SURE_J: Science Undergraduate Research Journal

Volume 4 | Issue 1 Article 1

January 2022

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An Investigation into use of Natural Mistletoe Extracts and their Formulations as Potential Anti-Cancer Treatments in Human Carcinomas

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Received 21st April 2022, Accepted for publication 14th June 2022, Published 14th October 2022.

Abstract
Cancer is a prevalent disease that is responsible for around 10 million deaths a year. Cases and deaths will continue to rise daily if nothing is done to find the most effective treatment. Mistletoe is an evergreen, semi-parasitic plant that grows on trees. This plant contains a number of biologically active compounds that contribute to its anti-cancer properties e.g., lectins. Mistletoe is obtained from nature and has been shown to be safe, potent, and effective in treating cancer. Herein we review the biological effects of mistletoe on cancer cells, formulations that can be used in cancer treatment and discuss the benefits and caveats of such treatments.

Keywords: mistletoe, cancer, therapy, *Viscum album*, *Viscum fraxini*

1. Introduction
In Ireland, the average number of 'registered tumours' in males and females is estimated to be just above 43,000 per year between 2017-2019 (National Cancer Registry Ireland, 2019). 55% of the registered tumours excluding non-invasive and non-melanoma skin cancers require extensive treatment as they are life-changing invasive cancers (National Cancer Registry Ireland, 2019). The treatment of cancer differs depending on type. The treatments a cancer patient can undergo are surgery, radiation therapy, chemotherapy, hormonal therapy, target therapy and immunotherapy (Miller *et al*., 2019).

As cancer is a prevalent disease a number of unconventional therapies have been discovered. One unconventional therapy is the use of mistletoe. Mistletoe is used in primary, adjuvant and palmitate treatment of cancer (Urech & Baumgartner, 2015). Mistletoe has been used to treat more than 80,000 patients between medical clinics in Switzerland and Germany since the 1920s (Kaegi, 1998). Iscador is the name of the mistletoe preparation that is commercially available in Europe and South Africa (Kaegi, 1998). Cancer patients undergo unconventional therapies to improve their quality of life (QoL) and to help manage symptoms such as nausea (Vickers & Cassileth, 2001).

2. Mistletoe
Mistletoe is an evergreen plant and is a semi-parasitic plant that grows on trees e.g., apple, elm, oak, and pine trees (Kaegi, 1998). Mistletoe is native to many parts of Europe but it rarely grows in northern and far eastern Europe as mistletoe cannot survive the low temperatures of -

20°C (Iscador AG, 2018). Mistletoe also has trouble growing in the south of Europe due to the strong sunlight and aridity (Iscador AG, 2018). White-berried mistletoe (*Viscum album L.*) is used in cancer treatment and is split into three botanical subspecies, pine mistletoe (*Viscum album ssp. austriacum*), fir mistletoe (*Viscum album ssp. abietis*) and deciduous mistletoe (*Viscum album ssp. Album*) (Becker, 1986). *Viscum fraxini* is mistletoe that grows on ash trees (Yang *et al,* 2019). *Viscum album* is the European species of mistletoe (Becker, 1986). Mistletoe species can only grow on specific host trees (Iscador AG, 2018).

Historically the use of mistletoe as a cancer therapy was documented in the early 20th century by Rudolf Steiner who used a preparation of white-berried mistletoe (Kaegi, 1998). In 1916, Ita Wegman following indications from Steiner, then developed Iscar, a parenteral, and treated the first cancer patient in 1917 (Iscador AG., 2018). The preparation was registered as ISCADOR after further development in 1926 and is now the most frequently used mistletoe preparation in cancer treatment (Iscador AG., 2018).

Chemical composition of mistletoe

Mistletoe contains a number of different types of biologically active compounds (e.g., lectins, polysaccharides, viscotoxins, kuttan's peptides, flavonoids) (see Table 1)(Iscador AG., 2018), each of which have the potential to interact with cancer cells (Marvibaigi *et al*., 2014). Plant lectins interact with cancer cells expressing aberrant glycan structures on their cell surface, resulting in cell death. Mistletoe contain three structurally similar lectins (ML-I, ML -II and ML-III) which differ in terms of their ability of carbohydrate binding (Pevzner et al., 2004). They belong to the type-2 ribosome-inhibiting proteins (RIPs) (Zänker & Kaveri, 2015). These lectins contain an A chain (which is cytotoxic) and a B chain (carbohydrate binding with immunomodulatory properties). Binding of the B chain is selective (Marvibaigi *et al*., 2014). For example, there are different targets (galactoside alone, galactoside and N-acetylgalactosamine glycans or N-acetylgalactosamine alone) depending on the lectin (ML-X)(Majeed *et al*., 2021). This variation in binding explains the selective cytotoxicity (activation of intrinsic and extrinsic apoptosis pathways) of lectins on cancer cells (Twardziok *et al*., 2016). Viscotoxins contain a phosphate specific binding site and are amphipathic in nature thereby augmenting their cytotoxicity in cancer cells by altering membrane integrity (Schaller *et al*., 1998)(Zänker & Kaveri, 2015). Flavanoid derivatives such as quercetin and quercetin methyl ester are also found in mistletoe (Pietrzak *et al*., 2017). These mainly exist in glycosylated form and induce apoptosis and exhibit scavenger properties. As these components have different effects in the body and the chemical composition of mistletoe varies depending on the location and species of host, season and type of harvest, extraction method and commercial producer (Marvibaigi *et al*., 2014) we present their distribution in various mistletoe species (Table 1) and detail their potential anti cancer effect in the remainder of this section.

Mistletoe lectins

Ribosome Inactivating Proteins are highly potent cytotoxins which can interfere with protein biosynthesis (Beztsinna *et al*., 2018). These include lectins. The 254 amino acid A-chain catalyses hydrolysis of the N-glycosidic bond at adenine -4324 in the 28S ribosome, thereby inhibiting protein elongation and resulting in cell death. The 264 amino acid B-chain binds carbohydrates which mediates cellular uptake of lectin (Marvibaigi *et al*., 2014). The B chain of lectin causes the secretion of cytokines and increases the activity of natural killer (NK) cells (Marvibaigi *et al*., 2014).

IL-2 is normally used for activation of NK or T cells isolated from cancer patients (Radulovic *et al*., 2003) and is approved by the FDA for the treatment of metastatic renal cell carcinoma

and metastatic melanoma (Jiang *et al*., 2016)(Konjevic *et al*., 2003). However due to its adverse effects at high dose there is a need to discover natural compounds capable of eliciting a favourable therapeutic response (Lu & Chen, 2010).

ML-I, ML-II and ML-III can be separated by their molecular weight and their specific sugar-binding characteristics (Urech *et al*, 2006). The lectin concentration in mistletoe varies depending on the season and the host tree (Iscador AG., 2018). In winter the mistletoe plant contains more lectins compared to in the summer (Iscador AG., 2018). In mistletoe that grows on oak, apple and poplar contain a lot of lectin with ML-I being the predominated one while mistletoe grown on pine trees have a low lectin concentration with most of the lectin present being ML-III and containing very little ML-I (Iscador AG., 2018).

Viscotoxins

Viscotoxins are polypeptides with immunogenic effects (Zänker and Kaveri, 2015). They contain 46 amino acids and three disulphide bridges (Marvibaigi *et al*., 2014), with a phosphate-binding site (Zänker & Kaveri, 2015). This binding site can interfere with a cell's membrane which destroys it . The cytotoxicity of viscotoxins has been demonstrated in bladder and breast carcinoma cell lines (Eggenschwiler *et al*., 2007) .The viscotoxins present in mistletoe are A1, A2, A3, B, 1-PS and U-PS (Urech *et al*, 2006). Mistletoe grown on oak trees, elm trees, apple trees and deciduous trees contain A2 and A3 in high concentrations but do not contain 1-PS and U-PS (Urech *et al*, 2006). Mistletoe grown on pine trees contain high levels of 1-PS and U-PS but does not contain A1 while mistletoe grown on fir trees contain high levels of A3, a substantial amount of 1-PS and very little A2 (Urech *et al*, 2006).*V. album ssp. austriacum* contains the lowest concentration of viscotoxins compared to other *V. album* subspecies (Urech *et al*, 2006). Different extracts from host trees in *abietis* and *album* show no difference in the viscotoxin concentration (Urech *et al*, 2006).

Kuttan's peptides

Kuttan's peptides are heat resistant peptides with a molecular weight of 5000Da (Kuttan & Kuttan, 1992). Kuttan's peptides have been shown to have similar properties to viscotoxins as they have cytotoxic and immunostimulatory properties (Kuttan & Kuttan, 1992) against tumour cells (Kuttan & Kuttan, 1993). *In vitro* and *in vivo* studies show that kuttan's peptides stimulate macrophages and lymphocytes towards the tumour when injected near the tumour site (Kuttan & Kuttan, 1992). Natural killer cells and antibody dependent cellular cytotoxicity are increased when administered with these peptides (Kuttan & Kuttan, 1992).

Polysaccharides and oligosaccharides

Polysaccharides and oligosaccharides concentration in mistletoe will change depending on the season and the part of the plant that is used (Urech & Baumgartner, 2015). Mistletoe berries contain polysaccharides, examples are rhamnogalacturonans with arabinogalactan side chains, arabinogalactans, and xyloglucans. Arabinogalactans selectively stimulates the proliferation of CD4+ T helper lymphocytes, while rhamnogalacturonans stimulate NK cells activity (Urech & Baumgartner, 2015). The ability of arabinogalactans to induce the intrinsic apoptotic pathway, increase ROS and decrease cellular proliferation was previously demonstrated in breast cancer cells (Moghtaderi *et al*., 2017).

Flavonoids

Flavonoids are present in *V. album* (Urech & Baumgartner, 2015) with known anti cancer properties (Kopustinskiene *et al*., 2020). Its derivatives induce apoptosis in cell culture models and have antioxidant effects (Iscador AG., 2018).

Table 1: Constituents present in different types of *V. album* subspecies.

		Constituents					
	***V. album* subspecies**	**Lectins***	**Viscotoxins**	**Kuttan's peptides**	**Polysaccharides and oligosaccharides**	**Flavonoids**	**References**
Mistletoe species	Pine *album*	ML-III, ML-II, ML-I	A1, A2, A3, B	Present	Arabinogalactans, galacturonans	Quercetin and quercetin methyl ester	Urech & Baumgartner, 2015
	Oak *album*	ML-I, ML-II, ML-III	A1, A2, A3, B	Present	Arabinogalactans, galacturonans	Quercetin and quercetin methyl ester	Urech & Baumgartner, 2015
	Elm *album*	ML-I, ML-II, ML-III	A1, A2, A3, B	Present	Arabinogalactans, galacturonans	Quercetin and quercetin methyl ester	Urech & Baumgartner, 2015
	Pine *austriacum*	ML-III, ML-II, ML-I	A2, A3, B, 1-PS, U-PS	Present	Arabinogalactans, galacturonans	Quercetin and quercetin methyl ester	Urech *et al*, 2006
	Fir *abietis*	ML-I, ML-II, ML-III	A1, A3, B, 1-PS	Present	Arabinogalactans, galacturonans	Quercetin and quercetin methyl ester	Urech *et al*, 2006

*ranked from highest concentration to least

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3. Mistletoe and Key Biological Pathways

Mistletoe uses a number of different biological pathways that cause its anti-cancer effects. The different components (see Table 2) of mistletoe result in different biological effects. These are: cytotoxicity and apoptosis in tumour cells and inhibition of angiogenesis (Yang *et al.*, 2019).

Cyclooxygenases-2 signalling pathway

Viscum Album preparations influence cyclooxygenases (COX)-2 activity (Oei *et al.*, 2019). COX-2 is an important enzyme involved in a number of different inflammatory reaction pathways (Oei *et al.*, 2019). *Viscum Album* preparations reduce the selectivity of COX-2 in preclinical studies (lung carcinoma) (Oei *et al.*, 2019). COX-2 upregulation is shown to be linked with poor colorectal cancer prognosis, and results in an overproduction of prostaglandin E_2 (PGE_2) which promotes cell growth, migration, invasion, and survival (Greenhough *et al*., 2009). *Viscum album* preparations can suppress COX-2 activities by posttranscriptional destabilization of its transcripts (Oei *et al.*, 2019). See Figure 1 for COX-2 pathway.

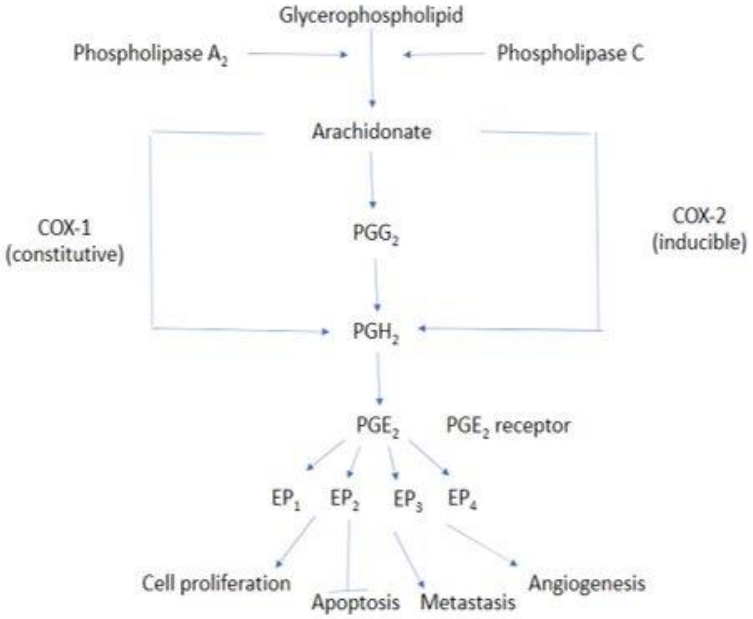

Figure 1: COX-2 signalling pathway. Arachidonate is released from cell membranes by cytoplasmic phospholipase A2. Free arachidonic acid is metabolized to eicosanoids. Arachidonic acid is converted to PGH2 by COX. PGH2 is subsequently metabolised to prostaglandins (PGs) by specific prostaglandin synthases.

Downregulation of c-Myc expression

Viscum fraxini, an aqueous extract of mistletoe, inhibits the proliferation of liver cancer cells by the downregulation of c-Myc expression as shown in *in vitro* (hepatocellular carcinoma) studies (Yang *et al.*, 2019)(see Figure 2). c-Myc is overexpressed in both viral and alcohol-related hepatocellular carcinoma and the greater the overexpression of c-Myc, the more aggressive the form of hepatocellular carcinoma (Yang *et al.*, 2019). This extract increases pro-apoptotic protein expression e.g., Bax, and inhibits anti-apoptotic protein expression e.g., XIAP. It also regulates cell signalling proteins e.g., c-Myc (by its suppression), in a dose-dependent manner (Yang *et al.*, 2019). *Viscum fraxini* affects the translation of c-Myc, not the transcription as the gene is not altered by its use (Yang *et al.*, 2019).

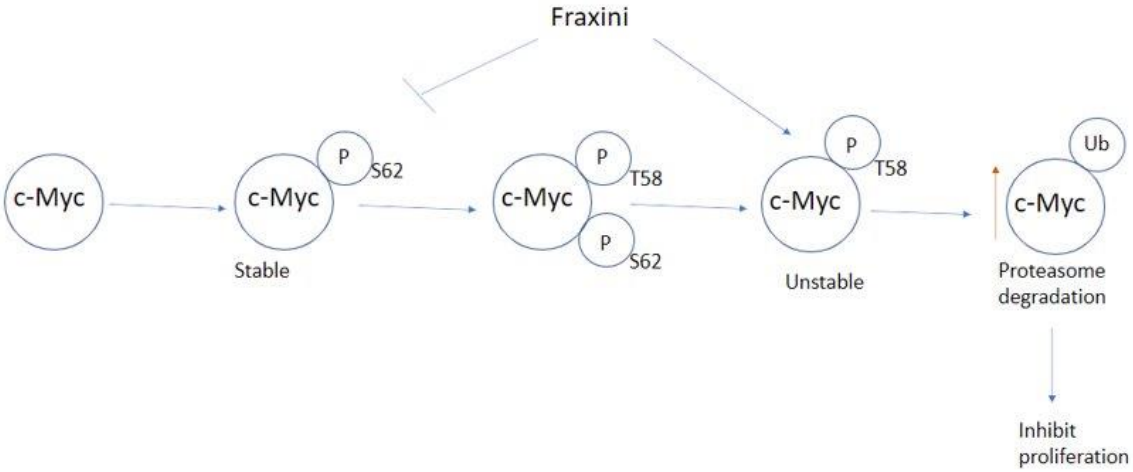

Figure 2: Mechanism of *V. fraxini* interaction with c-Myc pathway. Suppression of c-Myc by *V. fraxini* is via a decrease in c-Myc stability which may involve phosphorylation sites T58 and S62 on c-Myc.

MAPK

Mistletoe has been shown to use mitogen-activated protein kinase (MAPK) in *in vitro* (leukaemia) studies (Szurpnicka *et al.*, 2020)(see Figure 3). The MAPK pathway used by mistletoe plays a key role in tumour proliferation (Szurpnicka *et al.*, 2020). MAPKs are components that are used in signalling pathways to transfer extracellular stimulation into intracellular responses (Pae *et al*., 2001). There are three subfamilies of MAPKs which are p44 and p42 MAPKs, p54 and p46 stress-activated protein kinases (SAPK) and p38 MAPK (Pae *et al*., 2001). p44 and p42 MAPKs are referred to as extracellular signal-regulated protein kinase 1 and 2 (ERK1/2) as they control cell proliferation and differentiation while protecting cells from apoptotic cell death (Pae *et al*., 2001). p38 MAPK and SAPKs are activated by stress signals e.g., inflammatory cytokines and can promote leukaemia cell death by inhibiting cell proliferation (Pae *et al*., 2001). Mistletoe increases p38 MAPK and SAPKs activation and decreases ERK which leads to apoptosis and cancer cell death (Szurpnicka *et al.*, 2020). Mistletoe lectin II causes these effects (Pae *et al*., 2001).

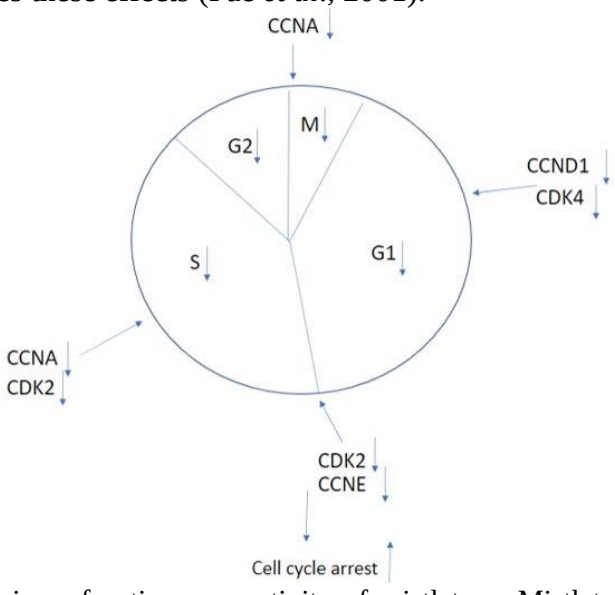

Figure 3: MAPK mechanism of anti-cancer activity of mistletoe. Mistletoe downregulates cyclins (CCND1, CCNE, CCNA) and cyclin-dependent protein kinases (CDK4, CDK2) inhibiting cell cycle.

PI3K / AKT

The phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway is one of the most important pathways that regulates cell growth, motility, survival, metabolism and angiogenesis (Katso *et al*., 2001). Activation of this pathway contributes to the development of tumours and resistance to anti-cancer therapies (Martini *et al*., 2014). Mistletoe targets the signalling pathway phosphatidylinositol 3-kinase (PI3K) (*in vitro* study in lung cancer) / protein kinase B (AKT) (*in vitro* study in submandibular gland squamous cell carcinoma) which is responsible for the growth and survival of cells (Szurpnicka *et al.*, 2020). AKT is a target for PI3K as when it is activated it inhibits apoptosis in cells (Choi *et al.*, 2004). Mistletoe has been shown to downregulates BCL1, BCL2, XIAP which are inhibitors of apoptosis (IAPs) and upregulates pro-apoptotic proteins (Bax) in *in vitro* lung cancer studies (Szurpnicka *et al.*, 2020). Mistletoe activates caspases and releases cytochrome c which leads to cell apoptosis (Szurpnicka *et al.*, 2020). See Figure 4 for mechanism of action.

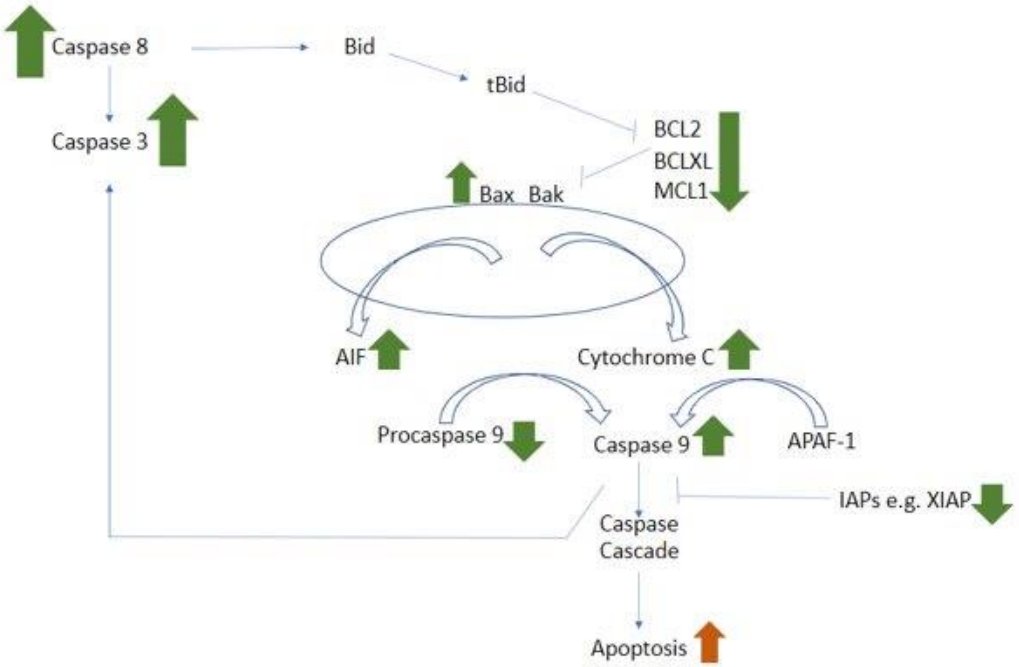

Figure 4: PI3K / AKT mechanism of anti-cancer activity of mistletoe. Mistletoe upregulates proapoptotic proteins (Bax, Bak) and downregulates inhibitors of apoptosis (IAPs) such as BCL2, BCL2, MCL1, XIAP. Additionally, mistletoe leads to release of cytochrome c and activation of caspases resulting in apoptosis.

Table 2: The effect of different mistletoe preparations on cancers.

***V. album* preparation**	**Type of cancer**	**Effect**	**Study type**	**References**
Iscador Quercus spezial	----------	Increase of leukocytes, granulocytes and eosinophils	Randomized clinical trial (RCT) placebo-controlled trial on healthy subjects	Huber *et al.*, 2005
Viscum album	Breast cancer	Increase activity in NK cells	Preclinical study and clinical trial	Tabiasco *et al.*, 2002 Hajto, 1986
Viscum album var. coloratum Iscador M	----------	Increase p38 MAPK and SAPKs activity and decreases ERK	*In vitro*	Szurpnicka *et al.*, 2020 Twardziok *et al.*, 2017
Viscum album var. coloratum Iscador M Iscador Quercus spezial	Lung cancer, tongue cancer, myeloid leukaemia	Downregulation of IAPs and upregulation of Bax	*In vitro*	Choi *et al.*, 2004 Park *et al.*, 2012 Klingbeil *et al.*, 2013 Fan *et al.*, 2019
Iscador Quercus spezial	Breast cancer	COX-2 signalling	*In vivo*	Hegde *et al.*, 2011
Viscum fraxini	Liver cancer	Downregulation of the c-Myc expression	*In vivo*	Yang *et al.*, 2019

4. Mistletoe and the Immune System

Tumours have different pathways that evade the immune system, these pathways include resistance to immune effector cells, induction of immune tolerance and causing paralysis of antigen-presenting cells (APCs) e.g., dendritic cells (DCs) (Elluru *et al*., 2008). DCs stimulate T cells therefore with tumours suppressing the maturation and activation of DC T-cells are not attacking the tumour (Elluru *et al*., 2008). Mistletoe has been shown to stimulate DCs which in turn stimulates T-cells which can then mount an immune response against the tumour (Elluru *et al*., 2008).

Mistletoe increase leukocytes, granulocytes, eosinophils, and lymphocytes, increase the activity of NK cells and increase cytokine secretion (Szurpnicka *et al.*, 2020) (Huber *et al*, 2005). NK cells inhibit tumour growth (Hajto, 1986). They kill cancer cells by releasing cytotoxic substance and inducing apoptosis (Iscador AG., 2018). Granulocytes, neutrophil and monocytes have been shown to resist tumour growth and metastasis *in vivo* (Hajto, 1986).

Lectin (mistletoe constituent) binds to monocytes and macrophages which stimulates the synthesis and release of cytokines (Iscador AG., 2018).

5. Pharmaceutical Processing

Pharmaceutical processing and the season the mistletoe is harvested influences the concentration of chemical substituents in the mistletoe extracts (Urech & Baumgartner, 2015). Extracts of mistletoe from summer and winter have different concentrations of constituents, therefore to avoid this, the summer and winter extract should be mixed together to avoid discrepancies (Urech *et al*., 2006).

Mistletoe formulation

There are a number of different formulations of mistletoe that are used to treat cancer. Iscador is a fermented aqueous extract of *Viscum album* and Helixor is an unfermented extract of *Viscum album*. These are marketed under different names depending on their mistletoe source: Iscador M : derived from apple trees; *Malus domestica*, Iscador P : derived from mistletoe grown on pine trees; *Pinus sylvestris*), Iscador Qu (from oak trees; *Quercus robur*), Iscador U (from elm trees; *Ulmus minor*), Iscador A : derived from mistletoe grown on fir trees. Helixor M is derived from mistletoe grow on apple trees and *Viscum fraxini,* Helixor P (from pine trees) and Helixor A (from spruce trees*; Picea abies)* (Becker, 1986) (Kleijnen & Knipschild, 1994). Eurixor is a mistletoe formulation that uses unfermented *Viscum album* from poplar trees (Kleijnen & Knipschild, 1994).

Mistletoe harvest

Mistletoe harvest takes place twice a year, in summer and in winter, and is separated depending on the host tree (Iscador AG., 2018). The harvest takes place in summer when mistletoe is at the peak of its vegetative development and in winter when the berries and fruit are fully developed (Iscador AG., 2018). This is to ensure that mistletoe ideal physiological stages are harvested (Urech *et al.*, 2006).

The parts of mistletoe that are harvested are one- to- two year old leaves, stems, buds, and ripened berries in winter (Iscador AG., 2018). These are then separated from the mistletoe bush and are taken to the manufacturing site (Iscador AG., 2018).

Preparation of Iscador

The mistletoe parts after sorting are crushed using rolling mills and are then mixed with water (Iscador AG., 2018). This aqueous extract from a *Viscum Album* plant is then fermented for three days (plant) to four days (berries) with *Lactobacillus plantarum* to make Iscador (Kaegi, 1998) (Iscador AG., 2018). The product is mixed and then filtered to remove *Lactobacillus plantarum* and insoluble plant residues (Kaegi, 1998) (Iscador AG., 2018). The summer and winter extract of the same mistletoe plant are mixed together at a 1:1 ratio (Iscador AG., 2018). This product is then standardised and packaged for injections (Kaegi, 1998). The only difference in formulations for Iscador M, Iscador P and Iscador A is the type of mistletoe used (Kaegi, 1998). See Figure 5.

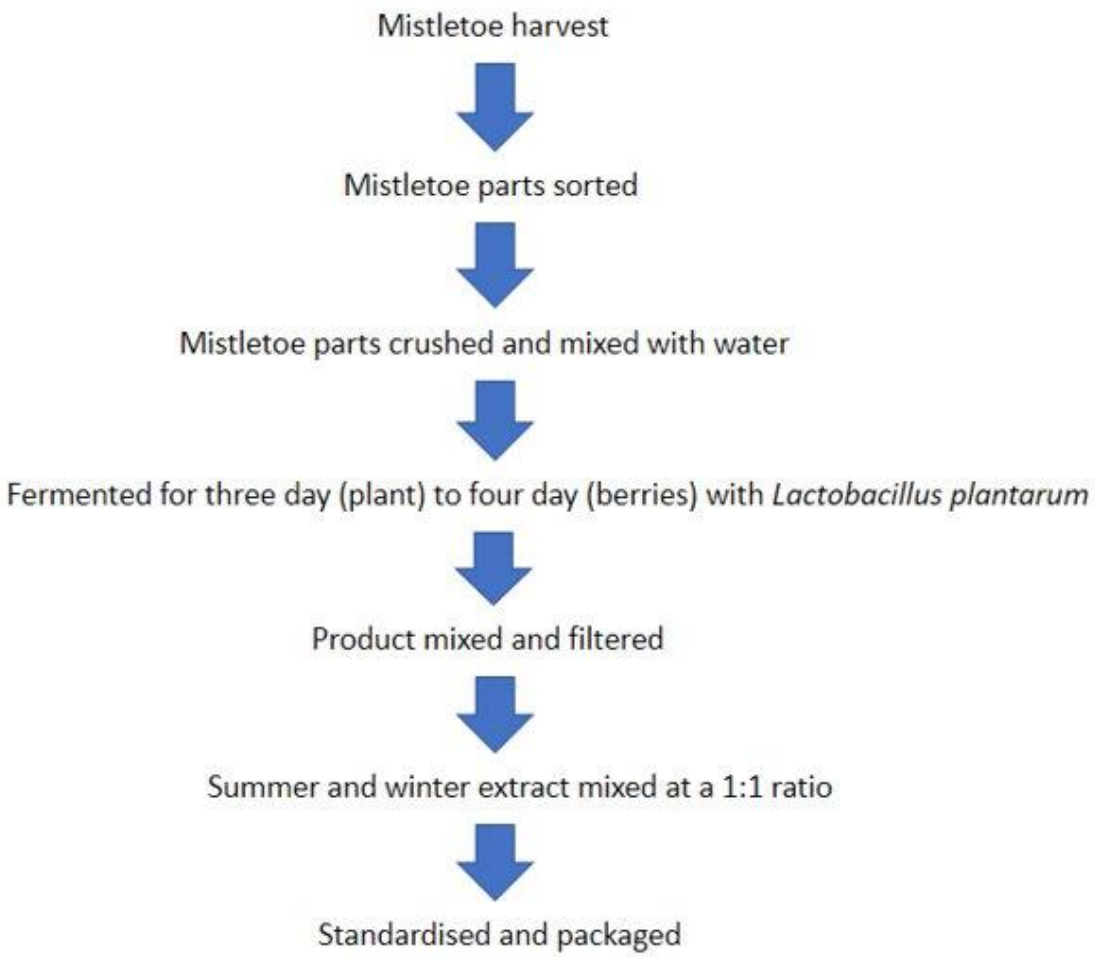

Figure 5: Harvest and preparation of Iscador. Mistletoe is harvested, sorted, crushed, fermented, filtered and packaged for use.

6. Clinical Treatment with Mistletoe Formulations

Mistletoe injections

Mistletoe extractions are administered as injectables as lectins structures are degraded in the gastrointestinal tract and have poor absorption (Lyu *et al*, 2004). Iscador is injected into the tumour or as near as possible to the tumour site by subcutaneous injections into the abdominal wall (Kaegi, 1998). This treatment usually takes place three to seven times a week over several weeks but the treatment is adjusted to the patient's general condition (Kaegi, 1998). Iscador treatment can be taken alongside chemotherapy and radiotherapy (Kaegi, 1998) (Oei *et al*, 2019).

Iscador

Viscum Album is thought to reduce tumour size, stimulate an immune response, and improve wellbeing and may improve survival in patients with breast, lung, liver, stomach, ovary, cervix, and colon cancer (Kaegi, 1998). Iscador M, Iscador P are used to treat breast, cervix, lung, stomach, ovary, and colon cancer (Marvibaigi *et al*., 2014) (Kaegi, 1998). Breast cancer patients that underwent treatment using Iscador M were found to have a decrease in tumour-related symptoms and fatigue, and health-related quality of life improved (HRQoL) (Marvibaigi *et al*., 2014). Breast cancer patients that underwent treatment using Iscador P were found to have a decrease in tumour-related symptoms, enhanced self-regulation and survival time increased (Marvibaigi *et al*., 2014).

Helixor

Helixor is a formulation made from *Viscum Album* (Lee *et al.*, 2018). As stated previously there are three types of Helixor: Helixor A, Helixor M and Helixor P (Kleijnen & Knipschild, 1994). Helixor A is used to treat brain tumours, lung, head and neck cancer. (Helixor, 2021) Helixor M is used to treat bladder and post-menopausal cancer (Helixor, 2021). Helixor P is used to treat skin, kidney, testicular and pre-menopausal breast cancer (Helixor, 2021). Breast cancer patients that underwent Helixor A treatment were found to have an improvement in HRQoL and a decrease in the side effects of chemotherapy (Marvibaigi *et al*., 2014) (Kienle & Kienle, 2007).

Isorel

Isorel is a formulation made from the entire plant of *Viscum Album* (Zarkovic *et al*., 2001). Isorel is produced by Novipharm GmbH (Zarkovic *et al*., 2001). Isorel M is made from mistletoe on a pine tree (Zarkovic *et al*., 2001). It inhibits the growth of malignant cells, stimulates the patient's immune system, and increase the effectiveness of chemotherapy and radiotherapy (Zarkovic *et al*., 2001). When Isorel is taken in conjunction with chemotherapy it is thought to reduce the harmful mutagenic effects of oxygen-free radicals (Zarkovic *et al*., 2001). It has been used to treat colorectal cancer (Cazacu *et al.*, 2003).

Lektinol

Lektinol is a formulation made from extracts of *Viscum Album* (Mengs *et al*., 2001). Lektinol is produced by Madaus AG (Mengs *et al*., 2001). Lektinol has been shown to be cytotoxic to breast, prostate, lung, and renal cancers in *in vitro* studies (Mengs *et al*., 2001).

Fermented versus unfermented mistletoe preparation

The fermentation status of preparations determines the overall effectiveness of their anti-cancer status. Fermented mistletoe preparation Iscador (used in cancer therapy for decades) and an unfermented mistletoe preparation of the same were compared to see which preparation of mistletoe is better for the treatment of cancer (Ribéreau-Gayon *et al.*, 1986). The mistletoe preparations were tested in two cell lines rat hepatoma tissue culture (HTC) cells and human leukaemia Molt 4 cells (Ribéreau-Gayon *et al.*, 1986). The concentration of lectins in unfermented mistletoe preparation is 10 times higher than in fermented mistletoe preparation which is approximately 100ng/ml (Ribéreau-Gayon *et al.*, 1986). Fermented mistletoe was more potent than unfermented mistletoe inhibiting cell growth of HTC cells while unfermented mistletoe has a stronger cytotoxic effect on Molt 4 cells than HTC cells (Ribéreau-Gayon *et al.*, 1986) .

In general the tolerability of mistletoe extracts (summarised in Table 3) is largely good and in preclinical trials, there was no toxicity observed at low doses (Kienle *et al*, 2009). However, ingestion of the raw plant could cause seizures and in the worst-case death (Kaegi, 1998).

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Table 3: Mistletoe formulation and cancer treatment.

Formulation name	**Administration**	**Cancer**	**Effect**	**Reference**
Iscador A	Injection	Breast, lung, colon, rectum, stomach cancer	Increase in HRQoL, increase in survival time	Kienle & Kienle, 2007
Iscador M	Injection	Breast, cervix, lung, ovary, stomach, and colon cancer	HRQoL improved, symptoms decreased, and fatigue decreased	Marvibaigi *et al*., 2014
Iscador P	Injection	Breast, cervix, lung, ovary, stomach, and colon cancer	A decrease in symptoms, enhanced self-regulation and an increase in survival time	Marvibaigi *et al*., 2014
Viscum fraxini	Injection	Liver and pancreatic cancer	Downregulation of the c-Myc expression	Yang *et al*, 2019
Helixor A	Injection	Brain tumours, lung, head and neck cancer	Improved HRQoL and a decrease in chemotherapy side effects	Marvibaigi *et al*., 2014, Helixor, 2021, Kienle & Kienle, 2007
Helixor M	Injection	Bladder and breast cancer (post-menopausal)	Inhibition of epidermal growth factor which induced the proliferation of cells	Marvibaigi *et al*., 2014, Helixor, 2021
Helixor P	Injection	Skin, kidney, testicular and breast cancer (pre-menopausal)	Cytotoxic activity on tumour cells	Marvibaigi *et al*., 2014, Helixor, 2021

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7. Benefits and Caveats of Mistletoe Treatment

Mistletoe as an add-on cancer treatment alongside chemotherapy and/or radiotherapy has a number of advantages and disadvantages. The patients and medical professional need to access the advantages and disadvantages and decide if mistletoe treatment is right for them.

Benefits of mistletoe treatment

The advantages of mistletoe treatment are improvement in QoL and survival time for the patient, reduction in the side effects and adverse drug reactions by chemotherapy and radiotherapy, increase in the immunological response, inhibition of cancer cell proliferation and patients that undergo mistletoe treatment have a reduction in hospitalization stay (Marvibaigi *et al*., 2014) (Matthes *et al*, 2010) (Oei *et al*, 2019) (Kienle *et al*, 2009) (Yang *et al*, 2019).

Caveats of mistletoe treatment

The disadvantages of the use of mistletoe as an add-on treatment for cancer are at the injection site, where there can be swelling and local pain. Side effects that can occur during mistletoe treatment are fever, flu-like symptoms, headaches, and chills (Kienle *et al*, 2009) (Kaegi, 1998). While there are a number of side effects for the use of mistletoe, they are not permanent and will subside over time and not every patient will experience them (Kienle *et al*, 2009).

8. Clinical Trials

Current clinical trial on Iscador Qu

As of the time of writing this article there is currently a clinical trial recruiting patients with primary and recurrent inoperable pancreatic cancer in Stockholm, Sweden (Wode, 2019). This is a phase three trial. It started in June 2016 and is ongoing as of May 2022. The trial is a randomized double-blinded multicentre parallel-group placebo-controlled clinical trial (Wode, 2019). The main aims of the clinical trials (see Table 4) are to determine the overall survival of patients, evaluate the quality of life, weight of patients and to see if the adverse effects of chemotherapy decrease (Wode, 2019).

Completed phase one clinical trial on Iscucin Populi and Viscum Mali

There are also a number of completed clinical trials for mistletoe as a cancer treatment. The University medical centre in Freiburg, Germany completed a phase one clinical trial study on two mistletoe preparations (*Iscucin Populi* (IP) and *Viscum Mali* (VM)) (Huber, 2011). The trial started in January 2008, there were seventy-one healthy participants aged between 18-45 years. The participants involved had no disease except hay fever, did not smoke or abuse drugs and were not pregnant (Huber, 2011). The trial was a three-armed randomized study (Huber, 2011). There were three different dose strengths of IP and VM, for IP the strengths were called F, G and H and for VM the dose strengths were called D3, D2 and 2% of each. Each dose of the mistletoe preparations was given twice a week for four weeks starting with the F for IP and then working up to H and for VM it started with D3 and working up to 2% of each and the results that were found were compared to the placebo results (Huber, 2011). The results found that there was a strong local reaction at the injection site for the IP strength G and H which caused distinct eosinophilia and there was a significant increase in the total amount of leucocytes (Huber *et al*, 2011). T-helper cell counts increased with the use of IP strength F, G and H compared to the placebo (Huber *et al*, 2011). There were only mild local reactions for VM but there was a small but insignificant increase of eosinophils (Huber *et al*, 2011). The clinical trial found that the IP strength G and H should only be given after a pre-treatment with

a low dose first to make them more tolerable (Huber *et al*, 2011). Both preparations were found to be safe (Huber *et al*, 2011). As the trial was only a phase one trial a further trial will be required on cancer patients to understand the effect of the mistletoe preparations on cancer.

Completed phase two clinical trial on abnoba viscum fraxini

On the 29th of January 2004, Abnoba Gmbh completed a phase two clinical trial on *abnoba viscum fraxini* (Ruebben, 2013). The trial was to determine the maximum dose, safety, and effectiveness of *viscum fraxini* after transurethral resection of nonmuscle invasive bladder cancer (Ruebben, 2013). There were thirty-seven participants with aged between 18-80 years (Ruebben, 2013). One patient was excluded since the patient had not been treated therefore thirty-six participants underwent the trial, five women and thirty-one men (Rose *et al*., 2015). The trial was a single group dose-escalation study (Ruebben, 2013). The dose started at 45mg of abnoba viscum fraxini 2 and went up to 675mg of abnoba viscum fraxini 2 (Rose *et al*., 2015). The participants were split into groups of three and were started at the lowest dose. (Rose *et al*., 2015) If no WHO grade III toxicity was observed the next dose level was tested in another group of three patients (Rose *et al*., 2015). The study found that there was no dose-limiting toxicity up to the administration of 675mg abnoba viscum fraxini 2 because there was no single grade three toxicity observed (Rose *et al*., 2015). Some side effects observed were urinary tract infection and irritations, and pyrexia. (Rose *et al*., 2015) Before the treatment began the participants underwent transurethral resection of bladder tumours and a marker tumour was left (Rose *et al*., 2015). 55.6% of patients had complete remission of the marker tumour which showed strong evidence of the efficacy of *abnoba viscum fraxini* 2 (Rose *et al*., 2015). As this was a phase II study further studies are required to confirm the efficacy of this treatment.

Completed phase four clinical trial on abnoba viscum quercus

In March 2006, Abnoba GmbH completed a phase four clinical trial on Abnoba-viscum Quercus (Kim, 2011). The trial was to determine the QoL, immunomodulation, and safety of adjuvant mistletoe treatment in patients with gastric carcinoma (stage Ib/II) receiving chemotherapy after operation (Kim, 2011). The study took place at the ASAN medical centre Seoul, Republic of Korea. There were thirty-two participants with an aged 19-70 years. The study was prospective, controlled, and randomized, comparing two arms. The patients were randomly allocated to the intervention group or the control group (no additional therapy), sixteen in each (Kim *et al*., 2012). Three participants dropped out, one from the intervention group and two from the control group (Kim *et al*., 2012). There were no significant differences in age, sex, height, weight, blood pressure, pulse rate, type of operation (total gastrectomy or distal gastrectomy) and pathologic classifications between the two cohorts. The number of male patients was significantly higher in both cohorts. There were 28 patients in stage Ib and one patient in stage II.

The intervention group underwent oral chemotherapy and mistletoe injection while the control group only underwent oral chemotherapy (Kim *et al*., 2012). Abnoba- viscum Quercus was injected subcutaneously three times a week with an increasing injection dose after a week (Kim *et al*., 2012). The dose started at 0.02mg eventually building to 20mg which continued till the end of the study (Kim *et al*., 2012). The trial found that there was an increase in the QoL of the patients who were in the intervention group and the patients' global health status increased (Kim *et al*., 2012). Aside from 3 QoL parameters (pain, eating restrictions, hair loss) and basophiles baseline of QoL, immunological parameters, hematology and liver function tests were not different between the groups. White blood cells count, and eosinophils increased in

the treatment group compared to the control group (Kim *et al*., 2012). The rate of diarrhoea decreased in the treatment group compared to the control (Kim *et al*., 2012).

Table 4: Summary of clinical trials

Mistletoe	**Clinical trial**	**Goal of study**	**Result**	**Reference**
Iscador Qu	Recruiting phase 3	To determine the overall survival of patients, evaluate the quality of life, weight of patients and to see if the adverse effects of chemotherapy decrease	---------	Wode, 2019
Iscucin Populi, *Viscum Mali*	Completed phase 1	To determine the safety of the preparation	Both preparations were found to be safe.	Huber *et al*., 2011
Abnoba viscum fraxini 2	Completed phase 2	To determine the maximum dose, safety, and effectiveness of viscum fraxini after transurethral resection of nonmuscle invasive bladder cancer	There was no dose-limiting toxicity up to the administration of 675mg and 55.6% of patients had complete remission of the marker tumour which showed strong evidence of the efficacy.	Rose *et al*., 2015
Abnoba viscum quercus	Completed phase 3	To determine the quality of life, immunomodulation, and safety of adjuvant mistletoe treatment in patients with gastric carcinoma receiving chemotherapy after operation	White blood cells count, and eosinophils increased, rate of diarrhoea decreased, increase in the quality of life of the patients and the patient's global health status increased	Kim *et al*., 2012

9. Final Remarks

It is apparent that mistletoe can be used to treat cancer as evident in the different pieces of literature cited in this article. Pharmaceutical companies should investigate developing mistletoe formulations as a treatment for cancer. There are several parameters that need to be

considered, which include the type of mistletoe, the parts of mistletoe that are harvested, the time and season of harvest and if the formulation is fermented or not. Pharmaceutical processing and the season the mistletoe is harvested influences the concentration of chemical substituents in mistletoe extracts (Urech & Baumgartner, 2015).

Mistletoe's anti-cancer properties are due to a number of different pathways. Mistletoe can also reduce the side effects and adverse drug reactions that are caused by chemotherapy and radiotherapy (Marvibaigi *et al*., 2014). Therefore fewer drugs are required to treat the side effects of the treatment and patients are also more likely to finish the medication as it is more convenient to take one drug than two or three. Mistletoe can improve the patients' health-related quality of life as it can reduce nausea and fatigue and reduce the hospitalization period of the patient (Marvibaigi *et al*., 2014). These positive effects on the patient mean that the patient is more likely to cooperate with treatment and achieving the desired outcome.

Mistletoe contains a number of constituents each of which target different pathways. The main constituent that causes mistletoes anti-tumour and immunomodulatory effects is lectin (Marvibaigi *et al*., 2014). Mistletoe lectin causes the excretion of cytokines and increases the activity of NK cells (Marvibaigi *et al*., 2014). Viscotoxins have a cytotoxic effect. Viscotoxins contain a phosphate-binding site. This binding site can interfere with a cell membrane and destroy it (Zänker & Kaveri, 2015). Mistletoe lectin stimulates the patient's immune system and viscotoxins can cause tumour cell death. These constituents help the cancer patient to have a better immune system which can help the patient avoid another illness which could have affected the patient if their immune system is compromised with chemotherapy or radiotherapy.

Mistletoe formulations reduce the proliferation of cancer cells by a number of different pathways which leads to the apoptosis of the cancer cells. The overproduction of PGE_2 leads to the promotion of cancer cell growth, migration, invasion, and survival (Greenhough *et al*., 2009). Mistletoe preparations suppress COX-2 activities by posttranscriptional destabilizing of its transcripts which stops the overproduction of PGE_2 causing apoptosis (Oei *et al.*, 2019). Mistletoe also increases the activity of NK cells, which release a cytotoxic substance that causes apoptosis (Iscador AG., 2018).

Further preclinical trials need to be undertaken *in vitro* to determine the anti-tumour activity of the mistletoe preparation. More clinical trials should be conducted on mistletoe formulations with a large number of participants to fully establish the safety and efficiency of the formulation. The clinical trials should also find the standard dose for the preparation. These trials should also take into account follow-up time to see the long-term effects, if any, of mistletoe formulations (Marvibaigi *et al*., 2014).

Currently, there are two mistletoe clinical trials recruiting patients. The first one is a phase 3 clinical trial on Iscador Qu effect on the survival and HRQoL on pancreatic cancer patients (Wode, 2019). The other trial is a phase 3 clinical trial on the efficacy of abnobaVISCUM 900 compared to mitomycin C monotherapy in patients with superficial bladder carcinoma by evaluation of the time to tumour recurrence and a secondary objective to evaluate the safety of abnobaVISCUM (Tschirdewahn, 2021). The mistletoe preparations Helixor M, and Iscador P are currently undergoing active clinical trials that are not recruiting patients.

The cancers that mistletoe has been used to treat are breast, cervix, colon, ovary, stomach, and lung cancers (Kaegi, 1998). In Ireland, lung, breast, ovary, and colon cancers are prevalent

therefore mistletoe treatment would give patients another treatment option to consider when deciding on a treatment plan.

Mistletoe has been proven safe and it has strong anti-cancer activity. Mistletoe formulation can be used as an add-on treatment with chemotherapy and/or radiotherapy as it reduces side effects. Pharmaceutical companies should consider producing an anti-cancer drug from mistletoe.

10. References

Becker H. (1986). Botany of European mistletoe (Viscum album L.). *Oncology*, 43(1), 2–7.

Beztsinna, N., de Matos, M., Walther, J., Heyder, C., Hildebrandt, E., Leneweit, G., Mastrobattista, E. and Kok, R. (2018). Quantitative analysis of receptor-mediated uptake and pro-apoptotic activity of mistletoe lectin-1 by high content imaging. *Scientific Reports*, 8(1).

Cazacu, M., Oniu, T., Lungoci, C., Mihailov, A., Cipak, A., Klinger, R., Weiss, T. and Zarkovic, N. (2003). The Influence of Isorel on the Advanced Colorectal Cancer. *Cancer Biotherapy and Radiopharmaceuticals*, 18(1), 27-34.

Choi, S., Lyu, S. and Park, W. (2004). Mistletoe lectin induces apoptosis and telomerase inhibition in human A253 cancer cells through dephosphorylation of akt. *Archives of Pharmacal Research*, 27(1), 68-76.

Eggenschwiler, J., von Balthazar, L., Stritt, B. *et al*. (2007). Mistletoe lectin is not the only cytotoxic component in fermented preparations of Viscum album from white fir (Abies pectinata). *BMC Complement Altern Med*. 7 (14).

Elluru, S., van Huyen, J., Delignat, S., Kazatchkine, M., Friboulet, A., Kaveri, S. and Bayry, J. (2008). Induction of maturation and activation of human dendritic cells: A mechanism underlying the beneficial effect of Viscum album complementary therapy in cancer. *BMC Cancer*, 8(1).

Fan, J., Wu, M., Wang, J., Ren, D., Zhao, J. and Yang, G. (2019). 1,7-Bis(4-hydroxyphenyl)-1,4-heptadien-3-one induces lung cancer cell apoptosis via the PI3K/Akt and ERK1/2 pathways. *Journal of Cellular Physiology*, 234(5), 6336-6349.

Greenhough, A., Smartt, H., Moore, A., Roberts, H., Williams, A., Paraskeva, C. and Kaidi, A. (2009). The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis*, 30(3), 377-386.

Hajto, T. (1986). Immunomodulatory Effects of Iscador: A *Viscum album* Preparation. *Oncology*, 43(1), 51-65.

Hegde, P., Maddur, M., Friboulet, A., Bayry, J. and Kaveri, S. (2011). Viscum album Exerts Anti-Inflammatory Effect by Selectively Inhibiting Cytokine-Induced Expression of Cyclooxygenase-2. *PLoS ONE*, 6(10).

Helixor.com. (2021). *Helixor - Healthcare professionals*. [online] Available at: <https://www.helixor.com/healthcare-professionals/mistletoe-therapy-helixor-a-m-p/> [Accessed 14th February 2021].

Huber, R., Lüdtke, H., Wieber, J. and Beckmann, C. (2011). Safety and effects of two mistletoe preparations on production of Interleukin-6 and other immune parameters - a placebo controlled clinical trial in healthy subjects. *BMC Complementary and Alternative Medicine*, 11(1).

Huber, R., Rostock, M., Goedl, R., Lüdtke, R., Urech, K., Buck, S., and Klein, R. (2005). 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. *European journal of medical research*, *10*(10), 411–418.

Huber, R. (2011). *Safety and Immunological Effects of Two Mistletoe Preparations in Healthy Volunteers* [online] Clinicaltrials.gov. Available at: https://www.clinicaltrials.gov/ct2/show/record/NCT01378702?term=mistletoe&recrs=e&draw=2&rank=1&view=record [Accessed 12th January 2021]

Iscador AG. (2018). *ISCADOR® In Cancer Therapy: Scientific Information and Study Results*. 1st ed. Lörrach: Iscador AG. Available at: https://static1.squarespace.com/static/5bb69fb37980b3398ec2c66b/t/5ce3312c48e42b0001237cb6/1558393133806/E-Version_F_WI_CH_DE_en_0.2_11.2018+1+%282%29.pdf [Accessed 7th November 2020]

Jiang T, Zhou C, Ren S. (2016). Role of IL-2 in cancer immunotherapy. Oncoimmunology. 5(6).

Kaegi, E. (1998). Unconventional therapies for cancer: Iscador. *Canadian Medical Association Journal*, 158(9), 1157-1159.

Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, Waterfield MD. (2001). Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. *Annu Rev Cell Dev Biol.* (17), 615–75.

Kienle, G., Glockmann, A., Schink, M., and Kiene, H. (2009). Viscum album L. extracts in breast and gynaecological cancers: a systematic review of clinical and preclinical research. *Journal of experimental & clinical cancer research: CR*, *28*(1), 79.

Kienle, G. and Kienle, H. (2007). Complementary cancer therapy: a systematic review of prospective clinical trials on anthroposophic mistletoe extracts. *European journal of medical research*, 12(3), 103-119.

Kim, B. (2011). *RCT With Adjuvant Mistletoe Treatment in Gastric Cancer Patients - Tabular View - ClinicalTrials.gov*. [online] Clinicaltrials.gov. Available at: <https://www.clinicaltrials.gov/ct2/show/record/NCT01401075?term=mistletoe&phase=3&draw=2&rank=1> [Accessed 6th March 2021].

Kim, K., Yook, J., Eisenbraun, J., Kim, B. and Huber, R. (2012). Quality of life, immunomodulation and safety of adjuvant mistletoe treatment in patients with

gastric carcinoma – a randomized, controlled pilot study. *BMC Complementary and Alternative Medicine*, 12(1).

Kleijnen, J. and Knipschild, P. (1994). Mistletoe treatment for cancer review of controlled trials in humans. *Phytomedicine*, 1(3), 255-260.

Klingbeil, M., Xavier, F., Sardinha, L., Severino, P., Mathor, M., Rodrigues, R. and Pinto, D. (2013). Cytotoxic effects of mistletoe (Viscum album L.) in head and neck squamous cell carcinoma cell lines. *Oncology Reports*, 30(5), 2316-2322.

Konjevic G, Jovic V, Jurisic V, Radulovic S, Jelic S, Spuzic I. (2003). IL-2-mediated augmentation of NK-cell activity and activation antigen expression on NK- and T-cell subsets in patients with metastatic melanoma treated with interferon-alpha and DTIC. *Clin Exp Metastasis*. (20),647-655.

Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. 2020. Flavonoids as Anticancer Agents. *Nutrients*. 12(2), 457.

Kuttan, G. and Kuttan, R. (1992). Immunological mechanism of action of the tumour reducing peptide from mistletoe extract (NSC 635089) cellular proliferation. *Cancer Letters*, 66(2), 123-130.

Kuttan G. & Kuttan R. (1993). Reduction of leukopenia in mice by "Viscum album" administration during radiation and chemotherapy. *Tumori*. 79(1),74–76.

Lee, Y., Jung, I., Koo, D., Kang, D., Oh, T., Oh, S. and Lee, S. (2018). Efficacy and safety of Viscum album extract (Helixor-M) to treat malignant pleural effusion in patients with lung cancer. *Supportive Care in Cancer*, 27(5), 1945-1949. Lu C.C. and Chen J.K., 2010. Resveratrol enhances perforin expression and NK cell cytotoxicity through NKG2D-dependent pathways. *J Cell Physiol*. 223, pp.343-351.

Lyu, S., Kwon, Y., Joo, H. and Park, W. (2004). Preparation of alginate/chitosan microcapsules and enteric coated granules of mistletoe lectin. *Archives of Pharmacal Research*, 27(1), 118-126.

Majeed, M., Hakeem, K.R., and Rehman, R.U. (2021) Mistletoe lectins: from interconnecting proteins to potential tumour inhibiting agents. *Phytomedicine Plus*, 1(3).

Martini M, De Santis M.C., Braccini L., Gulluni F., Hirsch E. (2014). PI3K/AKT signaling pathway and cancer: an updated review. 2014. *Ann Med*. (46). 372–83.

Marvibaigi, M., Supriyanto, E., Amini, N., Abdul Majid, F. and Jaganathan, S. (2014). Preclinical and Clinical Effects of Mistletoe against Breast Cancer. *BioMed Research International*, 2014, 1-15.

Matthes, H., Friedel, W., Bock, P., and Zänker, K. S. (2010). Molecular mistletoe therapy: friend or foe in established anti-tumor protocols? A multicenter, controlled, retrospective pharmaco-epidemiological study in pancreas cancer. *Current molecular medicine*, 10(4), 430–439.

Mengs, U., Burger, A., Wetzel, D., Weber, K. and Fiebig, H. (2001). The standardized mistletoe preparation Lektinol has antitumoral potencies. *Breast Cancer Research*, 3(S1).

Miller, K., Nogueira, L., Mariotto, A., Rowland, J., Yabroff, K., Alfano, C., Jemal, A., Kramer, J. and Siegel, R. (2019). Cancer treatment and survivorship statistics, 2019. *CA: A Cancer Journal for Clinicians*, 69(5), 363-385.

Moghtaderi, H., Sepehri, H., Attari, F. (2017) Combination of arabinogalactan and curcumin induces apoptosis in breast cancer cells in vitro and inhibits tumor growth via overexpression of p53 level in vivo. Biomedicine & Pharmacotherapy. 88, 582-594.

National Cancer Registry Ireland (2019) Cancer in Ireland 1994-2017 with estimates for 2017-2019: Annual report of the National Cancer Registry. NCRI. [online] Available at:https://www.ncri.ie/sites/ncri/files/pubs/NCRI_Annual%20Report2019_03102019.pdf [Accessed 19th January 2021]

Oei, S., Thronicke, A. and Schad, F., (2019). Mistletoe and Immunomodulation: Insights and Implications for Anticancer Therapies. *Evidence-Based Complementary and Alternative Medicine*, 2019(8), 1-6.

Pae, H., Oh, G., Kim, N., Shin, M., Lee, H., Yun, Y., Oh, H., Kim, Y. and Chung, H., 2001. Roles of Extracellular Signal-Regulated Kinase and p38 Mitogen-Activated Protein Kinase in Apoptosis of Human Monoblastic Leukemia U937 Cells by Lectin-II Isolated from Korean Mistletoe. *In Vitro & Molecular Toxicology*, 14(2), 99-106.

Park, Y., Do, Y. and Jang, B. (2012). Apoptosis of K562 leukemia cells by Abnobaviscum F®, a European mistletoe extract. *Oncology Reports*, 28(6), 2227-2232.

Pietrzak, W., Nowak, R., Gawlik-Dziki, U., Lemieszek, M.K., Rzeski, W. (2017). LC-ESI-MS/MS identification of biologically active phenolic compounds in mistletoe berry extracts from different host trees. Molecules, 22 (624).

Pevzner, I.B., Agapov, I.I., Niwa, H., Maluchenko, N.V., Moisenovich, M.M., Pfüller, U., Tonevitsky, A.G. (2004) Differences in amino acid sequences of mistletoe lectin I and III B-subunits determining carbohydrate binding specificity. *Biochem. Biophys. Acta*, 1675, 155-164.

Ribéreau-Gayon, G., Jung, M., Di Scala, D. and Beck, J., (1986). Comparison of the Effects of Fermented and Unfermented Mistletoe Preparations on Cultured Tumor Cells. *Oncology*, 43(1), 35-41.

Rose, A., El-Leithy, T., Dorp, F., Zakaria, A., Eisenhardt, A., Tschirdewahn, S. and Rübben, H. (2015). Mistletoe Plant Extract in Patients with Nonmuscle Invasive Bladder Cancer: Results of a Phase Ib/IIa Single Group Dose Escalation Study. *Journal of Urology*, 194(4), 939-943.

Roy, P., Saikia, B. (2016) Cancer and cure: A critical analysis. *Indian journal of cancer*, 53(3), 441-442.

Ruebben, H. (2013). *Maximum Tolerable Concentration of abnobaVISCUM Fraxini Intravesically in Patients With Superficial Bladder Cancer - Tabular View - ClinicalTrials.gov*. [online] Clinicaltrials.gov. Available at: <https://www.clinicaltrials.gov/ct2/show/record/NCT02007005?term=mistletoe&phase=1&draw=2&rank=5> [Accessed 6th March 2021].

Schaller, G., Urech, K., Grazi, G., and Giannattasio, M. (1998). Viscotoxin composition of the three European subspecies of Viscum album. Planta Med, 64, 677-678.

Szurpnicka, A., Kowalczuk, A. and Szterk, A. (2020). Biological activity of mistletoe: in vitro and in vivo studies and mechanisms of action. *Archives of Pharmacal Research*, 43(6), 593-629.

Tabiasco, J., Pont, F., Fournié, J. and Vercellone, A. (2002). Mistletoe viscotoxins increase natural killer cell-mediated cytotoxicity. *European Journal of Biochemistry*, 269(10), 2591-2600.

Tschirdewahn, S. (2021). *Therapeutic Instillation of Mistletoe*. [online] Clinicaltrials.gov. Available at: <https://www.clinicaltrials.gov/ct2/show/NCT02106572?term=mistletoe&recrs=a&draw=2&rank=2> [Accessed 18th March 2021].

Twardziok, M., Kleinsimon, S., Rolff, J., Jäger, S., Eggert, A., Seifert, G., Delebinski. C.I. (2016). Multiple active compounds from Viscum album L. synergistically converge to promote apoptosis in Ewing sarcoma. *PLoS One*, 11(9).

Twardziok, M., Meierhofer, D., Börno, S., Timmermann, B., Jäger, S., Boral, S., Eggert, A., Delebinski, C. and Seifert, G. (2017). Transcriptomic and proteomic insight into the effects of a defined European mistletoe extract in Ewing sarcoma cells reveals cellular stress responses. *BMC Complementary and Alternative Medicine*, 17(1).

Urech, K. and Baumgartner, S. (2015). Chemical Constituents of *Viscum album L.: Implications for the Pharmaceutical Preparations of Mistletoe*. *Translation research in biomedicine*. 4, 11-23.

Urech, K., Schaller, G. and Jäggy, C. (2006). Viscotoxins, Mistletoe Lectins and their Isoforms in Mistletoe (Viscum album L.) Extracts Iscador. *Arzneimittelforschung*, 56(06), 428-434.

Vickers, A. & Cassileth, B. (2001). Unconventional therapies for cancer and cancer-related symptoms. *The Lancet Oncology*, 2(4), 226-232.

Wode, K. (2019). *Mistletoe Therapy in Primary and Recurrent Inoperable Pancreatic Cancer* [online] Clinicaltrials.gov. Available at: <https://www.clinicaltrials.gov/ct2/show/NCT02948309?term=mistletoe&draw=2&rank=2> [Accessed 3rd December 2020].

Yang, P., Jiang, Y., Pan, Y., Ding, X., Rhea. P., Ding, J., Hawke, D., Felsher, D., Narla, G., Lu, Z. and Lee, R. (2019) Mistletoe extract Fraxini inhibits the proliferation of liver cancer by down-regulation c-Myc expression. *Scientific Reports*, 9(6428), 1-10.

Zarkovic, N., Vukovic, T., Loncaric, I., Miletic, M., Zarkovic, K., Borovic, S., Cipak, A., Sabolovic, S., Konitzer, M. and Mang, S. (2001). An Overview on Anticancer Activities of the Viscum Album Extract Isorel®. *Cancer Biotherapy and Radiopharmaceuticals*, 16(1), 55-62.

Zänker, K., and Kaveri, S. (eds) (2015) Mistletoe: From Mythology to Evidence-Based Medicine. *Journal of Biomedical and Translation*. 4, 11-23.