



HAL
open science

Modeling the relationship between circulating tumour cells number and prognosis of metastatic breast cancer

Edoardo Botteri, Maria Teresa Sandri, Vincenzo Bagnardi, Elisabetta Munzone, Laura Zorzino, Nicole Rotmensz, Chiara Casadio, Maria Cristina Cassatella, Angela Esposito, Giuseppe Curigliano, et al.

► To cite this version:

Edoardo Botteri, Maria Teresa Sandri, Vincenzo Bagnardi, Elisabetta Munzone, Laura Zorzino, et al.. Modeling the relationship between circulating tumour cells number and prognosis of metastatic breast cancer. *Breast Cancer Research and Treatment*, 2009, 122 (1), pp.211-217. 10.1007/s10549-009-0668-7 . hal-00535421

HAL Id: hal-00535421

<https://hal.science/hal-00535421>

Submitted on 11 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Modeling the relationship between circulating tumour cells number and prognosis of metastatic breast cancer

Edoardo Botteri · Maria Teresa Sandri · Vincenzo Bagnardi · Elisabetta Munzone · Laura Zorzino · Nicole Rotmensz · Chiara Casadio · Maria Cristina Cassatella · Angela Esposito · Giuseppe Curigliano · Michela Salvatici · Elena Verri · Laura Adamoli · Aron Goldhirsch · Franco Nolè

Received: 1 August 2009 / Accepted: 24 November 2009 / Published online: 6 December 2009
© Springer Science+Business Media, LLC. 2010

Abstract Circulating tumor cell (CTC) count has been shown to be an independent predictor of progression in metastatic breast, prostate, and colorectal cancer. A cutpoint is generally used to identify favorable and unfavorable response groups. In this study, we propose an approach in which the number of CTCs is analyzed as a continuous predictor, to detect the shape of the relationship between CTCs and prognosis of metastatic breast cancer. We evaluated the association of baseline CTC with progression-free survival (PFS) and overall survival (OS) in a series of 80 patients treated for advanced breast cancer at the European Institute of Oncology, Milan. The association between CTCs and prognosis was analyzed with standard categorical survival analysis and spline regression models. At baseline,

median age was 55 years; 33 patients were newly diagnosed with metastatic breast cancer (41%), while 28 (35%) and 19 (24%) were pretreated with one and two previous chemotherapy lines, respectively. After a median follow-up of 28 months, 76 disease progressions and 44 deaths were observed. Kaplan–Meier curves showed a clear association between CTCs and PFS (P -value 0.03) and OS (P -value < 0.01). Patients with no CTC at baseline had a significantly better prognosis. When analyzing the CTCs as a continuous variable, we found an increase in risk with increasing number of CTCs, for both PFS and OS. The increase rate lessened after approximately 5 CTCs. CTCs represent a robust prognostic factor in the metastatic breast cancer setting. A nonlinear increase in risk of both progression and death with increasing number of CTCs was observed, with a lessening increase after approximately 5 CTCs. If distinct prognostic groups are to be identified, women with no CTC could plausibly represent a distinct favorable one.

An invited commentary to this article can be found at doi:
[10.1007/s10549-010-0762-x](https://doi.org/10.1007/s10549-010-0762-x).

E. Botteri · V. Bagnardi · N. Rotmensz
Division of Epidemiology and Biostatistics, European Institute of Oncology, Via Ripamonti 435, 20141 Milan, Italy

M. T. Sandri · L. Zorzino · M. C. Cassatella · M. Salvatici
Unit of Laboratory Medicine, European Institute of Oncology, Milan, Italy

V. Bagnardi
Department of Statistics, University of Milan-Bicocca, Milan, Italy

E. Munzone · A. Esposito · G. Curigliano · E. Verri · L. Adamoli · A. Goldhirsch · F. Nolè (✉)
Division of Medical Oncology, European Institute of Oncology, Milan, Italy
e-mail: franco.nole@ieo.it

C. Casadio
Division of Pathology, European Institute of Oncology, Milan, Italy

Keywords Circulating tumor cells · Metastatic breast cancer · Prognosis

Introduction

Despite the essential contributions of screening programs in early detection of breast cancer and the continuous advances in treatment, 6–10% of women diagnosed with breast cancer present with metastatic disease [1], and others develop metastatic disease in the course of time, with a probability depending on tumor stage, biology, and treatments used. The 5-year overall survival (OS) rate among such patients rarely exceeds 20% [2]. Since no specific predictive factors for treatment response have been

satisfactorily validated for use in a standard clinical setting, patients are usually treated with a set of agents until progression becomes evident.

Circulating tumor cells (CTCs) have been shown to be an independent prognostic factor in metastatic breast cancer (MBC) [3–6], in metastatic prostate cancer [7–10] and in metastatic colorectal cancer [11, 12]. They represent a promising tool for therapeutic monitoring: an elevated number of CTCs measured at any time in the clinical course were associated with a high likelihood of a very short time to progression.

When studying the link between CTC count and evolution of disease, researchers generally use cut-off values to identify favorable and unfavorable response groups. For example, in studies on MBC, a cutpoint of 5 CTCs has been generally adopted [3, 4, 13, 14]. Dichotomization has some perceived advantages, such as simplicity, easy interpretation of the results, and functionality in clinical practice, but also some intrinsic problems, such as loss of information and statistical power, increased probability of false positive results, and impossibility of detecting non-linear relationships between the predictor and the outcome [15–18].

In this study, we will propose an approach in which the number of CTCs is analyzed as a continuous predictor, to detect the shape of the relationship between CTCs and prognosis of MBC patients.

Patients and methods

Details of study design are described elsewhere [4]. In brief, this was a prospective mono-institutional study conducted at the European Institute of Oncology in Milan aimed to evaluate the prognostic significance of CTCs, detection in patients with advanced breast cancer. In particular, the study investigated the association between the number of CTCs at baseline and patients' characteristics, as well as their modification during treatments, in women with histological diagnosis of breast cancer and evidence of metastatic disease, starting a new line of therapy and/or in those in patients already treated for the advanced disease with a maximum of two previous lines of chemotherapy. A baseline blood draw for enumeration of CTCs was performed; subsequently, blood samples were collected after 4 and 8 weeks, at the time of the first clinical evaluation, and every 2 months thereafter—until disease progression was documented. However, only baseline values are used in this analysis. In order to isolate and enumerate the CTCs, the CellSearch™ System (Veridex) was used. It consists of two instruments: (1) the CellTracks AutoPrep System for the isolation and staining of the cells, and (2) CellSpotter Analyzer, for the analysis and enumeration of the CTCs.

Results are expressed as the number of tumor cells/7.5 ml of blood. The conventional threshold of 5 CTCs/7.5 ml was used for the PFS and OS analysis at each of the blood draw time points. We additionally used 20 CTCs/7.5 ml as the second arbitrary cut-off point because it was a round number that guaranteed similar number of patients in all strata.

Statistical methods

Progression-Free Survival (PFS) and OS were defined as the length of time from the baseline blood draw to disease progression and death from breast cancer, respectively. PFS and OS were estimated using the Kaplan–Meier method. In the absence of events, PFS and OS were censored at the last follow-up visit. The log-rank test was used to test differences in PFS and OS between subgroup of patients, according to CTC count distribution. The following four categories were considered: 0, 1–4, 5–20, and >20.

In order to investigate the shape of the relationship between CTC and the hazard of progression, multivariable Cox proportional hazard models were fitted. CTC count was firstly modeled as a categorical variable. Then, restricted cubic spline models were used. Cubic splines are smoothly joined piecewise third-order polynomials [19]. Polynomials are fitted within intervals delimited by knots, and restrictions are placed on the resulting curve to ensure a smooth appearance at the knot points. Three knots were set at the 25th, 50th, and 75th percentile of the CTC distribution, corresponding to 1, 5, and 20 CTCs, respectively. Results were presented in terms of Hazard Ratio (HR), using 0 CTC as reference. Owing to the skewness of the distribution of CTCs, the log-transformed data were used in the analyses and reported on the log scale in the figures. Since the CTC count could be zero, the $\log(\text{CTC} + 1)$ transformation was used.

All analyses were carried out with the SAS software (SAS Institute, Cary, NC) and the R (<http://cran.r-project.org/>) software with the Harrell's Design and Hmisc libraries. All the reported *P*-values were two sided.

Results

A total of 80 patients were enrolled, from March 2005 to May 2006. Median age at diagnosis of primary tumor and at first blood draw was 49 years (range 29–71) and 55 years (range 32–77), respectively. Characteristics of the patients and association with number of CTCs are reported in Table 1. Thirty-three patients were newly diagnosed

Table 1 Characteristics of population and association with CTC

Classification	All no. 80 No. (%)	Number of CTC Median (range)	P-value ^b
Age at diagnosis			
≤35	7 (9)	10 (0–66)	0.57
36–50	39 (49)	6 (0–662)	
51+	34 (42)	7 (0–237)	
Age at first blood draw			
≤35	3 (4)	19 (0–66)	0.84
36–50	29 (36)	6 (0–662)	
51+	48 (60)	6 (0–237)	
Ca 15-3 at first blood draw			
≤31	32 (42)	4 (0–314)	0.08
>31	44 (58)	16 (0–662)	
Stage of tumor ^a			
T1	28 (39)	10 (0–237)	0.13
T2	27 (38)	4 (0–314)	
T3 or T4	17 (24)	16 (0–662)	
Positive lymph nodes ^a			
0	21 (28)	4 (0–166)	0.03
1–3	40 (53)	5 (0–314)	
≥4	14 (19)	17 (1–662)	
Distant Mts at diagnosis			
Positive	18 (23)	6 (0–662)	1.00
Negative	62 (77)	6 (0–314)	
Histotype			
Ductal	58 (75)	7 (0–662)	1.00 ^c
Lobular	6 (8)	6 (0–27)	
Other	13 (17)	2 (0–237)	
ER and PgR receptors			
At least one positive	63 (79)	7 (0–662)	0.43
Both negative	17 (21)	5 (0–32)	
Her2/neu			
Overexpressed	25 (33)	2 (0–68)	0.01
Not overexpressed	51 (67)	9 (0–662)	
Ki-67 (%)			
<20	21 (29)	7 (0–166)	0.17
≥20	52 (71)	5 (0–662)	
Recruitment line at baseline			
0	33 (41)	7 (0–314)	1.00
1	28 (35)	5 (0–343)	
2	19 (24)	7 (0–662)	
No. of metastatic sites at baseline			
1	36 (45)	5 (0–343)	0.06
2+	44 (55)	8 (0–662)	
Type of metastatic sites			
Bone	15 (19)	5 (0–343)	<0.01 ^d
Visceral	16 (20)	4 (0–27)	
Other type	8 (10)	2 (0–9)	

Table 1 continued

Classification	All no. 80 No. (%)	Number of CTC Median (range)	P-value ^b
Bone + Other type	8 (10)	4 (0–27)	
Visceral + Other type	8 (10)	7 (0–166)	
Bone + Visceral	10 (13)	29 (5–237)	
Bone + Visceral + Other type	15 (19)	19 (0–662)	

^a Six patients had clinical stage only

^b Differences between medians are tested using the Kruskal–Wallis Test

^c Ductal versus lobular

^d Bone + Visceral ± Other type *versus* Others

with MBC (41%), while 28 (35%) and 19 (24%) were pretreated with one and two previous chemotherapy lines, respectively. Regarding the final treatment, nine patients received only hormone therapy, 43 patients chemotherapy and 28 patients chemotherapy combined with HER2/Neu directed treatments. Patients who presented with both bone and visceral metastases had a statistically significant higher number of CTCs compared to other patients ($P < 0.01$). The associations between number of metastatic sites and number of CTCs and between Ca 15-3 and number of CTCs were borderline statistically significant ($P = 0.06$ and $P = 0.08$, respectively). With regard to the characteristics of the primary tumor, number of positive lymph nodes was positively associated with the number of CTCs ($P = 0.03$) and Her2/Neu patients had lower CTC values ($P = 0.01$).

Univariate survival analysis is reported in Table 2. After a median follow-up of 28 months (range 1–45), we observed 76 progressions and 44 deaths, all due to breast cancer. Median survival was 33 months from baseline. Number of CTCs at baseline, categorized as 0, 1–4, 5–20, and >20, resulted significantly associated with PFS ($P = 0.03$) and OS ($P < 0.01$). The corresponding Kaplan–Meier curves are shown in Fig. 1a, c, respectively. The 12 women with no CTC showed a longer median progression-free and overall median survival compared to women with ≥1 CTC ($P = 0.07$ and $P < 0.01$, respectively). When comparing 0 CTC to 1–4 CTCs, we found a borderline statistically significant difference for OS ($P = 0.08$), but not for PFS ($P = 0.61$). Stage of primary tumor was associated with OS, even if no interpretable trend emerged. Number of positive lymph nodes, Her2/neu, Ca 15-3, number of metastatic sites at baseline and type of metastatic sites resulted significantly associated with PFS and/or OS and were included in the multivariable survival models.

Table 2 Univariate analysis: clinicopathologic features associated with survival

Classification	No. at risk	Progression free survival		Overall survival	
		Events (median survival ^a)	<i>P</i> -value	Events (median survival ^a)	<i>P</i> -value
All patients	80	76 (6)		44 (33)	
CTCs at baseline					
0	12	12 (11)	0.028	1 (NE)	<0.001
1–4	19	18 (8)	0.010 ^b	7 (45)	<0.001 ^b
5–20	28	26 (5)		20 (27)	
≥21	21	20 (4)		16 (18)	
Ca 15-3 at baseline blood draw					
≤31	32	29 (8)	0.110	16 (35)	0.018
>31	44	43 (6)		26(24)	
Age at baseline blood draw					
≤50 years	32	29 (6)	0.349	17 (34)	0.700
>50 years	48	47 (7)		27 (33)	
Age at BC diagnosis					
≤50 years	46	42 (9)	0.075	24 (34)	0.397
>50 years	34	34 (5)		20 (29)	
Stage of tumor					
T1	28	28 (5)	0.662	22 (22)	0.008
T2	27	27 (8)		9 (45)	
T3 or T4	17	15 (6)		11 (27)	
Positive lymph nodes					
0	21	19 (8)	0.087	8 (35)	<0.001
1–3	40	39 (6)	0.044 ^c	23 (33)	
≥4	14	13 (4)		12 (18)	
Distant Mts at BC diagnosis					
M0	62	60 (6)	0.660	34 (34)	0.531
M1	18	16 (6)		10 (29)	
Histotype					
Ductal	58	56 (6)	0.604	33 (32)	0.741
Lobular	6	6 (9)		3 (34)	
ER and PgR receptors					
Positive	63	60 (8)	0.244	34 (34)	0.507
Negative	17	16 (5)		10 (29)	
Her2/neu					
Not overexpressed	51	49 (5)	0.063	35 (27)	0.011
Overexpressed	25	23 (9)		9 (NE)	
Ki-67 (%)					
<20	21	21 (9)	0.950	12 (35)	0.729
≥20	52	49 (6)		29 (29)	
Recruitment line at baseline					
0	33	31 (9)	0.183	17 (33)	0.614
1	28	26 (8)		15 (34)	
2	19	19 (5)		12 (33)	
Number of metastatic sites					
1	35	33 (10)	0.011	19 (34)	0.098
2+	45	43 (5)		25 (29)	
Type of metastatic sites					
Bone + Visceral ± Other type	25	25 (3)	<0.001	18 (18)	<0.001
Others	55	51 (10)		26 (35)	

NE not evaluable

^a Median survival (in months) is the time at which half of the population has survived

^b Trend test

^c ≥4 vs. <4

In Fig. 1, besides the standard Kaplan–Meier curves, the continuous relationship between CTCs and survival is represented, together with standard multivariable HRs from

Cox regression models (Fig. 1b, d). With regard to PFS, we observed an increasing risk of progression with increasing number of CTCs. The increase rate lessened after

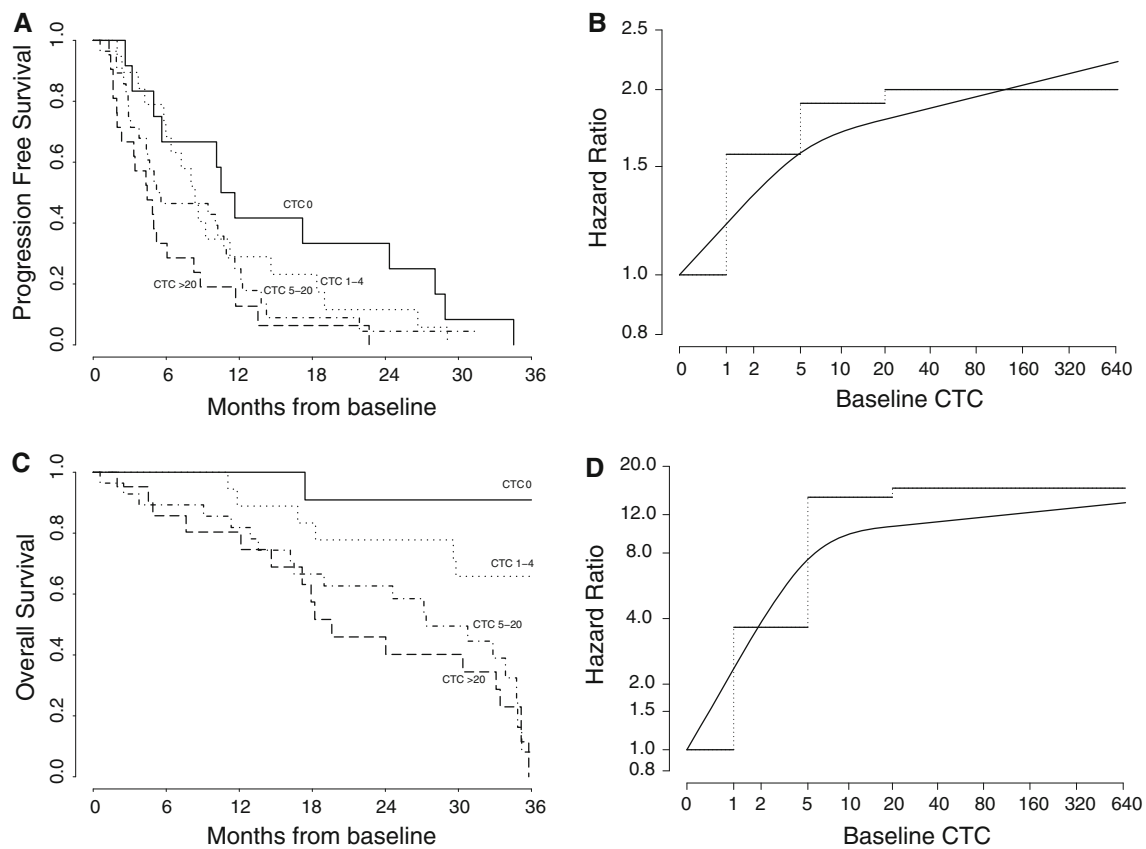


Fig. 1 Analysis of the relationship between CTCs and survival. **a** Kaplan–Meier curves of PFS. **b** The curve represents the continuous relationship between CTCs and PFS, based on a multivariable spline Cox regression model. Horizontal segments represent multivariable hazard ratios of PFS using cut-off values as in **a**. HR were adjusted for Ca 15-3, Her2/Neu, and type of metastatic sites. 0 CTC is the

reference. **c** Kaplan–Meier curves of OS. **d** The curve represents the continuous relationship between CTCs and OS, based on a multivariable spline Cox regression model. Horizontal segments represent multivariable hazard ratios of OS using cut-off values as in **c**. HR were adjusted for Ca 15-3, Her2/Neu, and type of metastatic sites. 0 CTC is the reference

approximately 5 CTCs. Furthermore, using the same cut-points as for the Kaplan–Meier curves, multivariable HRs gave similar information, with a little difference in risk increase between 5–20 CTCs and >20 CTCs. Similar results were observed for OS, with an increasing risk of death with increasing number of CTCs and a lessening increase rate after approximately 5 CTCs, almost vanishing after approximately 15–20 CTCs. However, the magnitude of the HRs for OS was greater than for PFS.

Discussion

In the first report on this series of women, with nine deaths (11%) and a median follow-up of 8 months, we showed that the number of CTCs at baseline and changes in CTC's number at different follow-up points represent a strong independent predictor of PFS, but we could not show a prognostic value of CTCs on OS [4]. This study, with 44

deaths (55%) and a median follow-up of 26 months, reinforces the observations on PFS and showed a strong predictive value of CTCs also on OS.

Assessment of CTC using CellSearch has been cleared by the Food and Drug Administration as a prognostic indicator, in conjunction with other clinical methods, for patients with metastatic breast, prostate, and colorectal cancers. Studies in metastatic breast, colorectal, and prostate cancer showed that patients about to start new lines of chemotherapy could be divided into groups with favourable and unfavourable prognosis on the basis of the number of CTCs measured with the analytically valid CellSearch system (Veridex, Raritan, NJ, USA) [3, 5–13, 20]. When studying the association between the number of CTCs and evolution of the disease, researchers generally use a single cutpoint to identify favorable and unfavorable response groups, such as 5 CTCs for MBC [3, 4, 14], three for metastatic colorectal cancer [11], and five for castration resistant prostate cancer [7]. Dichotomization is very common in clinical studies, since it

satisfies the general need in clinical practice to label individuals as having or not having a characteristic and to decide the treatment strategy. However, converting all patients into two categories has some intrinsic problems, such as loss of information and statistical power, increased probability of false positive results, and impossibility of detecting the shape of the relationship [15–18]. In addition, in the absence of a priori information, “optimal” cutpoints are usually obtained by trying many thresholds and choosing the one which, to some extent, gives the most satisfactory result, for example, choosing the value which minimizes the *P*-value of the Log-rank test [13]. The disadvantages of this approach, related to multiple testing and generalizability of the results, have been abundantly discussed in the literature [15, 16, 21–23]. Alternative strategies to dichotomization, in which continuous predictors are kept continuous and trends are investigated, have been proposed: among these, the most commonly used are fractional polynomials and spline regression models [23–28]. When using the restricted cubic spline regression method [24, 25], we observed a significant nonlinear increase in risk of both progression and death with increasing number of CTCs; this increase rate lessened after approximately 5 CTCs. Moreover, for OS, the increase rate became negligible after approximately 15–20 CTCs. Similar trends were found in previous reports [13, 29]. No exhaustive biological explanation has been proposed so far to explain why this plateau was reached. We think that this phenomenon might be explained through investigation of the underlying nonlinear dynamics [30, 31] of the population of CTCs, and we will focus on this point in further studies.

If researchers are not familiar with curve-fitting models, such as spline regression, then we recommend using two or three cutpoints rather than just one, to categorize individuals in more homogeneous response groups and to observe possible trends. With a categorical analysis, using 1, 5, and 20 as arbitrary cutpoints, we found that the group of women with no CTC detected could plausibly represent a distinct favorable prognostic group, showing a longer median progression-free and overall median survival compared to the other groups. Obviously, a detection of 0 CTC does not imply that there are no CTCs in circulation. For example, as reported by Tibbe et al. [29], when 500 CTCs are in circulation, using the CellSearchTM System and assuming a blood volume of 5 l, the estimated probability of collecting 0 CTC in a 7.5 ml aliquot is 50%, and the estimated probability of detecting 0 CTC is even greater, mainly depending on the ability of the operator. False negative probabilities decrease with increasing number of CTCs in circulation and/or with increasing number of blood samples.

In conclusion, CTCs represent a robust predictive factor in the MBC setting. A nonlinear increase in risk of both

progression and death with increasing number of CTCs was observed, with a lessening increase after approximately 5 CTCs.

References

1. Ries LA, MP Eisner, CL Kosary et al Seer cancer statistics review, 1975-2002. http://seer.cancer.gov/csr/1975_2002/
2. American Cancer Society (2008) Cancer facts and figures, 2008. American Cancer Society, Oakland
3. Cristofanilli M, Hayes DF, Budd GT, Ellis MJ, Stopeck A, Reuben JM, Doyle GV, Matera J, Allard WJ, Miller MC, Fritsche HA, Hortobagyi GN, Terstappen LW (2005) Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. *J Clin Oncol* 23:1420–1430
4. Nole F, Munzone E, Zorzino L, Minchella I, Salvatici M, Botteri E, Medici M, Verri E, Adamoli L, Rotmensz N, Goldhirsch A, Sandri MT (2008) Variation of circulating tumor cell levels during treatment of metastatic breast cancer: prognostic and therapeutic implications. *Ann Oncol* 19:891–897
5. Hayes DF, Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Miller MC, Matera J, Allard WJ, Doyle GV, Terstappen LW (2006) Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. *Clin Cancer Res* 12:4218–4224
6. Budd GT, Cristofanilli M, Ellis MJ, Stopeck A, Borden E, Miller MC, Matera J, Repollet M, Doyle GV, Terstappen LW, Hayes DF (2006) Circulating tumor cells versus imaging—predicting overall survival in metastatic breast cancer. *Clin Cancer Res* 12:6403–6409
7. de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, Doyle GV, Terstappen LW, Pienta KJ, Raghavan D (2008) Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. *Clin Cancer Res* 14:6302–6309
8. Scher HI, Jia X, de Bono JS, Fleisher M, Pienta KJ, Raghavan D, Heller G (2009) Circulating tumour cells as prognostic markers in progressive, castration-resistant prostate cancer: a reanalysis of IMMC38 trial data. *Lancet Oncol* 10:233–239
9. Shaffer DR, Leversha MA, Danila DC, Lin O, Gonzalez-Espinoza R, Gu B, Anand A, Smith K, Maslak P, Doyle GV, Terstappen LW, Lilja H, Heller G, Fleisher M, Scher HI (2007) Circulating tumor cell analysis in patients with progressive castration-resistant prostate cancer. *Clin Cancer Res* 13:2023–2029
10. Danila DC, Heller G, Gignac GA, Gonzalez-Espinoza R, Anand A, Tanaka E, Lilja H, Schwartz L, Larson S, Fleisher M, Scher HI (2007) Circulating tumor cell number and prognosis in progressive castration-resistant prostate cancer. *Clin Cancer Res* 13:7053–7058
11. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Picus J, Morse MA, Mitchell E, Miller MC, Doyle GV, Tissing H, Terstappen LW, Meropol NJ (2009) Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. *Ann Oncol* 20(7):1223–1229
12. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Picus J, Morse M, Mitchell E, Miller MC, Doyle GV, Tissing H, Terstappen LW, Meropol NJ (2008) Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J Clin Oncol* 26:3213–3221
13. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW, Hayes

- DF (2004) Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 351:781–791
14. De Giorgi U, Valero V, Rohren E, Dawood S, Ueno NT, Miller MC, Doyle GV, Jackson S, Andreopoulou E, Handy BC, Reuben JM, Fritsche HA, Macapinlac HA, Hortobagyi GN, Cristofanilli M (2009) Circulating tumor cells and [18F]fluorodeoxyglucose positron emission tomography/computed tomography for outcome prediction in metastatic breast cancer. *J Clin Oncol* 27(20):3303–3311
 15. Royston P, Altman DG, Sauerbrei W (2006) Dichotomizing continuous predictors in multiple regression: a bad idea. *Stat Med* 25:127–141
 16. Altman DG, Lausen B, Sauerbrei W, Schumacher M (1994) Dangers of using optimal cutpoints in the evaluation of prognostic factors. *J Natl Cancer Inst* 86:829–835
 17. Austin PC, Brunner LJ (2004) Inflation of the type I error rate when a continuous confounding variable is categorized in logistic regression analyses. *Stat Med* 23:1159–1178
 18. Faraggi D, Simon R (1996) A simulation study of cross-validation for selecting an optimal cutpoint in univariate survival analysis. *Stat Med* 15:2203–2213
 19. Durrleman S, Simon R (1989) Flexible regression models with cubic splines. *Stat Med* 8:551–561
 20. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, Tibbe AG, Uhr JW, Terstappen LW (2004) Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res* 10:6897–6904
 21. MacCallum RC, Zhang S, Preacher KJ, Rucker DD (2002) On the practice of dichotomization of quantitative variables. *Psychol Methods* 7:19–40
 22. Leeflang MM, Moons KG, Reitsma JB, Zwinderman AH (2008) Bias in sensitivity and specificity caused by data-driven selection of optimal cutoff values: mechanisms, magnitude, and solutions. *Clin Chem* 54:729–737
 23. Greenland S (1995) Avoiding power loss associated with categorization and ordinal scores in dose-response and trend analysis. *Epidemiology* 6:450–454
 24. Royston P (2000) A strategy for modelling the effect of a continuous covariate in medicine and epidemiology. *Stat Med* 19:1831–1847
 25. Greenland S (1995) Dose-response and trend analysis in epidemiology: alternatives to categorical analysis. *Epidemiology* 6:356–365
 26. Royston P, Reitz M, Atzpodien J (2006) An approach to estimating prognosis using fractional polynomials in metastatic renal carcinoma. *Br J Cancer* 94:1785–1788
 27. Sauerbrei W, Royston P, Binder H (2007) Selection of important variables and determination of functional form for continuous predictors in multivariable model building. *Stat Med* 26:5512–5528
 28. Bagnardi V, Zambon A, Quatto P, Corrao G (2004) Flexible meta-regression functions for modeling aggregate dose-response data, with an application to alcohol and mortality. *Am J Epidemiol* 159:1077–1086
 29. Tibbe AG, Miller MC, Terstappen LW (2007) Statistical considerations for enumeration of circulating tumor cells. *Cytometry A* 71:154–162
 30. d’Onofrio A (2009) Fractal growth of tumors and other cellular populations: linking the mechanistic to the phenomenological modeling and vice versa. *Chaos Solitons Fractals* 41:875–880
 31. Wheldon TE (1988) Mathematical model in cancer research. A. Hilger, Bristol