Course of Mitogen-stimulated T Lymphocytes in Cancer Patients Treated with *Viscum album* Extracts

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Abstract. *Background: In a prospective observational study, the impact of two different dose regimes of a commercially available fermented Viscum album L. extract (VA-E, Iscador®) on the function of T lymphocytes from cancer patients was investigated. Patients and Methods: A total of 71 cancer patients were enrolled. These patients attended two different sections of a tumor outpatient clinic which are used to apply different VA-E escalation schemes. Our hypothesis was that a rapid dose escalation of subcutaneously applied VA-E may induce strong local reactions at the injection side (>3 cm diameter) and may have an effect on the functional competence of T lymphocytes (mitogen-activated interleukin-2 receptor alpha chain), which was recorded over an observation period of six month. Results: Within this observation period, a decline of stimulated T cell function was observed, particularly in patients with colorectal or prostate cancer; this decline was not seen in patients with breast cancer (who received lower mean concentrations per month) nor in patients with dose adaptation in response to too strong local reactions. Conclusion: With respect to T-cell function, our results indicate that in patients without local reactions, a long lasting mistletoe extract application should be withheld periodically to allow T-cell reactivity to recover.*

Extracts from *Viscum album* (VA-E) are widely used as complementary cancer treatment particularly in Germany and Switzerland. Several clinical studies, including historical, retrospective, prospective and randomized trials, reported extended survival times, improved quality of life, or tumor regression with mistletoe therapy (for review see 1, 2). The pharmacological effects of VA-E are defined not only by the induction of apoptosis (3-7), but also by indirect immune activation (for an overview see 1, 8, 9).

In breast cancer patients, Hajto (10) observed an increased number and function of granulocytes 6 h after intravenous application of VA-E. Within 24 h, uptake of [^{3}H]-thymidine in the DNA of mitogen-stimulated lymphocytes, natural killer and antibody-dependent cell-mediated cytotoxicity in the peripheral blood, and numbers of large granular lymphocytes increased, but returned to baseline levels by 48 to 72 h. Our group observed a prevention of surgery-induced depression of the oxidative burst in granulocytes from breast cancer patients by intravenous application of VA-E (18).

After subcutaneous administration of VA-E to breast cancer patients, an increase of peripheral T helper cells, NK- and T-cells with expression of interleukin-2-receptor α chains (CD25) was observed within 4 weeks in 10 patients (11, 12). In other studies, however, no significant changes were observed in the lymphocyte subsets after subcutaneous application of the same VA-E (13, 14). Investigations of long-term treated tumor patients showed that the relative proportion of lymphocytes and the number of NK-cells increased within 7 months of subcutaneous application at increasing VA-E concentrations, while the number of other lymphocyte subsets (*i.e.* $CD19^+$ B-cells, $CD4^+$ T helper cells, $CD8^+$ $CD28^-$ suppressor cells, $CD8^+$ $CD28^+$ cytotoxic cells) and the proportion of $CD25^+$ (activated) T-lymphocytes showed only some statistically remarkable trends (15). For $CD19^+$ B-cells, $CD4^+$ T helper cells, $CD8^+$ $CD28^+$ cytotoxic cells and $CD16^+/CD56^+$ NK-cells, we observed statistically remarkable peaks within the 2nd and the 3rd month of therapy (15).

The results are obviously conflicting, but may depend on different schemes of dosage and different preparations of the drug. In fact, the dose applications of VA-E remain a matter of discussion. Preparatory studies have shown that

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strong local reactions after subcutaneous injection of VA-E, which are in most cases due to a rapid increase of applied VA-E concentrations, call forth a physiological "reaction reduction" from T-cells in some patients. In this prospective observational study, with defined inclusion and exclusion criteria, we thus aimed to observe the impact of two different dose regimes of the commercially available fermented VA-E Iscador® M or Iscador® Qu on tumor patients with regards to the function of T lymphocytes, a matter which is ignored in most studies.

Patients and Methods

Design. All patients came to the outpatient clinic of the Gemeinschaftskrankenhaus Herdecke run by Professor Schietzel and Dr. Stumpf to receive mistletoe therapy. Thus, due to ethical reasons, we were unable to randomize them. Nevertheless, as both medical doctors used different dose escalation schemes for several years, we were able to compare two groups within this observational study. The study was registered according the German law and was carried out according to the ICH-GCP guidelines.

The primary aim of this observational study was to investigate the functional competence of stimulated T lymphocytes, while the secondary aims were the course of peripheral lymphocyte subsets, quality of life and body temperature of the patients in response to the subcutaneous VA-E injections. Because it was not designed as a confirmatory study to prove efficacy, but as an explorative study to judge safety of two different dosage schemes, we had no control group without any mistletoe extract application.

Patients. All patients were informed about the purpose of the study. They gave informed consent to participate and were recruited consecutively between March 2002 and November 2004. The patients were not selected and were enrolled consecutively as they attended the clinics (Figure 1).

The inclusion criteria were: Patients with breast cancer, colorectal cancer, or prostate cancer, adequate blood counts (*i.e.* leukocytes >3,000/μl, thrombocytes >100,000/μl), adequate kidney and liver function, Karnofsky's Index >60%, at least 2 weeks after a surgical intervention, at least 4 weeks after chemotherapy and/or radiation, no mistletoe therapy within the previous 12 weeks, and informed consent for data collection.

The exclusion criteria were: pregnancy or lactating patients, significant pre-existing medical or psychiatric conditions (including history of *e.g.* heart disease, leukemia, autoimmune diseases), patients in final stages of their disease, persisting toxicity of a prior chemotherapy or radiation at the onset of the study, protein allergy (especially against proteins from mistletoe), use of other immunomodulating drugs, or participation in another study during the previous 4 weeks.

A total of 71 patients were enrolled (Figure 1). Among them, the data from 4 patients were not analyzed because they left the study within the first 3 months. Among the remaining 67 patients (Table I) all could be investigated within the time frame of the 3rd to the 6th month (none of the 67 patients left the study. There were 36 patients with breast cancer 14 with prostate cancer and 17 with colorectal tumors (8 colon, 8 rectum, 1 sigma). Forty-four of the patients were female and 23 male.

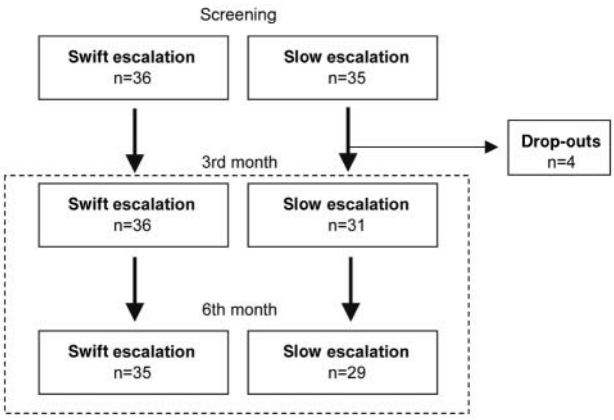

Figure 1. *Set of patients analyzed within the two dosage groups. The patients were not selected and were enrolled consecutively as they attended the clinics of the two medical doctors following different dosage schemes. The reasons for the drop-outs are described in the text.*

In order to establish a connection between immune modulation and conventional outcome parameters (*i.e.* relapse and metastasis frequency, survival time), patients will be followed over a period of at least five years. The immunological tests were performed monthly during the first six months.

Mistletoe extract. Women received a commercially available fermented drug extract from mistletoe grown on apple trees, Iscador® M (Weleda AG, Schwäbisch Gmünd, Germany), while men received a standardized fermented drug extract from mistletoes grown on oak trees, Iscador® Qu. All patients received the VA-E subcutaneously 2 to 3 times per week in the morning (7 to 10 o'clock).

Thirty-six patients were treated in the trial section of Dr. Stumpf with "series packs" of Iscador® ("swift" escalation scheme; VA-E swift), *i.e.* series pack 0: 2 ampoules 0.01 mg subcutaneously, 2x0.1 mg, 3x1.0 mg; series package 1: 2x0.1 mg, 2x1.0 mg, 3x10 mg, and series package 2: 2x1.0 mg, 2x10 mg, 3x 20 mg.

Thirty-one of the tumor patients were intended to be treated with a "slow" escalation schema (intention-to-treat set, ITT; VA-E slow) by Prof. Dr. Schietzel: 8 ampoules 0.01 mg for 4 weeks, 4 weeks 0.01 mg and 0.1 mg alternating, 4 weeks 0.1 mg, *etc.* Because of the fact that all medical doctors were free to diverge from their own intention, the mean concentration of applied VA-E per month and injection were calculated (Tables II-IV). In fact, in the slow escalation group, within the first 4 weeks most patients received 0.04 mg as a mean concentration per month, within the 2nd month most had 0.6 mg (2 patients had >2 mg/month), at the 3rd month 1.0 mg (2 patients had >10 mg/month), at the 4th month 0.85 mg (2 patients had >10 mg/month), at the 5th month 1.0 mg (6 patients had >10 mg/month), and 1 mg at the 6th month (6 patients had >10 mg/month).

Thirty-six patients were intended to be treated by the swift escalation scheme. In 14 of them the local reaction was too strong and the doses had to be reduced (= per protocol analysis set, PP). Although not planned, the numbers of patients VA-E swift *vs.* VA-E slow were comparable: Among the women, 24 received VAE-swift and 20 VA-E slow; among the men, 12 had VA-E swift and 11

VA-E slow. With respect to the tumor localization, 20 patients with breast cancer received VA-E swift and 16 VA-E slow. However, most patients with prostate cancer received VA-E slow (n=11) and only 3 VA-E swift, while most patients with colorectal tumors received VA-E swift (n=13) and only 4 VA-E slow.

Local reactions. It was postulated that a swift increase of VA-E dosage would lead to stronger local reactions and thus may impair the stimulation capacity of T lymphocytes taken *ex vivo* and incubated for 72 hours in the presence of the mitogenic lectin phytohemagglutinine. To obtain more exact data, the patients received a "side-effect diary" in which they documented the concentration of each Iscador® ampoule and the observed local reactions (size, redness, swelling, itching) at the injection site.

In vitro cultures. Peripheral blood cells from the patients taken each month within an observation period of 6 months were cultured in the presence of the mitogenic lectin phytohemagglutinine (PHA) (2.5 μg/ml) as described elsewhere (16, 17). After 72 h of incubation at 37˚C, mononuclear antibodies (Coulter-Immunotech, Krefeld, Germany) against the CD25 and CD3 epitopes on the surface of lymphocytes were added. After adequate gating, the cultured lymphocytes were analyzed by flow cytometry (EPICS XL-MCL, Coulter, Krefeld, Germany) as described (16, 17). The analysis of the low affinity interleukin-2 receptor expression (CD25) on the surface of $CD3^+$ T lymphocytes from whole blood cultures correlates with the uptake of tritiated thymidine in lymphocyte cultures (16, 17), and seems to be a much more "*in vivo*-like" method to measure activation and proliferation than methods which rely on isolated lymphocytes alone. T-cell function was thus defined as mitogen-induced expression of interleukin-2 receptors on the surface of $CD3^+$ T lymphocytes (% $CD25^+$ in T-cells).

Peripheral blood lymphocytes. Peripheral blood lymphocytes taken from the patients each month were differentiated using monoclonal antibodies (CD3, CD4, CD8, CD16, CD19, CD25, CD28, CD56, CD62L, HLA-DR; Coulter-Immunotech, Krefeld, Germany, and Becton Dickinson, Heidelberg, Germany) by flow cytometry (EPICS XL-MCL, Coulter, Krefeld, Germany) according to the standard procedures.

Data management. Data were documented in case report forms. After monitoring by an independent institution, the data were transferred into an ACCESS database twice by two different typists. A plausibility control of each parameter was performed. The monitor corrected all errors by reviewing the source data. Missing data were not replaced.

Statistical analysis. All parameters were compared at a significance level $p=0.05$ between the groups or times according to their distribution. A value of $0.05<p<0.10$ was judged as a trend. The normal distribution was tested using the Kolmogorov-Smirnov Test, and continuous data were tested using Student's *t*-test. The impact of several variables on the T-cell function and for differences between the distinct groups was measured with using analyses of variance (ANOVA) and cross tabulations (Pearson's Chi-square), respectively. All results within the explorative part of the analysis are interpreted as being solely descriptive. Data analysis was performed using SPSS 13.0.

Results

Local reactions. In 32 cases (48%) no local reactions were observed in response to subcutaneous VA-E application within the first 8 weeks, while in 21 cases (31%) moderate local reactions (1-3 cm) and in 14 cases (21%) strong ones (>3 cm) occurred. Among the 36 patients treated with VA-E swift (ITT, Table I), 27 had local reactions (75%) and 9 had none (25%), while among the patients treated with VA-E slow only 8 had local reactions (26%) and 23 had none (74%).

In the VA-E slow group, 6 had local reaction of 1-3 cm size (19%) and two had >3 cm (6%), while within the VA-E swift group, 15 patients (42%) had a reaction of 1-3 cm and 12 (33%) had >3 cm in size. These differences between the escalation groups are statistically significant ($p<0.001$; Chi-square). Because several of the patients had very strong local reactions, in 39% of patients treated VA-E swift the doses had to be reduced. Among these patients (n=14), 10 (71%) had a reaction 1-3 cm in size, while 4 (29%) had a reaction >3 cm in size.

However, there were no significant differences between the escalation groups with respect to body temperature and quality of life (data not shown) as measured with the Herdecke questionnaire on quality of life (HQL; (19).

Peripheral T lymphocytes. No significant differences between escalation groups were found in the absolute number of $CD3^+$ T lymphocytes, $CD4^+$ T helper/inducer cells, $CD8^+$ suppressor/cytotoxic cells, or the CD4/CD8 ratio, which all remained more or less stable within time (data not shown). Only the proportion of $CD25^+$ (activated) cells in the T-cell subset marginally decreased, from 12% to 8% in the group with a slow escalation, and from 21% to 16-18% in the swift group), which can be regarded as a normalization. The adjusted values of %$CD25^+$ in T-cells did not differ between groups (data not shown).

However, a significant difference was observed between the escalation groups with respect to the HLA-DR^+ (activated) T-cells ($p<0.05$, *t*-test). In the group treated with VA-E swift, the proportion of (adjusted) HLA-DR^+ T-cells decreased in the peripheral blood, while in theVA-E slow group a slight increase in the proportion of these cells was detected within the observation period of 6 months (data not shown).

Mitogen-stimulated T lymphocytes. To measure the impact of demographic (*i.e.* gender, age, tumor, TNM status) or medical decision-associated variables (*i.e.* dose escalation scheme [ITT/PP] and local reactions) on the course of PHA-stimulated T-cell function, we first performed univariate analyses of variance. The baseline level of T-cell function within all cancer patients was 78.1±9.0% (healthy individuals: 72.9±10.6%). Neither the tumor group, tumor

Table I. *Data of investigated patients.*

Patient No.	Gender	Age (years)	Tumour localisation	T	N	M	G	Metastases	Escalation scheme ITT	Escalation scheme PP	Size of local reaction (cm)	PHA-activated T-cells within the first 3 month
1	f	50	Breast	4	1	0	2	-	slow	slow	0	Incr.
3	m	72	Prostate	.	.	.	.	-	slow	slow	0	Incr.
5	m	65	Prostate	.	.	.	.	-	slow	slow	0	Nc
6	m	46	Colorectal	4	1	1	1	mul.	swift	swift	0	Incr. -> Decr.
7	f	48	Breast	1	0	0	2	-	swift	reduction	1-3	Decr.
8	m	80	Prostate	2	.	.	2	-	swift	swift	1-3	Decr.
9	f	64	Breast	2	0	0	2	-	swift	swift	>3	Decr.
10	f	71	Breast	1	0	0	2	-	swift	reduction	1-3	Decr.
11	m	49	Prostate	.	.	1	3	oss	swift	reduction	>3	Decr.
12	f	61	Colorectal	3	1	0	2	-	swift	swift	1-3	Decr.
13	f	60	Breast	3	2	1	2	oss	swift	swift	1-3	Incr.
14	f	62	Breast	1	0	0	2	-	swift	swift	0	Decr.
15	f	62	Breast	1	0	0	1	-	swift	swift	0	Nc
16	f	62	Breast	2	0	0	3	-	swift	swift	1-3	Incr.
17	f	61	Breast	1	2	0	3	-	swift	reduction	1-3	Decr.
18	f	53	Breast	1	0	0	2	-	swift	swift	>3	Decr.
19	f	58	Colorectal	2	1	1	3	hep	swift	swift	>3	Decr.
20	f	69	Breast	1	0	0	2	-	swift	swift	>3	Decr.
21	f	53	Colorectal	2	0	1	3	mul.	slow	slow	1-3	Nc
22	f	62	Colorectal	.	.	.	.	-	slow	slow	>3	Incr.
23	f	57	Breast	1	0	0	2	-	swift	reduction	>3	Incr. -> Decr.
24	m	69	Colorectal	3	0	0	3	-	swift	swift	1-3	Incr. -> Decr.
25	f	44	Breast	1	0	1	.	hep	swift	swift	>3	Nc
26	m	60	Colorectal	3	1	0	2	-	swift	reduction	1-3	Incr. -> Decr.
27	m	64	Colorectal	2	0	0	2	-	swift	swift	>3	Decr.
28	f	44	Breast	1	0	0	2	-	swift	reduction	>3	Incr.
29	m	59	Colorectal	2	0	0	2	-	swift	reduction	>3	Nc -> Incr.
30	m	69	Colorectal	2	1	0	3	-	swift	swift	0	Decr.
31	f	63	Colorectal	3	1	0	2	-	swift	reduction	1-3	Decr.
32	m	70	Colorectal	3	0	0	3	-	swift	swift	>3	Decr. -> nc
33	m	55	Colorectal	1	.	1	3	oss	swift	swift	0	Decr.
34	f	76	Colorectal	3	1	0	2	-	swift	swift	0	Decr.
35	m	65	Colorectal	2	0	0	2	-	swift	swift	>3	Decr.
41	f	65	Breast	2	0	0	2	-	swift	slow	0	Nc -> Decr.
42	f	76	Breast	2	0	0	2	-	slow	slow	0	Decr.
43	f	50	Breast	2	0	0	3	-	slow	slow	0	Decr.
44	f	70	Breast	1	0	0	1	-	slow	slow	0	Nc
45	f	49	Breast	1	0	0	2	-	slow	slow	1-3	Decr.
51	m	65	Prostate	2	0	0	.	-	slow	slow	0	Incr. -> Decr.
52	f	58	Breast	1	0	0	2	-	slow	slow	0	Incr
53	m	65	Prostate	.	.	.	1	-	slow	slow	0	Decr.
54	f	49	Colorectal	3	0	0	2	-	slow	slow	>3	Incr. -> Decr.
55	f	50	Breast	1	0	0	1	-	slow	slow	1-3	Decr.
56	m	62	Prostate	3	0	0	2	-	slow	slow	0	Nc -> Decr.
57	m	57	Prostate	2	0	0	2	-	slow	slow	0	Decr.
58	m	70	Prostate	.	.	.	.	-	slow	slow	0	Decr.
59	m	70	Prostate	.	.	1	2	oss	slow	slow	0	Decr.
60	f	78	Breast	1	0	0	2	-	slow	slow	0	Decr.
61	m	65	Prostate	4	0	0	2	-	slow	slow	0	Incr.
62	f	65	Breast	1	1	0	1	-	slow	slow	0	Decr.
63	f	45	Breast	2	0	0	3	-	slow	slow	0	Nc
64	f	42	Breast	1	0	0	2	-	slow	slow	1-3	Incr.
65	f	64	Colorectal	3	2	0	2	-	slow	slow	0	Incr.
66	f	69	Prostate	.	.	1	3	oss	slow	slow	0	Decr.

Table I. *continued*

Table I. *continued*

Patient No.	Gender	Age (years)	Tumour localisation	T	N	M	G	Metastases	Escalation scheme ITT	Escalation scheme PP	Size of local reaction (cm)	PHA-activated T-cells within the first 3 month
67	m	68	Prostate	2	0	0	2	-	slow	slow	0	Decr.
68	f	50	Breast	1	0	0	1	-	swift	reduction	1-3	Decr.
69	f	38	Breast	2	1	0	2	-	swift	reduction	1-3	Nc
70	f	52	Breast	2	1	0	3	-	slow	slow	1-3	Inc. -> Decr.
71	m	68	Prostate	2	0	0	2	-	swift	swift	0	Nc -> Incr.
72	f	62	Breast	2	0	0	2	-	swift	reduction	1-3	Decr.
73	f	68	Breast	1	1	0	2	-	slow	slow	0	Decr.
74	f	60	Breast	1	0	0	2	-	slow	slow	0	Incr.
75	f	64	Breast	2	1	0	2	-	swift	reduction	1-3	Decr.
76	f	43	Breast	2	0	0	2	-	swift	reduction	1-3	Nc -> Incr.
77	f	70	Breast	2	0	0	3	-	slow	slow	1-3	Decr.
78	f	63	Breast	1	1	0	2	-	slow	slow	0	Incr.
79	f	70	Breast	2	0	0	3	-	swift	swift	0	Incr.

Course of mitogen-stimulated T-cells: Incr. – increase; Decr. – decrease; NC – no change; gender: f – female; m – male; OSS – bones; hep – hepatic; mul – multiple.

Table II. *Course of PHA-activated T lymphocytes with respect to VA-E concentration and cancer type.*

	Breast cancer		Colon cancer		Prostate cancer	
Month	Mean VA-E concentration (mg/month)	% T-cell function	Mean VA-E concentration (mg/month)	% T-cell function	Mean VA-E concentration (mg/month)	% T-cell function
0	0	78.2±9.0	0	78.3±8.6	0	77.4±10.2
1	0.29±0.35	79.5±9.8	0.35±0.16	76.2±9.4	0.14±0.16	77.3±11.4
2	1.62±2.30	80.0±9.6	3.72±2.63	78.5±7.8	1.39±0.18	75.5±9.5
3	2.49±3.89	78.4±9.8	7.14±5.37	74.7±12.8	4.24±3.40	71.8±9.4*
4	2.55±4.00	78.0±14.7	8.03±5.62	77.1±7.3	6.03±5.03	72.0±6.0*
5	3.24±3.70	75.7±12.1	8.56±5.63	75.0±8.6	7.27±6.27	74.7±9.8
6	4.45±4.22	76.7±9.8	9.19±6.20	67.6±10.7**	8.86±7.93	72.4±10.6*
Regression 0-6	B=0.721	B=–0.504	B=1.725	B=–1.282	B=1.624	B=–0.846

Results are means±standard deviation (%). The regression refers to the course between month 0 and month 6 (unstandardized B coefficient).*$p<0.05$;**$p=0.001$ (2-tailed *t*-test; as compared to month 0 (baseline)).

stage (TNM or G), gender, age nor PP dose escalation scheme (or local reactions) had a significant impact on the observed variance (all variables F<1.6); however, the intention to be treated in the slow or swift arm (ITT set) explained some of the variance but had no significant impact (F=2.337, $p=0.122$; UNIANOVA).

The PHA-stimulated T-cell function of all patients was 78.1±10.0, 78.6±9.2, 76.1±10.8, 76.7±11.9, 75.3±10.7 and 73.4±10.6% at the 1st, 2nd, 3rd, 4th, 5th and 6th month, respectively. As compared to the baseline level of these patients, T-cell function at month 5 was in trend lower ($p=0.059$, *t*-test), while at the 6th month it was significantly lower ($p=0.0001$). There were no significant differences between male and female cancer patients, except a remarkable trend (F=3.667, $p=0.060$; ANOVA) for lower T-cell function in men at month 6.

To analyze the causes for the observed variances, the effect of the variables age, gender, PP set and local reactions, and the variables T, N, M and G on the T-cell function within the 6 month observation period; at 3 months the PP set had an effect (F=3.581, $p=0.038$; not significant, because of a significant Levene's test of equality of

Table III. *Course of PHA-activated T lymphocytes with respect to local reactions.*

Month	No local reactions within the first 3 month	1-3 cm size within the first 3 month	>3 cm size within the first 3 month
0	75.2±9.2	79.5±8.2	82.5±8.1
1	76.8±10.0	77.7±10.5	82.0±9.1
2	76.4±9.9	79.1±8.5	82.3±7.8
3	72.3±11.8	78.5±10.0	80.4±6.7
4	73.3±10.4	79.1±7.7	79.8±17.5
5	71.9±10.8	77.4±8.8	80.0±11.3
6	69.2±9.4**	78.1±9.8	75.0±11.3**
Regression			
0-6	B=–1.104	B=–0.182	B=–1.036
0-3	B=–0.910	B=–0.130	m=–0.60

Results are means±standard deviation (%). The regression refers to the course between 0 and months 6 and 3, respectively (unstandardized B coefficient). **$p<0.01$ (2-tailed *t*-test).

variance), and at 4 months the PP set ($F=5.457$, $p=0.009$) and PP set * age group ($F=7.028$, $p<0.0001$) had a highly significant effect. Thus, the differences in the dose escalation schemes and dose reductions of VA-E were the main relevant variables.

We next measured the course of T-cell function within time with respect to the 3 main types of cancer. As shown in Table II, the baseline level of patients with breast cancer, colorectal cancer and prostate cancer was similar. In breast cancer patients, the mean applied concentration of VA-E increased moderately and the T-cell function slightly declined within the 6 month observation period (not significant). In colorectal cancer patients, the dose escalation was much more rapid and yielded higher final concentrations at the 3rd month (which insignificantly reduced the T-cell function), and a highly significant decline of T-cell function was observed in the 6th month. In prostate cancer patients, we also had a more rapid increase of the mean VA-E concentration per month and observed significantly lower T-cell functions at months 3, 4 and 6. However, within the group of colorectal cancer patients, no significant differences between women and men were observed within the observation period ($F<1.4$; $p>0.2$). Thus, the results indicate that some patients may respond to a stronger increase of VA-E concentration with a decline of T-cell function within time.

To test the possibility that the responsiveness of the patients towards the antigens of the injected VA-E may have an impact on the course of stimulated T-cell function, we analyzed this in detail. As shown in Table III, the local reactions (as observed within the first 3 months) had no significant impact on the course of T-cell function, because a decline was observed both in the group without any local reactions and within the group with strong reactions. However, the group with moderate local reactions had a stable course of functional T-cells in response to the VA-E injection. It is worth mentioning that the group with the strongest reactions had stable T-cell function within the first 5 months, which significantly decreased at the 6th month, while the group without any reactions towards the VA-E antigens revealed a decrease of T-cell function at the 3rd month (which was not significant), and significantly at the 6th month. Nevertheless, an important observation was that the primary (baseline) T-cell function might be associated with the responsiveness towards the VA-E antigens, because the best primary T-cell function was found in patients with strong local reactions, while the lowest function was observed in patients without any local reaction within the first 3 months.

Next we analyzed the two different dose escalation schemes with respect to T-cell function in the PP set (similar results were found for the ITT set, but are not shown). Both, swift and slow escalation reduced the T-cell function at the 5th and 6th month, while dose adaptation in response to strong local reactions resulted in a stable course of T-cell function within the observation period (Table IV). The resulting escalation of VA-E doses in this reduction group was less than in the slow escalation group which in turn revealed a decrease of T-cell function within time. This indicates that individual dose adaptation might be appropriate.

A marginal increase of T-cell function at month 1 was noted in the slow escalation group (statistically trend), which did not occur in the swift or the dose reduction group. This small increase corresponds with the improvement of the maximal stimulated T-cell function of breast cancer patients within the first 2 months (Table II).

Discussion

As VA-Es are widely used in complementary cancer treatment, it is highly important that the applied concentrations are optimal for the patients. At present, two different escalation courses of VA-E are recommended by different pharmaceutical companies, *i.e.* one constant concentration for a few months as recommended by phytotherapeutical companies, and increasing concentrations for several months as recommended by companies with an anthroposophical background (8). But even when using the regimen with increasing concentrations, one has the option of appling the drugs with a swift escalation scheme resp. a slow escalation scheme. In the absence of clinical outcome results to judge adequate dosage escalation, one may focus on the reaction of relevant immunocompetent cells in cancer patients and on the local reactions at the injection

Table IV. *Course of PHA-activated T lymphocytes with respect to escalation scheme and applied VA-E concentration.*

	Slow doses escalation		Swift doses escalation		Doses reduction	
Month	Mean VA-E concentration (mg/month)	% T-cell function	Mean VA-E concentration (mg/month)	% T-cell function	Mean VA-E concentration (mg/month)	% T-cell function
0	0	74.6±9.1	0	80.8±8.4	0	81.7±7.4
1	0.04	77.6±10.4 (*)	0.50±0.33	77.3±7.9 (*)	0.38±0.12	80.7±12.0
2	0.57	75.9±9.2	4.88±2.16	80.3±8.5	0.80±0.59	81.9±9.0
3	1.00	72.6±9.8	7.43±4.49	77.3±12.7	2.15±2.79	81.5±6.8
4	0.89	73.2±10.1	8.34±4.86	77.1±14.9	1.10±1.21	83.2±7.6
5	1.06	73.4±9.4	7.93±3.98	74.6±13.0*	1.84±1.88	81.2±8.1
6	4.60	69.9±9.6*	8.32±3.96	74.4±10.8*	2.23±2.13	79.4±9.9
0-6	B=0.577	B=–0.900	B=1.546	B=–0.993	B=0.354	B=–0.164

Results are means±standard deviation (%). The regression refers to the course between month 0 and month 6 (unstandardized B coefficient). (*) $0.05<p<0.10$; *$p<0.05$; **$p=0.001$ (2-tailed *t*-test).

site. As reported previously, intravenous application of the VA-E used for this investigation prevented a surgery-induced immunosuppression of granulocytes (18) and stimulated natural killer cells (21). Now we have investigated long-term effects on the functional competence of T lymphocytes. Our hypothesis was that a rapid escalation of high VA-E concentrations may impair their function. In fact, in patients with colorectal or prostate cancer, we observed higher VA-E doses and an impairment of T-cell function within time, while in breast cancer patients (which had more moderate mean concentrations of VA-E) the course of T-cell function remained stable. Moreover, an individual approach seems to be more suited, because the most stable T-cell function was observed in the group with dose adaptation (reduction in response to excessively local reactions) and in the group with moderate local reactions.

Thus, in some groups of patients, one may observe a significant decline of interleukin-2 receptor expression on mitogen-stimulated T-cells, which reflects an impairment of acute immune responses. Because it is well known that a significant degree of impairment of immune functions found in breast cancer patients can be associated with poorer prognosis (20), such a situation should be avoided. Nevertheless, the observed T-cell impairment is a physiological down-regulation of the immune response towards the injected antigens of *Viscum album* L. Because this effect can be observed even in patients with a slow escalation, the VA-E application should be paused periodically to recover T-cell reactivity.

One may argue that the observed decline of T-cell function in several patients could reflect a tumor-associated immunosuppression, but so far only two patients had a progressive course of disease within the 6 month observation period (one from the slow group had an increased T-cell function without local reactions, and another from the swift group had a decrease of T-cell function and local reaction >3 cm diameter). Nevertheless, the most stable course of T-cell function was found in the group with dose adaptation (*i.e.* reduction group) on grounds of excessive local reactions and in the group of patients with moderate local reactions. These patients are treated best according to the guidelines of the VA-E producer. Patients with no local reaction (slow dose escalation or non-reactivity of patients) should be screened immunologically.

Taken together, our results indicate that VA-E should be applied on a more individual basis (particularly in adaptation to the local reactions) than on the basis of fixed escalation schemes. Whether the observed differences may have an effect on relevant outcome parameters of the patients, such as survival and relapse, cannot be judged as yet, but all of the patients from this study will be followed for at least 5 years to determine their outcome.

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