advanced and metastasised stage do not receive any curative treatment. Palliative mistletoe therapy is offered at this stage to improve quality of life.

Mistletoe therapy in patients with advanced non-small cell lung cancer

(Bar-Sela et al. 2013)

Patients and methods: Patients with inoperable, non-small cell lung cancer (NSCLC) receiving therapy with gemcitabine/carboplatin or with pemetrexed/carboplatin were included in a randomised Phase II study. Patients were allocated to two groups by 1:1 randomisation. One group also received ISCADOR Qu (n = 33), the other group (n = 39) did not receive any additional therapy.

Study objectives: This study examined the extent to which mistletoe therapy with ISCADOR can reduce chemotherapy-related side effects and improve quality of life.

Results: It was possible to administer significantly more chemotherapy cycles in the ISCADOR group. The progression-free time was 4.8 months in the control group, 6 months in the ISCADOR group, and the overall survival period was 13.3 or 15.9 respectively. However, these differences and the reduction of chemotherapy-related side effects were not significant

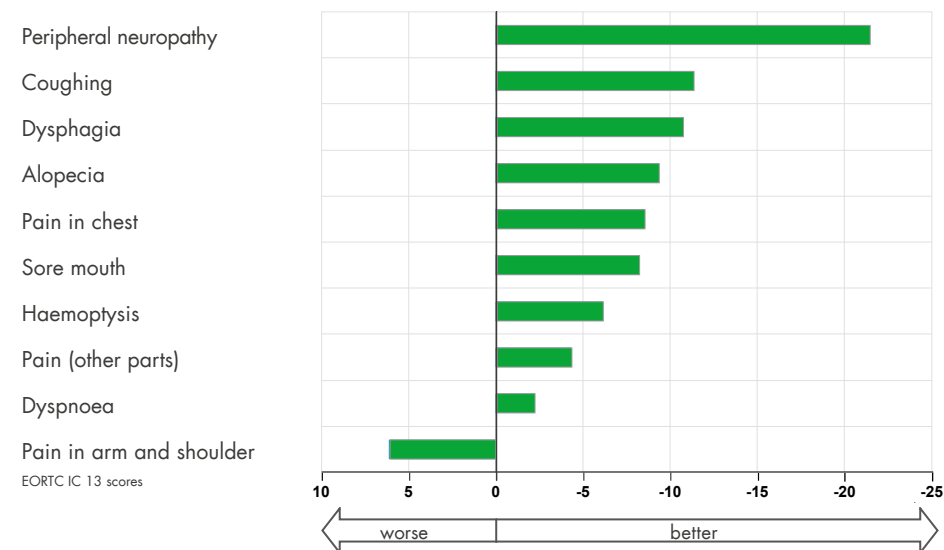


Fig. 34: Quality of life in patients with NSCLC during chemotherapy, with and without ISCADOR Qu as additional therapy. A difference of more than 5 points is clinically relevant.

due to the small number of cases in the study. Quality of life measurements (Fig. 34) always showed benefits for the patients in the ISCADOR group, except for the «pain in the shoulder» criterion, but not all differences were significant.

There was only one side effect of ISCADOR therapy. This was a grade II oversize local reaction at the injection site. The therapy could therefore be classified as safe.

Conclusions: Additional administration of ISCADOR alongside chemotherapy is a promising combination for patients in the late stage of NSCLC, reducing side effects related to chemotherapy. These results should be verified in a larger Phase III study.

6.3.3 Colorectal cancer

With around 62,000 new diagnoses in 2012, colorectal cancer is the third most common type of cancer in Germany in men, and the second most common in women. Around 26,000 patients die from this each year. The risk of the disease increases with age of the population. More than half of patients first diagnosed with this condition are over 70 years of age, and only 10% are younger than 55. Almost two-thirds of cases of the disease occur in the colon, and around 30% are localised in the rectum. The remaining incidence of disease is distributed between the rectosigmoid and the anal canal (Katalinic et al. 2015). Colorectal cancer has

a moderate prognosis; the five-year survival rate is around 63%. Early diagnosis and surgical removal of the tumour are the most important measures in this case. Chemotherapy is recommended only for R₀-resected patients preferably with 1-4 affected lymph nodes.

Postoperative ISCADOR treatment in patients with primary, non-metastasised colorectal cancer (Friedel et al. 2009, 2009b)

Patients and methods: The study was a multicentre, controlled, retrospective, epidemiological cohort study, conducted according to GEP guidelines with 804 patients with primary, non-metastasised colorectal cancer of UICC stage I to III. Of the 804 patients from 26 centres in Germany and Switzerland, 429 patients received ISCADOR as part of a supportive long-term therapy after surgery. ISCADOR was applied subcutaneously two to three times a week, in addition to conventional chemotherapy and/or radiotherapy and during oncological aftercare. From the per-protocol analysis of the ISCADOR group, a subgroup of 106 patients who received ISCADOR Qu was analysed separately. The 375 patients from the control group were treated with conventional therapies or received passive aftercare without further medication. The median follow-up time in this study was approximately 5 years.

Study objectives: The primary outcome parameter was the frequency of side effects related to chemo- or radiotherapy. Secondary outcome parameters were the persistence of disease-related symptoms in the ISCADOR group, the Karnofsky index, the duration of hospitalisation during the observation period and disease-free survival (DFS).

Results: The patients in the ISCADOR group showed significantly fewer side effects related to chemo- or radiotherapy than the patients in the control group. Only 19.1% of the patients in the ISCADOR group but 48,3% of the patients in the control group developed side effects (Fig. 35).

The subgroup analysis of 106 patients with ISCADOR Qu showed an even bigger difference: only 7.5% of the patients in the ISCADOR Qu group developed these side effects related to chemotherapy or radiotherapy, in comparison to almost 48,3% of the patients in the control group (Fig. 36).

The patients in the ISCADOR group developed fewer disease-related symptoms such as nausea or vomiting, appetite loss, depression, fatigue (tiredness), irritability or weariness, sleep disturbances, mucositis or skin reactions.

The Karnofsky index also significantly improved in the ISCADOR group ($p < 0.001$). The performance of the patients in the ISCADOR group increased continuously and significantly to

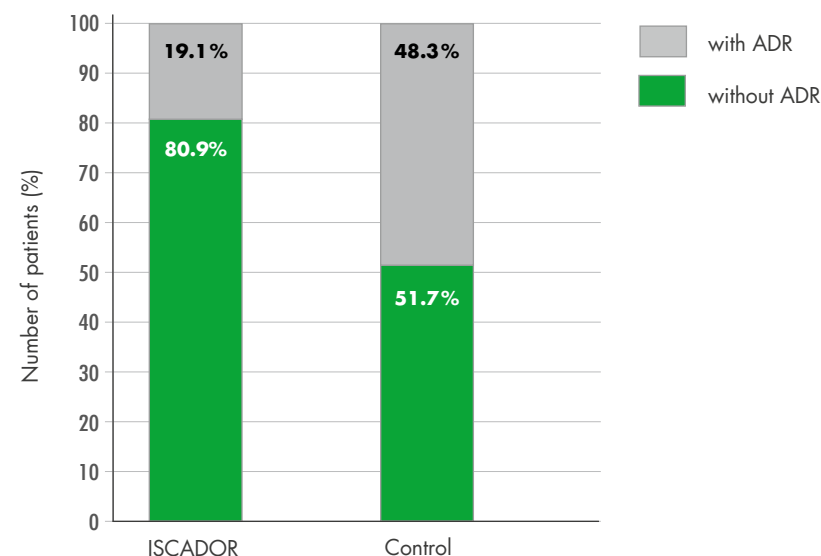


Fig. 35: Proportion of colorectal cancer patients with adverse drug reactions (ADR) after conventional therapy, $p < 0.001$ (only patients who received chemo- and/or radiotherapy were evaluated)

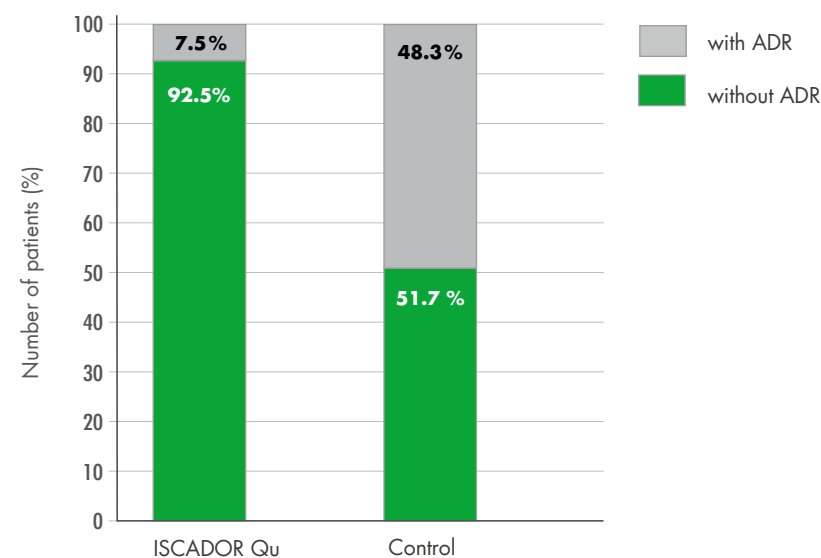


Fig. 36: Proportion of colorectal cancer patients with adverse drug reactions (ADR) after conventional therapy who received ISCADOR Qu compared to the control group, $p < 0.001$ (only patients who received chemo- and/or radiotherapy therapy were evaluated)

86.8% during the first chemotherapy cycle and then increased after chemotherapy to 93.7%. In contrast to this, the control group showed a slight fall in performance after the first cycle of therapy, which rose again almost to the original value of 84.6% again after therapy.

Additionally, the average hospitalisation time was 35.5 days in the ISCADOR group. This was significantly shorter ($p = 0.015$) than the control group, which spent 41.2 days in hospital. Furthermore, a benefit was observed for disease-free survival (DFS) (Fig. 37). Accordingly, the adjusted relative hazard rate (hazard ratio, HR) for disease-free survival in the ISCADOR group was 0.68 (0.51–0.92, $p = 0.013$), which corresponds to a significant reduction in the estimated relative risk of around 32% and a reduction in the relapse rate of around a third.

The adverse drug reactions related to ISCADOR were assessed to check the safety of therapy. Ten patients (2.3 %) reacted to ISCADOR with systemic side effects such as dizziness, exhaustion, depression, tinnitus, nausea, slight fever or itching. All of these side effects were slight to moderate (WHO/CTC grade 1–2). Therapy with ISCADOR was stopped early due to these systemic side effects in five cases. One patient showed an acute allergic reaction. In 100 patients (23.3%), local reactions such as indurations, erythema, itching or oedema occurred

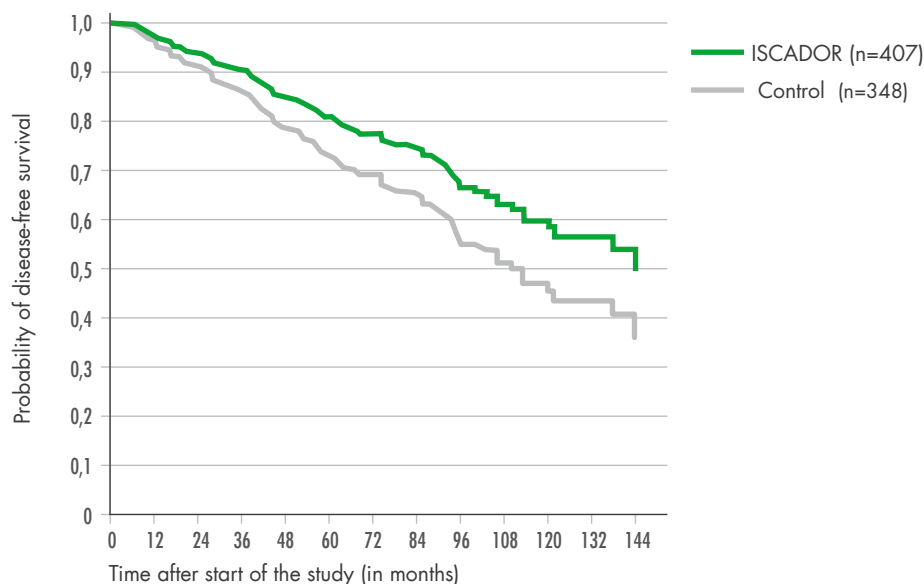


Fig. 37: Disease-free survival (DFS) in colorectal cancer, Cox proportional hazard regression, HR (95% CI) = 0.68 (0.51–0.92), $p = 0.013$

at the injection sites. The reactions were always of slight or moderate level, and then fully subsided. However, they still led to two patients stopping therapy. No life-threatening side effects occurred.

Conclusions: The study results prove that therapy with the mistletoe extract ISCADOR in addition to conventional oncological basic treatment significantly reduces the side effects of the latter. Furthermore, ISCADOR can help to prolong survival.

ISCADOR reduces cancer-related fatigue syndrome in patients with primary, non-metastasised colorectal cancer (Bock et al. 2014)

Patients and methods: In a second analysis, patients were selected from the study groups as described above who had cancer-related fatigue at the start of the study.

Results: 143 patients without and 181 patients with additional ISCADOR therapy were included. Sixteen patients with ISCADOR therapy and 86 patients without ISCADOR therapy still suffered from fatigue at the end of the observation (Fig. 38).

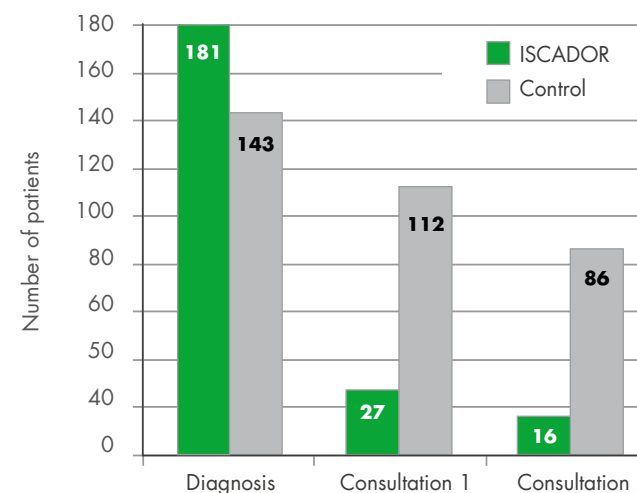


Fig. 38: Cancer-related fatigue in patients with stage III non-metastasised colorectal cancer. Consultations 1 and 2 took place after an average of 3.5 and 7 months.

Conclusions: ISCADOR therapy represents a measure very suitable for reducing cancer-related fatigue in patients with colorectal cancer during treatment with chemo-/radiotherapy.

6.3.4 Pancreatic cancer

Pancreatic cancer is the fourth most common cancer-related cause of death in the Western world. It is also one of the ten most common type of tumour in Germany, where, in 2012, around 17,000 people were newly diagnosed with the disease (Katalinic et al. 2015). Early symptoms of this indication can include upper abdomen or back pain. However, they rarely occur at the beginning of the disease and are often not identified as such for a long time. In 90% of cases, therefore, pancreatic cancer is detected at an advanced stage. The majority of pancreatic tumours show marked local invasion when diagnosed. In three-quarters of patients, the tumour has already spread: only to the adjoining lymph nodes in a quarter of patients, but in half of the cases distant metastases are diagnosed. As a result, many patients already have a poor prognosis at first diagnosis, which is reflected in a five-year survival rate of only 8% for men and 9% for women. Pancreatic cancer is therefore one of the most malignant cancers. In the advanced stage of pancreatic cancer, palliative mono-chemotherapy with gemcitabine is generally used. However, the Folfirinx protocol achieves significantly better results than gemcitabine in selected patients. Due to patients' poor general condition, often only passive after-care or best supportive care is given, so that treatment with mistletoe extracts can be very helpful in improving quality of life.

Improvement of supportive therapy using ISCADOR in patients with pancreatic cancer (Matthes et al. 2009, 2010, Stauder et al. 2009)

Patients and methods: The study was a multicentre, controlled, epidemiological, retrospective cohort study. It was conducted according to GEP guidelines with patients suffering from pancreatic cancer at all stages of severity (UICC stages I to IV). 396 patients at 17 centres in Germany and Switzerland took part in the study. Of these 396 patients, 201 received ISCADOR subcutaneously two to three times a week as part of supportive long-term therapy, in addition to adjuvant chemotherapy (and/or radiotherapy), or in addition to best supportive care. From the per-protocol analysis from the ISCADOR group, a subgroup of 75 patients who received ISCADOR Qu was analysed separately. The 195 patients from the control group received chemotherapy only, in most cases gemcitabine (and/or radiotherapy) or best supportive care without further oncological medication. The therapy or subsequent median observation time was around 15 months in the ISCADOR group, in the ISCADOR Qu group around 20 months, and in the control group around 10 months. The median duration of chemotherapy was seven months in the ISCADOR group and five months in the control group.

Study objectives: The frequency of side effects related to chemotherapy (and/or radiotherapy) was assessed as a primary outcome parameter of efficacy. The persistence of disease-related symptoms in the ISCADOR group compared to the control group, the Karnofsky index, the

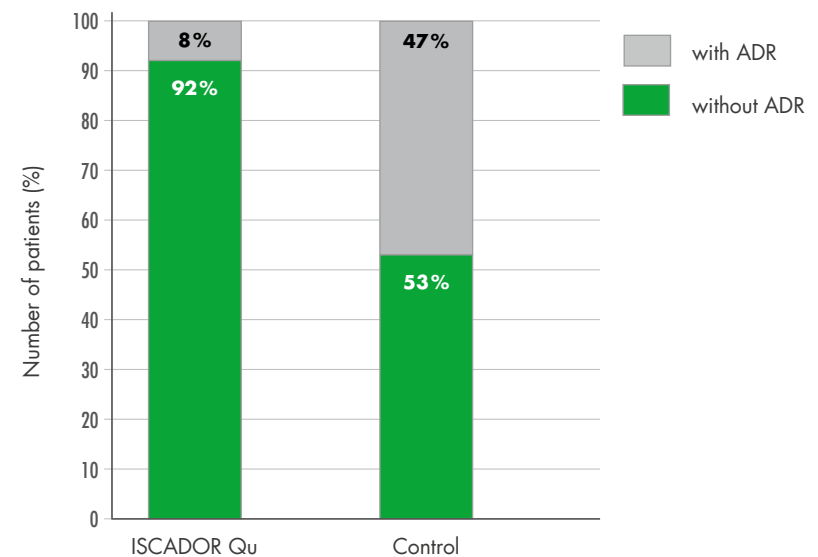


Fig. 39: Proportion of pancreatic cancer patients with adverse drug reactions (ADR) from conventional therapy, $p < 0.001$ (subgroup analysis of ISCADOR Qu, only patients who received chemotherapy or radiotherapy were assessed)

duration of hospitalisation during the observation period and overall survival (OS) were secondary outcome parameters. Safety parameter was the number of patients with documented adverse drug reactions (ADR) related to ISCADOR or ISCADOR Qu therapy.

Results: Significantly fewer side effects related to conventional chemotherapy (and/or radiotherapy) were observed in the ISCADOR groups compared to the control group. In the ISCADOR group, 14% developed these side effects, and only 8% in the ISCADOR Qu group. By contrast, almost 50% of the control group suffered from side effects. The adjusted relative risk of developing therapy-related side effects was significantly lower in the ISCADOR and ISCADOR Qu group (ISCADOR group: Odds ratio (OR, 95% CI) = 0.46 (0.28 – 0.77), $p = 0.003$; ISCADOR Qu group: OR (95% CI) = 0.17 (0.06 – 0.50), $p < 0.001$) than in the control group. This is a risk reduction of 54% in the ISCADOR group and 83% in the ISCADOR Qu group (Fig. 39).

The patients in both ISCADOR groups also showed fewer disease and therapy-related symptoms after the first oncological therapy cycle. Nausea, vomiting, appetite loss, depression, fatigue/tiredness, sleep disorders and back pain occurred much less frequently in the ISCADOR group than in the control group.

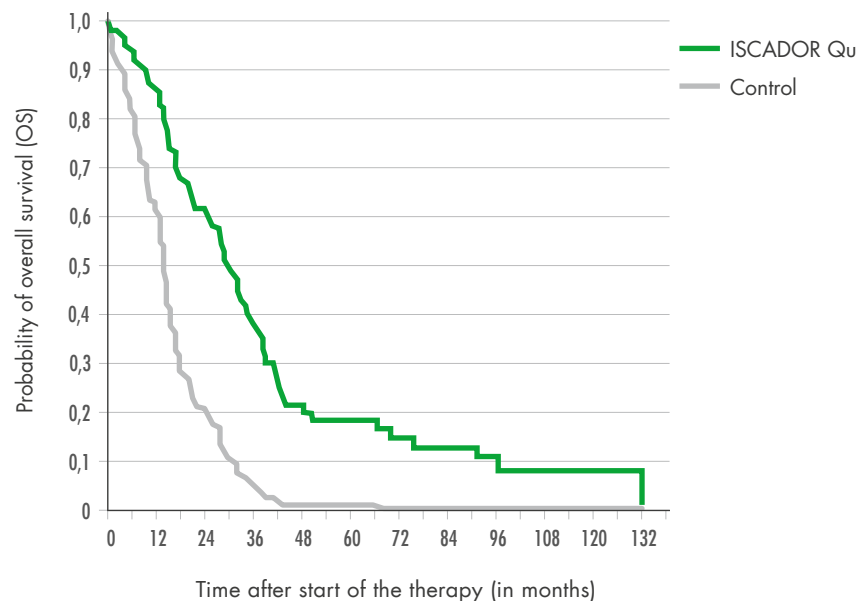


Fig. 40: Overall survival (OS) for pancreatic cancer, Cox proportional hazard regression, HR (95% CI) = 0.31 (0.18 - 0.54), $p = 0.001$

An excellent study result was obtained when calculating survival time (overall survival, OS). For all stages of severity of pancreatic cancer, this was significantly longer in both ISCADOR groups than in the control group. The adjusted hazard ratio (relative risk) for the mortality rate was 0.58 in the ISCADOR group and 0.31 in the ISCADOR Qu group (Fig. 40), so that the relative risk of death in the ISCADOR group was reduced by around 42% and reduced by around 69% in the ISCADOR Qu group compared to the control group.

The Karnofsky index significantly improved in the ISCADOR group. The performance of the patients in the ISCADOR group increased from 74.1 to 79.1% after the first chemotherapy cycle. In contrast, the control group had a fall in performance from 80.3% to 74.7%. The average hospitalisation time was 40 days in the ISCADOR group. This was significantly shorter than in the control group, with 54 days.

The adverse drug reactions related to ISCADOR were assessed to check the safety of therapy. Only 3 patients (0.8%) from the ISCADOR group reacted with slight, non-specific systemic side effects such as dizziness, exhaustion or slight fever. No systemic side effects were observed in the ISCADOR Qu group. In 45 patients (22.4%) in the ISCADOR group and 11 patients

(14.7%) in the ISCADOR Qu group, local side effects occurred at injection sites such as erythema, induration, oedemas, itching or localised pain. These were always slight to moderate. No severe, life-threatening side effects occurred. Therapy with ISCADOR could therefore be evaluated as safe and well tolerated.

Conclusions: In this study, the safety and efficacy of ISCADOR as a part of supportive therapy for patients with pancreatic cancer of all stages of severity could be shown. Patients in the ISCADOR group had significantly fewer side effects related to conventional therapy, fewer disease-related symptoms, a better general condition, a shorter hospitalisation time and a significantly higher probability of survival than the control group. The described effects were even more pronounced in the ISCADOR Qu subgroup. The therapy with ISCADOR was well tolerated and safe.

Mistletoe therapy in locally advanced or metastasised pancreatic cancer (Tröger et al. 2013, 2014)

Patients and methods: This study was an open, monocentre, randomised phase III study in patients with locally advanced or metastasised pancreatic cancer. It was conducted at the Liver and Gallbladder Department of the First Surgical Hospital at the Clinical Centre of Serbia in Belgrade. The patients were observed over a period of twelve months. Half of the patients received only best supportive care (BSC) for acute treatment of cancer-related symptoms, but no antineoplastic therapy; the other half received BSC and injected ISCADOR Qu subcutaneously three times a week. Observed efficacy in relation to overall survival (OS) and quality of life (QOL) in the two treatment groups was then compared. QOL was recorded using the questionnaire of the European Organization for Research and Treatment of Cancer (EORTC QLQ-C30). This is completed by the patient and includes a scale for general health/quality of life, five functional scales and nine symptom scales.

The group sequential study design involved an independent board of experts (Independent Data Monitoring Board, IDMC) to assess overall survival and drug safety of the 220 patients, who were included. This board recommended ending the study early due to proven efficacy, and endorsed publication.

Results: The baseline characteristics of both study groups were statistically well balanced. The analysis yielded a median survival time in the ISCADOR group of 4.8 months in comparison to 2.7 months in the control group (Hazard Rate (HR) = 0.49; $p < 0.0001$). Patients were stratified into prognosis groups described in the following two subgroup analyses. The median survival time in the group with «good prognosis» for patients receiving ISCADOR treatment was

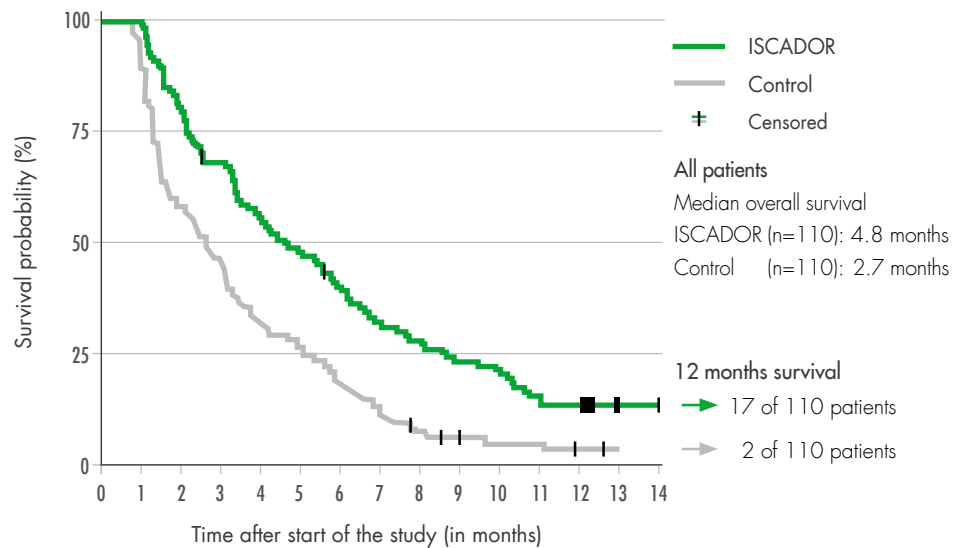


Fig. 41: Overall survival in the ISCADOR group compared to the control group for all patients

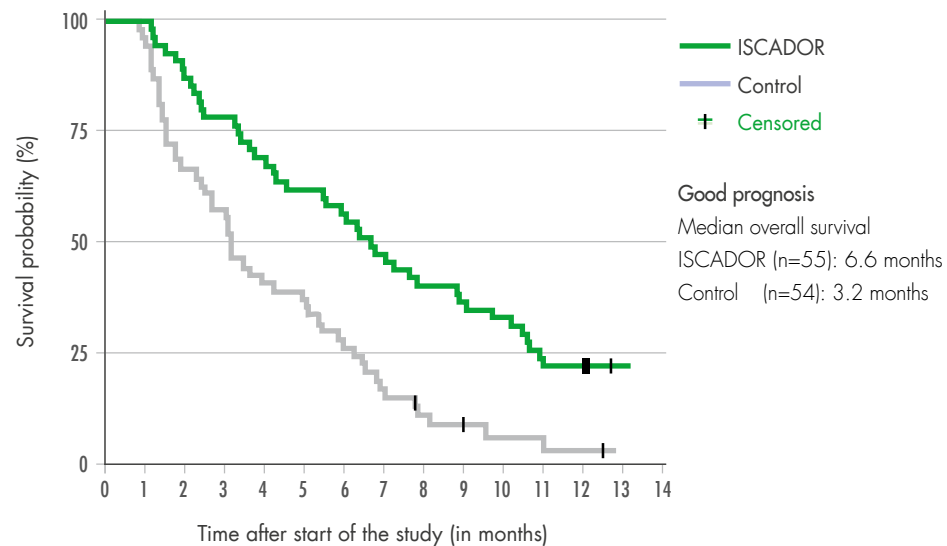


Fig. 42: Overall survival in the ISCADOR group compared to the control group for patients with a «good» prognosis

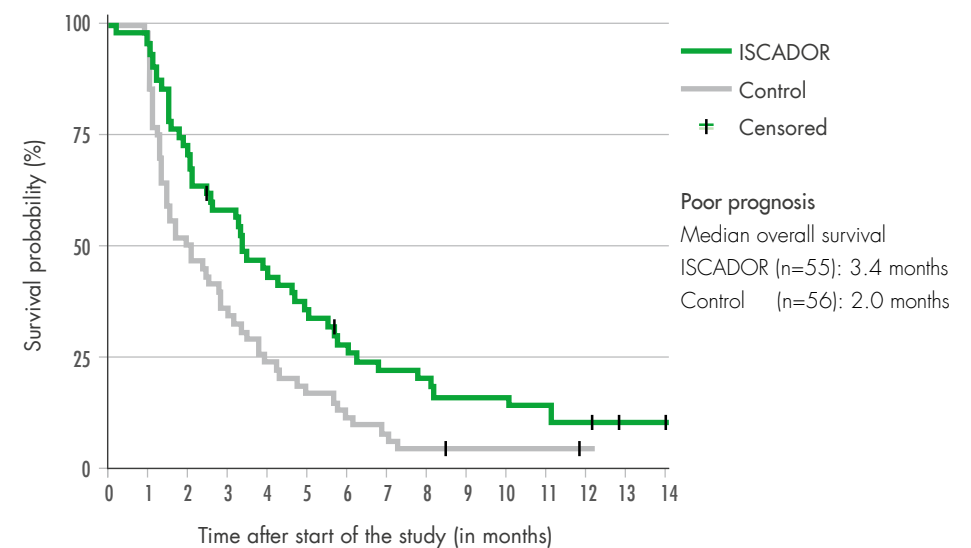


Fig. 43: Overall survival in the ISCADOR group compared to the control group for patients with a «poor» prognosis

6.6 months, but for the control group only 3.2 months (HR = 0.43; $p < 0.0001$). In the patient group with «poor» prognosis, the corresponding times were, respectively, 3.4 and 2.0 months (HR = 0.55; $p = 0.0031$) (Fig. 41 – 43).

Likewise, for the parameters by which quality of life was recorded, there were mostly marked differences between the treatment groups. In EORTC QLQ-C30, 13 of the 15 parameters showed ISCADOR treatment as superior to a significant and clinically relevant degree. The patients in the ISCADOR group assessed their general health condition as significantly better than in the control group. The parameters of the five functional scales in the ISCADOR group were also much less impaired than in the control group (Fig. 44).

The marked success of the therapy with ISCADOR was also reflected in fewer cancer-related symptoms and their reduced intensity, in particular pain, nausea and vomiting, and energy and appetite loss (Fig. 45). In contrast to all expectations, the investigators were even able to observe a slight weight gain in patients in the ISCADOR group (Fig. 46). ISCADOR therapy was well tolerated in all cases; no serious or severe side effects were detected.

Conclusions: This study showed significant and clinically relevant benefits in overall survival and a significant improvement in quality of life for patients receiving ISCADOR therapy. ISCADOR

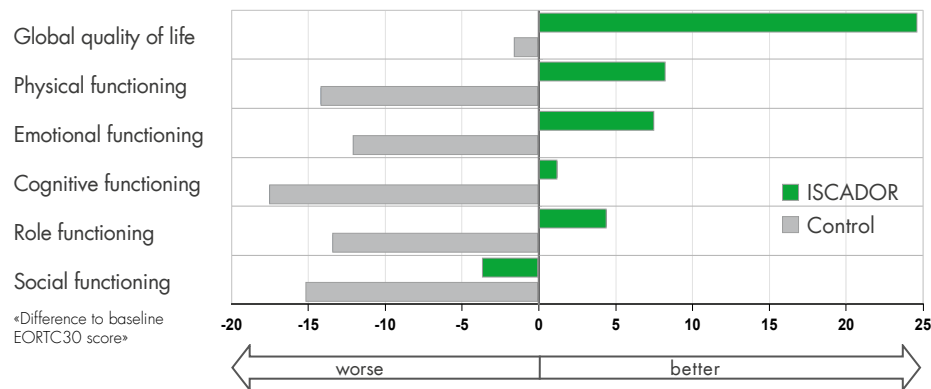


Fig. 44: Quality of life (mean changes in functions in accordance with EORTC QLQ-C30)

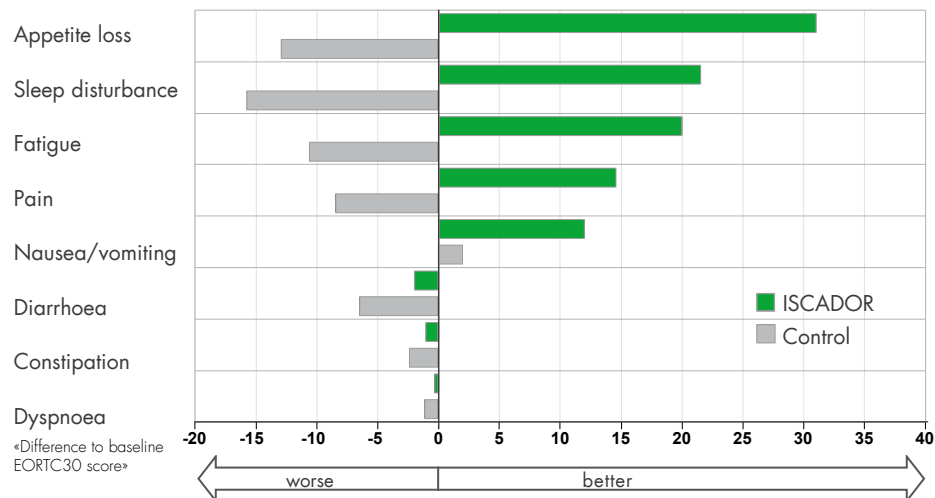


Fig. 45: Quality of life (mean changes in symptoms in accordance with EORTC QLQ-C30)

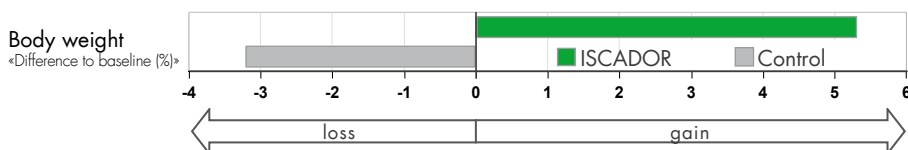


Fig. 46: Body weight (mean change in body weight in %)

is therefore a non-toxic second-line treatment for patients with locally advanced or metastasised pancreatic cancer, which extends overall survival and effectively improves quality of life.

6.3.5 Malignant melanoma

Malignant melanoma is a tumour of melanocytes, with a high lymphogenic and haematogenous metastasising tendency. Incidence has continuously increased worldwide in the last 40 years, so that malignant melanoma now represents the fifth most common cancer in Germany, where there were around 21,000 diagnoses of the disease (men and women in roughly equal numbers) in 2012. The average age of onset for women is 59, which is comparatively low. Onset for men is eight years later, on average (Katalinic et al. 2015). Most important in melanoma is prevention and early diagnosis, since ten-year survival is 75% in patients without metastases, falling to 20 to 40% for patients with local/regional metastases. The prognosis is usually very unfavourable for distant metastasis, and the median survival without treatment is only four to six months.

Efficacy and safety of ISCADOR in long-term treatment of patients with primary malignant melanoma (Augustin et al. 2005, Friedel et al. 2009a)

Patients and methods: The therapeutic efficacy and safety of long-term therapy with ISCADOR, and in a subgroup with ISCADOR P, was examined. A comparison was done with an untreated (watchful waiting) control group for patients with malignant melanoma with a moderate to high risk of AJCC/UICC stage II and III during post-surgical aftercare. The study was a multicentre, controlled, epidemiological, retrospective cohort study with a parallel group design conducted in accordance with GEP (Good Epidemiological Practice) guidelines. In total, 686 patients from 35 centres in Germany and Switzerland participated in the study. The ISCADOR group comprised 329 patients, of which 274 patients (83.3%) were treated with ISCADOR P. The control group comprised 357 patients. The median ISCADOR therapy duration with three injections a week was about 30 months. The median aftercare/subsequent observation period in the ISCADOR group and the control group was, respectively, 81 and 52 months.

Study objectives: Adjusted overall survival (OS) was selected as the primary outcome parameter of efficacy. The secondary outcome parameters concerned disease-related survival as well as the adjusted risk of developing brain metastases. The outcome parameter for safety was the number of patients with documented adverse drug reactions (ADRs) caused by ISCADOR or ISCADOR P therapy.

Results: The adjusted hazard ratio (HR, relative risk) for dying of any cause during the therapy and subsequent observation period (= overall survival, OS) was significantly lower in the ISCADOR group and ISCADOR P group than in the control group (Fig. 47). The adjusted HR in the ISCADOR P group was 0.60, which represents an estimated relative risk reduction of 40%.

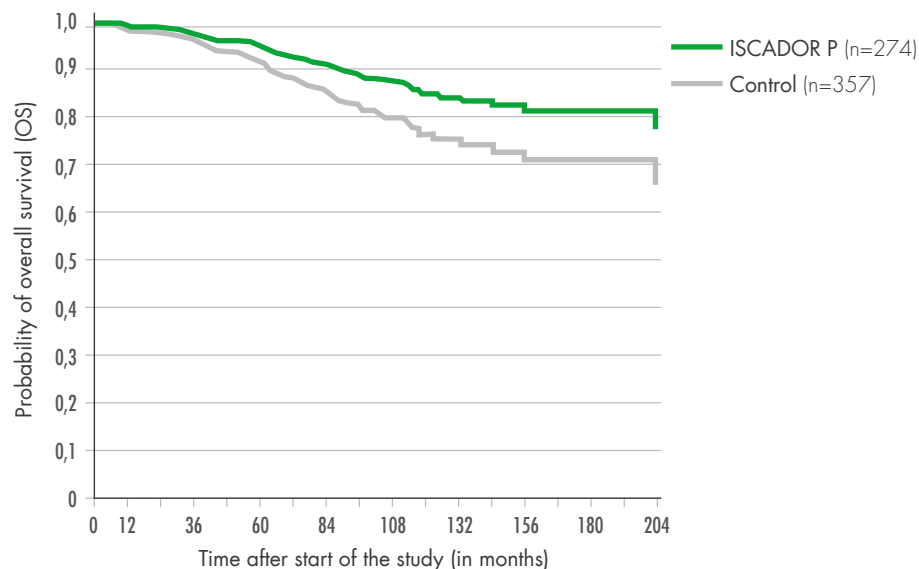


Fig. 47: Overall survival (OS) for malignant melanoma, multivariate adjusted Cox regression analysis, HR (95% CI) = 0.60 (0.38– 0.93), p = 0.024

Comparable results were obtained for disease-related survival (Fig. 48). The HR in the ISCADOR group was 0.41 and 0.38 in the ISCADOR P group; these correspond to a relative risk reduction of, respectively, 59 and 62%. The frequency of metastases, particularly brain, lung and lymph node metastases, was significantly less in the ISCADOR group than in the control group.

Eleven of 329 (3.3%) patients in the ISCADOR group, and 8 patients (2.9%) in the ISCADOR P group experienced systemic therapy-related adverse drug reactions (ADRs), which were unspecific and only slight to moderate. The reactions spontaneously subsided within a week in most cases. In one case, treatment was stopped early due to moderate headaches and tiredness. No life-threatening side effects occurred. Local reactions at the injection site could be observed in 42 patients (12.8%) in the group treated with ISCADOR, and in 23 (8.4%) patients treated with ISCADOR P. This primarily involved erythema, oedema, itching or localised pain. The localised side effects were predominantly slight to moderate (WHO/CTC grades 1-2) and spontaneously subsided in most cases. Therapy was stopped early due to these systemic side effects in 5 cases with ISCADOR, and in 4 cases with ISCADOR P.

Conclusions: Postsurgical supportive long-term treatment with ISCADOR, and in a subgroup with ISCADOR P, in patients with primary malignant melanoma with a moderate to high risk of

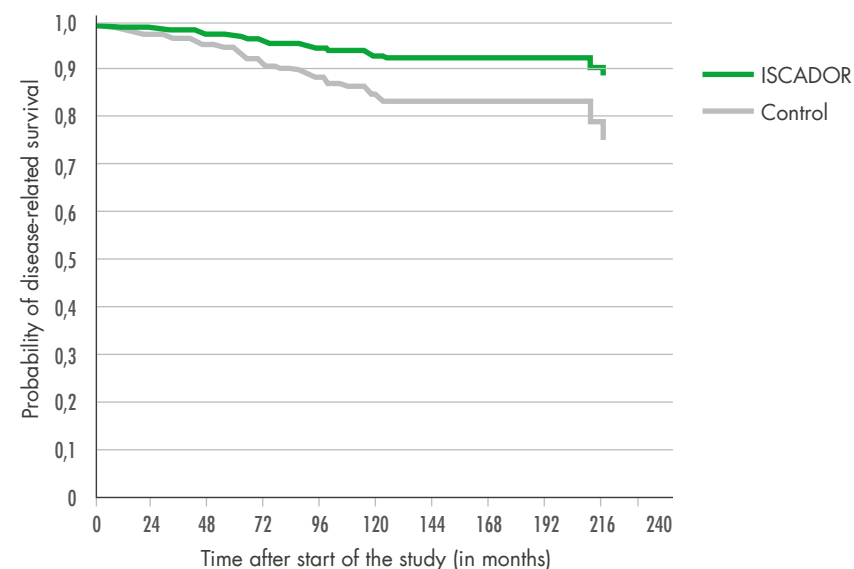


Fig. 48: Adjusted disease-related survival for malignant melanoma (disease-related survival) Hazard Ratio (95% CI): HR = 0.41 (0.23 – 0.71), p = 0.002

UICC/AJCC stage II to III, showed a considerable survival benefit compared to a control group from the same cohort without ISCADOR. Additionally, a delay of metastasis formation could be observed in the ISCADOR group. This clearly contradicts conjectures in connection with the interim analysis of an EORTC study by Kleeberg et al. from 2004, that treatment with ISCADOR might increase the risk of brain metastases. (In the study in question, most of the patients treated with ISCADOR were screened for brain metastasis, whereas patients in the control group were not.) In contrast to this speculative hypothesis, the present study impressively documented a reduction in the risk of metastases with ISCADOR treatment.

6.3.6 Osteosarcoma

Osteosarcoma is the most common primary malignant bone tumour. Its proliferative cells are capable of forming bones and osteoids. Osteosarcoma shows aggressive growth with destruction of the surrounding bone and in some cases joints, and early metastases in the lungs. At the time of diagnosis, 20% of patients already have metastases, and it is estimated that another 60% have non-visible micro-metastases. 60 to 75% of patients can be cured by extensive surgery with pre- and postsurgical chemotherapy. However, historical data shows that the risk of recurrence increases after the second relapse and that relapse-free survival decreases below 20% after twelve months.

ISCADOR versus oral etoposide as adjuvant therapy in osteosarcoma patients after a second relapse (Longhi et al. 2009, 2014)

Patients and methods: A randomised, open study was conducted at the Musculoskeletal Oncology Department, Istituto Ortopedico Rizzoli, Bologna, Italy. Osteosarcoma patients who received surgery after the second, metastasis-related relapse, and who were classified as disease-free, received subcutaneously administered ISCADOR P or orally administered etoposide. Disease-free survival (DFS) was defined as a primary outcome parameter. Both groups were additionally compared with a historic patient group to estimate the external validity of this small study.

Results: Of 20 patients included in the study, 9 patients were randomised into the Iscador P group and 11 into the etoposide group. The DFS average was 39 months in the ISCADOR group and 4 months in the etoposide group (Fig. 49). The patients in the ISCADOR group developed fewer side effects than in the etoposide group.

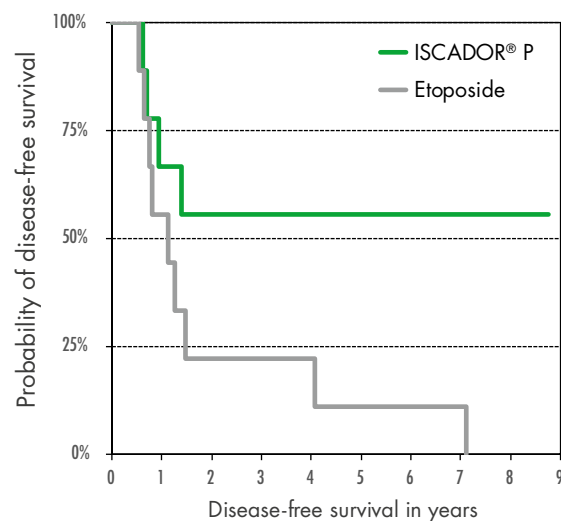


Fig. 49: Probability of disease-free survival after 2nd relapse of osteosarcoma

Conclusions: Therapy with ISCADOR P is a promising option to extend survival in osteosarcoma patients who have developed a second relapse. Therapy with ISCADOR is also more economical for osteosarcoma treatment than Interferon- α or mifamurtide. Despite the small number of patients in the study, the results are significant due to the large difference from the control group. A study with about 100 patients will be undertaken to corroborate the efficacy of ISCADOR therapy for patients with osteosarcoma.

6.4 ISCADOR in cancer-related fatigue syndrome

Cancer-related fatigue syndrome (CRF) is a serious side effect of cancer from which numerous tumour patients suffer. Symptoms are related to many factors and may be bodily, such as dyspnoea, emotional, such as depression, mental, such as memory disorders, and social, such as withdrawal. In contrast to normal tiredness, fatigue is not compensated by sufficient sleep. The condition is partially triggered by the tumour itself. Exhaustion also often occurs during and due to chemotherapy or radiation. After these therapies have finished, fatigue may last from weeks to months and even years after the last treatment. The quality of life of those affected is therefore impaired in an enduring way, sometimes even after the curing of a tumour disease. Treatment options such as physical training, supportive psycho-oncological therapies and medicinal treatments exist, but they often seem to fail to achieve sufficient improvement in daily circumstances. This means that many patients resign themselves to seeing no potential improvement in their exhaustion (Lawrence et al. 2004, Rüffer and Flechtner 2006, Sood et al. 2007, Heim and Feyer 2011). Examinations show that mistletoe therapy with ISCADOR can positively impact cancer-related fatigue syndrome. A 36-year-old patient who had suffered from recurring breast cancer for 10 years and had severe CRF, received complementary treatment with ISCADOR M for two and a

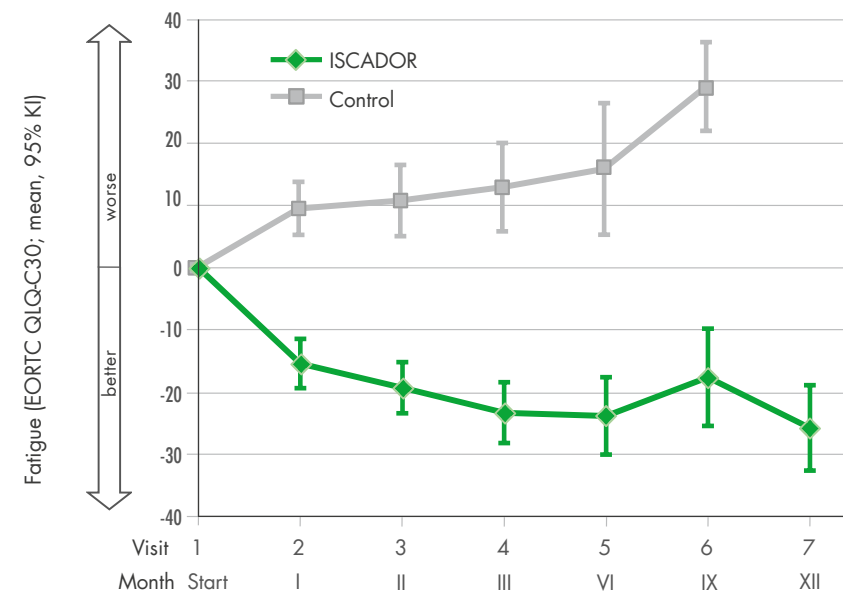


Fig. 50: Example of fatigue progression during therapy with ISCADOR Qu. The results originate from a randomised study by Tröger et al. (2014) of 220 pancreatic cancer patients who received either ISCADOR Qu or no additional therapy.

half years. An inverse correlation between the intensity of the mistletoe therapy and the extent of her fatigue could be identified during this period. A dose reduction, prescribed by the doctor, and a therapy break that was decided by the patient, led to worsening of the fatigue and her general condition, while an increase in the dose or the resumption of treatment led to an improvement in her fatigue and general condition (Wode et al. 2009). Similar results could be seen in the previously mentioned studies from Bock et al. 2004, 2014, Tröger et al. 2009, 2014, Friedel et al. 2009, Matthes et al. 2010. In these, patients treated with ISCADOR suffered significantly less from cancer-related fatigue syndrome or tiredness than patients in the control group (Fig. 50). ISCADOR is therefore a promising option for treatment of cancer- or therapy-related fatigue.

6.5 ISCADOR therapy and immune checkpoint inhibitors

6.5.1 Principles

Since authorisation of the first immune checkpoint inhibitors in 2011 in the USA, cancer therapy has focused on these new therapeutic approaches (Petrausch 2016). In principle, the immune system's ability is used to destroy tumours. The immune checkpoint inhibitors target the «tumour immune escape» by means of which a tumour evades the body's immune defences.

Strengthening the immune system was previously a domain of complementary medicine and of mistletoe therapy, in particular. However, mistletoe extracts have additional cytotoxic effects (Kienle and Kiene 2003) and therefore even more potential to act upon tumour cells.

After the induction of immunogenic tumour cell death, specific tumour antigens and PAMPs (pathogen-associated molecular patterns) are released, and these are internalised by activated pro-inflammatory antigen-presenting dendritic cells or macrophages and presented to the cytotoxic T cells. In consequence, the tumour-specific T lymphocytes activated by this can now perform their specific cytotoxic effector functions (see also section 5).

Mistletoe extracts could therefore serve as an adjuvant for immunotherapy, since they can trigger apoptosis (through which tumour antigens and PAMPs are released) and act via toll-like receptor interactive pathways on macrophages, which are thus activated. Mistletoe extracts could therefore have a complementary effect in coordination with anti-immunosuppressing therapies. The initial need here is to investigate the safety of this combination.

6.5.2 Practical use exemplified in the treatment of non-small cell lung cancer

Therapy for non-small cell lung cancer (NSCLC) has transformed in the last few years. In addition to conventional intravenous chemotherapy, more carefully targeted growth inhibitors such as checkpoint inhibitors are used in tablet form.

So far, there are no systematic studies of simultaneous or sequential therapy with checkpoint inhibitors and mistletoe preparations. However, knowledge of the effects of mistletoe preparations and initial clinical findings by users seems to justify a combination therapy: ISCADOR contributes its cytotoxic effect on tumour cells and the checkpoint inhibitor triggers the immune system brake. Finally, it is possible that undesired autoimmune effects arising in specific immune therapy could be reduced by using additive mistletoe therapy.

First clinical experiences with the combination of immune checkpoint inhibitors and ISCADOR

The first clinical experiences with additive mistletoe therapy (ISCADOR Qu) alongside immune checkpoint inhibitors involved lung cancer patients. At Havelhöhe lung cancer centre, in Berlin, investigations have been conducted since authorisation of these checkpoint inhibitors. The studies involved sequential and simultaneous combination immunotherapy of mistletoe extracts (including ISCADOR Qu) together with nivolumab and pembrolizumab.

No noticeable effects differing from those recorded in authorisation studies were observed. No indications of increased adverse effects have been found at this point. First evaluations of this combination therapy are currently being undertaken, and publication (Thronike et al., submitted) is expected soon.

7 Therapy with ISCADOR

7.1 General points

After removal from the refrigerator, the ISCADOR ampoule should be briefly warmed up in the hand. Always ensure that subcutaneous injection technique is strictly adhered to, at an angle between 30° and 45°. To avoid overreactions, in the initial phase treatment should start with a slow dose escalation in very low concentrations (series 0), increasing until the individual, optimum dose response is reached (maintenance treatment). The injection is administered in the abdominal region or alternatively (e.g. irradiation of the abdomen or dermatitis) in the thigh. When appropriate, injections can also be given in the region of the tumour. Injections into irradiated or inflamed areas of skin should always be avoided. Subcutaneous injections can usually be performed by the patient after instructions from the doctor.

Research has shown that patients benefit most when ISCADOR treatment starts immediately after diagnosis, ideally before surgery, with treatment continuing during radio- or chemotherapy in doses that have been reached at the beginning of this therapy [Bock et al. 2004, Augustin et al. 2005, Friedel et al. 2009, Matthes et al. 2009, Tröger et al. 2009, 2013]. Increased local reactions may occur if radio-/chemotherapy is started simultaneously, particularly with ISCADOR M. This means that a slower increase in dosage is recommended in this case. Therapy with series 0 has proven successful in practice.

The treating doctor should decide on the treatment duration. In principle, duration of treatment is unlimited and depends on the patient's particular risk of recurrence and individual condition, or the wellbeing and state of the patient. In most cases, this means continuation of the therapy for around five years from the point of diagnosis, and potentially longer, or from the start of primary therapy, since this can reduce the risk of recurrence.

If the course of therapy has a positive outcome, and from the second year of treatment onwards, optional treatment pauses of 1 week can be introduced following application of 2 packs of ISCADOR (2 x 7 ampoules). These interruptions can be extended with increasing duration of the therapy. In general, if the therapy is interrupted for a half year or longer, series 0 should be used to avoid overreactions.

The choice of suitable ISCADOR preparation depends largely on the localisation of the tumour. The type of tumour, disease stage, constitution and general condition of the patient, as well as the course of therapy, also play a role in choosing the ISCADOR preparation. You can find detailed information on this in the brochure «ISCADOR in cancer therapy – recommendations for treatment».

Each ISCADOR therapy is divided into an initial phase and a maintenance phase. The maintenance phase can be carried out in a rhythmically alternating or constant dose.

7.2 Initial phase

Following choice of host tree, every ISCADOR therapy starts with an initial phase, also called dose-finding phase. In this phase, low, rhythmically changing doses (series 0) are used to determine the optimum individual dose response of the patient and to prevent overreactions. To do this, 2 packs of the corresponding ISCADOR series 0 (= 2 x 7 injection ampoules with 1 ml content) are injected subcutaneously at lukewarm temperature two to three times a week. An injection rhythm of three times a week has proven to be the best in the first year (e.g. Monday, Wednesday, Friday). If series 0 is well-tolerated and if the patient has not yet reached their individual dose response (e.g. by having local reactions at the injection site), the dose can be increased.

7.3 Optimum dose response

The optimum individual dose response at the beginning of treatment can be recognised by at least one of the following reactions. It is to be expected that these local and temperature reactions will weaken or disappear entirely during the course of ISCADOR therapy.

- **Changes to subjective wellbeing** indicate that the dosage has been administered in an optimum range. These may include an improvement in general wellbeing, increase in appetite and body weight, normalisation of sleep, sensation of warmth, improved performance and mental state (such as brightening of mood), greater courage in the face of problems, increased initiative, and pain relief.
- **Temperature response** in the form of an increase in body temperature around 5 hours after injection, restoration of the physiological morning/evening difference of at least 0.5°C



Fig. 51: Typical local skin reaction with induration and local hyperthermia

(0.9°F), or an increase in mean body temperature during treatment through to normalisation of thermoregulation (< 38°C/100.4°F).

- **Immunological reaction:** leukocytes ↑; lymphocytes ↑; initially also eosinophils ↑ (± NK CD16/56). Blood sampling takes place at the start of therapy and then on the day after the 7th and 14th injection in the morning (24 hours after the ISCADOR injection).
- **Local inflammatory reaction:** erythema with a maximum diameter of 5 cm or 2 inches at the injection site, also with induration, itching, swelling or local hyperthermia. These reactions generally spontaneously disappear after one to two days. But the absence of a local reaction is not a sign of reduced efficacy.

7.4 Maintenance phase

The initial phase is followed by long-term use of the individual optimum concentration. During this maintenance phase therapy, a rhythmically changing dose (ISCADOR series) or constant dose (ISCADOR single strengths) can be chosen, whereby both therapy concepts are recommended for all tumour localisations and stages.

The target dose in the maintenance phase has been reached when at least one of the responses described under point 7.3 occurs. Therapy is continued with the series which represents the highest strength for the optimum dose response. For example, if treatment is undertaken with series I and a local reaction is observed with the last 3 ampoules of this series (10 mg), then series I is the preparation of choice. Treatment continues with one ampoule from ISCADOR series I, subcutaneously injected two to three times a week. After the second year a treatment pause of one week after every 14 injections can be introduced. If the course of therapy is successful, the treatment-free intervals may be extended with increasing duration of treatment (see also 7.1).

Constant dosage with ISCADOR single strengths is specifically used if an overreaction occurs at the highest concentration level of the respective series, and if continuation with the next

lower series is not seen as sufficient (e.g. overreaction with 10 mg from series I → transfer to ISCADOR 5 mg special). If indicated by the individual dose response (e.g. excessive local reactions) and the course of disease (e.g. if concomitant immunological analysis favours an increase or reduction of dose), partial amounts of one ampoule can also be injected. At advanced stages of the disease, or if the patient feels worse on ISCADOR-free days, it may be useful to inject 1 ampoule daily without a treatment pause.

If the therapy is interrupted for a half year or longer, a restart with series 0 is recommended to prevent overreactions (see also 7.1).

Every 3 to 6 months the dosage should be reviewed in the light of the patient's dose response and the tumour response.

7.5 Indications

Application is guided according to the anthroposophic understanding of the human being and nature. In adults, this includes stimulation of formative and integrating forces in order to dissolve and reintegrate growth processes that have become autonomous; for example in*:

- malignant tumours, also with accompanying impairment of hematopoietic organs
- benign tumours
- defined precancerous disorders
- reducing the risk of tumour recurrence after surgery

* These indications are based on product information provided to the German Health Authorities, and may differ depending on country.

7.6 Side effects

One hundred years of experience and studies conducted with ISCADOR (see section 6) show that therapy with ISCADOR is safe. Only a few patients react with systemic side effects such as dizziness, exhaustion, fever or pruritus; local reactions including indurations, erythema, itching or oedemas occur at or around the injection site in approximately 20% of patients. These reactions are always of mild to medium severity, and subsequently resolve completely.

A slight increase in body temperature and locally restricted inflammatory reactions are seen almost as a matter of course at the subcutaneous injection site at the beginning of therapy and are a sign of the patient's response. Slight, transitory swellings of regional lymph nodes are likewise harmless. If fever increases above 38 °C (possibly with exhaustion, shivering, general feeling of being unwell, headaches and transient dizziness) or if local reactions exceed 5 cm in diameter, the next injection should only be given after these symptoms have abated, and in a reduced strength or dosage. The fever caused by ISCADOR injections should not be suppressed by antipyretics. If fever is persistent for more than 3 days, the possibility of infectious processes or tumour fever should be considered. Excessive local reactions may be avoided by using a lower strength of the preparation or a smaller volume of ISCADOR.

In very rare cases, local or systemic allergic reactions or allergoid reactions may occur (normally as generalised pruritus, urticaria or exanthema, sometimes also as Quincke's oedema, chills, dyspnoea and bronchospasm, sporadically with shock or as erythema exsudativum multiforme) which require discontinuation of the preparation and where appropriate, medical treatment. Activation of previous existing inflammations and inflammatory irritations of superficial veins in the area of injection are possible. In such cases a temporary discontinuation of treatment is necessary until the inflammatory reaction has abated.

Chronic granulomatous inflammations (sarcoidosis, erythema nodosum) and autoimmune diseases (dermatomyositis) have been described during mistletoe therapy. Symptoms of increased intra-cerebral pressure were also described in brain tumours/metastases during mistletoe therapy.

7.7 Combination with standard therapies

7.7.1 Chemotherapy

At optimum dosage, ISCADOR reduces the side effects of chemotherapy and alleviates treatment with cytostatic agents (Bock et al. 2004, Friedel et al. 2009, Kienle and Kiene 2010, Matthes et al. 2009, 2010, Tröger et al. 2009, 2012). It has been found that many patients undergoing chemotherapy no longer needed to be prescribed leukocyte growth factors to avoid neutropenia (Tröger et al. 2009). Their bone marrow had already been sufficiently stimulated by the mistletoe extract to synthesise neutrophil granulocytes. Thus, chemotherapy could be administered without dose reduction. Chemotherapy-induced vomiting, which often significantly limits patients' quality of life, was mitigated in many treatments with ISCADOR (Löwe-Mesch et al. 2008). This means that

many patients do not need any, or hardly any, antiemetics during chemotherapy. ISCADOR also reduces gastrointestinal and cutaneous side effects, which are also a major problem of chemotherapy (Friedel et al. 2009). Local reactions at the injection site may be intensified when combined with chemotherapy, in which case a lower dose of ISCADOR might be indicated (series O).

Interactions between ISCADOR and chemotherapeutics

Subcutaneous administration of mistletoe preparations such as ISCADOR has often been used simultaneously with chemotherapy to improve its therapeutic tolerability and to reduce disease- and therapy-related symptoms (Bock et al. 2004, Friedel et al. 2009, Kienle and Kiene 2004, Kienle 2010, Matthes et al. 2010, Tröger et al. 2009, 2012). This raises the question of interactions between cytostatics and mistletoe preparations, and consequently increasing numbers of studies on this have been conducted recently. It has been known for some time that the constituents of mistletoe preparations are metabolised in the liver and excreted renally as well as via the intestinal tract. Radioactively labelled mistletoe proteins accumulate in the liver and spleen and are also excreted via the intestinal and the urinary tract (Pfeiffer-Wüstinger 1980). However, there is hardly any research on exactly which metabolic pathways are taken, or which catabolic products occur, since mistletoe extracts are multicomponent compounds, and data are mainly available for individual substances.

In a study by Matthes et al. (2005), the metabolic induction activity of mistletoe extracts on liver cells (HepG2 cells) was investigated. For this, extracts of AbnobaVISCUM Quercus, Helixor M and ISCADOR M and Qu special were supplied to the HepG2 cells. Analysis was undertaken of their metabolism in relation to the cytochrome P450-dependent monooxygenase (phase I)-system, as well as in relation to the conjugation of lipophilic substances with glucuronic acid and sulphates as an expression of the phase-II reaction. For the phase-I reaction, the phenoxazone derivative 7-ethoxyresorufin as a specific substrate for CYP 1A1 and 2, was chosen, along with amidopyrine-demethylation, specific for CYP 3A1 and 2. The phase-II reaction was measured by means of p-nitrophenol-UDP-glucuronyltransferase. None of the mistletoe preparations significantly affected the conversion rate by CYP 3A1 and 2. Equally, no induction of CYP 1A1 and 2 occurred. It was found that all preparations enhance p-nitrophenol conjugation. For further questions regarding metabolism of mistletoe extracts in total, it thus appears useful to consider phase-II biotransformation.

An investigation by Engdal and Nilsen (2009) tested whether an inhibition of CYP 3A4 could be induced by herbal medicinal products, including mistletoe extracts (ISCADOR). In this study

it was shown *in vitro* that CYP IIIA4 metabolism was slightly inhibited by ISCADOR; however, no clinical relevance caused by systemic or intestinal interactions was apparent. These results were reproduced in a study by Doehmer and Eisenbraun (2012).

An *in vitro* study by Weissenstein et al. (2014) examined whether clinically relevant ISCADOR concentrations influenced the activity of conventional chemotherapeutic drugs such as doxorubicin, gemcitabine, docetaxel, cisplatin etc. The results showed no impairment by ISCADOR M and Qu special of the cytostatic and cytotoxic effects of these common chemotherapeutic agents in breast, prostate and pancreatic carcinoma cell lines in the experimental configurations used. Another *in vitro* study by Weissenstein et al. (2016) investigated whether ISCADOR has an inhibitory effect on Trastuzumab. Here, the Her-2 positive human breast-cancer line SK-BR-3 was treated with Trastuzumab. Different concentrations of the drug were combined with ISCADOR M in clinically relevant doses. These *in vitro* results showed no risk of ISCADOR inhibiting the antitumoural action of Trastuzumab. On the contrary, it was found that, *in vitro*, ISCADOR and Trastuzumab exert complementary anticancer effects.

While these *in vitro* data are not precisely transferrable to the complex *in vivo* situation, especially in respect of medicinal products administered subcutaneously on the one hand and intravenously or orally on the other, they can contribute to current findings on the safety of cancer patients who receive mistletoe therapy in combination with a chemotherapy.

Pfeifer et al. (2006) confirm that complementary mistletoe therapy is safe in combination with simultaneous chemo-, radiotherapy and/or hormone therapy as well as before, during and after tumour surgery. In the follow-up study by Tröger et al. (2012), likewise, there was no indication of reduced efficacy of chemotherapy for disease-free survival up to five years after the end of therapy when mistletoe extracts were given simultaneously (see also 6.3.1).

7.7.2 Radiotherapy

In principle, a combination of radiotherapy and ISCADOR is useful and to be recommended as ISCADOR reduces the side effects of radiotherapy. However, injection sites must not be in the irradiated areas.

7.7.3 Hormone/antihormone therapy

ISCADOR can be combined with hormone/antihormone therapy, as it does not limit their effectiveness (Tröger et al. 2012). Side effects of this treatment can also be reduced by ISCADOR. However, dosage has to be assessed and may need adjusting (reducing).

7.8 Interactions with other drugs

When ISCADOR is applied simultaneously with immunosuppressive therapies such as corticosteroids or bisphosphonates, immune monitoring should be carried out or the dosage should be adjusted (reduced). Studies on interactions with other immunomodulatory agents are not available. If such preparations are used in close chronological sequence, it is advisable to exercise caution with dosage and to monitor the appropriate immune parameters (see 7.3). Nothing contraindicates simultaneous therapy with monoclonal antibodies (e.g. Trastuzumab). In fact, this combination appears appropriate given the different immunological approaches involved (see 7.7.1).

7.9 Contraindications

- Known allergy to mistletoe preparations
- Acute inflammatory diseases or high fever: treatment should be discontinued until the signs of inflammation subside
- Chronic granulomatous diseases, florid autoimmune diseases, and diseases treated with immunosuppressive drugs
- Hyperthyroidism with tachycardia

8 References

- Antony S et al.: Effect of *Viscum album* in the inhibition of lung metastasis in mice induced by B16F10 melanoma cells. J Exp Clin Cancer Res 16, 159-162 (1997)
- Antony S et al.: Inhibition of lung metastasis by adoptive immunotherapy using Iscador. Immunol Invest 28, 1-8 (1999)
- Antony S et al.: Role of natural killer cells in Iscador mediated inhibition of metastasis by adoptive immunotherapy. Immunol Invest 29, 219-231 (2000)
- Augustin M et al.: Safety and efficacy of the long-term adjuvant treatment of primary intermediate- to high-risk malignant melanoma (UICC/AJCC stage II and III) with a standardized fermented European mistletoe (*Viscum album* L.) extract. Results from a multicenter, comparative, epidemiological cohort study in Germany and Switzerland. Arzneim-Forsch/Drug Res 55, 38-49 (2005)
- Barbieri L et al.: Polynucleotide adenosine glycosidase activity of ribosome-inactivating proteins: effect on DNA, RNA and poly(A). Nucleic Acids Res 25, 518-522 (1997)
- Bar-Sela G et al.: 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 49(5), 1058-64. (2013)
- Baumgartner S et al.: Investigation of the Iscador production process in model systems of cytotoxicology and phytopathology. In Scheer R et al. (Ed.): Fortschritte in der Misteltherapie. KVC Verlag, Essen, 169-179 (2005)
- Becker H: Botany of European mistletoe (*Viscum album* L.). Oncology 43 (Suppl. 1), 2-7 (1986)
- Becker H and Scher JM: Short survey of the main natural components of European mistletoe (*Viscum album* L.). In Scheer R et al. (Ed.): Fortschritte in der Misteltherapie. KVC Verlag, Essen, 3-11 (2005)
- Berg PA and Stein GM: Beeinflusst die Misteltherapie die Abwehr epithelialer Tumoren? Eine kritische immunologische Analyse. Dtsch Med Wschr 126, 339-345 (2001)
- Bock PR et al.: Efficacy and safety of long-term complementary treatment with standardised European mistletoe extract (*Viscum album* L.) in addition to the conventional adjuvant oncological therapy in patients with primary non-metastatic breast cancer. Arzneim-Forsch/Drug Res 54 (8), 456-466 (2004)
- Bock PR et al.: Treatment with a fermented mistletoe (*Viscum album* L.) extract as part of long-term supportive care in patients with primary non-metastatic colorectal carcinoma. Phytomedicine 14 (2), 43 (2007)
- Bock PR et al.: Targeting inflammation in cancer-related-fatigue: a rationale for mistletoe therapy as supportive care in colorectal cancer patients. Inflammation & Allergy - Drug Targets 13, 105-111 (2014)
- Braedel-Ruoff S: Immunomodulatory effects of *Viscum album* extracts on natural killer cells: review of clinical trials. Forsch Komplementmed 17, 63-73 (2010)
- Büssing A et al.: DNA-stabilisierende Wirkungen von *Viscum album* L. – Sind Mistelextrakte als Adjuvans während der konventionellen Chemotherapie indiziert? Forsch Komplementärmed 3, 244-248 (1996)
- Büssing A et al.: Entwicklung lymphozytärer Subpopulationen bei Tumorpationen nach subkutaner Applikation von Mistelextrakten. Forsch Komplementärmed 6, 196-204 (1999)
- Büssing A: Biological and pharmacological properties of *Viscum album* L. In Büssing A (Ed.): Mistletoe - The Genus *Viscum*. Harwood Academic Publishers, Amsterdam, 123-182 (2000)
- Büssing A: Die Mistel in der adjuvanten Tumorthherapie. In Heiß G (Ed.): Krebs was nun? Leben mit Krebs. Perspektiven in das 21. Jahrhundert. Merz-Verlag, Darmstadt, 551-560 (2001)
- Büssing A: *Viscum album* L. – mechanisms of cytotoxicity. In Scheer R et al. (Ed.): Die Mistel in der Tumorthherapie. KVC Verlag, Essen, 121-134 (2001)
- Büssing A et al.: Granulozytenfunktions-Beeinflussung durch eine einmalige perioperative Mistelextrakt-Infusion. Deutsche Zeitschrift für Onkologie 36, 148-153 (2004)
- Büssing A et al.: Prevention of surgery-induced suppression of granulocyte function by intravenous application of a fermented extract from *Viscum album* L. in breast cancer patients. Anticancer Res 25, 4753-4758 (2005)
- Büssing A et al.: No stimulation of *in vitro* cultured tumour cells by mistletoe lectin. In Scheer R et al. (Ed.): Fortschritte in der Misteltherapie. KVC Verlag, Essen, 281-290 (2005)
- Büssing A and Schietzel M: Apoptosis-inducing properties of *Viscum album* L. extracts from different host trees, correlate with their content of toxic mistletoe lectins. Anticancer Res. 19, 23-28 (1999)
- Carlsson M et al.: A five-year follow-up of quality of life in women with breast cancer in anthroposophic and conventional care. eCAM Evidence-based Complementary and Alternative Medicine 3 (4), 523-531 (2006)
- Concato J et al.: Randomized, controlled trials, observational studies, and the hierarchy of research designs. N Engl J Med 342, 1887-1892 (2000)
- Debreczeni JE et al.: Structure of viscotoxin A3: disulfide location from weak SAD data. Acta Cryst D59, 2125-2132 (2003)
- Doehmer J and Eisenbraun J: Assessment of extracts from mistletoe (*Viscum album*) for herb-drug interaction by inhibition and induction of cytochrome P450 activities. Phytother Res 26, 11-17 (2012)
- Dold U et al. (Ed.): Krebszusatztherapie beim fortgeschrittenen nicht kleinzelligen Bronchialkarzinom. Georg Thieme Verlag, Stuttgart, New York, 1-144 (1991)
- Elluru S et al.: Molecular Mechanisms underlying the immunomodulatory effects of mistletoe (*Viscum album* L.) extracts Iscador. Arzneim-Forsch/Drug Res 56 (6a), 461-466 (2006)
- Elluru S et al.: Tumor regressive effects of *Viscum album* preparations – Exploration of immunomodulatory mechanisms. Medicina 67 (Suppl. II), 85-89 (2007)
- Elluru S et al.: Induction of maturation and activation of human dendritic cells: A mechanism underlying the beneficial effect of *Viscum album* as complementary therapy in cancer. BMC Cancer 8, 161-168 (2008)
- Elsässer-Beile U et al.: Biological effects of natural and recombinant mistletoe lectin and an aqueous mistletoe extract on human monocytes and lymphocytes *in vitro*. J Clin Lab Anal 14, 255-259 (2000)
- Engdal S and Nilsen OG: *In vitro* inhibition of CYP3A4 by herbal remedies frequently used by cancer patients. Phytother Res 23, 906-912 (2009)
- Fischer A: Charakterisierung der immunmodulatorischen Wirkung von Mistelpräparaten auf Zellen des Immunsystems von Rindern. Dissertation, Hannover (2006)
- Fischer S: *In vitro*-Versuche zur T-Zellaktivität. Hippokrates Verlag, Stuttgart, 168 (1996)
- Flückiger H and Baumgartner S: Auswirkungen des Iscador-Maschinenprozesses auf Mistelextrakte in botanischen Untersuchungssystemen. Merkurstab 56 (3), 114-121 (2003)
- Franz M et al.: Modulation of mistletoe lectins cytotoxicity by carbohydrates and serum glycoproteins. Arzneim-Forsch/Drug Res 50, 471-478 (2000)
- Franz M: Struktur und biologische Aktivitäten der chitinbindenden Mistellektine. Dissertation, Tübingen (2003)
- Friedel WE et al.: Fermented European mistletoe (*Viscum album* L.) extract in supportive care in patients with primary non-metastatic colorectal carcinoma. In Scheer R et al. (Ed.): Die Mistel in der Tumorthherapie 2. KVC Verlag, Essen, 563-576 (2009)
- Friedel WE et al.: Clinically relevant survival benefit from supportive treatment with fermented European pine tree mistletoe extract in primary malignant melanoma patients. 7th World Congress on Melanoma and 5th Congress of the European Association of Dermato-Oncology (EADO), Vienna May (2009a)
- Friedel WE et al.: 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. J Soc Integrat Oncol 7 (4), 137-145 (2009b)

Giudici M et al.: Membrane activity of viscotoxins A3 and B – interactions with membrane model systems. In Scheer R et al. (Ed.): Fortschritte in der Misteltherapie. KVC Verlag, Essen, 99-107 (2005)

Grossarth-Maticek R et al.: 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. *Altern Ther Health Med* 7, 57-78 (2001)

Grossarth-Maticek R and Ziegler R: Prospective controlled cohort studies on long-term therapy of breast cancer patients with a mistletoe preparation (Iscador). *Forsch Komplementärmed* 13, 285-292 (2006)

Grossarth-Maticek R and Ziegler R: Wirksamkeit und Unbedenklichkeit einer Langzeitbehandlung von Melanompatienten mit einem Mistelpräparat (Iscador). *Schweiz Z GanzheitsMed* 19, 325-332 (2007)

Grossarth-Maticek R and Ziegler R: Randomized and non-randomized prospective controlled cohort studies in matched pair design for the long-term therapy of corpus uteri cancer patients with a mistletoe preparation (Iscador). *Eur J Med Res* 13, 107-120 (2008)

Hajtő T et al.: Difficulties and perspectives of immunomodulatory therapy with mistletoe lectins and standardized mistletoe extracts in evidence-based medicine. *eCAM*, 1-6 (2009)

Heim ME, Feyer P: Das tumorassoziierte Fatigue-Syndrom. *Journal Onkologie* 1, 42-47 (2011)

Heinzerling L et al.: Immunologic effector mechanisms of a standardized mistletoe extract on the function of human monocytes and lymphocytes *in vitro*, *ex vivo*, and *in vivo*. *J Clin Immunol* 26, 347-359 (2006)

Huber R et al.: Häufigkeit grippaler Infekte bei Gesunden unter Gabe eines lektinreichen und eines lektinarmen Mistelpräparats im Rahmen einer randomisierten, doppelblinden, placebokontrollierten Studie. *Forsch Komplementärmed* 8, 354-358 (2001)

Huber R et al.: Mistletoe treatment induces GM-CSF- and IL-5 production by PBMC and increases blood granulocyte and eosinophil counts: a placebo controlled randomized study in healthy subjects, *Eur J Med Res* 10, 411-418 (2005)

Huber R et al.: *In vitro* immunoreactivity towards lectin-rich or viscotoxin-rich mistletoe (*Viscum album* L.) extracts Iscador applied to healthy individuals. *Arzneim-Forsch/Drug Res* 56 (6a), 447-456 (2006)

Huyen van JPD et al.: Interleukin-12 is associated with the *in vivo* anti-tumor effect of mistletoe extracts in B16 mouse melanoma. *Cancer Letters* 243, 32-37 (2006)

Iscador AG: Iscador in cancer therapy – recommendations for treatment, 1st Edition (2016)

Kabisch M et al.: Randomized controlled trials: part 17 of a series on evaluation of scientific publications. *Dtsch Arztebl Int*;108(39): 668 (2011). DOI: 10.3238/arztebl.2011.066

Katalinic A et al.: Krebs in Deutschland 2011/2012, 10. Ausgabe. Robert Koch-Institut und Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V. (Ed.), Berlin (2015)

Kelter G and Fiebig HH: Absence of tumor growth stimulation in a panel of 26 human tumor cell lines by mistletoe (*Viscum album* L.) extracts Iscador *in vitro*. *Arzneim-Forsch/Drug Res* 56 (6a), 435-440 (2006)

Kienle GS et al.: Mistletoe in cancer – a systematic review on controlled clinical trials. *Eur J Med Res* 8, 109-119 (2003)

Kienle GS: Informationsseiten zur Misteltherapie bei Krebs. www.mistel-therapie.de, wissenschaftlicher Teil (2014)

Kienle GS and Kiene H: Die Mistel in der Onkologie – Fakten und konzeptionelle Grundlagen. Schattauer Verlag, Stuttgart (2003)

Kienle GS and Kiene H: Klinische Studien zur Misteltherapie onkologischer Erkrankungen. *Deut Zeitschr Onkol* 1, 17-24 (2004)

Kienle GS and Kiene H: Influence of *Viscum album* L. (European mistletoe) extracts on quality of life in cancer patients: a systematic review of controlled clinical studies. *Integr Cancer Ther* 9 (2), 142-157 (2010)

Kjell Benson BA and Hartz AJ: A comparison of observational studies and randomized, controlled trials. *N Engl J Med* 342, 1878-1886 (2000)

Kleeberg U et al.: Final results of the EORTC 18871/DKG 80-1 randomised phase III trial: rIFN- α 2b versus rIFN- γ versus Iscador M versus observation after surgery in melanoma patients with high-risk primary (thickness > 3 mm) or regional lymph node metastasis. *Eur J Cancer* 40, 390-402 (2004)

Klein R et al.: *In vivo*-induction of antibodies to mistletoe lectin-1 and viscotoxin by exposure to aqueous mistletoe extracts: a randomised double-blinded placebo controlled phase I study in healthy individuals. *Eur J Med Res* 7, 155-163 (2002)

Klein R et al.: Demonstration of antibodies to the chitin-binding mistletoe lectin (cbML) in tumor patients before and during therapy with an aqueous mistletoe extract. *Eur J Med Res* 9 (3), 316-322 (2004)

Klein R: Effects of mistletoe extracts on immunocompetent cells *in vitro* and *in vivo* and their relevance for the therapy of tumor diseases and other clinical entities. In Scheer R et al. (Ed.): Fortschritte in der Misteltherapie. KVC Verlag, Essen, 207-221 (2005)

Klein R: Effects of mistletoe extracts on immunocompetent cells *in vitro* and *in vivo*. In Scheer R et al. (Ed.): Die Mistel in der Tumorthherapie 2. KVC Verlag, Essen, 185-193 (2009)

Kovacs E et al.: Cytostatic and cytotoxic effects of mistletoe (*Viscum album* L.) quercus extract Iscador. *Arzneim-Forsch/Drug Res* 56 (6a), 467-473 (2006)

Krauspenhaar R et al.: Crystal structure of mistletoe lectin I from *Viscum album*. *Biochemical and Biophysical Research Communications* 257, 418 – 424 (1999)

Kreis W: Advances in structure elucidation of mistletoe constituents. In Scheer R et al. (Ed.): Die Mistel in der Tumorthherapie 2. KVC Verlag, Essen, 17-29 (2009)

Kuehn JJ: Treatment responses to *Viscum album* pini (Iscador P) in non-Hodgkin's lymphoma exploring a new therapeutic route. *Medicina* 67 (Suppl. II), 107-114 (2007)

Kuehn JJ: Efficacy and risk estimation of subcutaneous mistletoe treatment (*Viscum album* L. Pini) in patients with non-Hodgkin's Lymphoma, a retrospective controlled case study. In Scheer R et al. (Ed.): Die Mistel in der Tumorthherapie 2. KVC Verlag, Essen, 353-373 (2009)

Kuehn JJ and Fornalski M: Beeinflussung immunkompetenter Zellen des peripheren Blutes durch *Viscum album* (Iscador M) bei Patientinnen mit Mammakarzinom. In Kropiunigg U and Stacher A (Ed.): Ganzheitsmedizin und Psycho-Neuroimmunologie. Vierter Wiener Dialog. Facultas, Wien, 396-407 (1997)

Kuttan G et al.: Isolation and identification of a tumor reducing component from mistletoe extract (Iscador). *Cancer Lett* 41, 307-314 (1988)

Lawrence DP et al.: Evidence report on the occurrence, assessment, and treatment of fatigue in cancer patients. *J Natl Cancer Inst Monogr*, 40-50 (2004)

Lee RT et al.: The sugar-combining area of the galactose-specific toxic lectin of mistletoe extends beyond the terminal sugar residue: comparison with a homologous toxic lectin, ricin. *Carbohydr Res* 254, 269-276 (1994)

Loewe-Mesch A et al.: Adjuvante simultane Mistel-/Chemotherapie bei Mammakarzinom – Einfluss auf Immunparameter, Lebensqualität und Verträglichkeit. *Forsch Komplementmed* 15, 22-30 (2008)

Longhi A et al.: A randomized study with adjuvant mistletoe versus oral Etoposide on post relapse disease-free survival in osteosarcoma patients. *Eur J Integr Med* 1, 31-39 (2009)

Longhi et al.: A randomized study on postrelapse disease-free survival with adjuvant mistletoe versus oral Etoposide in osteosarcoma patients. *Evidence-Based Complementary and Alternative Medicine*, Volume 2014, Article ID 210198, 9 pages, <http://dx.doi.org/10.1155/2014/210198>

Maldacker J: Preclinical investigations with mistletoe (*Viscum album* L.) extract Iscador. *Arzneim-Forsch/Drug Res* 56 (6a), 497-507 (2006)

Matthes B et al.: Metabolic characterisation of mistletoe preparations in the liver cell (HepG2). In Scheer R et al. (Ed.): Fortschritte in der Misteltherapie. KVC Verlag, Essen, 191-203 (2005)

Matthes H et al.: Improvement of the palliative supportive care in pancreatic carcinoma patients with a fermented mistletoe (*Viscum album* L.) extract treatment. *Phytomedicine* 14 (2), 54 (2007)

Matthes H et al.: Supportive care in pancreatic carcinoma patients treated with a fermented mistletoe (*Viscum album* L.) extract. In Scheer R et al. (Ed.): Die Mistel in der Tumorthherapie 2. KVC Verlag, Essen, 551-562 (2009)

Matthes H et al.: Molecular mistletoe therapy: friend or foe in established anti-tumour protocols? A multi-center, controlled, retrospective pharmaco-epidemiological study in pancreas cancer. *Current Molecular Medicine* 10, 430-439 (2010)

Ostermann T et al.: Survival of cancer patients treated with mistletoe extract (Iscaidor): a systematic literature review. *BMC Cancer* 9, 451-460 (2009)

Pal A: Structure determination of Viscotoxin A1, Tendamistat and Tri Peptidyl Peptidase-I. Dissertation, Göttingen (2008)

Petrusch U.: Eine kritische Betrachtung neuer Immuntherapien. *Leading Opinions Hämatologie & Onkologie. ASCO* 4, 53-55 (2016)

Pfeifer B et al.: Onkologie integrativ. Elsevier, Urban & Fischer Verlag, München (2006)

Pfeiffer-Wüstinger G: „Mistelprotein“: Markierung mit Radiojod und Biodistribution an Ratten. *Swiss Pharma* 2, 21-22 (1980)

Pfüller U: Chemical constituents of European mistletoe (*Viscum album* L.). In Büssing A (Ed.): Mistletoe – The Genus *Viscum*. Harwood Academic Publishers, Amsterdam, 101-122 (2000)

Ramm H: Zur Entwicklung der weißbeerigen Mistel (*Viscum album* L.): Bildung des Haustorialsystems. *Mistilteinn* 1, 2-13 (2000)

Ramm H: Zur Entwicklung der weißbeerigen Mistel (*Viscum album* L.): Bildeprozesse im Mistelspross. *Mistilteinn* 2, 2-11 (2001)

Ramm H: Zur Entwicklung der weißbeerigen Mistel (*Viscum album* L.): Bildeprozesse in Blüte, Frucht und Same. *Mistilteinn* 3, 32-41 (2002)

Ramm H: Zum Verhalten mistelverbreitender Vögel. *Mistilteinn* 5, 54-67 (2005)

Ramm H: Mistletoe through Cultural and Medical History: The All-Healing Plant Proves to Be a Cancer-Specific Remedy. In: Zänker KS, Kaveri ZV (Ed.): Mistletoe: From Mythology to Evidence-Based Medicine. Karger, Basel, 1-10 (2015)

Ramm H: Geisteswissenschaftliche Aspekte zur Mythologie und Biologie der Mistel. In: Selg P, Orange M, Ramm H, Poehrtger S (Ed.): Mistelforschung und Krebstherapie. Verlag des Ita Wegman Instituts, Arlesheim, 125-154 (2016)

Ramm H: Zum Verhalten mistelverbreitender Vögel. In: Ramm H, Urech K (Ed.): Aus der Arbeit mit der Mistel. Erfahrungen, Ergebnisse, Perspektiven. Verlag des Ita Wegman Instituts, Arlesheim, 145-161 (2017)

Ramm H et al.: Cultivation and development of *Viscum album* L. In Büssing A (Ed.): Mistletoe – The Genus *Viscum*. Harwood Academic Publishers, Amsterdam, 75-94 (2000)

Ramm H et al.: Weißbeerrige Mistel (*Viscum album* L.). In: Meyer U, Pedersen AP (Ed.): Anthroposophische Pharmazie. Grundlagen, Herstellungsprozesse, Arzneimittel. Salumed Verlag, Berlin, 239-261 (2016)

Reinmuth N: Immuntherapie beim Lungenkarzinom. *Journal Onkologie* 7, 530-532 (2016)

Ribereau-Gayon G et al.: Effects of mistletoe (*Viscum album* L.) extracts on cultured tumor cells. *Experientia* 42, 594-599 (1986)

Rothschild, S: Targeted therapies in non-small cell lung cancer – beyond EGFR and ALK. *Cancer* 7 (2), 930-949 (2015)

Rüfer JU, Flechtner H: Fatigue – Diagnostik, klinische Implikationen und Therapie. *Onkologie* 12, 36-40 (2006)

Saller R et al.: Misteltherapie – Erwünschte und unerwünschte Wirkungen in der wissenschaftlichen Diskussion, Teil 1. *Praxismagazin* 12, 6-14 (2004)

Saller R et al.: Misteltherapie – Erwünschte und unerwünschte Wirkungen in der wissenschaftlichen Diskussion, Teil 2. *Praxismagazin* 2, 6-17 (2005)

Sander F: Wirkung des Mistlektin-I auf das Wachstums- und Metastasierungsverhalten der humanen Melanomzelllinie MV3 *in vivo* in einem Xenograftmodell mit scid- und pfp/rag2-Mäusen. Dissertation, Hamburg 2008

Salzer G: Pleura Carcinosis. Cytomorphological findings with the mistletoe preparation Iscaidor and other pharmaceuticals. *Oncology* 43, 66-70 (1986)

Schink M and Borowsky M: Cytotoxic effects of mistletoe lectins and a mistletoe preparation on human natural killer cells *in vitro*. In Scheer et al. (Ed.): Die Mistel in der Tumorthherapie. KVC Verlag, Essen, 163-176 (2001)

Schink M et al: Zusammenhang der NK-Zellaktivität gegen autologe Tumor- und K562-Zellen mit dem klinischen Verlauf unter Misteltherapie. *Forsch Komplementärmed* 13, 147-155 (2006)

Schink M et al.: Mistletoe extracts reduces the surgical suppression of natural killer cell activity in cancer patients. A randomized phase III trial. *Forsch Komplementärmed* 14, 9-17 (2007)

Schink M et al.: Effect of a perioperative infusion of mistletoe extract on surgery-induced immunosuppression in colorectal cancer patients. In Scheer et al. (Ed.): Die Mistel in der Tumorthherapie 2. KVC Verlag, Essen, 441-454 (2009)

Schleyerbach PW: Verlauf immunologischer Parameter bei Mammakarzinom unter Therapie mit Mistel-Extrakten: eine Anwendungsbeobachtung. Dissertation, Tübingen (2004)

Seidel D: A Genomics-based classification of human lung tumors. *Science Translational Medicine* 5, 209ra153 (2013)

Selg P: Mensch und Mistel. Die Begründung der onkologischen *Viscum*-Behandlung durch Rudolf Steiner und Ita Wegman. Band 1, 1917-1925. Salumed Verlag, Berlin (2016)

Sood A et al.: A critical review of complementary therapies for cancer-related fatigue. *Integr Cancer Ther* 6 (1), 8-13 (2007)

Stauder G et al: Improved quality of life and survival time by supportive long-term treatment with fermented mistletoe (*Viscum album* L.) extract from oak tree (*quercus*) in patients with pancreatic cancer on any stage. ESMO Conference and 11th World Congress on Gastrointestinal Cancer, Barcelona June (2009)

Stein GM et al.: Induction of anti-mistletoe lectin antibodies in relation to different mistletoe-extracts. *Anti-Cancer Drugs* 8 (Suppl. 1), 57-59 (1997)

Stein GM et al.: Thionins from *Viscum album* L: influence of the viscotoxins on the activation of granulocytes. *Anticancer Res* 19, 1037-1042 (1999)

Stein GM et al.: Polysaccharides and viscotoxins – immunological effects and interactions. In Scheer R et al. (Ed.): Die Mistel in der Tumorthherapie. KVC Verlag, Essen, 145-161 (2001)

Stein G and Berg PA: Mistletoe extract-induced effects on immunocompetent cells: *in vitro* studies. *Anticancer Drugs* 8, Suppl 1, 39-42 (1997)

Steiner R: Aus Besprechungen mit praktizierenden Ärzten, Dornach, 22. April 1924. In Zbinden (Ed.): Physiologisch-Therapeutisches auf Grundlage der Geisteswissenschaft (GA 314). Verlag der Rudolf-Steiner-Nachlassverwaltung, Dornach, 285-288 (1965)

Stettin A et al.: 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*. *Klin Wschr* 68, 896-900 (1990)

Stumpf C and Büssing A: Stimulation of antitumour immunity by intrapleural instillation of a *Viscum album* L. extract. *Anticancer Drugs*, Suppl 1, 23-26 (1997)

Tabiasco J et al.: Mistletoe viscotoxins increase natural killer cell-mediated cytotoxicity. *Eur J Biochem* 269, 2591-2600 (2002)

Thronicke A. et al: Clinical safety of combined therapy of immune checkpoint inhibitors and *Viscum album* L. therapy in patients with advanced or metastatic cancer (submitted 2016)

Tröger W et al.: 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 – Basic and Clinical Research* 3, 35-45 (2009)

Tröger W et al.: Five-year follow-up of patients with early stage breast cancer after a randomized study comparing additional treatment with *Viscum album* (L.) extract to chemotherapy alone. *Breast Cancer: Basic and Clinical Research* 1, 173-180 (2012)

Tröger W et al.: *Viscum album* [L.] extract therapy in patients with locally advanced or metastatic pancreatic cancer: A randomised clinical trial on overall survival. *Eur J Cancer* 49, 3788-3797 (2013)

Tröger W et al.: Quality of life of patients with advanced pancreatic cancer during treatment with mistletoe. *Dtsch Arztebl Int* 111 (29-30), 493-502 (2014)

Unger C and Hildenbrand B: Das Immunsystem und die Immunabwehr von Krebs. In Unger C and Weis J (Ed.): *Onkologie – Unkonventionelle und supportive Therapiestrategien*. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 79-96 (2004)

Urech K: Viscin – verstecktes pharmakologisches Potenzial der Mistel (*Viscum album* L.). *Mistilteinn* 4, 16-29 (2003)

Urech K et al.: Apoptosis inducing activity of viscin, a lipophilic extract from *Viscum album* L. *J Pharm Pharmacol* 57, 101-109 (2005)

Urech K et al.: Triterpenes of mistletoe (*Viscum album*) in the "bird-lime" viscin and its antiproliferative activity. In Scheer R et al. (Ed.): *Fortschritte in der Misteltherapie*. KVC Verlag, Essen, 133-144 (2005)

Urech K et al.: Viscotoxins, mistletoe lectins and their isoforms in mistletoe (*Viscum album* L.) extracts Iscador. *Arzneim-Forsch/Drug Res* 56 (6a), 428-434 (2006)

Urech K et al.: Viscotoxin and mistletoe lectin contents in *Viscum album* L. – pharmaceutical implications. In Scheer R et al. (Ed.): *Die Mistel in der Tumorthherapie* 2. KVC Verlag, Essen, 67-77 (2009)

Wagner R: *Fragen und Antworten zur Misteltherapie*. Verlag Johannes M. Meyer, Stuttgart (2007)

Weissenstein U et al.: Interaction of standardized mistletoe (*Viscum album*) extracts with chemotherapeutic drugs regarding cytostatic and cytotoxic effects *in vitro*. *BMC Complementary and Alternative Medicine* 14, 6 (2014)

Weissenstein U et al.: Interaction of a standardized mistletoe (*Viscum album*) preparation with antitumor effects of Trastuzumab *in vitro*. *BMC Complementary and Alternative Medicine* 16, 271 (2016)

Willich S: Randomisierte kontrollierte Studien - Pragmatische Ansätze erforderlich. *Dtsch Arztebl*; 103(39): A 2524-9 (2006)

Wode K et al: Mistletoe treatment in cancer-related fatigue: a case report. *Cases Journal* 2, 77 (2009)

Wong CK et al.: Intracellular signal transduction in eosinophils and its clinical significance. *Immunopharmacol Immunotoxicol* 24, 165-186 (2002)

Zaenker KS et al. A specific mistletoe preparation (Iscador Qu) in colorectal cancer (CRC) Patients: More than just supportive care? *J Cancer Sci Ther* 4, 264-270 (2012). doi:10.4172/1948-5956.1000153

Ziegler R and Grossarth-Maticek R: Individual patient data meta-analysis of survival and psychosomatic self-regulation from published prospective controlled cohort studies for long-term therapy of breast cancer patients with a mistletoe preparation (Iscador). *eCAM*, 1-10 (2008)

9 Drug information

Based on the product information submitted to the German Health Authorities

ISCADOR solution for injections

Active ingredient: fermented, aqueous mistletoe extract.

Composition: fermented, aqueous extract from *Viscum album* from various host trees. Other ingredients: sodium chloride and water for injection.

Indications: according to the anthroposophic understanding of human being and nature. This includes in adults: stimulation of formative and integrating forces in order to dissolve and reintegrate growth processes that have become autonomous, for example in malignant tumours, also with accompanying impairment of haematopoietic organs; in benign tumours; in defined precancerous disorders; for prevention of tumour recurrence after surgery.

Contraindications: known allergy to preparations of European mistletoe (*Viscum album* L.). Disorders accompanied by acute inflammation or high fever. Chronic granulomatous diseases, florid autoimmune diseases, and diseases treated with immunosuppressive drugs. Hyperthyroidism with tachycardia.

Side effects: a slight increase in body temperature, locally restricted inflammatory reactions around the subcutaneous injection site occur at the beginning of therapy regularly and are signs of the response situation of the patient. Transient, slight swellings of regional lymph nodes are also harmless phenomena. Fever induced by ISCADOR injection should not be repressed with antipyretics. Should fever last longer than 3 days the possibility of infectious processes or tumour fever must be taken into consideration. If fever increases over 38°C or 100.4°F (possibly with lassitude, shivering, generally feeling unwell, headache and transient dizziness) or if local skin reactions exceed 5 cm or 2 inch in diameter, the next injection should only be given after these symptoms have abated and at a reduced strength or dose. Local or systemic allergic or allergoid reactions may occur (normally as generalised pruritus, urticaria or exanthema, occasionally also with Quincke's oedema, chills, dyspnoea and bronchospasm, sporadically with shock or as erythema exsudativum multiforme which require discontinuation of the preparation and immediate medical treatment. Activation of previously existing inflammations as well as inflammatory irritations of superficial veins in the area of injection are possible. In such cases also a temporary discontinuation of treatment is necessary until the inflammatory reaction subsided. Chronic granulomatous inflammations (sarcoidosis, erythema nodosum) and autoimmune diseases (dermatomyositis) have been described during mistletoe therapy. Symptoms of increased intracerebral pressure during mistletoe therapy were also described in patients with brain tumours/brain metastases.

Pharmaceutical forms and packs: solution for injection in series packs: 2 x 7 ampoules cont. 1 ml (bundle package)*, series 0 also 1 x 7 ampoules cont. 1 ml*. Solution for injection in single strength packs: 1 x 7 ampoules cont. 1 ml.

Special precautions for storage: Store in a refrigerator at temperatures between + 2°C and + 8°C or 36 °F and 46 °F.

State of information: November 2015

* not available in all countries

Iscador AG, 79539 Lörrach

10 Imprint and contact

© Iscador AG, 1st edition, August 2018



Editor	Iscador AG		
Headquarter	Iscador AG Kirschweg 9 CH-4144 Arlesheim Switzerland	Phone	+41 (0)61 706 72 22
		Fax	+41 (0)61 706 72 33
			info@iscador.ch
			www.iscador.ch
Branch office	Iscador AG Spitalstraße 22 DE-79539 Lörrach Germany	Phone	+49 (0)7621 162 26 00
		Fax	+49 (0)7621 162 26 01
			info@iscador.de
			www.iscador.de

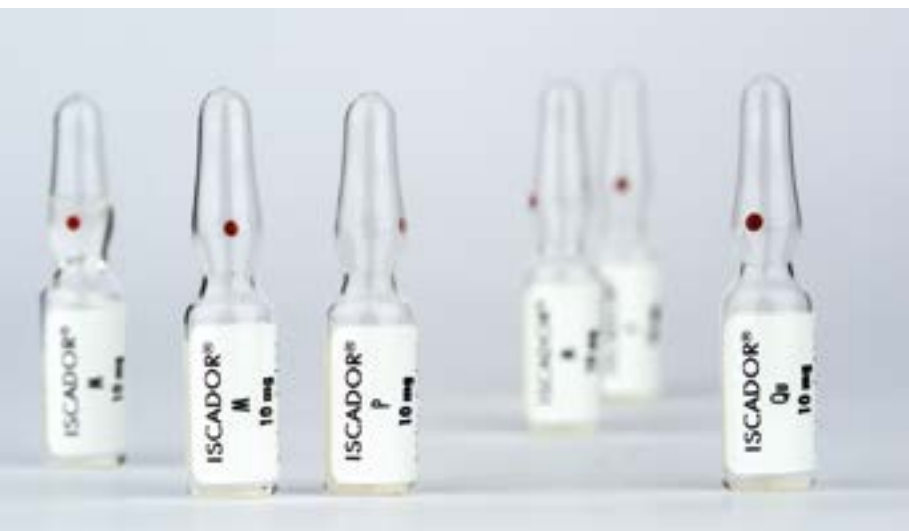
Infoline for professionals & Pharmacovigilance (free of charge)

Switzerland	Phone	0800 706 700
	Fax	+41 (0)61 706 75 33
		infoline@iscador.ch
		pv@iscador.ch
Germany	Phone	0800 706 700 0
	Fax	+49 (0)7621 162 26 02
		infoline@iscador.de
		pv@iscador.de
International	Phone	00800 706 700 00
		infoline@iscador.com
		www.iscador.com

Note

This brochure «ISCADOR in Cancer Therapy – Scientific Information and Study Results» is subject to copyright laws. Reprint, registration with online services and internet as well as reproduction on data storage media, also in part, only with prior written consent of Iscador AG.

All information and recommendations were compiled with utmost care in accordance with current state of the art. Errors and misprints expected. Guarantee and liability claims of any kind are excluded.



F_WLDE_en_1.0_11.2018

ISCADOR  AG

Solutions for Integrative Oncology

www.iscador.com