AMT: Preclinical pharmacology studies

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Abstract. Auron-Misheil-Therapy (AMT) consisting of aqueous camomile extract supplemented with calcium, vitamins, the antihistamine chlorpheniramine and human insulin is under development as anti-cancer treatment. AMT was preclinically investigated in tumour cell lines and tumour xenografts to guide clinical phase I/II studies. AMT was tested against 56 human tumour cell lines, in a clonogenic assay in 98 patient-derived xenografts and in *in vivo* studies. AMT showed *in vitro* cytotoxic activity with highest susceptibility in cervical cancer, glioblastoma and colon cancers. In the clonogenic assay, anti- cancer activity of AMT was most active in cervical and uterine tumours, in colon cancer, glioblastoma, leukaemia, melanoma and pancreatic cancer. *In vivo*, AMT showed slight activity in tumour xenograft models of colon and mammary cancer. It also showed immune stimulatory effects by induction of IL-6- and TNF-α secretion in human PBMCs. The immune stimulatory potential of AMT, together with slight anti-tumour efficacy observed in the present study, indicates a role of AMT in tumour therapy.

Introduction

In the search for novel, effective anti-cancer treatments not only pure compounds, usually with known modes of action are investigated, but also natural products or more complex preparations, whose substantial anti-tumour activity may be demonstrated before the precise mechanism of action is fully understood. As an example for the latter ones, aqueous extracts of the European mistletoe (*Viscum album* L.) have been widely used for decades as alternative or complementary treatment and adjuvant cancer therapy particularly in Germany, Austria and Switzerland (1-5).

Auron-Misheil-Therapy (AMT) is another representative of a more complex formulation. From compassionate use programs among end-stage cancer patients since 1989, preliminary data have provided evidence of benefits in terms of improved quality of life, sometimes together with body weight gain and reduction of pain. AMT is at present under development as an anti-cancer agent in phase II clinical trials.

Preclinical research with AMT revealed pronounced anti-cancer activity in a range of human tumour models, including bladder, brain, colon, lung, breast, melanoma, prostate and cervix, as well as in cells derived from haematological malignancies, including leukaemias and lymphomas. At the same time, the precise mode of action of AMT remains to be determined. The evidence thus far indicates that its action is predominantly cytotoxic (i.e. actively killing cells) rather than cytostatic (inhibiting mitosis), but the anti-tumour activity of AMT is likely to depend on more than one mechanism, because of the number of different constituents.

This study summarizes the preclinical research with AMT with focus on its anti-tumour and immune stimulatory properties.

Materials and methods

Test compounds. Auron-Misheil-Therapy (AMT) was provided as sterile, ready-to-use solution by Micro Carrier Systems, Neuss and was stored at 4˚C until use. The single components of AMT, aqueous camomile extract (derived from 1.8 g camomile blossom), Calcium Sandoz + Vitamin C® (Novartis), Becozyme® (vitamin B complex, Roche), Allerfin® (chlorpheniramin maleat, The Arab Pharmaceutical Manufacturing Co. Ltd.), and phenol were provided by Micro Carrier Systems, Neuss. Actrapid HM® (human insulin, Novo Nordisk) was obtained from the pharmacy. All compounds were stored at 4˚C.

For *in vitro* studies AMT was tested at concentrations ranging from 0.0001% v/v to 10.0% v/v. The single compo-

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Abbreviations: AMT, Auron-Misheil-Therapy; ATCC, American Type Culture Collection; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen; IC_{50}, 50% inhibitory concentration; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; NCI, National Cancer Institute; PBMC, peripheral blood mononuclear cells; PMA, phorbol-12-myristate-13-acetate; T/C, test/control; TNF, tumour necrosis factor

Key words: anti-tumour activity, immune stimulation, Auron-Misheil-Therapy

Table I. Constitution of AMT for testing individual components.

Component	Amount in 100 ml MISHEIL (ml)	Volume of experimental approach (ml)	Amount of component (ml)	Amount of Aqua injectabilia (ml)
Camomile extract	36.146	1,000	361.46	638.54
Calcium Sandoz + Vitamin C®	35.95	1,000	359.95	640.5
Phenol	0.25	10,000	25.0	9975.0
Becozyme®	16.76	1,000	167.6	832.4
Allerfin®	8.38	1,000	83.8	916.2
Actrapid®	2.514	1,000	25.14	974.86

nents were tested at their respective final concentrations as they appear in AMT (see Table I). A dilution scheme to prepare AMT is given in Table I. AMT (100 ml) consisted of 36.146 ml aqueous camomile extract, 35.95 ml Calcium Sandoz + Vitamin C®, 0.25 ml Phenol, 16.67 ml Becozyme®, 8.38 ml Allerfin®, 2.514 ml Actrapid HM®. Therefore, the single components were diluted with Aqua injectabilia according to Table I and were tested in a dose range of 0.0001% v/v to 10.0% v/v. Final dilutions were prepared in cell culture medium.

For *in vivo* studies the sterile, ready-to-use solution of AMT was administered intramuscularly at doses of 0.12 and 0.06 ml/mouse daily for up to 22 days. The daily dose was split and applied at hour 0 and 6. Control mice received saline solution as vehicle (0.12 ml/mouse/day) intramuscularly, using the corresponding administration schedule.

5-Fluorouracil (5-FU medac®, Medac, Hamburg), dacarbazine (Detimedac®, Medac, Hamburg), paclitaxel (Taxol®, Bristol-Myers Squibb, Munich), gemcitabine (Gemzar®, Lilly, Giessen), cyclophosphamide (Endoxan®, Baxter, Frankfurt) were obtained as clinical formulations from the pharmacy and tested at the maximum tolerated dose in an optimized schedule.

Monolayer assay cytotoxicity assay. A modified propidium iodide assay was used to determine the cytotoxic activity of AMT against human tumour cell lines. The test procedure has been described elsewhere (6). Cell lines tested were derived from patient tumours engrafted as a subcutaneously growing tumour in NMRI nu/nu mice, or obtained from the American Type Culture Collection, Rockville, MD, USA, National Cancer Institute, Bethesda, MD, USA, or Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. Briefly, human tumour cell lines were grown at 37˚C in a humidified atmosphere (95% air, 5% CO_2) in monolayer cultures in RPMI-1640 medium supplemented with 10% fetal calf serum and phenol red (PAA, Cölbe, Germany). Cells were trypsinized and maintained weekly. Cells were harvested from exponentially growing cultures by trypsination, counted and plated in 96-well flat-bottomed micro plates (140 μl cell suspension, $5x10^3$ to $10x10^3$ cells/well). After a 24 h recovery to allow cells to resume exponential growth, 10 μl of culture medium (6 control wells per plate) or medium containing the test drug were added to the wells. Each drug concentration was plated in triplicate. After 4 days of incubation, the culture medium was replaced by fresh medium containing 6 μg/ml of propidium iodide. Microplates were then kept at -18˚C for 24 h, to give a total cell kill. After thawing of the plates, fluorescence was measured using the Cytofluor 4000 microplate reader (Perseptive Biosystems) (excitation 530 nm, emission 620 nm).

Clonogenic assays with human tumour xenografts and haematopoietic stem cells. Effects of AMT on clonogenicity of tumour cells were investigated in a clonogenic assay. Tumour xenografts were derived from patient tumours engrafted as a subcutaneously growing tumour in NMRI nu/nu mice obtained from Oncotest's breeding facility (7,8). Details of the test procedure have been described earlier (9). Briefly, solid human tumour xenografts were removed from mice under sterile conditions, mechanically disaggregated and subsequently incubated with an enzyme cocktail consisting of collagenase type IV (41 U/ml), DNase I (125 U/ml), hyaluronidase type III (100 U/ml) and dispase II (1.0 U/ml) in RPMI-1640 medium at 37˚C for 45 min. Cells were passed through sieves of 200 and 50 μm mesh size and washed twice with sterile PBS-buffer. The percentage of viable cells was determined in a Neubauer-hemocytometer using trypan blue exclusion. The bottom layer consisted of 0.2 ml/well Iscove's Modified Dulbecco's Medium (Life Technologies), supplemented with 20% (v/v) fetal calf serum (Sigma), 0.01% (w/v) gentamicin (Life Technologies) and 0.75% (w/v) agar (BD Biosciences). Cells $1.5x10^4$ to $4x10^4$ were added to 0.2 ml of the same culture medium supplemented with 0.4% (w/v) agar and plated in 24-multiwell dishes onto the bottom layer. The test compounds were applied by continuous exposure (drug overlay) in 0.2 ml of culture medium. Every dish included 6 untreated control wells and drug-treated groups in triplicate at 6 concentrations. Cultures were incubated at 37˚C and 7.5% CO_2 in a humidified atmosphere for 7-20 days and monitored closely for colony growth using an inverted microscope. Within this period, *in vitro* tumour growth led to the formation of colonies with a diameter of >50 μm. At the time of maximum colony formation, counts were performed with an automatic image analysis system (OMNICON 3600, Biosys GmbH). Twenty-four hours prior to evaluation, vital colonies were stained with a sterile aqueous solution of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (1 mg/ml, 100 μl/well).

For testing haematopoietic stem cells, samples of bone marrow (2 donors) or cord blood (1 donor) were diluted 2- to 3-fold with PBS containing 0.1% (w/v) BSA. Mononuclear

cells were enriched from the respective samples by Ficoll Paque® density gradient centrifugation and washed twice with PBS containing 0.1% (w/v) BSA. The resulting cell suspension was stored in aliquots in a freezing medium consisting of 90% v/v fetal calf serum and 10% v/v DMSO at -80˚C. Aliquots were thawed for testing as appropriate. The colony forming test was performed using 24-well plates and MethoCult GF (Stem Cell Technologies) as culture medium. Twenty-five thousand cells/ml of the above-mentioned preparation were seeded in a final volume of 500 μl per well. Solutions of the test compounds were added directly to the medium. Every dish included 6 untreated control wells and drug-treated groups in triplicate at 5 concentrations. Three wells of the test plate were filled with 1 ml of sterile water to ensure that maximum humidity was attained during the subsequent incubation period. Cultures were incubated at 37˚C and 7.5% CO_2 in a humidified atmosphere for 12 days. Colony growth was evaluated using an inverted microscope (10).

In vivo evaluation in nude mice carrying tumour xenografts. *In vivo* efficacy of AMT was determined in mice carrying human tumour xenografts, and murine tumours, respectively. All experiments were conducted according to the guidelines of the German Animal Health and Welfare Act (Tierschutzgesetz). Animal health was examined at the day before tumour implantation and before randomization to ensure that only animals of good health were selected to enter testing procedures. The test procedure has been described elsewhere (11). Briefly, tumour fragments of human tumour xenografts were implanted into the flanks of immune-deficient mice of NMRI nu/nu genetic background (Oncotest breeding facility, Freiburg, Germany). Tumour fragments of murine tumours were implanted into the flanks of C57/black6 mice (Bomholtgard Breeding and Research Center, Ry, Denmark). The fragments were obtained from tumours in serial passage, established either from direct implantation of patient material, or from injection of tumour cell lines obtained from the American Type Culture Collection, Rockville, MD, USA, National Cancer Institute, Bethesda, MD, USA, or Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. Tumour growth was assessed by serial calliper measurements of two perpendicular tumour diameters. Treatment was started when tumours were palpable and reached a median volume of ~100 mm^3, depending on tumour type. Each treatment group consisted of 8-11 mice. Animals with appropriate tumour volumes were randomly distributed into treatment and control groups (day 0). Tumour diameters and body weights were recorded twice weekly. For the evaluation of treatment efficacy, tumour volumes were calculated for each time point according to the formula (length x $width^2$)/2 by using specifically designed software and by plotting the median relative tumour volume against time. Relative tumour volumes were calculated for each single tumour by dividing the tumour volume on day X by the initial tumour volume on day 0 at the time of randomization. A median body weight loss of >20% without recovery was considered not evaluable for anti-tumour efficacy. The U-Test by Mann-Whitney-Wilcoxon was used for the statistical analysis of the data based on median relative tumour volume parameters.

Immune stimulation. Peripheral blood mononuclear cells (PBMC) were isolated from freshly drawn human blood treated with EDTA as an anti-coagulant. Cells were diluted with 3 volumes of CliniMACS PBS/EDTA buffer (Miltenyi, Bergisch Gladbach), carefully layered over FicollPaque (GE Healthcare) in a conical tube and centrifuged at 400 x g for 40 min at 20˚C in a swinging-bucket rotor without brake. The upper layer was aspirated, leaving the mononuclear cell layer undisturbed at the interphase. The interphase cells (lymphocytes, monocytes and thrombocytes) were carefully transferred into a new conical tube. This was filled with CliniMACS PBS/EDTA buffer and centrifuged at 300 x g for 10 min at 20˚C. The supernatant was completely removed. For removal of platelets the cell pellet was resuspended in 50 ml buffer and centrifuged at 200 x g for 10 min at 20˚C. The supernatant was completely removed and the last washing step was repeated. Cells were resuspended in AIMV-Medium (Life Technologies, Karlsruhe) and counted in a Neubauer-haemocytometer.

For the stimulation of PBMC 250,000 cells per well were seeded in a 96-well plate. PBMC of 5 different healthy donors were stimulated with AMT (0.3% v/v), or a stimulation cocktail containing PMA 10 ng/ml (Sigma, Deisenhofen), Ionomycin 1 μg/ml (Sigma) and LPS 1 μg/ml (Linaris, Wertheim-Bettingen), respectively. Cells were incubated at 37˚C and 5% CO_2 in a humidified atmosphere for 24 h.

The supernatants from the stimulated PBMC were taken immediately before use and tested for cytokines by a cytometric bead assay (CBA). The supernatant from PBMC stimulated with the cocktail containing PMA, Ionomycin and LPS was used as an internal positive control. Before the cytokines were measured, the internal positive control and the AMT supernatants were diluted with CBA assay diluent. The cytokines interleukin (IL)-2, IL-4, IL-6, IL-10, tumour necrosis factor α (TNF-α) and interferon γ (IFN-γ) were quantitatively measured with a Coulter Cytomics FC500 cytometer using the CBA (Becton-Dickinson, San Diego) according to the manufacturer's instructions. The results were analyzed with the Coulter Cytomics Bead Array Analysis program.

Results

Auron-Misheil-Therapy (AMT). AMT is a mixture of approved pharmaceuticals in low therapeutic doses and consists of an aqueous extract of camomile supplemented with calcium, vitamins, the antihistamine chlorpheniramine, and human insulin (Table II). AMT was developed as a supportive therapy for patients with malignancies.

In vitro efficacy tests. In order to investigate its anti-tumour efficacy, AMT was tested in a broad panel of 56 permanent human tumour cell lines reflecting 12 different tumour types *in vitro* using a proliferation inhibition assay. The cell line panel comprised commercial available cell lines, as well as cell lines established from solid tumour xenografts which were directly derived from patient material. The ready-to-use AMT solution was added at concentrations ranging from 0.001% v/v to 10% v/v to the cell cultures, and inhibition of proliferation was measured. AMT showed concentration-dependent and

Table II. Formulation of AMT showing final concentrations of single constituents.

Compound	Amount/100 ml	Conc. (mg/ml)
Aqueous camomile extract of 1.8 g blossom	36.146 ml	-
Phenol	0.25 ml	2.65
Calciumglubionate	4943.0 mg	49.43
Vitamin C	1797.5 mg	17.975
Vitamin B1	83.8 mg	0.838
Vitamin B2	45.84 mg	0.4584
Nicotinamide	335.2 mg	3.352
Vitamin B6	33.52 mg	0.3352
Vitamin B5 (Dexpanthenol)	50.28 mg	0.5028
Chlorpheniramin-maleate	83.8 mg	0.838
Human insulin (251.4 I.E.)	8.8 mg	0.088

selective inhibition of tumour cell growth. The concentrations of AMT, where 50 or 70% of the cells were inhibited in proliferation, are given in Table III for each cell line. Cytotoxic activity was most pronounced in the ovarian cancer cell line A2780 (IC_{50} = 0.16% v/v), the cervical cancer cell line SW-756 (IC_{50} = 0.17% v/v), and the colon cancer cell line SW-480 (IC_{50} = 0.17% v/v). Further, above average activity was observed towards tumour cell lines of glioblastoma (CNXF 498NL, LN-229, SF-268, SF-295, U-87 MG; IC_{50} values ranging from 0.32% v/v to 0.52% v/v), colon cancer (COLO-205, DLD-1; IC_{50} = 0.35% v/v and 0.51% v/v respectively), and towards cell lines of leukaemia and lymphoma (CCRF-CEM, HL-60, K-562, MOLT-4, RPMI-8226, U-937; IC_{50} values ranging from 0.18% v/v to 0.39% v/v) (Table III). Tumour cell lines of pancreatic cancer (PAXF 1657L, BXPC3), and renal cancer (RXF 1781L, RXF 393NL, RXF 486L, RXF 944L, CAKI-1) were generally less sensitive (Table III).

Inhibition of clonogenicity of tumour cells by AMT was evaluated in additional tumour models using a clonogenic assay. The anti-proliferative activity of AMT was evaluated in cell suspensions freshly prepared from 98 different human tumour xenografts of 19 tumour types which grew as solid tumours in serial passage on immune-deficient nude mice. In addition, AMT was tested in 3 preparations of haematopoietic stem cells as a model system for non-malignant tissue. AMT inhibited anchorage-independent growth and *in vitro* colony formation of tumour cells in semi-solid medium. IC_{50} values in the clonogenic assay ranged from 0.02% v/v to 6.61% v/v (Table IV). Cytotoxic selectivity of AMT for cervical cancer (CEXF 633 and CEXF 773, IC_{50} = 0.35% v/v and 0.38% v/v, respectively), glioblastomas (CNXF 498, IC_{50} = 0.48% v/v), and leukaemias (CCRF-CEM and JURKAT, IC_{50} = 0.02% v/v and 0.19% v/v, respectively) could be confirmed (Tables IV and V). In addition, considerable inhibition of colony formation was observed against tumour models of melanoma (MEXF 462, MEXF 514, MEXF 535, MEXF 989; IC_{50} values ranging from 0.06% v/v to 0.5% v/v), pancreatic cancer (PAXF 1657 and PAXF 546; IC_{50} = 0.32% v/v and 0.38% v/v respectively), gastric cancer (GXF 251 and GXF 97; IC_{50} = 0.08% v/v and 0.44% v/v respectively), prostate cancer (PRXF 1369 and PRXF MRI-H-1579; IC_{50} = 0.18% v/v and 0.39% v/v respectively), as well as against individual models of renal cancer (RXF 393 and RXF 423; IC_{50} = 0.46% v/v and 0.31% v/v respectively), and colon cancer (CXF 1034, CXF 158, CXF 1753; IC_{50} values ranging from 0.24% v/v to 0.41% v/v) (Tables IV and V). Colony formation of haematopoietic stem cells derived from bone marrow or cord blood was inhibited by AMT with IC_{50} values between 0.24% v/v and 0.34% v/v (Table IV). Less activity was seen in tumour types such as liver cancer, non-small cell lung cancer and ovarian cancer (Tables IV and V).

The single components of AMT at their respective final concentrations were evaluated separately for anti-tumour activity in comparison to the total AMT preparation in the clonogenic assay. Table II shows the concentrations of the single components as they occur in AMT, when the product is manufactured according to the composition shown in Table I. Four human tumour models, responsive towards AMT, were selected according to the above-mentioned test results, namely LXFA 629 (adeno lung cancer), MAXF 401 (adenocarcinoma of the breast), MEXF 462 (amelanotic melanoma), PAXF 1657 (adenocarcinoma of the pancreas). Fig. 1 shows the results at each drug concentration. AMT showed clear antitumour activity in a concentration range between 0.1% v/v and 10.0% v/v, which is in accordance to the response described above. At 1.0% v/v colony formation was inhibited by >70% in 3/4 tumour models, and at 3.0 and 10.0% v/v, in all tumour models tested. Calcium Sandoz + Vitamin C was active at concentrations of 3.0% v/v (inhibition of 2/4 tumour models) and 10.0% v/v (4/4). The aqueous camomile extract was active in 3/4 tumour models at the highest test concentration of 10% v/v (Fig. 1).

Slight inhibitory effects were obtained for Allerfin and Becozyme. Allerfin inhibited colony growth at the highest test concentration in the lung cancer model LXFA 629 and the mammary cancer model MAXF 401 with T/C = 30% in each case, but was inactive in the two other tumour models. Becozyme effected inhibition in colony growth at the highest test concentration in the melanoma model MEXF 462 with T/C = 38%, but was totally inactive in all other tumour models. No anti-tumour activity was seen in case of Actrapid HM (Fig. 1). Overall, the activity of AMT was strikingly higher than that of any single component.

In vivo efficacy tests. AMT was tested in six human xenograft models which have been identified to be sensitive to AMT treatment *in vitro* (see above), as well as in three different models of murine tumours. Anti-tumour efficacy was measured as tumour volume inhibition and growth delay over time. Side effects were determined as lethality and body weight loss. AMT was administered intramuscularly at dose levels of 0.12 and 0.06 ml/mouse daily for up to 22 days. The daily dose was split and applied at hour 0 and 6. Standard anti-cancer drugs, administered at their maximum tolerated dose and optimal schedule, were used as positive controls. AMT was well tolerated in all experiments with median body weight increase in the range of 2-7%. AMT given at 0.06 ml/mouse/day showed slight anti-tumour activity in the human mammary

Table III. Cytotoxic activity of AMT against 56 human tumour cell lines tested *in vitro* in a monolayer proliferation assay.

Tumour type	Tumour cell line	IC_{50} (% v/v)	
Colon	COLO-205	0.35	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	DLD-1	0.51	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	HCC-2998	0.66	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	HCT-116	1.61	IC_{50} >2 mean IC_{50}
	HT-29	1.61	IC_{50} >2 mean IC_{50}
	SW-480	0.17	IC_{50} ≤1/2 mean IC_{50}
Cervix/Uterus	DoTc2	0.39	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	SW-756	0.17	IC_{50} ≤1/2 mean IC_{50}
	UXF 1138L	0.43	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Central nervous system	CNXF 498NL	0.52	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	LN-229	0.49	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	SF-268	0.32	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	SF-295	0.33	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	U-87 MG	0.45	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Leukaemia	CCRF-CEM	0.28	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	HL-60	0.22	IC_{50} ≤1/2 mean IC_{50}
	K-562	0.19	IC_{50} ≤1/2 mean IC_{50}
	MOLT-4	0.20	IC_{50} ≤1/2 mean IC_{50}
Lymphoma	RPMI-8226	0.18	IC_{50} ≤1/2 mean IC_{50}
	U-937	0.39	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Lung, non-small cell	LXF 1121L	0.42	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	LXF 289L	0.51	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	LXF 526L	0.34	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	LXF 529L	0.97	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	LXF 629L	0.71	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	LXF 66NL	1.25	IC_{50} >2 mean IC_{50}
	LXF 923L	1.72	IC_{50} >2 mean IC_{50}
	H460	1.86	IC_{50} >2 mean IC_{50}
Breast	MAXF 401NL	0.29	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MCF-7	0.59	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MDA-MB-231	0.49	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MDA-MB-453	0.51	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MDA-MB-486	0.47	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Melanoma	MEXF 276L	0.24	IC_{50} ≤1/2 mean IC_{50}
	MEXF 394NL	0.54	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MEXF 462NL	0.48	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MEXF 514L	0.76	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MEXF 520L	0.55	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Ovary	OVXF 1619L	0.49	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	OVXF 899L	0.90	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	A2780	0.16	IC_{50} ≤1/2 mean IC_{50}
	OVCAR-3	0.66	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	OVCAR-5	0.57	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	SK-OV-3	1.82	IC_{50} >2 mean IC_{50}
Pancreas	PAXF 1657L	1.87	IC_{50} >2 mean IC_{50}
	BxPC3	0.91	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	PANC-1	0.63	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Prostate	DU-145	0.35	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	PC3M	0.80	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	22RV1	0.52	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	LNCAP	1.42	IC_{50} >2 mean IC_{50}

Table III. Continued.

Tumour type	Tumour cell line	IC_{50} (% v/v)	
Kidney	RXF 1781L	1.74	IC_{50} >2 mean IC_{50}
	RXF 393NL	0.75	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	RXF 486L	1.12	IC_{50} >2 mean IC_{50}
	RXF 944L	0.61	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	CAKI-1	1.64	IC_{50} >2 mean IC_{50}
Mean IC_{50}		0.55	

Compound	Tumour	T/C (%) at test concentration % v/v					
		0.001	0.01	0.1	1.0	3.0	10.0
AMT	LXFA 629	84	76	92	31	19	1
	MAXF 401	97	78	51	0	0	0
	MEXF 462	81	82	80	19	2	0
	PAXF 1657	99	90	86	22	0	0
Camomile extract	LXFA 629	80	80	73	66	64	9
	MAXF 401	82	83	81	63	79	4
	MEXF 462	95	91	68	65	49	26
	PAXF 1657	100	96	100	94	105	84
Calcium Sandoz + Vitamin C®	LXFA 629	89	81	75	60	31	8
	MAXF 401	70	82	84	66	15	3
	MEXF 462	94	89	103	86	2	1
	PAXF 1657	85	82	89	121	100	15
Phenol	LXFA 629	89	81	94	93	89	23
	MAXF 401	93	71	92	91	75	2
	MEXF 462	89	59	87	84	83	29
	PAXF 1657	78	83	78	89	82	77
Allerfin®	LXFA 629	83	82	84	103	125	30
	MAXF 401	84	88	79	97	91	30
	MEXF 462	88	93	94	86	109	115
	PAXF 1657	97	101	87	93	90	91
Becozyme®	LXFA 629	85	93	93	81	84	83
	MAXF 401	95	85	92	86	95	90
	MEXF 462	80	90	88	98	96	38
	PAXF 1657	84	95	94	94	98	93
Actrapid®	LXFA 629	83	77	78	80	88	82
	MAXF 401	89	90	86	98	115	114
	MEXF 462	89	87	91	94	98	70
	PAXF 1657	89	75	76	73	83	87

Figure 1. Anti-tumour activity of AMT and single components thereof *in vitro* using a clonogenic assay.

Table IV. Cytotoxic activity of AMT against 98 human tumour xenografts tested *in vitro* in a clonogenic assay.

Tumour type	Tumour xenograft	IC_{50} (% v/v)	
Haematopoietic stem cells	KMGLZ	0.27	IC_{50} ≤1/2 mean IC_{50}
	KMSPL	0.34	IC_{50} ≤1/2 mean IC_{50}
	NSB001	0.24	IC_{50} ≤1/2 mean IC_{50}
Bladder	BXF 1036	0.38	IC_{50} ≤1/2 mean IC_{50}
	BXF 1218	0.77	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Cervix/uterus	CEXF 633	2.35	IC_{50} >2 mean IC_{50}
	CEXF 773	0.35	IC_{50} ≤1/2 mean IC_{50}
	SK-UT-1B	0.38	IC_{50} ≤1/2 mean IC_{50}
Glioblastoma	CNXF 498	0.30	IC_{50} ≤1/2 mean IC_{50}
	SF-268	0.48	IC_{50} ≤1/2 mean IC_{50}
Colon	CXF 1034	0.64	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	CXF 1044	0.41	IC_{50} ≤1/2 mean IC_{50}
	CXF 1103	4.53	ICv >2 mean IC_{50}
	CXF 1297	0.64	IC_{50} ≤1/2 mean IC_{50}
	CXF 1299	6.61	IC_{50} >2 mean IC_{50}
	CXF 158	0.28	IC_{50} ≤1/2 mean IC_{50}
	CXF 1729	1.15	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	CXF 1753	0.24	IC_{50} ≤1/2 mean IC_{50}
	CXF 1784	4.55	IC_{50} >2 mean IC_{50}
	CXF 243	1.36	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	CXF 264	4.92	IC_{50} >2 mean IC_{50}
	CXF 280	0.77	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	CXF 647	1.36	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	CXF 676	3.07	IC_{50} >2 mean IC_{50}
	CXF 886	0.55	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Stomach	GXF 1172	1.03	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	GXF 209	3.92	IC_{50} >2 mean IC_{50}
	GXF 251	0.08	IC_{50} ≤1/2 mean IC_{50}
	GXF 97	0.44	IC_{50} ≤1/2 mean IC_{50}
Head and neck	HNXF 536	5.27	IC_{50} >2 mean IC_{50}
	HNXF 908	0.57	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Leukaemia	CCRF-CEM	0.02	IC_{50} ≤1/2 mean IC_{50}
	JURKAT	0.19	IC_{50} ≤1/2 mean IC_{50}
Lymphoma	RAJI	1.62	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Myeloma	L-363	0.71	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Liver	LIXF 1788	0.50	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	LIXF 1791	5.55	IC_{50} >2 mean IC_{50}
	LIXF 575	2.02	IC_{50} >2 mean IC_{50}
	HEP-G2	2.47	IC_{50} >2 mean IC_{50}
Lung, adenocarcinoma	LXFA 1012	0.23	IC_{50} ≤1/2 mean IC_{50}
	LXFA 289	3.54	IC_{50} >2 mean IC_{50}
	LXFA 297	5.18	IC_{50} >2 mean IC_{50}
	LXFA 526	2.03	IC_{50} >2 mean IC_{50}
	LXFA 592	0.44	IC_{50} ≤1/2 mean IC_{50}
	LXFA 629	0.12	IC_{50} ≤1/2 mean IC_{50}
	LXFA 677	2.30	IC_{50} >2 mean IC_{50}
	LXFA 749	3.07	IC_{50} >2 mean IC_{50}
	LXFA 923	4.41	IC_{50} >2 mean IC_{50}
	LXFA 983	1.98	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	A-549	5.40	IC_{50} >2 mean IC_{50}
	PC-14	1.63	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}

Table IV. Continued.

Tumour type	Tumour xenograft	IC_{50} (% v/v)	
Lung, squamous cell	LXFE 211	1.89	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	LXFE 397	1.57	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	LXFE 409	3.54	IC_{50} >2 mean IC_{50}
Lung, large cell	LXFL 1072	1.61	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	LXFL 1176	1.76	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	LXFL 430LX	0.29	IC_{50} ≤1/2 mean IC_{50}
	LXFL 529	0.88	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Breast	MAXF 1162	0.78	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MAXF 1322	2.05	IC_{50} >2 mean IC_{50}
	MAXF 1384	1.00	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MAXF 401	0.19	IC_{50} ≤1/2 mean IC_{50}
	MAXF 449	1.30	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MAXF 574	0.56	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MAXF 583	5.19	IC_{50} >2 mean IC_{50}
	MAXF 857	1.57	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MCF-7	1.54	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MDA-MB-231	1.53	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MX1	0.52	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Melanoma	MEXF 1341	1.72	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MEXF 276	1.90	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MEXF 462	0.43	IC_{50} ≤1/2 mean IC_{50}
	MEXF 514	0.50	IC_{50} ≤1/2 mean IC_{50}
	MEXF 535	0.33	IC_{50} ≤1/2 mean IC_{50}
	MEXF 672	1.17	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MEXF 989	0.06	IC_{50} ≤1/2 mean IC_{50}
	HT-144	1.53	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Ovary	OVXF 1023	1.60	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	OVXF 550	2.17	IC_{50} >2 mean IC_{50}
	OVXF 899	2.34	IC_{50} >2 mean IC_{50}
	SK-OV-3	2.06	IC_{50} >2 mean IC_{50}
Pancreas	PAXF 1657	0.32	IC_{50} ≤1/2 mean IC_{50}
	PAXF 546	0.38	IC_{50} ≤1/2 mean IC_{50}
	PAXF 736	0.66	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MIA PaCa-2	1.74	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Prostate	PRXF 1369	0.18	IC_{50} ≤1/2 mean IC_{50}
	22RV1	2.80	IC_{50} >2 mean IC_{50}
	DU-145	1.17	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	MRI-H-1579	0.39	IC_{50} ≤1/2 mean IC_{50}
	PC3M	4.99	IC_{50} >2 mean IC_{50}
Kidney	RXF 1220	1.87	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	RXF 1393	2.43	ICv >2 mean IC_{50}
	RXF 393	0.46	IC_{50} ≤1/2 mean IC_{50}
	RXF 423	0.31	IC_{50} ≤1/2 mean IC_{50}
	RXF 631	1.56	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	RXF 944LX	2.11	IC_{50} >2 mean IC_{50}
Sarcoma	SXF 1186	5.04	IC_{50} >2 mean IC_{50}
	SXF 1301	1.73	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	SXF 1410	0.35	IC_{50} ≤1/2 mean IC_{50}
	SXF 463	1.97	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
	SXF 627	1.87	1/2 mean IC_{50} <IC_{50} ≤2 mean IC_{50}
Mean IC_{50}		1.0	

Table V. Tumour type selectivity of AMT in human tumour xenografts tested *in vitro* in a clonogenic assay.

Tumour type	Anti-tumour selectivity	
	Sensitivity[a]	Resistance[b]
Cervix/Uterus	3/3	0/3
Leukaemia	2/2	0/2
Melanoma	4/8	0/8
Stomach	2/4	1/4
Pancreas	2/4	0/4
CNS	1/2	0/2
Prostate	2/5	2/5
Kidney	2/6	2/6
Bladder	1/3	1/3
Colon	3/14	5/14
Lung NSCL	4/19	8/19
Sarcoma	1/5	1/5
Mammary	1/11	2/11
Head and neck	0/2	1/2
Lymphoma	0/2	0/2
Liver	0/4	3/4
Ovary	0/4	3/4
28/98	29/98	

[a]Individual IC_{50} <1/2 mean IC_{50} of all tumours and [b]individual IC_{50}.

cancer model MAXF 401 (optimal T/C of 71%), as well as in the murine melanoma B-16 (optimal T/C of 63%), and given at 0.12 ml/mouse/day in the murine colon adenocarcinoma colon 38 (optimal T/C of 65%) (Table VI). AMT revealed the greatest inhibitory effect in the human colon cancer model CXF 1753 with an optimal T/C of 67% at 0.06 ml/mouse on day 28 which was significant ($p<0.05$) (Table VI, Fig. 2). Anti-tumour efficacy was generally not strictly dose-dependent. CXF 1753 responded to the positive control 5-fluorouracil which was given at 100 mg/kg/d i.p. on days 0, 7 and 14 with an optimal T/C value of 27% on day 28 (Fig. 2).

The human melanoma MEXF 989, the lung adenocarcinoma LXFA 629, the pancreatic adenocarcinoma PAXF 1657, and the leukaemia CCRF-CEM (optimal T/C values ranging from 78 to 100%) and the murine Lewis lung adenocarcinoma (optimal T/C of 84%) were resistant to AMT treatment (Table VI). The standard anti-cancer drugs used as positive controls, dacarbazine (MEXF 989), paclitaxel (LXFA 629), gemcitabine (PAXF 1657), cyclophosphamide (CCRF-CEM), showing significant inhibition of tumour growth in each case (Table VI).

Immune stimulatory effects. The immune stimulating of AMT was investigated on PBMCs of five different healthy donors. AMT was tested at a subtoxic concentration of 0.3% v/v. The secretion of IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ was quantitatively measured by flow cytometry. AMT clearly

Table VI. Inhibition of tumour growth by AMT tested *in vivo* in tumour-bearing mice.

Tumour type	Tumour model	AMT		Positive controls	
		Dose (ml/mouse/day)[a]	Optimum T/C (%)	Compound[b]	Optimum T/C (%)
Human colon cancer	CXF 1753	0.12	68	5-Fluorouracil	27
		0.06	67	(5-FU medac®)	
Human lung cancer	LXFA 629	0.12	87	Paclitaxel	4
		0.06	96	(Taxol®)	
Human breast cancer	MAXF 401	0.12	93	Paclitaxel	0
		0.06	71	(Taxol®)	
Human melanoma	MEXF 989	0.12	100	Dacarbazine	0
		0.06	100	(Detimedac®)	
Human pancreatic cancer	PAXF 1657	0.12	86	Gemcitabine	53
		0.06	95	(Gemzar®)	
Human leukaemia	CCRF-CEM	0.12	100	Cyclophosphamide	0
		0.06	100	(Endoxan®)	
Murine colon carcinoma	Colon 38	0.12	65	n.d.	n.a.
		0.06	76		
Murine lung cancer	Lewis lung	0.12	92	n.d.	n.a.
		0.06	84		
Murine melanoma	B-16	0.12	71	n.d.	n.a.
		0.06	63		

[a]AMT was given daily for up to 22 consecutive days, with the daily dose split into two administrations. [b]Doses of positive controls: 5-Fluorouracil 100 mg/kg/d i.p. on days 0, 7, 14; Paclitaxel 20 mg/kg/d i.v. on days 0, 7, 14; Dacarbazine 300 mg/kg/d i.p. on day d 0; Gemcitabine 300 mg/kg/d i.v. on days 1 and 15; Cyclophosphamide 200 mg/kg i.p. on days 0 and 14. n.d., not done and n.a, not available.

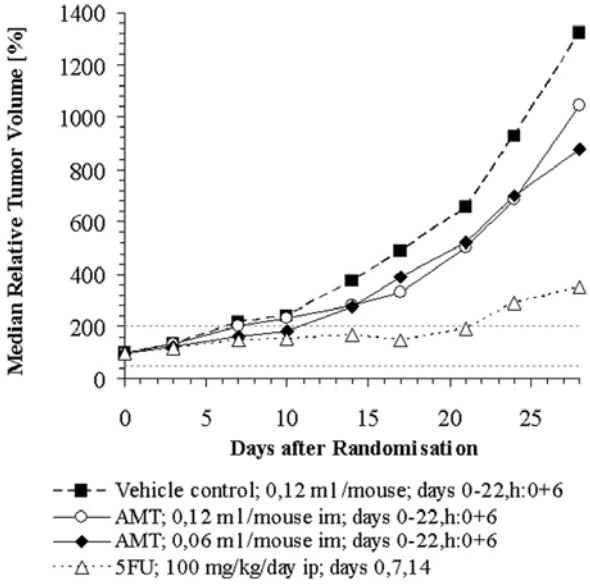

Figure 2. *In vivo* anti-tumour efficacy of AMT in the human colon adenocarcinoma model CXF 1753.

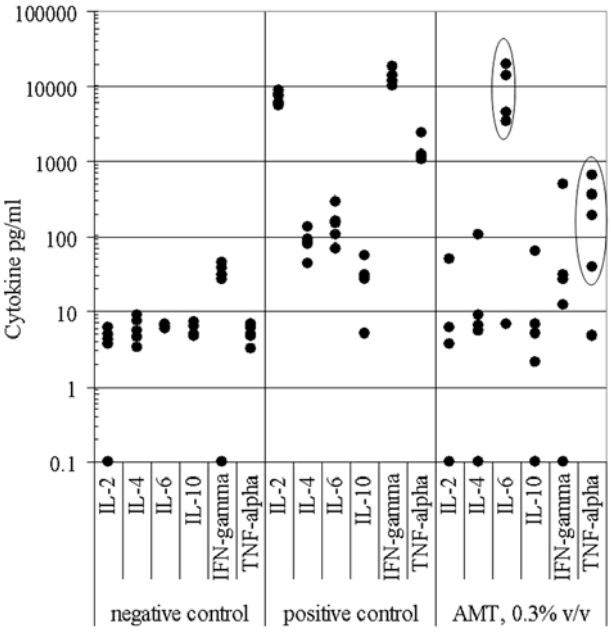

Figure 3. Induction of cytokine secretion by AMT compared to background and positive control (LPS, PMA, ionomycin).

induced the secretion of the cytokines IL-6 and TNF-α in 4/5 donors. Levels of detected IL-6 were in the range between 3.4 and 20.0 ng/ml at 0.3% v/v AMT, compared to a IL-6 secretion range of 0.067 to 0.29 ng/ml in case of the positive control (a stimulation cocktail of LPS, PMA and ionomycin) in these donor preparations (Fig. 3). TNF-α levels in successfully stimulated blood samples were between 0.04 and 0.65 ng/ml in case of AMT (0.3% v/v) and between 1.1 and 2.4 ng/ml in case of the positive control (Fig. 3). Background levels of IL-6 and TNF-α in unstimulated PBMCs were <0.007 ng/ml (Fig. 3). In addition, a moderate elevation of IL-2, IL-4 and IL-10 could be detected in individual blood samples, but this effect was not consistent among all samples investigated (Fig. 3).

Discussion

In contrast to pure compounds with defined chemical structure, the basis of AMT is an aqueous extract of camomile, supplemented with calcium, several vitamins, the antihistamine chlorpheniramine, and human insulin. Despite the difficulty of allocating an active principle in such a complex compound mixture with the therapeutic use of such preparations, the therapeutic use of phytotherapeutic medicines is generally accepted, e.g. the use of St. John's Wort as antidepressant for treatment of mild to moderately severe depression (12-14), the sleep enhancing effect of valerian (15), or the use of aqueous extracts of the European mistletoe (*Viscum album* L.) as alternative treatment and adjuvant cancer therapy particularly in Germany, Austria and Switzerland (1-5). The objective of the present study was to investigate anti-tumour efficacy of AMT *in vitro* and *in vivo* in tumour cell lines and tumour xenografts in order to understand the therapeutic potential of the preparation.

The *in vitro* anti-proliferative activity of AMT was evaluated in panels of permanent human tumour cell lines and human tumour xenografts growing as colonies in soft agar. AMT showed concentration-dependent and selective inhibition of proliferation in several human tumour cell lines of cervical cancer, colon cancer, glioblastoma, ovarian cancer, leukaemia, and lymphoma. These findings provide evidence for the anti-neoplastic potency of AMT. Using a clonogenic assay, AMT was further characterized for its ability to inhibit anchorage-independent growth and *in vitro* colony formation of tumour cells in semi-solid medium. The test panel of 98 tumours, representing 19 different tumour types, aimed to reflect the heterogeneity of human tumours seen in clinical studies. The tumour cell preparations for the clonogenic assay were prepared directly from human tumour xenografts growing in nude mice. Based on the heterogeneity of growing solid tumour, these preparations comprised tumour stem cells to a certain extent which are discussed to be responsible for the metastatic and infiltrative potential of a tumour (16-20). Hence, the clonogenic assay reflects better the *in vivo* situation than *in vitro* assays using permanent tumour cell lines and has been found to be a predictive test for further *in vivo* evaluation of anti-cancer drugs (9,21-24). AMT inhibited colony formation of tumour cells in semi-solid medium, and based on the distribution of IC_{50} values, tumour types such as cervical cancer, colon cancer, glioblastoma, leukaemia, melanoma, and pancreatic cancer were identified to be most sensitive towards AMT. Based on IC_{50} values, these sensitive tumour models were on average ~4-fold more sensitive than the mean IC_{50} of all models tested, indicating a tumour selective way of growth inhibition. Moreover, these sensitive tumour models were slightly more sensitive than haematopoietic stem cells as representative model system for non-malignant tissue. Thus, inhibition of colony formation of haematopoietic stem cells was in an acceptable range.

In order to allocate the anti-tumour activity of AMT to an active constituent, the single components of AMT were tested in comparison to the whole formulation in four different

tumour models. There was clear anti-tumour activity of part, but not all of the components. Calcium Sandoz + Vitamin C, the aqueous camomile extract and phenol showed most inhibition of colony formation. However, the activity of AMT was strikingly higher than that of any single component. Thus, the anti-tumour activity of AMT benefits from the combination of all single components. Besides the aqueous camomile extract, calciumglubionate is present in the mixture at the highest concentration of all single compounds, and activity of Calcium Sandoz + Vitamin C could be demonstrated in the present study. Calcium as a ubiquitous second messenger regulates many cellular functions, including cell growth, differentiation and apoptosis (25-27). In addition, it was shown by several groups, that calcium can slow down cancer cell division, and administration of calcium offers an approach to the primary prevention of intestinal neoplasia and/or therapy of colorectal cancer metastases (28-30). In this manner, calcium probably contributes to the anti-tumour activity of AMT. But clearly, progress towards the clinical use of AMT will require detailed investigation into the precise role of each of its constituents.

In subsequent *in vivo* studies in tumour xenografts, established in immune-deficient nude mice, administrations of AMT at doses of 0.12 or 0.06 ml/mouse/day, respectively, were well tolerated. The dose level of 0.12 ml/mouse/day corresponds to 3.6 ml/kg/day, equal to 6.48 ml/m^2 body surface (31). This dose is in agreement with the dose used in the clinical set-up with cancer patients who are treated with 5 ml AMT twice daily, equal to 10 ml/patient/day, or 5.56 ml/m^2 body surface. Slight anti-tumour activity was observed in a well differentiated, papillary breast adenocarcinoma, derived from a lung metastasis of a 51-year old female patient (MAXF 401), as well as in the murine melanoma B-16, and the murine colon adenocarcinoma colon 38. However, tumour growth inhibition was not significant, and not as pronounced as the respective positive control paclitaxel in case of MAXF 401. Significant inhibition of tumour growth was observed in a colon adenocarcinoma xenograft model, derived from an abdominal wall metastasis of a 61-year old male patient (CXF 1753), but tumour growth inhibition was not as pronounced as the positive control 5-fluorouracil in this model.

The immune stimulating potency of AMT was shown in experiments with isolated PBMCs, where AMT induced the secretion of IL-6 and TNF-α. IL-6, also known as interferon-$ß_2$ (IFN-$ß_2$), T-cell replacing factor (TRF)-like factor, B cell differentiation factor (BCDF), B-cell stimulatory factor-2 (BSF-2), hybridoma-plasmacytoma growth factor (HPGF), hepatocyte stimulating factor (HSF), or monocyte-granulocyte inducer type-2 (MGI-2), is a multifunctional cytokine which is produced by a variety of cells including T- and B cells and monocytes. IL-6 regulates immune responses, acute phase reactions and haematopoiesis (32-35), and is involved in T cell activation, growth and differentiation (35,36). It is known to induce serine esterase and perforin which are required for target cell lysis in the granules of cytotoxic T cells (37,38) which are described to play an important role in the direct recognition and killing of immunogenic tumour cells. IL-6 has shown activity against cancer cells (39,40), but was also found to be a growth factor for myeloma cells (41,42). TNF-α is produced by immune cells including T cells, B cells, NK cells and monocytes. TNF-α was originally characterized as an anti-tumour protein inducing necrosis of MethA sarcomas *in vivo* (43). TNF-α has been extensively studied and considered as an anti-tumour drug. It was applied intra-tumourally in cancer patients and some efficacy could be observed (44,45). However, the use of TNF-α as a single systemic agent in high doses has shown toxic side effects (46,47). Mechanisms of action, that have been suggested for the anti-tumour effect of TNF-α, include direct cytotoxicity against tumour cells, activation of immune anti-tumour response and selective damage of tumour blood vessels (48). In acute phase reactions which occur e.g. during early phases of an infection IL-6 and TNF-α have a wide spectrum of biological activities that help to coordinate the body's response. Some of the effects e.g. increased activation of B- and T lymphocytes and phagocytosis, are also important in anti-tumoural immune responses. The secretion of IL-6 and TNF-α was induced during incubation of PBMCs with AMT. It has been described that aqueous extracts of the European mistletoe (*Viscum album* L.) also stimulate immunological relevant immune effector cells like macrophages, natural killer cells, as well as B- and T lymphocytes with subsequent release of cytokines, such as IL-1, IL-6, IL-10, TNF-α and granulocyte macrophage colony stimulating factor (49-53). These effects contributed to the established use of such extracts for stimulation of the immune system in patients suffering from malignant tumours (54).

At present, AMT is under the development as an anti-cancer agent. From compassionate use programs among end-stage cancer patients in the Middle East since 1989, preliminary data have provided evidence of benefits in terms of improved quality of life, sometimes together with body weight gain and reduction of pain. During clinical studies, it would now be of interest also to monitor cytokine levels in serum of patients before and after treatment. The immune stimulatory potential of AMT together with the slight anti-tumour efficacy observed in the present study indicate a role of AMT in tumour therapy.

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