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ORIGINAL REPORT

Unique Localization of Circulating Tumor Cells in Patients With Hepatic Metastases

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ABSTRACT

Purpose
There are few data on the impact of immediate and differing surgical interventions on circulating tumor cells (CTCs), nor their compartmentalization or localization in different anatomic vascular sites.

Patients and Methods
CTCs from consecutive patients with colorectal liver metastases were quantified before and immediately after open surgery, laparoscopic resection, open radiofrequency ablation (RFA), or percutaneous RFA. For individuals undergoing open surgery, either hepatic resections or open RFA, CTCs were examined in both systemic and portal circulation by measuring CTCs in samples derived from the peripheral vein, an artery, the hepatic portal vein, and the hepatic vein.

Results
A total of 29 consecutive patients with colorectal liver metastases with a median age of 55 years (range, 30 to 88 years) were included. CTCs were localized to the hepatic portosystemic macrocirculation with significantly greater numbers than in the systemic vasculature. Surgical procedures led to a statistically significant fall in CTCs at multiple sites measured. Conversely, RFA, either open or percutaneous, was associated with a significant increase in CTCs.

Conclusion
Surgical resection of metastases, but not RFA, immediately decreases CTC levels. In patients with colorectal liver metastases, CTCs appear localized to the hepatic (and pulmonary) macrocirculations. This may explain why metastases in sites other than the liver and lungs are infrequently observed in cancer.

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INTRODUCTION

The prognostic role of circulating tumor cells (CTCs) is now established[1,2] and there are data supporting their role as more reproducible indications of disease status than current imaging methods.[3] A number of methodologies have been proposed for their measurement, including quantitative real-time polymerase chain reaction–based assays,[4-6] immunomagnetic separation, and laser scanning cytometry.[7-10] Following these data, the US Food and Drug Administration has now approved the prognostic use of the flow cytometry–based Veridex CellSearch (Warren, NJ) system in a number of clinical settings including breast and prostate cancer, diseases that are considered incurable when metastatic.[11-15]

In colorectal cancer. approximately 50% of patients develop metastatic liver disease and 25% have evidence of this at the time of initial diagnosis.[16] If untreated, the median survival of these patients is less than 12 months; however, with curative liver resection in those for whom it is suitable, the 5-year survival increases to 40% to 60%.[17] Increasingly, a variety of locoregional therapeutic modalities have been advocated to treat patients with unresectable liver metastases although intraoperative manipulation of organs has been reported to promote tumor spread.[18-20]

Radiofrequency ablation (RFA), which converts radiofrequency waves into heat resulting in tissue desiccation and coagulative necrosis, has emerged over the last decade as an accepted method for treatment of liver metastases, although there have been no prospective randomized trials comparing RFA with other therapeutic modalities.[21-24] We therefore wished to compare the impact of surgical or RFA-based intervention on CTC measurements using a standardized US Food and Drug Administration–approved immunomagnetic flow-cytometry based system, immediately before and after these procedures. In doing so, we also wished to

Table 1. Patient Characteristics of 29 Patients With Colorectal Liver Metastases Included in This Study: Histology Confirmed Adenocarcinomas in All Patients

Therapeutic Modality	No.	Male	Female	Age (years) Median	Age (years) Range
Open resection	11	8	3	53	30-72
Laparoscopic resection	4	3	1	55	41-76
Open RFA	5	3	2	60	37-78
Percutaneous RFA	9	7	2	59	42-78

Abbreviation: RFA, radiofrequency ablation.

utilize these surrogate biomarkers to gain insights into the differential localization or compartmentalization of these cells, likely to be en route to further metastasis, an as yet unconfirmed assertion.

PATIENTS AND METHODS

Consecutive patients with metastatic colorectal liver metastases, confirmed as adenocarcinomas by histology, were recruited to this study from January to November 2008; appropriate local ethics committee approval was obtained. Samples were blinded for analysis and patients understood that the results would not be made available to them.

The CellSearch system was used to enrich and enumerate the CTCs, as described previously.[15] A 7.5-mL blood sample was taken in a CellSave preservative tube (Veridex, Warren, NJ), kept at room temperature, and processed within 72 hours. The system enriched for epithelial cell adhesion molecule (EpCAM) –positive epithelial cells by incubating the sample with ferrofluid conjugated to anti-EpCAM antibodies. Cells were stained with the following fluorescent-labeled monoclonal proprietary antibodies: CD45-APC to distinguish the CTCs from leukocytes and pan-cytokeratin 8, 18, and 19 to stain epithelial cells, and epidermal growth factor-receptor (EGFR) antibodies as we have recently described.[13] Nucleic acids were stained using 4,6-diamidino-2-phenylindole (to exclude RBCs). Samples were then scanned on the CellTracks analyzer II fluorescent microscope (Veridex) for analysis.

In patients undergoing percutaneous RFA (PRFA), 7.5 mL of blood was taken from the peripheral circulation only (the antecubital fossa) before and 1day after PRFA.

For patients undergoing surgery (ie, those not undergoing PRFA), 7.5 mL of blood was taken from peripheral venous (PV) and arterial circulations, and hepatic (HV) and portal veins (PoV), intraoperatively, before and 20 minutes after resection or open RFA (ORFA). The arterial sample was taken from an indwelling cannula in a radial artery inserted during routine anesthesia for intraoperative monitoring. The portal and hepatic venous blood was obtained from a direct venous puncture after mobilization of portal triads and liver to exposed portal vein and right hepatic vein during operation. For those undergoing laparoscopic liver resections, 7.5 mL of blood was taken from peripheral venous and the arterial circulation before and after resection after introduction of pneumoperitoneum with a CO pressure of 12 mmHg. All patients were received follow-up with our standard practice with microbubble ultrasound at 6 weeks after RFA, then a computed tomography (CT) scan at 3 months and 6 months, and with a CT scan at 3 months and 6 months for those after resection.

To exclude the possibility that CTC changes observed were not due to periodic fluctuations in their release, in six individuals undergoing open liver resections we obtained two samples from the HV and PoV to ensure the reliability and reproducibility of measurements described. No patients recruited here were undergoing synchronous resections of primary lesions.

Results are expressed as means, medians, standard deviations, and ranges. Comparisons between numbers of CTCs before and after procedures for each therapeutic modality were made using the Wilcoxon signed ranks test. A $P < .05$ was considered statistically significant. Statistical analysis was carried out using SPSS for Windows (version16.0, SPSS Inc. Chicago, IL).

RESULTS

A total of 29 consecutive patients requiring intervention for their colorectal liver metastases were recruited into this study. These comprised individuals for open liver resection (n = 11), ORFA (n = 5), laparoscopic liver resection (n = 4), and PRFA (n = 9); there were no significant differences in baseline characteristics between the groups except patients undergoing RFA were generally older (Table 1). One additional patient with metastatic carcinoid tumor was also recruited to this study as a negative control and no CTCs were detected in this individual, as anticipated. The majority patients (27 of 29) were white, one was Indian, and one was African. The numbers of CTCs before intervention, and the difference after intervention, in both the systemic and portosystemic circulations are presented in Tables 2 and 3.

Localization of CTCs and the Impact of Chemotherapy

Localization of CTCs in the circulation was examined by measuring them at different sites in both the systemic circulation as measured

Table 2. Impact of Interventions on CTCs at Different Vascular Sites

		Before Intervention								Differences After Intervention (pre minus post)											
		Systemic Circulation				Portosystemic Circulation				Systemic Circulation						Portosystemic Circulation					
		PV		PA		PoV		HV		PV			PA			PoV			HV		
Therapeutic Modality	No. of Patients	Median No. of CTCs	Range	Median No. of CTCs	Range	Median No. of CTCs	Range	Median No. of CTCs	Range	Median No. of CTCs	Range	P	Median No. of CTCs	Range	P	Median No. of CTCs	Range	P	Median No. of CTCs	Range	P
Open resection	11	1	0-3	1	0-6	87	0-500	187	0-500	1	0-3	.034	1	−5-5	.005	26	−3-275	.007	172	0-238	.011
Laparoscopic resection	4	1	0-7	0		NA		NA		1	−1-7	.026	0	0-0	1.0	NA			NA		
Open RFA	5	2	0-7	3	0-16	11	0-18	6	0-102	−11	−15-1	.04	−1	−4-1	.20	0	−26-1	.66	−28	−56-0	.07
Percutaneous RFA	9	0	0-4	NA		NA		NA		−4	−17-0	.012	NA			NA			NA		

NOTE. CTCs are measured per 7.5 mLs of blood.
Abbreviations: CTC, circulating tumor cell; PV, peripheral vein; PA, peripheral artery; PoV, portal vein; HV, hepatic vein; RFA, radiofrequency ablation; NA, not applicable.

Table 3. Impact of Interventions on CTCs at Different Vascular Sites

Therapeutic Modality	Before Intervention								Differences After Intervention (pre minus post)							
	Systemic Circulation				Portosystemic Circulation				Systemic Circulation				Portosystemic Circulation			
	PV		PA		PoV		HV		PV		PA		PoV		HV	
	Mean No. of CTCs	SD	Mean No. of CTCs	SD	Mean No. of CTCs	SD	Mean No. of CTCs	SD	Mean No. of CTCs	SD	Mean No. of CTCs	SD	Mean No. of CTCs	SD	Mean No. of CTCs	SD
Open resection (n = 11)	1.45	1.04	1.82	1.7	126	141	174	147	1.4	1.1	1.4	1.8	63	86	151	121
Laparoscopic resection (n = 4)	2.25	3.2	0		NA		NA		2	3.5	0	0	NA		NA	
Open RFA (n = 5)	3	2.9	5.2	6.3	10	6.9	24	44	−10	5.7	−1.4	2.1	−13	31	−35	23
Percutaneous RFA (n = 9)	0.67	1.3	NA		NA		NA		–5	5.2	NA		NA		NA	

NOTE. CTCs are measured per 7.5 mLs of blood.
Abbreviations: CTC, circulating tumor cell; PV, peripheral vein; PA, peripheral artery; PoV, portal vein; HV, hepatic vein; SD, standard deviation; RFA, radiofrequency ablation; NA, not applicable.

with peripheral venous and arterial blood samples, and the portosystemic circulation as measured with portal venous and hepatic venous blood samples, in patients undergoing an open surgical resection procedure (n = 11). This demonstrated that the median number of CTCs immediately before intervention in PV, arterial circulations, PoV, and HV measured 1 (range, 0 to 3), 1 (range, 0 to 6), 87 (range, 0 to 500) and 187 (range, 0 to 500), respectively. Thus, a much larger number of CTCs was observed in the liver macrocirculation, compared with elsewhere, a possible reflection of disease bulk and volume at this site.

Of the 11 patients who had open surgical liver resections, nine with synchronous liver metastases had preoperative chemotherapy, and two with metachronous solitary metastases detected approximately 2 years after the initial resection for the colonic primary did not have preoperative chemotherapy. Irrespective of whether chemotherapy was given or not, or the type of response to systemic cytotoxics, the number of CTCs in the peripheral circulation remained remarkably low in patients who did or did not receive chemotherapy, compared with the PoV and HV indicating that the liver macrocirculation (and also the lungs) appear to be sites where CTCs are pooled.

We caution however against drawing conclusions in this subgroup based on the small number of patients. Overall, however, it appeared that chemotherapy reduced the number of CTCs in the portosystemic system, we suggest reducing the risk of both lung and systemic spread although long-term clinical outcomes are still awaited.

Impact of Procedural Interventions on CTCs

In the case of surgical resection, the number of CTCs in any blood vessel sampled was significantly lower after the procedure than before it ($P < .05$). After the ORFA procedure, the number of CTCs significantly increased for PV ($P = .026$), but not for any other blood vessel ($P > .05$). After surgical resection (either open or laparoscopic), the number of CTCs in the peripheral blood (PV) decreased ($P = .005$), whereas it increased following RFA (percutaneous or open; $P = .001$) as presented in Table 2 and Figure 1. The decrease in CTCs throughout the circulation after open resection has been mentioned above, and it is interesting to note that a decrease was also observed after laparoscopic resection, although numbers of both patients and CTCs are small. Similarly, it is difficult to compare CTCs between ORFA and PRFA. Figure 2 demonstrates how CTC levels change for individual patients in the peripheral vein before and after surgery, and Figure 3 shows changes in the pulmonary arterial CTC levels.

The median follow-up period here measured 154 days (range, 70 to 284 days). Although this was a short period of follow-up, two patients developed recurrent liver metastases after open liver resection at 3 and 6 months. Both of them received preoperative chemotherapy and had a higher number of before intervention PV CTCs (both had

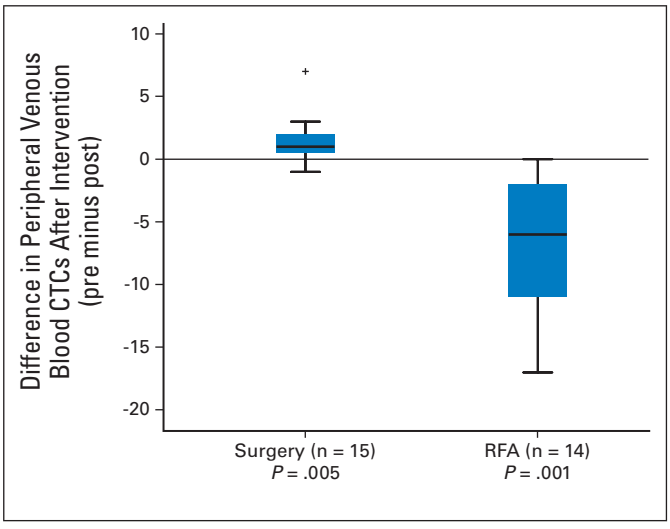

Fig 1. Changes in peripheral venous blood (antecubital fossa cannulation) before and after surgery (open or laprascopic) or radiofrequency ablation (RFA), open or percutaneous. Box and Whisker plots are displayed, showing the minimum, first quartile, median, third quartile, and maximum (+ = outlier). CTC, circulating tumor cell.

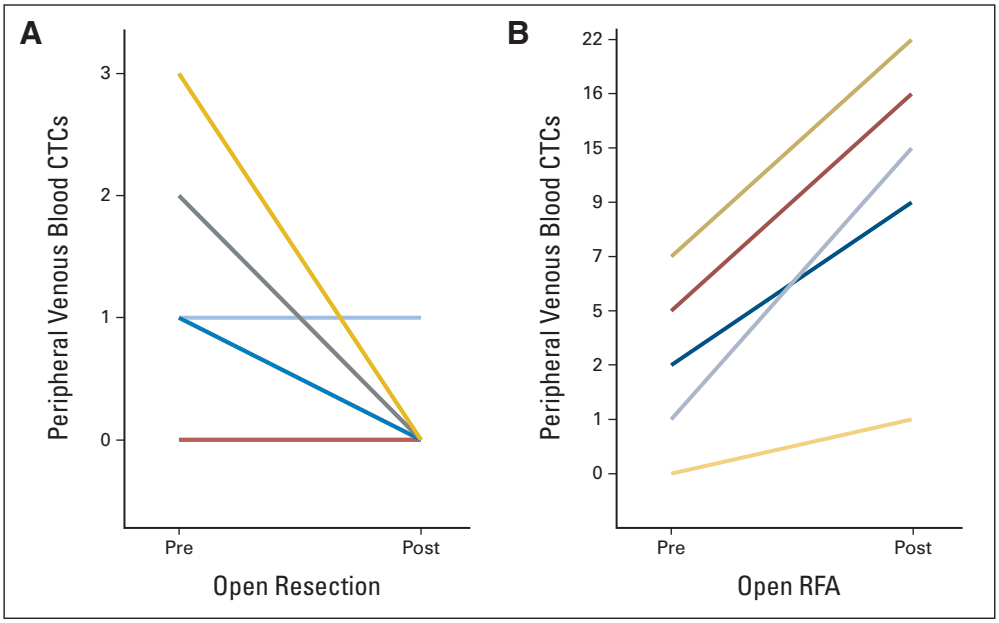

Fig 2. Individual patient changes in peripheral venous blood (antecubital fossa cannulation) before and after (A) open surgery or (B) open radiofrequency ablation (RFA). Each line represents one or more patients. CTC, circulating tumor cell.

three CTCs) before intervention compared with those without recurrence, suggesting that CTCs may be used as a surrogate maker for determining who should have postoperative chemotherapy to prevent recurrence. Overall, however, this period of follow-up is too short to draw conclusions regarding prognosis in these patients.

CTCs throughout the vasculature were stained for EGFR as described previously.[13] Within all of the individuals, when CTCs were obtained from different sites before or after procedures, the percent that stained positive for the EGFR was consistent, indicating that CTCs were homogenous for positive expression of EGFR, within an individual patient. In six individuals undergoing open liver resections in whom we measured two samples from the same site (HV or PoV), results showed no differences between samples indicating the reproducibility of these data.

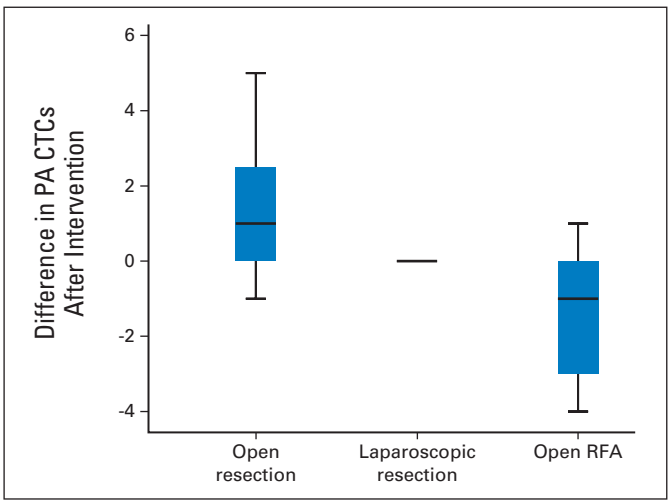

Fig 3. Differences in pulmonary arterial (PA) circulating tumor cells (CTCs) after intervention. Box and Whisker plots are displayed, showing the minimum, first quartile, median, third quartile, and maximum. RFA, radiofrequency ablation.

DISCUSSION

To our knowledge, we show for the first time the contrasting impacts of procedures on CTCs immediately measured at different sites in the vasculature. In this study of a consecutive series of patients with colorectal liver metastases, the impact of intervention using either liver resection or RFA was examined by measuring the number of CTCs in both the systemic and portosystemic circulations, using an automated approach. While the measurement of CTCs historically has been controversial with issues regarding reliability and reproducibility,[8] the system described is now approved by the US Food and Drug Administration for use in patients with metastatic colon, breast, and prostate cancer. The results herein demonstrate that surgical resection immediately reduced the number of CTCs throughout the circulation compared with RFA, which was associated with an increased number of CTCs (Tables 2 and 3 and Fig 1).

To our knowledge, this is the first study which has examined the differential localization of CTCs in the vasculature, by measuring them at different sites in both the systemic circulation as measured with peripheral venous and arterial blood samples, and portosystemic circulation measured with portal and hepatic venous blood samples. Some of these procedures were difficult to undertake: the hepatic vein is friable and obtaining blood samples directly is difficult requiring extensive mobilization of both the liver and vein. These data however show that CTCs are localized to the hepatic macrocirculation, while the lungs appear to sieve CTCs and significantly fewer enter the peripheral circulation. While these effects may be dilutional in their origin and reflect a concentration gradient close to the main cancer site, this provides insights into the clinical picture observed in cancer, in which metastases in the limbs are seldom seen, compared to liver and lung secondary cancers. Although the precise fate of these CTCs remains unknown, it is clear from these data that they inevitably played a role in cancer spread.

This nonrandomized study recruited all consecutive patients and assigned therapeutic options on the basis of individual clinical considerations, thus minimizing the potential for any bias. However, as with any nonrandomized study, selection or ascertainment biases may lead to erroneously attributed observations, and so our results should therefore be treated with appropriate caution. In addition, the number of patients recruited here are small and we thus do not wish to draw conclusions regarding prognosis, an aim of future larger trials; during this study, it became apparent that the procedures were often technically difficult to perform and thus we did not increase recruitment beyond 29 patients. Surgical excision remains the only potentially curative therapy for hepatic malignancies[16] and recent data suggest that the open approach is superior to RFA in terms of overall survival.[25,26] We do not know it the increase in CTCs observed here after RFA is more likely to lead to metastases, nor do we know the mechanism for this increase although it is tempting to suggest that RFA leads to some live tumor dissolution. Importantly, it is unknown whether this post-RFA increase in CTC contributes to the increased local recurrence rates observed by others.[25-28]

Despite years of research and hundreds of reports on tumor markers in oncology, the number of biomarkers that have emerged as clinically useful is very small with initial promise replaced by inconsistent data.[29,30] The development of guidelines for the reporting of tumor marker studies will encourage transparent and complete reporting so that relevant information will be broadly available to others and conclusions can be objectively ascertained. We have attempted to biologically characterize CTCs here by measuring their gene expression but the amount of RNA obtained was insufficient for RT-PCR or expression microarray analyses. Further research into the molecular biology of CTCs, and importantly establishing whether these cells are dead or alive, will increase our understanding of their role in tumor spread, and improved methods to eradicate this cancer cell reservoir, including the potential mechanism of CTC removal by the liver. Ongoing prospective clinical studies will also address whether changes in CTC levels predict real time changes in disease status. Based on these data this appears likely to be the case, and the utility of their measurement in the pre- and postsurgical settings requires further investigation.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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REFERENCES

1. Cristofanilli M, Budd GT, Ellis MJ, et al: Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 351:781-791, 2004
2. Cristofanilli M, Hayes DF, Budd GT, et al: Circulating tumor cells: A novel prognostic factor for newly diagnosed metastatic breast cancer. J Clin Oncol 23:1420-1430, 2005
3. Budd GT, Cristofanilli M, Ellis MJ, et al: Circulating tumor cells versus imaging–predicting overall survival in metastatic breast cancer. Clin Cancer Res 12:6403-6409, 2006
4. Stathopoulou A, Vlachonikolis I, Mavroudis D, et al: Molecular detection of cytokeratin-19-positive cells in the peripheral blood of patients with operable breast cancer: Evaluation of their prognostic significance. J Clin Oncol 20:3404-3412, 2002
5. Benoy IH, Elst H, Philips M, et al: Real-time RT-PCR detection of disseminated tumour cells in bone marrow has superior prognostic significance in comparison with circulating tumour cells in patients with breast cancer. Br J Cancer 94:672-680, 2006
6. Gervasoni A, Monasterio Munoz RM, Wengler GS, et al: Molecular signature detection of circulating tumor cells using a panel of selected genes. Cancer Lett 263:267-279, 2008
7. Smith BM, Slade MJ, English J, et al: Response of circulating tumor cells to systemic therapy in patients with metastatic breast cancer: Comparison of quantitative polymerase chain reaction and immunocytochemical techniques. J Clin Oncol 18:1432-1439, 2000
8. Ring AE, Zabaglo L, Ormerod MG, et al: Detection of circulating epithelial cells in the blood of patients with breast cancer: Comparison of three techniques. Br J Cancer 92:906-912, 2005
9. Pachmann K, Clement JH, Schneider CP, et al: Standardized quantification of circulating peripheral tumor cells from lung and breast cancer. Clin Chem Lab Med 43:617-627, 2005
10. Pachmann K, Camara O, Kavallaris A, et al: Monitoring the response of circulating epithelial tumor cells to adjuvant chemotherapy in breast cancer allows detection of patients at risk of early relapse. J Clin Oncol 26:1208-1215, 2008
11. Apostopolous C, Giamas G, Stebbing J: Clinical significance of circulating tumour cells. Biomarkers 1:9-11, 2007
12. Rack BK, Schindlbeck A, Schneeweiss J, et al: Prognostic relevance of circulating tumor cells (CTCs) in peripheral blood of breast cancer patients before and after adjuvant chemotherapy: The German SUCCESS trial. J Clin Oncol 26:7s, 2008 (abstr 503)
13. Payne R, Yague E, Apostolopolous A, et al: Measurements of EGFR expression on circulating tumour cells are reproducible over time in metastatic breast cancer patients. Pharmacogenomics 10:51-57, 2009
14. Jiao L, Apostolopolous C, Stebbing J: Detection of minimal residual disease and follow-up: Circulating tumour cells as tumour markers. Advances in Breast Cancer. September 2008:59-61
15. Slade MJ, Payne R, Riethdorf S, et al: Comparison of bone marrow, disseminated tumour cells and blood-circulating tumour cells in breast cancer patients after primary treatment. Br J Cancer 100: 160-166, 2009
16. Windsor AC, Cohen R, Jiao LR, et al: Cetuximab in the first-line therapy of metastatic colorectal carcinoma: Not so CRYSTAL clear. Future Oncol 4:741-744, 2008
17. Meyerhardt JA, Mayer RJ: Systemic therapy for colorectal cancer. N Engl J Med 352:476-487, 2005
18. Gutt CN, Riemer V, Kim ZG, et al: Impact of laparoscopic colonic resection on tumour growth and spread in an experimental model. Br J Surg 86:1180-1184, 1999
19. Gutt CN, Riemer V, Kim ZG, et al: Impact of laparoscopic surgery on experimental hepatic metastases. Br J Surg 88:371-375, 2001
20. Schmidt T, Koch M, Antolovic D, et al: Influence of two different resection techniques (conventional liver resection versus anterior approach) of liver metastases from colorectal cancer on hematogenous tumor cell dissemination: Prospective randomized multicenter trial. BMC Surg 8:6, 2008
21. Lee WS, Yun SH, Chun HK, et al: Clinical outcomes of hepatic resection and radiofrequency ablation in patients with solitary colorectal liver metastasis. J Clin Gastroenterol 42:945-949, 2008

22. Hur H, Ko YT, Min BS, et al: Comparative study of resection and radiofrequency ablation in the treatment of solitary colorectal liver metastases. Am J Surg 197:728-736, 2008

23. Nikfarjam M, Shereef S, Kimchi ET, et al: Survival outcomes of patients with colorectal liver metastases following hepatic resection or ablation in the era of effective chemotherapy. Ann Surg Oncol 16:1860-1867, 2008

24. Reuter NP, Woodall CE, Scoggins CR, et al: Radiofrequency ablation vs. resection for hepatic colorectal metastasis: Therapeutically equivalent? J Gastrointest Surg 13:486-491, 2008

25. Gleisner AL, Choti MA, Assumpcao L, et al: Colorectal liver metastases: Recurrence and survival following hepatic resection, radiofrequency ablation, and combined resection-radiofrequency ablation. Arch Surg 143:1204-1212, 2008

26. Eisele RM, Neumann U, Neuhaus P, et al: Open surgical is superior to percutaneous access for radiofrequency ablation of hepatic metastases. World J Surg 33:804-811, 2009

27. Hong SN, Lee SY, Choi MS, et al: Comparing the outcomes of radiofrequency ablation and surgery in patients with a single small hepatocellular carcinoma and well-preserved hepatic function. J Clin Gastroenterol 39:247-252, 2005

28. Molinari M, Helton S: Hepatic resection versus radiofrequency ablation for hepatocellular carcinoma in cirrhotic individuals not candidates for liver transplantation: A Markov model decision analysis. Am J Surg 198:396-406, 2009

29. McShane LM, Altman DG, Sauerbrei W, et al: Reporting recommendations for tumor MARKer prognostic studies (REMARK). Nat Clin Pract Urol 2:416-422, 2005

30. McShane LM, Altman DG, Sauerbrei W, et al: REporting recommendations for tumor MARKer prognostic studies (REMARK). Breast Cancer Res Treat 100:229-235, 2006

