# Effects of Whole Body Hyperthermia (41.8°C) on the Frequency of Tumor Cells in the Peripheral Blood of Patients with Advanced Malignancies[1]

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## ABSTRACT

***Purpose:*** **Combining heat with antineoplastic drugs has produced evidence of antitumor synergism. An increasing number of trials are investigating whole body hyperthermia (WBH) in combination with chemotherapy in patients with advanced malignancies. Here we investigated whether the hyperdynamic state of the circulation as a consequence of WBH may stimulate dissemination of malignant cells.**

***Experimental Design:*** **WBH in combination with chemotherapy was administered by a radiant heat device to 20 consecutive patients with advanced epithelial malignancies. One WBH session lasted for ~4 h (90 min heating time, 60 min plateau at 41.8°C, and 60–80 min cooling). Peripheral blood was drawn before WBH treatment (baseline), at the end of the plateau (1 h), and 24 h and 48 h thereafter. After removal of leukocytes using anti-CD45 magnetic beads, circulating tumor cells were detected immunocytochemically using the monoclonal antibody A45-B/B3, which binds to a common epitope present on various cytokeratins.**

***Results:*** **The method used to detect tumor cells in the peripheral blood proved to be specific and very sensitive (detection limit 1 tumor cell per $1.7 \times 10^5$ peripheral blood mononuclear cell). Before WBH, 6 of 20 patients had cytokeratin-positive cells in their blood. A treatment-induced increase in the number of circulating tumor cells became statistically significant at 24 h after WBH ($P = 0.043$) and was detected in a total of 9 patients, 5 of whom had no detectable malignant cells at baseline. There was no evidence of a correlation between an increase in the number of circulating tumor cells and increased metastasis frequency.**

***Conclusions:*** **Our findings suggest that WBH might induce a temporary release of tumor cells into the circulation, but this spread appears to be clinically not significant in patients with advanced malignancies.**

## INTRODUCTION

Preclinical and clinical studies have attributed a number of beneficial effects to WBH[3] including a potentiation of the tumoricidal effects of specific cytotoxic agents and a stimulation of different parameters of the immune system (1–7). During the last decade there has been a revival of interest in the treatment of cancer by WBH. A number of encouraging Phase I studies helped to form a rationale for Phase II studies (8–11), and an increasing number of institutions are currently investigating the efficacy of WBH in combination with chemotherapy in the treatment of different malignancies using intense hyperthermia as well as fever-like hyperthermia (12–16). This was made possible by a new heating technology, termed radiant heat WBH. By adding a humidification system to minimize evaporative heat loss, this system provides a safe and effective means of gently heating the patient to the target temperature of 41.8°C with no significant hyperthermia-related toxicity (17).

One of the major controversies in the hyperthermia immunobiology literature, based on a number of animal studies mainly performed in the 70s and 80s has been the possibility of promotion of tumor metastasis by hyperthermia. Results have been controversial with some groups reporting on an increased incidence of bone and pulmonary metastasis (18–23), and others reporting on a reduced potential of inoculated tumor cells for tumorous growth and the promotion of metastases (24). Interpretation of the data has been additionally impaired by the diversity of heating modalities, target temperatures, and heating times applied in these studies. None of the studies investigated hematogenous spread of tumor cells but rather focused on WBH-induced changes in immune reactivity. Because of these preclinical data the utilization of WBH in humans resides in its use as an adjunct to chemotherapy.

It has been demonstrated that surgical manipulation of tumors entails the risk of intraoperative tumor cell dissemination (25–27). The hyperdynamic state of the circulation at elevated temperatures, the locally active hyperaemia, and interdependent

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[3] The abbreviations used are: WBH, whole body hyperthermia; MNC, mononuclear cell; RT-PCR, reverse transcription-PCR; PR, partial response; mAb, monoclonal antibody; SD, stable disease; PD, progressive disease; CK, cytokeratin.

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*Table 1* Patient characteristics and status of circulating tumor cells

| Patient no. | Diagnosis | Sex | Age (years) | Best response to WBH+CT[a] | Duration | Detection of CK positive cells over time[b] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Before WBH | After WBH | | |
| | | | | | | | 0 h | 24 h | 48 h |
| Patients positive for circulating tumor cells | | | | | | | | | |
| 2 | Colorectal carcinoma | Male | 38 | SD | 18 weeks | 1 | 0 | 5 | ND |
| 3 | Pleuramesothelioma | Male | 64 | SD | 30 weeks | 5 | 3 | 5 | ND |
| 5 | Pleuramesothelioma | Male | 46 | SD | 26 weeks | 0 | 0 | 1 | ND |
| 6 | Colorectal carcinoma | Male | 60 | PD | 8 weeks | 0 | 2 | 8 | ND |
| 7 | Colorectal carcinoma | Male | 56 | SD | 46 weeks | 1 | 0 | 5 | ND |
| 9 | Ovarian carcinoma | Female | 44 | SD | 16 weeks | 5 | 0 | 3 | ND |
| 13 | Cholangiocellular carcinoma | Female | 60 | SD | 31 weeks | 8 | 0 | 10 | 0 |
| 14 | Colorectal carcinoma | Male | 56 | MR | 16 weeks | 1 | 4 | 0 | 0 |
| 16 | Ovarian carcinoma | Female | 62 | SD | 28 weeks | 0 | 1 | 35 | 0 |
| 19 | Colorectal carcinoma | Male | 63 | SD | 13 weeks | 0 | 0 | 3 | 0 |
| 20 | Cholangiocellular carcinoma | Female | 59 | SD | 18 weeks | 0 | 0 | 0 | 3 |
| Patients negative for circulating tumor cells | | | | | | | | | |
| 1 | Colorectal carcinoma | Female | 61 | SD | 18 weeks | 0 | 0 | 0 | ND |
| 4 | Colorectal carcinoma | Male | 46 | PR | 10 weeks | 0 | 0 | 0 | ND |
| 8 | Colorectal carcinoma | Male | 62 | SD | 42 weeks | 0 | 0 | 0 | ND |
| 10 | Colorectal carcinoma | Male | 61 | PD | 9 weeks | 0 | 0 | 0 | ND |
| 11 | Colorectal carcinoma | Male | 59 | PD | 8 weeks | 0 | 0 | 0 | 0 |
| 12 | Colorectal carcinoma | Male | 61 | PR | 30 weeks | 0 | 0 | 0 | 0 |
| 15 | Colorectal carcinoma | Male | 65 | PD | 7 weeks | 0 | 0 | 0 | 0 |
| 17 | Colorectal carcinoma | Male | 54 | PD | 8 weeks | 0 | 0 | 0 | 0 |
| 18 | SCLC | Female | 60 | SD | 22 weeks | 0 | 0 | 0 | 0 |

[a] CT, chemotherapy; ND, not done; MR, minor response.
[b] Total number of tumor cells detected.

changes in tumor blood flow may have a comparable effect and predispose to an increased release of tumor cells into the circulation (28, 29). Thus, we were interested to investigate tumor cell spread in patients with different malignancies being treated with WBH in combination with chemotherapy.

Recent developments in assay technology have improved the detection of micrometastases in bone marrow or detection of circulating tumor cells in the peripheral blood (30). On a molecular basis the amplification of tissue-specific mRNA transcripts by RT-PCR has been used in numerous studies to detect smallest quantities of tumor cells. Specific targets included carcinoembryonic antigen, prostate-specific antigen, MUC 1, and p53 mutations, as well as CKs (30, 31). Nevertheless, a number of problems have to be taken into account when interpreting RT-PCR results: (*a*) solid tumors may show genetic heterogeneity, thus hampering the detection of tumor-specific genomic changes at the level of a single cell; (*b*) the method is difficult to quantify; and (*c*) it depends highly on sample preparation and assay conditions. In contrast the immunocytochemical detection of occult tumor cells bears the advantage of identifying the actual circulating tumor cell. The sensitivity of this method has been additionally improved by the selective enrichment for tumor cells (32). The prognostic importance of the immunocytochemical identification of occult tumor cells in the bone marrow has been confirmed in various prospective clinical trials (30, 33). Many studies have used CKs as markers for epithelial cells, including carcinoma cells. These proteins are stably and abundantly expressed in a majority of epithelial tumors and, therefore, are suitable for an investigation of hematogenous spread of different tumors (34). Thus, enrichment of malignant cells using magnetic beads combined with subsequent immunohistochemical staining was used to isolate epithelial cells from the circulating blood of patients treated with WBH in combination with chemotherapy.

## PATIENTS AND METHODS

**Patients and Healthy Volunteers.** A total of 20 consecutive patients undergoing WBH was included in the study. The patients were treated on different protocols approved by the ethics committee of our institution. WBH was always applied in combination with a platinum-based chemotherapy, and all of the patients received the chemotherapy at 41.8°C, which had been defined as the target temperature. Informed consent was obtained from all of the patients before study entry. Eligibility criteria included a WHO performance status of 0–2, a life expectancy of >3 months, and measurable advanced metastatic disease. Patients with a severely compromised respiratory status, a history of angina pectoris, congestive heart failure, or serious dysrhythmias were ineligible. The majority of patients were male and suffered from colorectal carcinoma ($n = 13$). Other diagnoses included cholangiocellular carcinoma ($n = 2$), pleural mesothelioma ($n = 2$), ovarian carcinoma ($n = 2$), and small-cell lung cancer ($n = 1$; Table 1). A volume of 20 ml of heparinized blood was obtained before WBH treatment was started, immediately after the 60 min plateau at 41.8°C (1 h), and 24 h and 48 h after WBH. For control experiments, blood was drawn from 10 patients with advanced malignancies receiv-

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*Table 2* Characteristics of the control patients not treated with WBH

| Patient no. | Diagnosis | Sex | Age | Detection of CK-positive cells[a] | |
|---|---|---|---|---|---|
| | | | | Portion 1 | Portion 2 |
| 1 | Gastric cancer | Male | 63 | 1 | 1 |
| 2 | Gastric cancer | Male | 65 | 0 | 0 |
| 3 | Gastric cancer | Male | 65 | 0 | 0 |
| 4 | Esophageal cancer | Male | 54 | 0 | 0 |
| 5 | Head and neck cancer | Male | 61 | 0 | 0 |
| 6 | Gastric cancer | Female | 55 | 0 | 0 |
| 7 | NSCLC[b] | Male | 63 | 0 | 0 |
| 8 | NSCLC | Male | 63 | 26 | 94 (cluster) |
| 9 | Gastric cancer | Male | 64 | 0 | 0 |
| 10 | Breast cancer | Female | 69 | 46 | 43 |

[a] 40 ml of peripheral blood was drawn from each patient and analyzed in parallel in two equal portions.
[b] NSCLC, non-small cell lung cancer.

ing platinum-based chemotherapy alone (gastric cancer, $n = 5$; non small-cell lung cancer, $n = 2$; esophageal carcinoma, $n = 1$; breast cancer, $n = 1$; and head and neck cancer, $n = 1$; Table 2).

For control experiments to test for the sensitivity and specificity of the assay, 20–40 ml of heparinized blood was drawn from 12 healthy volunteers.

**WBH Treatment Procedure.** The WBH treatment procedure at our institution and the radiant heat system for delivering WBH have been described previously in detail (15, 17, 35). In brief WBH was administered by a humidified radiant heat device (RHS-7500; Enthermics Medical Systems, Inc., Menomonee Falls, WI) exposing the patient to a low-density radiant heat while preventing evaporative heat loss. A hyperthermia treatment session was defined as raising the core temperature of the patient to 41.8°C. A typical WBH treatment session lasted about 3.5–4 h, including a mean of 100 min to reach target temperature, 60 min at 41.8°C, and a 1-h cooling period.

As soon as the target temperature was achieved, the patient was removed from the radiant heat chamber, and wrapped into a blanket and a plastic sheet that served as a vapor barrier to prevent heat loss and maintain a stable plateau phase. Esophageal, rectal, skin, and ambient air temperatures were monitored continuously using Mallinckrodt temperature probes (Mon-a-Therm TM Skin and Mon-a-Therm Thermistor 400; Mallinckrodt Medical, St. Louis, MO) and Enthermics thermometry software. All of the WBH treatments were performed under general anesthesia.

**Preparation and Immunostaining on Adhesion Slides.** MNCs from blood samples were isolated by density centrifugation through NycoPrep (Nycomed Pharma AS, Oslo, Norway). Cells were collected from the interphase and washed twice in PBS/1%BSA and resuspended in 3 ml of PBS/1% BSA. For the immunomagnetic depletion of CD45-positive cells from the cell suspensions Dynabeads M450 CD45 (Pan Leukocyte; Dynal, Hamburg, Germany) was used. Members of the CD45 family of proteins are expressed on all of the nucleated hematopoietic cells but not on epithelial tumor cells. The depletion as well as the successive detection of epithelial cancer cells with the EPI-MET kit (Baxter, Unterschleiβheim, Germany) was performed according to the manufacturer's instructions with slight modifications. In brief, MNCs were incubated with anti-CD45-conjugated Dynabeads M450 at a bead:cell ratio of 5:1. The bead:cell-suspension was incubated under gentle rotation for 30 min at 2–8°C. The solution was then diluted to 8 ml in PBS/1% BSA, and the test tube was placed on the magnet to collect CD45-positive cells and unbound Dynabeads. The supernatant containing the enriched epithelial cells was collected, and the procedure was repeated twice. The CD45-negative cell fraction was centrifuged on cell adhesion slides. Routinely six slides were analyzed for each patient and each time point.

The identification of epithelial tumor cells with the EPI-MET kit is based on the reactivity of the murine mAb A45-B/B3, directed against a common epitope on CK polypeptides, including the CK heterodimers 8–18 and 8–19 (36). The anti-CK mAb A45-B/B3 was validated in a recent clinical study of >500 breast cancer patients (33). The cells were permeabilized, fixed, and stained with the antibody according to the manufacturer's instructions using the alkaline phosphatase antialkaline phosphatase technique. The color reaction was developed by incubation with New Fuchsin/naphtol-AS-BI phosphate/levamisole, followed by incubation in hematoxylin for nuclear counterstaining. Cells of the human breast and colon cancer cell lines MCF-7 and HT-29, respectively, served as positive controls. The specificity of the antibody reaction was confirmed by the addition of an unrelated mouse control antibody at an appropriate dilution. The slides were examined by light microscopy, evaluated separately by two of the authors (M. O. and K. B.), and consensus was obtained. The total number of tumor cells counted in all of the slides generated the tumor concentration for that sample. Thus, data are given as the total number of tumor cells detected in 20 ml of the patient's blood.

**Statistical Analysis.** The Wilcoxon signed-rank test was used to compare the detection of epithelial-cell events. A $P < 0.05$ was considered to indicate a statistically significant difference.

## RESULTS

**Sensitivity and Specificity.** The average sensitivity of the assay was one tumor cell in $1.7 \times 10^5$ nuclear blood cells as determined by seeding cells of the human breast cancer cell line

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MCF-7 into 20 ml of peripheral blood of normal volunteers. To investigate the possibility of false positive results, MNCs of 20 ml of peripheral blood of 10 normal volunteers were treated using the same assay conditions. All of the slides proved to be negative. Furthermore, to test the reliability of the assay 40 ml of peripheral blood was obtained from 10 control patients suffering from different advanced malignancies. Samples were divided into two portions of 20 ml, and analysis was performed in parallel. In 7 patients both portions were without evidence of circulating tumor cells. In the remaining 3 patients we found a comparable number of tumor cells in both portions (pt no. 1: 1 tumor cell in each portion; pt no. 8: 26 and 94 tumor cells including a cluster; and pt no. 10: 46 and 43 tumor cells; Table 2).

**Detection of Circulating Tumor Cells in Patients Treated with WBH.** Before WBH 6 of 20 (30%) patients had evidence of CK-positive cells in their blood. At 1 h, 24 h, and 48 h after WBH treatment CK-positive cells were detected in 4 of 20 patients (20%, not significant), 9 of 20 patients (45%, $P = 0.043$), and 1 of 10 patient (10%, not significant), respectively. A treatment-induced increase in the number of circulating tumor cells was detected in 9 patients, 5 of whom had no detectable malignant cells at baseline. Four of 6 initially positive patients had a slight increase in the number of circulating tumor cells at 24 h as compared with their baseline values, and 5 initially negative patients became positive at least at one point in time after WBH (Table 1). There was no evidence for a correlation between an increase in the number of circulating tumor cells and accelerated disease progression. Ten of 11 patients with CK-positive cells in at least one of the samples remained stable or had a minor response for a median of 22 weeks (13–46 weeks), and 1 progressed at 8 weeks. In those patients negative for CK-positive cells, tumor growth control was obtained in 5 patients (2 PR and 3 SD) for a median of 22 weeks (10–42 weeks), and 3 progressed at 7, 8, and 8 weeks, respectively. Because all of the patients except those with cholangiocellular carcinoma or pleural mesothelioma received WBH in combination with chemotherapy only as second or third line treatment, these results are within the expected range.

## DISCUSSION

Considering the small amount of circulating tumor cells detected in our samples, specificity has to be a major concern for techniques aiming on the detection of disseminated tumor cells. The CK-specific antibody used in this study is not expressed in normal peripheral blood cells. Many studies have shown that CK antigens are rarely detected in hematopoietic cells (30). In bone marrow samples the malignant nature of CK-positive cells has been additionally confirmed through genomic analysis (37–39).

Braun *et al.* (33) analyzed 191 patients with nonmalignant conditions, using the same mAb that we applied in our study. CK-positive cells were detected in only 1% of all of the bone marrow specimens analyzed. This is in contrast to other antibodies such as epithelial membrane antigen or tumor-associated glycoprotein 12, also expressed by plasmacytes and erythroid precursors (33). We analyzed blood samples of 10 normal healthy volunteers all of whom tested negative with the established staining procedure. Thus, false-positive results can be considered unlikely in the context of our study.

Initially, most of the work concerning the prognostic significance of micrometastatic disease has been performed in the bone marrow (30). Several prospective clinical trials have confirmed the presence of occult tumor cells as an independent risk factor in patients with mammary (33), colorectal (40–42), and non-small-cell lung carcinoma (42). Surgical manipulation of tumors always bears the risk of intraoperative tumor cell dissemination. An increasing number of studies has thus focused on the detection of circulating tumor cells in peripheral blood before surgery and at different points in time thereafter in colorectal cancer, breast cancer, melanoma, and prostate cancer (25, 26, 43–46). Using CKs as marker antigens either combined with a RT-PCR assay or immunomagnetic enrichment and immunocytochemistry, these studies were able to demonstrate that surgery enhances the release of tumor cells into the circulation. Furthermore, a risk of concomitant tumor cell recruitment on mobilization of peripheral blood progenitor cells used for autografting has also been shown (47). In addition, the potential of circulating tumor cells to form solid metastases *in vivo* has been demonstrated recently (48, 49).

Data concerning the effect of WBH on metastasis frequency in animal models are inconsistent, and the fact that these observations were mainly generated in animal models using fast growing tumor models impairs the applicability to what may be seen in humans (18–23). None of the studies investigated hematogenous spread of tumor cells but rather focused on WBH-induced changes in immune reactivity (18, 21). Furthermore, only one of these studies has combined WBH with chemotherapy (23). In that study, an increase in lung metastasis observed in Lewis lung carcinoma bearing C57BL/6 mice subjected to 42°C WBH alone could be prevented by the combined use of anticancer drugs and WBH.

Urano *et al.* (22) observed an increase in the frequency of lung metastasis as a consequence of WBH (42.5°C) in mice only in large, very weakly immunogenic murine tumors but not in moderately immunogenic tumors. Local hyperthermia did not increase the metastatic rate of both tumor types. Others found a significant delay in primary tumor growth of Lewis lung carcinoma in mice exposed to 42°C WBH. Nevertheless, this was accompanied by a more rapid dissemination of tumor cells demonstrated by a significant increase in the average grade of lung metastasis (23). WBH at 40°C neither inhibited nor potentiated tumor growth in that model. In VX2 tumor-bearing rabbits exposed to 42°C WBH, a temporary restraint of tumor growth was observed followed by a return to exponential increase in tumor volume and rapid death (50), whereas these animals could be cured with local hyperthermia (47–50°C/30 min). Finally, Dickson and Ellis (20) reported that a stimulation of tumor cell dissemination by raised temperatures (42°C) in rats with transplanted Yoshida tumors led to direct, lymphatic, and blood-borne spread of tumor cells. The significance of these findings is nevertheless questionable, because only 16 of 167 animals survived the heating procedure.

This is the first human study to investigate the effects of WBH (41.8°C) on the frequency of tumor cells in the peripheral blood. Our findings suggest that WBH in combination with chemotherapy might result in a temporary hematogenous

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spreading of tumor cells. At present, we are not able to dissect the influence of these two treatment modalities on tumor cell spreading. Experimental studies suggest that mechanisms contributing to the observed spread of tumor cells remain speculative but may include WBH-induced changes in tumor blood flow and local hyperemia.

The clinical significance of tumor cell spread induced by WBH remains to be determined. Nevertheless, our study does not provide evidence to support the assumption that WBH promotes tumor metastases, and the relevance of this phenomenon in patients with advanced malignancies might be rather limited. This might be different in patients treated in the adjuvant setting.

The possibility of hyperthermia-induced tumor cell spread should also be considered when other hyperthermic treatment modalities are applied, such as locoregional hyperthermia in combination with radiation (51) or a fever-range WBH where treatment is extended over several hours (16).

## REFERENCES


1. Hettinga, J. V. E., Lemstra, W., Meijer, C., Dam, W. A., Uges, D. R., Konings, A. W., de Vries, E. G., and Kampinga, H. H. Mechanism of hyperthermic potentiation of cisplatin action in cisplatin-sensitive and -resistant tumour cells. Br. J. Cancer, *75:* 1735–1743, 1997.

2. Rietbroeck, R. C., van de Vaart, P. J. M., Havemann, J., Blommaert, F. A., Geerdink, A., Bakker, P. J. M., and Veenhof, C. H. N. Hyperthermia enhances the cytotoxicity of platinum-DNA adduct formation of lobaplatin and oxaliplatin in cultured SW 1573 cells. J. Cancer Res. Clin. Oncol., *123:* 6–12, 1997.

3. Wiedemann, G., d'Oleire, F., Knop, E., Eleftheriadis, S., Feddersen, S., Klouche, M., Geisler, J., Mentzel, M., Schmucker, P., Feyerabend, T., Weiss, C., and Wagner, T. Ifosfamide and Carboplatin combined with 41.8°C whole-body hyperthermia in patients with refractory sarcoma and malignant teratoma. Cancer Res., *54:* 5346–5350, 1994.

4. Wilke, A. V., Jenkins, C., Milligan, A. J., Legendre, A., and Frazier, D. L. Effect of hyperthermia on normal tissue toxicity and on adriamycin pharmacokinetics in dogs. Cancer Res., *51:* 1680–1683, 1991.

5. Burd, R., Dziedzic, T. S., Xu, Y., Caligiuri, M. A., Subjeck, J. R., and Repasky, E. A.: Tumor cell apoptosis, lymphocyte recruitment and tumor vascular changes are induced by low temperature, long duration whole body hyperthermia. J. Cell Physiol., *177:* 137–147, 1998.

6. Multhoff, G., Botzler, C., Wiesner, M., Muller, E., Meier, T., Wilmanns, W., and Issels, R. D. A stress-inducible 72-kDa heat-shock protein (HSP72) is expressed on the surface of human tumor cells, but not on normal cells. Int. J. Cancer, *61:* 272–279, 1995.

7. Atanackovic, D., Corovic, A., Nierhaus, A., Neumeier, M., Hossfeld, D. K., and Hegewisch-Becker, S. 41.8 °C whole body hyperthermia as an adjunct to chemotherapy induces prolonged T cell activation in patients with various malignant diseases. J. Cancer Immunol. Immunother., in press, 2002.

8. Robins, H. I., Cohen, J. D., Schmitt, C. L., Tutsch, K. D., Feierabend, C., Arzoomanian, R. Z., Alberti, D., d'Oleire, F., Longo, W., Heiss, C., Rushing, D., and Springs, D. Phase I clinical trial of carboplatin and 41.8°C whole-body hyperthermia in cancer patients. J. Clin. Oncol., *11:* 1787–1794, 1993.

9. Robins, H. I., Rushing, D., Kutz, M., Tutsch, KD., Tiggelaar, C. L., Paul, D., Spriggs, D., Kraemer, C., Gillis, W., Feierabend, C., Arzoomanian, R. Z., Longo, W., Alberti, D., d'Oleire, F., Qu, R. P., Wilding, G., and Stewart, J. A. A Phase I clinical trial of melphalan and 41, 8°C whole-body hyperthermia in cancer patients. J. Clin. Oncol., *15:* 158–164, 1997.

10. Bull, J. M., Whang-Peng, J., Lees, D. E., Smith, R., Schuette, W., Kim, Y. D., and DeVita, V. T. A Phase I trial of systemic heat and adriamycin. Proc. Am. Soc. Clin. Oncol., *20:* 398, 1979.

11. Barlogie, B., Corry, P. M., Yip, E., Lippmann, L., Johnston, D. A., Khalil, K., Tenzynski, T. F., Reilly, E., Lawson, R., Dosik, G., Rigor, B., Hankenson, R., and Freireich, E. J. Total-body hyperthermia with and without chemotherapy for advanced human neoplasms. Cancer Res., *39:* 1481–1489, 1979.

12. Westermann, A. M., Grosen, E. A., Katschinski, D. M., Jager, E., Rietbroek, R., Schink, J. C., Tiggelaar, C. L., Jager, D., Zum Vorde sive Vording, P., Neuman, A., Knuth, A., Van Dijk, J. D., Wiedemann, G. J., and Robins, H. I. A pilot study of whole body hyperthermia and carboplatin in platinum-resistant ovarian cancer. Eur. J. Cancer, *37:* 1111–1117, 2001.

13. Wiedemann, G. J., Katschinski, D. M., Westerman, A. M., Jager, D., Zum Vorde sive Vording, P., Van Dijk, J., Bailey, H., Fine, J., Longo, W., Bakhshandeh, A., Grosen, E., and Robins, H. I. A Systemic Hyperthermia Oncology Working Group trial: Ifosfamide (IFO), carboplatin (CBDCA) and etoposide (VP-16) combined with aquatherm induced 41, 8°C whole body hyperthermia (WBH) for refractory sarcoma. Proc. Am. Soc. Clin. Oncol., *19:* 562a, 2000.

14. Wiedemann, G. J., Robins, H. I., Gutsche, S., Katschinski, D. M., Mentzel, M., Deeken, M., Eleftheriadis, S., Crahe, R., Weiss, C., Storer, B., and Wagner, T. Ifosfamide, carboplatin and etoposide (ICE) combined with 41.8°C whole-body hyperthermia in patients with refractory sarcoma: A phase II study. Eur. J. Cancer, *32A:* 888–892, 1996.

15. Hegewisch-Becker, S., Gruber, Y., Corovic, A., Pichlmeier, U., Atanackovic, D., Nierhaus, A., and Hossfeld, D. K. Whole body hyperthermia (41.8°C) combined with bimonthly oxaliplatin, high-dose leucovorin and 5-Fluorouracil 48h-continuous infusion in pretreated metastatic colorectal cancer: a phase II study. Ann. Oncol., *13:* 1197–1204, 2002.

16. Kraybill, W. G., Olenki, T., Evans, S. S., Ostberg, J. R., O'Leary, K. A., Gibbs, J. F., and Repasky, E. A. A phase I study of fever-range whole body hyperthermia (FR-WBH) in patients with advanced solid tumours: correlation with mouse models. Int. J. Hyperth., *18:* 253–266, 2002.

17. Robins, H. I., Woods, J. P., Schmitt, C. L., and Cohen, J. D. A new technological approach to radiant heat whole body hyperthermia. Cancer Lett., *79:* 137–145, 1994.

18. Dickson, J. A., and Muckle, D. S. Total body hyperthermia versus primary tumour hyperthermia in the treatment of the rabbit VX2 carcinoma. Cancer Res., *32:* 1916–1923, 1972.

19. Lord, P. F., Kapp, D. S., and Morrow, D. Increased skeletal metastases of spontaneous canine osteosarcoma after fractioned systemic hyperthermia and local x-irradiation. Cancer Res., *41:* 4331–4334, 1981.

20. Dickson, J. A., and Ellis, H. A. Stimulation of tumour cell dissemination by raised temperature (42 °C) in rats with transplanted Yoshida tumours. Nature (Lond.), *248:* 354–358, 1974.

21. Yerushalmi, A. Influence on metastatic spread of whole body or local tumor hyperthermia. Exp. J. Cancer, *12:* 455–463, 1976.

22. Urano, M., Rice, L., Epstein, R., Suit, H. D., and Chu, A. M. Effect of whole body hyperthermia on cell survival, metastasis frequency, and host immunity in moderately and weakly immunogenic murine tumors. Cancer Res., *43:* 1039–1043, 1983.

23. Oda, M., Koga, S., and Maeta, M. Effects of total-body hyperthermia on metastases from experimental mouse tumours. Cancer Res., *45:* 1532–1535, 1985.

24. Shen, R., Hornback, N. B., Shidnia, H., Shupe, R. E., and Brahmi, Z. Whole body hyperthermia decreases lung metastases in lung tumor-bearing mice, possibly via a mechanism involving natural killer cells. J. Clin. Immunol., *7:* 246–253, 1987.

25. Hansen, E. N., Untch, M., Pache, L., and Eiermann, W. Tumor cells in blood shed from the surgical field. Arch. Surg., *130:* 387–393, 1995.

26. Weitz, J., Kienle, P., Lacroix, J., Willeke, F., Benner, A., Lehnert, T., Herfarth, C., and von Knebel Doeberitz, M. Dissemination of tumor cells in patients undergoing surgery for colorectal cancer. Clin. Cancer Res., *4:* 343–348, 1998.

27. Eschwège, P., Dumas, F., Blanchet, P., Le Maire, V., Benoit, G., Jardin, A., Lacour, B., and Loric, S. Haematogenous dissemination of


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prostatic epithelial cells during radical prostatectomy. Lancet, *346:* 1528–1530, 1995.

28. Gautherie, M. (ed.). Whole Body Hyperthermia: Biological and Clinical Aspects. Berlin Heidelberg: Springer Verlag, 1992.

29. Waterman, F. M., Tupchong, L., Nerlinger, R., and Matthews, J. Blood flow in human tumors during local hyperthermia. Int. J. Radiat. Oncol. Biol. Phys., *20:* 1255–1262, 1991.

30. Pantel, K., Cote, R. J., and Fodstad, Ø. Detection and clinical importance of micrometastatic disease. J. Natl. Cancer. Inst., *91:* 1113–24, 1999.

31. Ghossein, R. A., Bhattacharya, S., and Rosai, J. Molecular detection of micrometastases and circulating tumor cells in solid tumors. Clin. Cancer Res., *5:* 1950–1960, 1999.

32. Naume, B., Borgen, E., Nesland, J. M., Beiske, K., Gilen, E., Renolen, A., Ravnas, G., Qvist, H., Karesen, R., and Kvalheim, G. Increased sensitivity for detection of micrometastases in bone-marrow/peripheral-blood stem-cell products from breast-cancer patients by negative immunomagnetic separation. Int. J. Cancer., *78:* 556–560, 1998.

33. Braun, S., Pantel, K., Müller, P., Janni, W., Hepp, F., Kentenich, C. R. M., Gastroph, S., Wischnik, A., Dimpfl, T., Kindermann, G., Riethmüller, G., and Schlimok, G. Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II or III breast cancer. N. Engl. J. Med., *342:* 525–533, 2000.

34. Moll, R., Franke, W. W., Schiller, D. L., Geiger, B., and Krepler, R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell, *31:* 11–24, 1982.

35. Robins, H. I., Dennis, W. H., Neville, A. J., Shecterle, L., Martin, P. A., Grossmann, J., Davis, T. E., Gillis, W., and Rusey, B. F. A nontoxic system for 41.8°C whole-body hyperthermia: results of a phase I study using a radiant heat device. Cancer Res., *45:* 3937–3944, 1985.

36. Stigbrand, T., Andrés, C., Bellanger, L., Bishr Omory, M., Bodenmuller, H., Bonfrer, H., Brundell, J., Einarsson, R., Erlandsson, A., Johansson, A., Leca, J. F., Levin, M., Meier, T., Nap, M., Nustad, K., Seguin, P., Sjodin, A., Suudstrom, B., van Dalen, A., Wioebelhaus, E., Wiklund, B., Arlestig, L., and Hilgers, J. Epitope specificity of 30 monoclonal antibodies against cytokeratin antigens: The ISOBM TD5–1 Workshop Tumor Biol., *19:* 132–152, 1998.

37. Klein, C. A., Schmidt-Kittler, O., Schardt, J. A., Pantel, K., Speicher, M. R., and Riethmüller, G. Comparative genomic hybridization loss of heterozygosity, and DNA sequence analysis of single cells. Proc. Natl. Acad. Sci. USA, *96:* 4494–4499, 1999.

38. Dietmaier, W., Hartmann, A., Wallinger, S., Heinmoeller, E., Kerner, T., Endl, E., Jauch, K. W., Hofstadter, F., and Ruschoff, J. Multiple mutation analysis in single tumor cells with improved whole genome amplification. Am. J. Pathol., *154:* 83–95, 1999.

39. Müller, P., Weckermann, D., Riethmüller, G., and Schlimok, G. Detection of genetic alterations in micrometastatic cells in bone marrow of cancer patients by fluorescence *in situ* hybridization. Cancer Genet. Cytogenet., *88:* 8–16, 1996.

40. Lindemann, F., Schlimok, G., Dirschedl, P., Witte, J., and Riethmüller, G. Prognostic significance of micrometastatic tumour cells in bone marrow of colorectal cancer patients. Lancet, *340:* 685–689, 1992.

41. Jauch, K. W., Heiss, M. M., Gruetzner, U., Funke, I., Pantel, K., Babic, R., Eissner, H. J., Riethmueller, G., and Schildberg, F. W. Prognostic significance of bone marrow micrometastases in patients with gastric cancer. J. Clin. Oncol., *14:* 1810–1817, 1996.

42. Pantel, K., Izbicki, J., Passlick, B., Angstwurm, M., Häussinger, K., Thetter, O., and Riethmüller, G. Frequency and prognostic significance of isolated tumour cells in bone marrow of patients with non-small-cell lung cancer without overt metastases. Lancet, *347:* 649–653, 1996.

43. Krag, D. N., Ashikaga, T., Moss, T. J., Kusminsky, R. E., Feldmann, S., Carp, N. Z., Moffat, F. L., Beitsch, P. D., Frazier, T. G., Gaskin, T. A., Shook, J. W., Harlow, S. P., and Weaver, D. L. Breast cancer cells in the blood: a pilot study. Breast J., *5:* 354–358, 1999.

44. Leather, A. J. M., Gallegos, N. C., Kocjan, G., Savage, F., Smales, C. S., Hu, W., Boulos, P. B., Northover, J. M. A., and Phillips, R. K. S. Detection and enumeration of circulating tumour cells in colorectal cancer. Br. J. Surg., *80:* 777–780, 1993.

45. Denis, M. G., Tessier, M. H., and Lustenberger, P. Circulating micrometastases following oncological surgery. Lancet, *347:* 913, 1996.

46. Brandt, B., Junker, R., Griwatz, C., Heidl, S., Brinkmann, O., Semjonow, A., Assmann, G., and Zänker, K. S. Isolation of prostate-derived single cells and cell clusters from human peripheral blood. Cancer Res., *56:* 4556–4561, 1996.

47. Brugger, W., Bross, K. J., Glatt, M., Weber, F., Mertelsmann, R., and Kanz, L. Mobilization of tumor cells and hematopoietic progenitor cells into peripheral blood of patients with solid tumors. Blood, *83:* 636–640, 1994.

48. Pretlow, T. G., Schwartz, S., Giaconia, J. M., Wright, A. L., Grimm, H. A., Edgehouse, N. L., Murphy, J. R., Markowitz, S. D., Jaminson, J. M., Summmers, J. L., Hamlin, C. R., Mac Lennon, G. T., Resnick, M. I., Pretlow, T. P., and Connell, C. F. Prostate cancer and other xenografts from cells in peripheral blood of patients. Cancer Res., *60:* 4033–4036, 2000.

49. Hosch, S. B., Kraus, J., Scheunemann, P., Zbicki, J. R., Schneider, C., Schumacher, U., Witter, K., Speicher, M. R., and Pantel, K. Malignant potential and cytogenetic characteristics of occult disseminated tumor cells in esophageal cancer. Cancer Res., *60*: 6836-, 2000.

50. Shah, S. A., and Dickson, J. A. Effect of hyperthermia on the immunocompetence ov BS2 tumor-bearing rabbits. Cancer Res., *38:* 3523–3531, 1978.

51. Wust, P., Hildebrandt, B., Sreenivasa, G., Rau, B., Gellermann, J., Riess, H., Felix, R., and Schlag, P. M. Hyperthermia in combined treatment of cancer. Lancet Oncol., *3:* 487–497, 2002.

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