Vitamin J kills Circulating Tumor Cells (CTCs) from the blood as effectively as chemotherapy (Taxane) in vitro.

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Abstract

Background: Metastatic disease is the most common cause of cancer-related death in patients with solid tumors. Cells escaping from the primary tumor are called Circulating Tumor Cells (CTCs).

Objectives: We tested the in vitro killing efficacy of 16 commonly used chemotherapies and Vitamin J on CTCs in blood samples from cancer patients. Vitamin J is a combination of Choline, Vitamin C, L-Arginine and Vitamin D3.

Methods: EDTA blood samples from cancer patients were analyzed as follows: 1-ml blood mixed with 10-ml lysis buffer (Quiagen) was spun 10-min at 700g at room temperature. Epithelial cells were detected using EpCAM (CD326) conjugated with fluorescein isothionate (FITC). Dying cells were detected using propidium iodide (PI), which stains only the nuclei of dying cells. Killing effectiveness was determined by exposing white cells from the cell pellet to different agent concentrations. The concentration calculated to be present in the blood of patients under treatment was set as one, and tenfold lower and higher concentrations were tested in comparison to control suspension without agent. The cytotoxic effect was measured at three, six and nine hours of culture. Cells were characterized as follows: 1) Live blood cells appearing only in transmitted light with no fluorescence staining. 2) Dead blood cells where the membrane had become permeable to PI, staining the nucleus orange-red fluorescent. 3) Live epithelial (presumably tumor) cells staining with green fluorescence due to reactivity with FITC-conjugated anti-EpCAM. 4) Dead CTCs with simultaneous green and red fluorescent staining, resulting in either a clear green cap with red nucleus, or later during disintegration with an orange combination stain. The numbers of live and dead CTCs without and with therapeutic agent and the increase in dead cells over control was calculated. Quantitative analysis was performed using the ScanR (Olympus) image system, allowing repeated scanning of the same area.

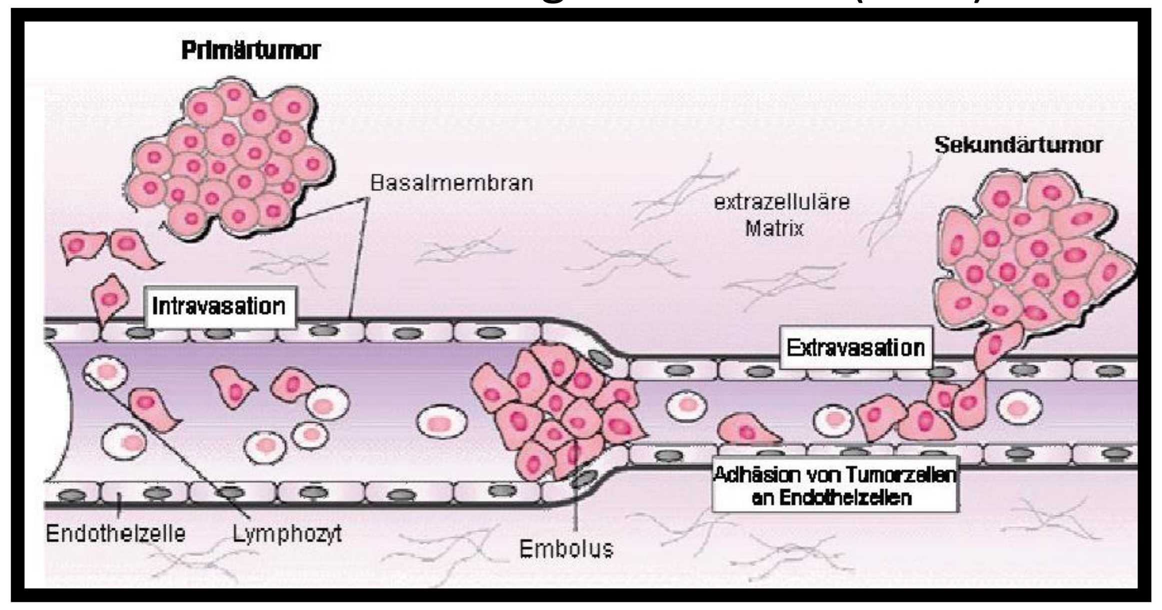
Results: Of 16 antitumor substances tested, Taxane was the most effective in killing CTCs. On average about 40% of existing CTCs (range 5-90%) were killed by Taxane. In comparison, Vitamin J killed between 10% and 95% of CTCs, with an overall average of about 50%. Vitamin J and Taxane in combination resulted in a much further increase of killing activity.

Conclusions: Vitamin J kills CTCs in EDTA blood samples from cancer patients as efficiently as the most effective antitumor substance, Taxane. The CTC killing activity and thus therapeutic action can be increased combining Vitamin J and Taxane.

Key Words:
Cancer • Circulating Tumor Cells (CTCs) • early detection • metastases • chemotherapy

Background

Metastatic disease is the most common cause of cancer-related death in patients with solid tumors. A considerable amount of evidence indicates that tumor cells are shed from a primary tumor mass at the earliest stages of malignant progression. Some of these cells will travel via the peripheral blood to sites anatomically distant from the primary tumor and form metastases. The cells that escape from the primary tumor settle down at a secondary site to cause metastasis are called Circulating Tumor Cells (CTCs).

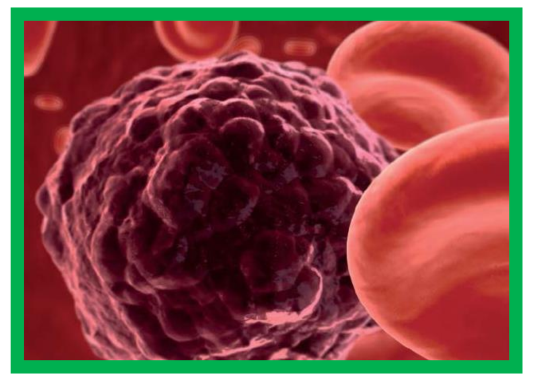


Objectives

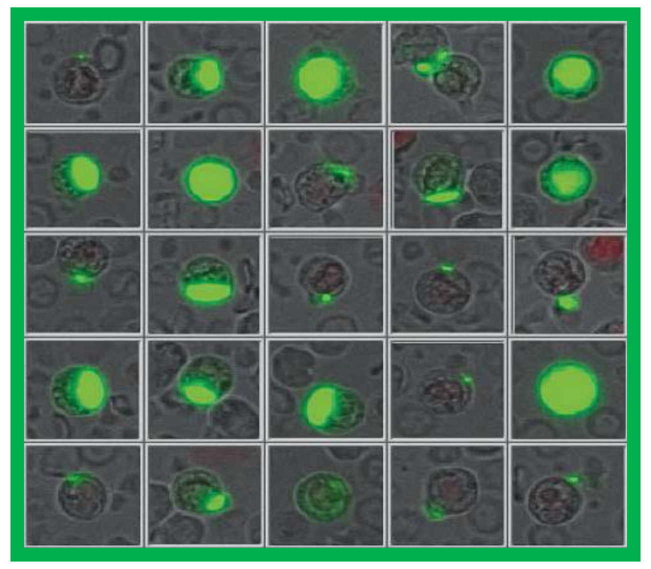
We tested the in vitro killing efficacy of 16 commonly used chemotherapies and Vitamin J on CTCs in blood samples from cancer patients. Vitamin J is a combination of Choline, Vitamin C, L-Arginine and Vitamin D3.

Methods

EDTA blood samples were taken from cancer patients and prepared according to the published method, using only red blood cell lysis and one centrifugation step. In short, 1ml of blood was mixed with 10ml of lysis buffer (Quiagen) for ten minutes in the cold and spun at 700g for ten minutes at room temperature. Epithelial cells were detected using EpCAM (CD326), an anti-epithelial antibody, conjugated with fluorescein isothionate (FITC). Dying cells were detected using propidium iodide (PI) which only stains the nuclei of dying cells with a permeable membrane. The cell suspension was subsequently incubated under cell culture conditions with the drugs in question. Cell kill effectiveness was determined by exposing all white cells from the cell pellet of 100µl of blood to different concentrations of the respective agent in medium, where the concentration calculated to be present in the blood of patients under treatment was set as one and a tenfold lower and a tenfold higher concentration was tested in comparison to the control suspension containing the cells without addition of the agent. Cells were short-time cultured under these conditions for up to nine hours and the cytotoxic effect measured at three, six and nine hours. Cells could be characterized as follows: 1.) Live blood cells appearing only in transmitted light with no fluorescence staining. 2.) Dead blood cells where the membrane had become permeable showing propidium iodide entering the cell, staining the nucleus orange-red fluorescent. 3.) Live epithelial (presumably tumor) cells staining with green fluorescence, preferentially as a cap, due to reactivity with FITC-conjugated anti-EpCAM. 4.) Dead CTCs with simultaneous green and red fluorescent staining, resulting either in a clear green cap with a red nucleus, or later during nucleic and cell disintegration with an orange combination stain. The numbers of live and dead CTCs were determined at each point in time without and with the indicated concentrations of the therapeutic agent and the percentage increase in dead cells over the control was calculated. Quantitative analysis of the samples at different times after incubation with the respective drugs was performed using the image analysis system of the ScanR (Olympus Hamburg, Germany), allowing repeated scanning of the same area.



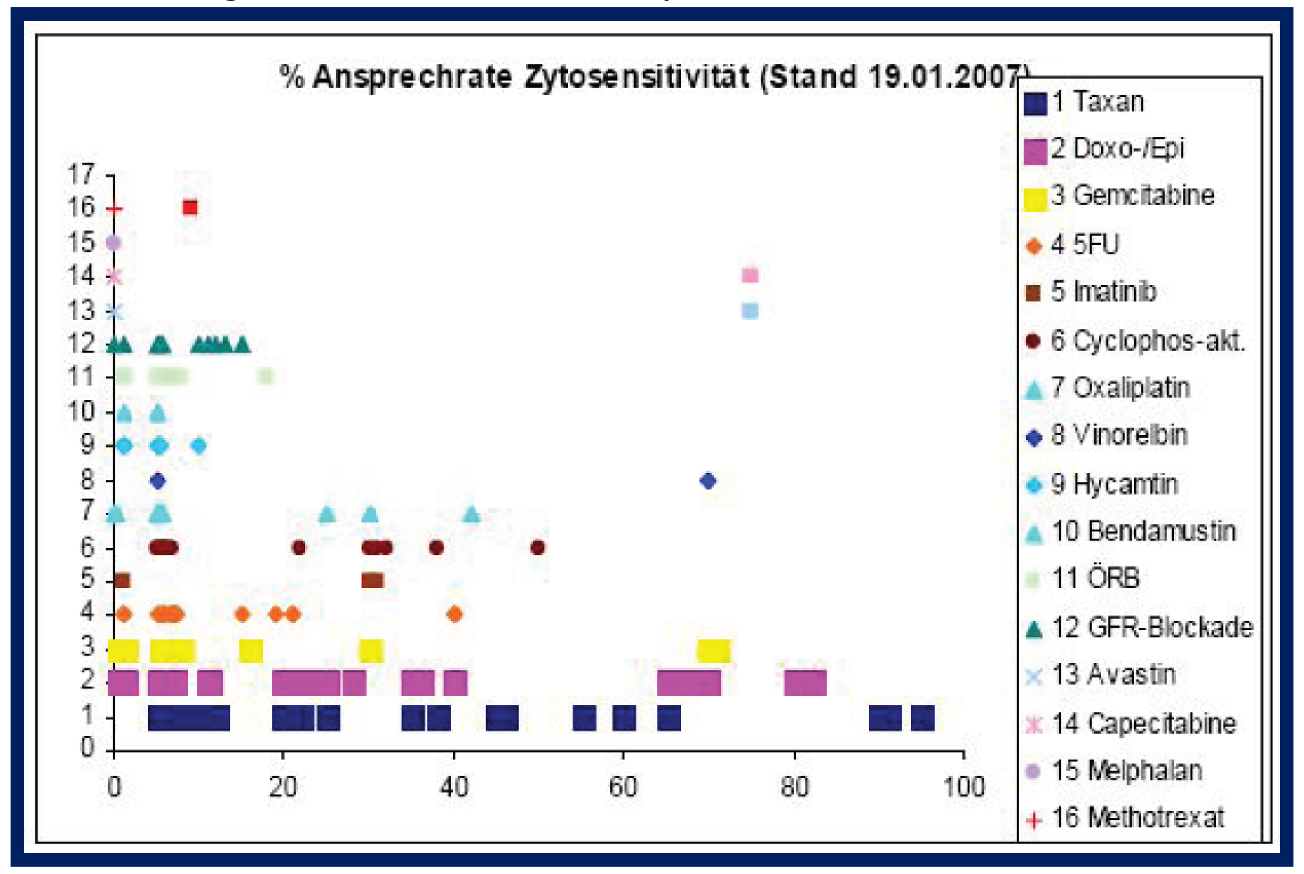
A) Circulating Tumor Cell (CTC)



B) EpCAM (Epithelial cytoadhesion molecule) signals on CTCs

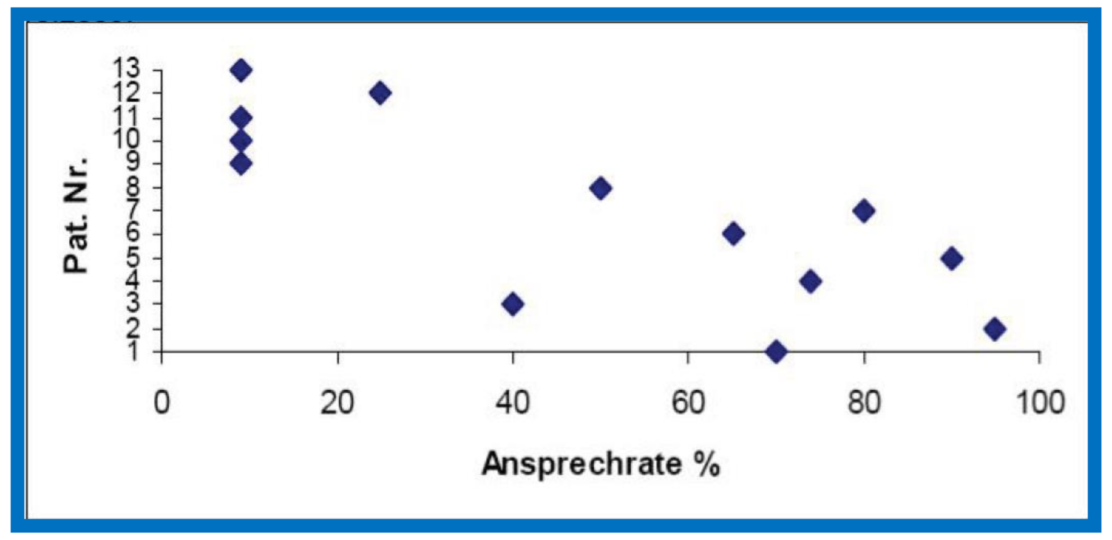
Result I

Of the 16 most commonly used antitumor substances -chemotherapies tested, Taxane appeared to be the most effective in killing circulating tumor cells. The range of killing in the EDTA patient blood samples was between 5 % and up to 90 % of the existing CTCs. In average about 40 % of the existing CTCs were killed by Taxane.



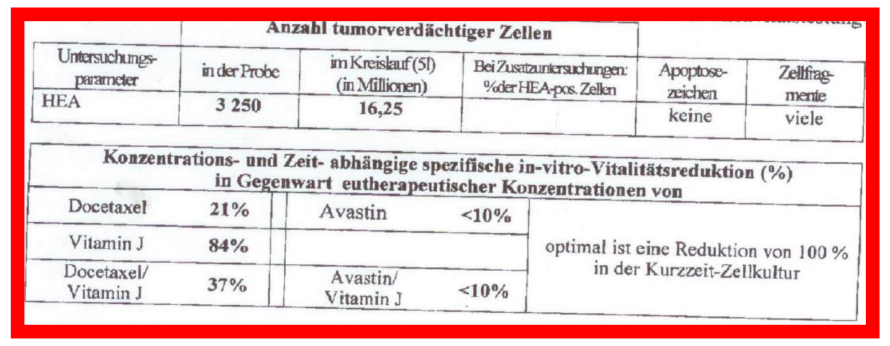
Result II

In comparison Vitamin J killed also between 10 % and up to 95 % of the existing CTCs per blood sample with an average of about 50 % in the overall patient blood samples.



Result III

A therapeutic combination of Vitamin J and Taxane in vitro resulted in a much further increase of the killing activity - additionally more than 60%.



Untersuchungs-parameter	Anzahl tumorverdächtiger Zellen: in der Probe	im Kreislauf (5l) (in Millionen)	Bei Zusatzuntersuchungen: % der HEA-pos. Zellen	Apoptose-zeichen	Zellfrag-mente
HEA	3 250	16,25		keine	viele

Konzentrations- und Zeit- abhängige spezifische in-vitro-Vitalitätsreduktion (%) in Gegenwart eutherapeutischer Konzentrationen von				
Docetaxel	21%	Avastin	<10%	optimal ist eine Reduktion von 100 % in der Kurzzeit-Zellkultur
Vitamin J	84%			
Docetaxel/ Vitamin J	37%	Avastin/ Vitamin J	<10%	

Conclusions

The Vitamin J combination kills in vitro circulating tumor cells in EDTA blood samples from cancer patients as epithelial cytoadhesion molecule shows as efficient as the most effective antitumor substance Taxane. The CTC killing activity and thus therapeutic action can even be increased combining Vitamin J and Taxane.

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Vitamin J eradicates Circulating Tumor Cells (CTCs) completely from the blood of cancer patients in vivo.

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Abstract

Background: Metastatic disease is the most common cause of cancer-related death in patients with solid tumors. Cells escaping from the primary tumor are called Circulating Tumor Cells (CTCs).

Study objectives: An acute Vitamin J infusion therapy 3-5 hours duration was performed in 12 cancer patients (breast, pancreas, stomach, melanoma and sarcoma). Vitamin J is a combination of Choline, Vitamin C, L-Arginine and Vitamin D3.

Methods: Prior to and following each Vitamin J infusion, serum and EDTA blood samples were analyzed for circulating tumor cells (CTCs) by MainTrac-Method and standards such as liver-, kidney- and metabolic values. Vital parameters (RR, pulse, O2Sat, ECG, temp.) were determined every quarter-hour prior to, during and subsequent to infusions. Depending on dose, infusion therapy was applied for 3-5 hours. During and subsequent to infusions, no patient showed relevant changes in vital parameters**.** **MainTrac-Method:** 1-ml blood mixed with 10-ml lysis buffer (Quiagen) was spun 10-min at 700g at room temperature. Epithelial cells were detected using EpCAM (CD326) conjugated with fluorescein isothionate (FITC). Dying cells were detected using propidium iodide (PI), which stains only the nuclei of dying cells. Cells were characterized as follows: 1) Live blood cells appearing only in transmitted light with no fluorescence staining. 2) Dead blood cells where the membrane had become permeable to PI, staining the nucleus orange-red fluorescent. 3) Live epithelial (presumably tumor) cells staining with green fluorescence due to reactivity with FITC-conjugated anti-EpCAM. 4) Dead CTCs with simultaneous green and red fluorescent staining, resulting in either a clear green cap with red nucleus, or later during disintegration with an orange combination stain. Quantitative analysis was performed using the ScanR (Olympus) image system, allowing repeated scanning of the same area.

Results: CTCs were reduced by 57% on average in only 3 to 5 hours of treatment with Vitamin J. In 3 cases CTCs were completely erased after Vitamin J infusion. These 3 patients had CTCs between 1,000 and 10,000 per ml prior to therapy. In one patient with liver cancer this eradication even continued for more than 4 weeks without any further treatment. This clearly indicates continuity of action beyond the acute treatment interval.

Conclusions: Vitamin J infusion therapy is generally very well tolerated. Vitamin J infusion therapy is a very effective antitumor therapy, reducing circulating tumor cells in the blood of cancer patients. Vitamin J killing activity continues over at least four weeks. After only a few intravenous administrations, patients undergoing Vitamin J therapy regularly report a significant improvement in their health condition as well their physical resilience.

Key Words:
Cancer • Circulating Tumor Cells (CTCs) • early detection • metastases • chemotherapy

Background

The underlying biology of the CTC is poorly understood. The demonstration that CTCs are associated with outcome using a range of platforms and across different malignancy sites is suggestive that CTCs have direct, or indirect, clinical relevance. Several studies have reported that CTCs are independent of other prognostic factors such as disease stage, tumor burden or the site of metastases. This may support the hypothesis that they are biologically distinct, with “aggressive” or even “stem cell like” characteristics. However, not all patients with CTCs experience disease relapse, and in some cases continue to have CTC positive blood years after initial curative surgery. Whether these cells represent a stable equilibrium arising from the continual replenishment of short lived CTCs from an occult metastatic deposit, or a small pool of circulating dormant tumor cells is under debate.

Objectives

An acute Vitamin J infusion therapy 3-5 hours duration was performed in 12 cancer patients (breast, pancreas, stomach, melanoma and sarcoma). Vitamin J is a combination of Choline, Vitamin C, L-Arginine and Vitamin D3.

Methods

Prior to and following each Vitamin J infusion, serum and EDTA blood samples were analyzed for circulating tumor cells (CTCs) by MainTrac-Method and **standards such as liver-, kidney- and metabolic values. Vital parameters** (RR, pulse, O2Sat, ECG, temp.) were determined every quarter-hour prior to, during and subsequent to infusions. Depending on dose, infusion therapy was applied for 3-5 hours. During and subsequent to infusions, no patient showed relevant changes in vital parameters**. MainTrac-Method:** 1-ml blood mixed with 10-ml lysis buffer (Quiagen) was spun 10-min at 700g at room temperature. Epithelial cells were detected using EpCAM (CD326) conjugated with fluorescein isothionate (FITC). Dying cells were detected using propidium iodide (PI), which stains only the nuclei of dying cells. Cells were characterized as follows: 1) Live blood cells appearing only in transmitted light with no fluorescence staining. 2) Dead blood cells where the membrane had become permeable to PI, staining the nucleus orange-red fluorescent. 3) Live epithelial (presumably tumor) cells staining with green fluorescence due to reactivity with FITC-conjugated anti-EpCAM. 4) Dead CTCs with simultaneous green and red fluorescent staining, resulting in either a clear green cap with red nucleus, or later during disintegration with an orange combination stain. Quantitative analysis was performed using the ScanR (Olympus) image system, allowing repeated scanning of the same area.

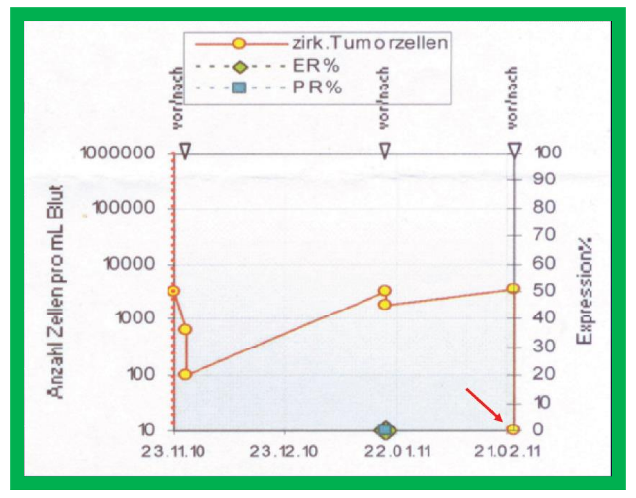
Result I

CTCs were reduced by 57% on average in only 3 to 5 hours of treatment with the Vitamin J infusion combination of Choline, Vitamin C, L-Arginine and Vitamin D3.

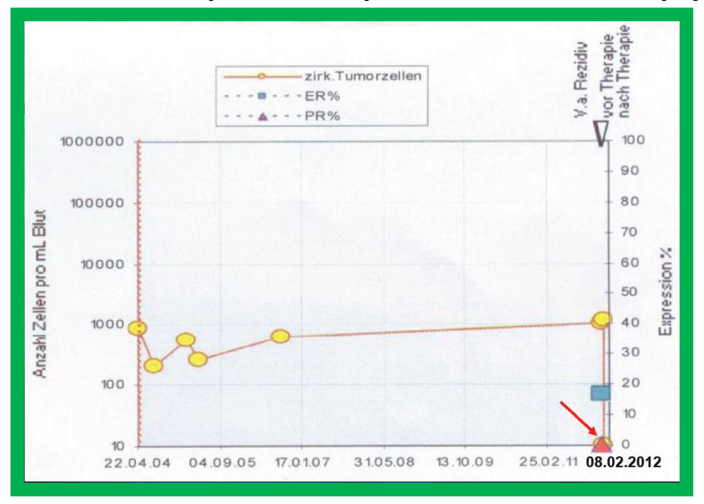
Tumorart	Werte	In Vivo Wirkung Normwerte	Vor im Vergleich vor Therapie	Nach I.V.-Therapie nach Therapie	nach-vor Differenz (Δ)	nach-vor Δ % VOR
Mamma-CA	MainTrac	0 bis 1 Mio TU-Zellen	18,75	10,00	-8,75	-46,00%
Mamma-CA	MainTrac	0 bis 1 Mio TU-Zellen	5,25	2,00	-3,25	-61,00%
Mamma-CA	MainTrac	0 bis 1 Mio TU-Zellen	1,75	1,25	-0,50	-28,00%
Mamma-CA	MainTrac	0 bis 1 Mio TU-Zellen	15,25	8,75	-6,50	-42,00%
Mamma-CA	MainTrac	0 bis 1 Mio TU-Zellen	3,75	0,25	-3,50	-93,00%
Pankreas-CA	MainTrac	0 bis 1 Mio TU-Zellen	32,50	3,00	-29,50	-90,00%
Pankreas-CA	MainTrac	0 bis 1 Mio TU-Zellen	3,00	2,75	-0,25	-8,00%
Magen-CA	MainTrac	0 bis 1 Mio TU-Zellen	14,00	1,75	-12,25	-87,00%
Prostata-CA	MainTrac	0 bis 1 Mio TU-Zellen	12,50	8,75	-3,75	-30,00%
Malignes Melanom	MainTrac	0 bis 1 Mio TU-Zellen	3,75	0,00	-3,75	-100,00%
Malignes Melanom	MainTrac	0 bis 1 Mio TU-Zellen	3,50	3,00	-0,50	-14,00%
Histiozytom-Sarkom	MainTrac	0 bis 1 Mio TU-Zellen	372,50	65,00	-307,50	-82,00%
Mittelwerte			**40,54**	**8,875**	**-31,67**	**-56,75%**

Result II

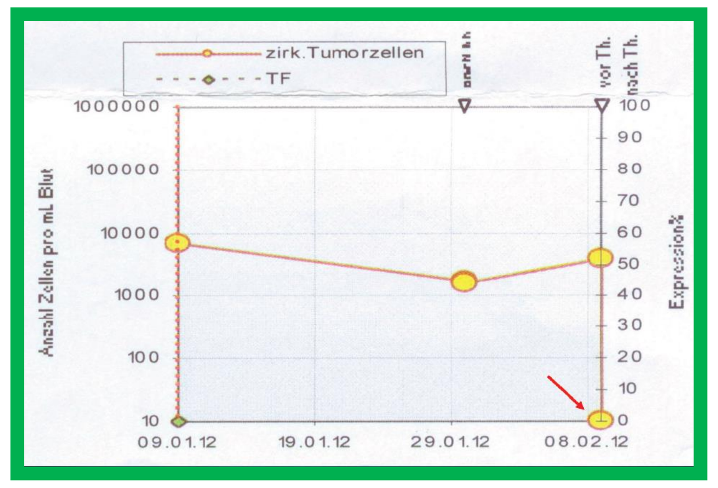
In 3 cases CTCs were completely erased after Vitamin J infusion. These 3 patients had CTCs between 1,000 and 10,000 per ml prior to therapy.



Case 1: 57 year old breast cancer patient – extended disease!



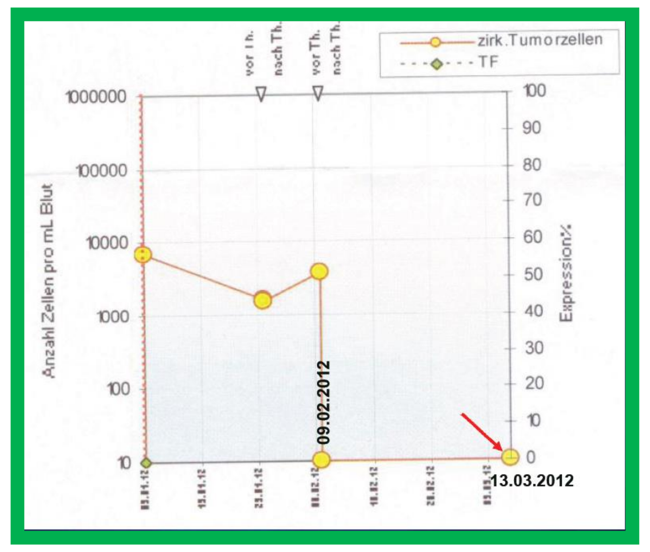
Case 2: 52 year old breast cancer patient – extended disease!



Case 3: 63 year old liver cancer patient – no therapeutic options!

Result III

In this patient with liver cancer, the eradication even continued for more than 4 weeks without any further treatment. This clearly indicates continuity of action beyond the acute treatment interval.



Conclusions

Vitamin J infusion therapy is generally very well tolerated. Vitamin J infusion therapy is a very effective antitumor therapy, reducing circulating tumor cells in the blood of cancer patients. Vitamin J killing activity continues over at least four weeks. After only a few intravenous administrations, patients undergoing Vitamin J therapy regularly report a significant improvement in their health condition as well their physical resilience.

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