Predictive Biomarkers and Personalized Medicine

Establishment and Validation of Circulating Tumor Cell–Based Prognostic Nomograms in First-Line Metastatic Breast Cancer Patients

Antonio Giordano[1], Brian L. Egleston[3], David Hajage[5], Joseph Bland[3], Gabriel N. Hortobagyi[2], James M. Reuben[1], Jean-Yves Pierga[6], Massimo Cristofanilli[4], and Francois-Clement Bidard[6]

Abstract

Purpose: Circulating tumor cells (CTC) represent a new outcome-associated biomarker independent from known prognostic factors in metastatic breast cancer (MBC). The objective here was to develop and validate nomograms that combined baseline CTC counts and the other prognostic factors to assess the outcome of individual patients starting first-line treatment for MBC.

Experimental Design: We used a training set of 236 patients with MBCs starting a first-line treatment from the M.D. Anderson Cancer Center (Houston, TX) to establish nomograms that calculated the predicted probability of survival at different time points: 1, 2, and 5 years for overall survival (OS) and 6 months and 1 and 2 years for progression-free survival (PFS). The covariates computed in the model were age, disease subtype, visceral metastases, performance status, and CTC counts by CellSearch. Nomograms were independently validated with 210 patients with MBCs from the Institut Curie (Paris, France) who underwent first-line chemotherapy. The discriminatory ability and accuracy of the models were assessed using Harrell c-statistic and calibration plots at different time points in both training and validation datasets.

Results: Median follow-up was of 23 and 29 months in the MD Anderson and Institut Curie cohorts, respectively. Nomograms showed good c-statistics: 0.74 for OS and 0.65 for PFS and discriminated OS prediction at 1, 2, and 5 years, and PFS prediction at 6 months and 1 and 2 years.

Conclusions: Nomograms, which relied on CTC counts as a continuous covariate, easily facilitated the use of a web-based tool for estimating survival, supporting treatment decisions and clinical trial stratification in first-line MBCs. *Clin Cancer Res; 19(6); 1–7. ©2013 AACR.*

Introduction

The 5-year relative overall survival (OS) rate for women diagnosed with metastatic breast cancer (MBC) is low, around 23% (1). Several prognostic clinical and pathologic characteristics have been reported to be independently associated with OS in MBCs (2–4).

In 2004, the enumeration of circulating tumor cells (CTC) by CellSearch in the peripheral blood of patients with MBCs was found to be a strong independent prognostic factor for survival and disease progression (5). These seminal findings were independently confirmed in other cohorts (6–15). In 2011, the IC2006-04 study conducted by the Institut Curie (Paris, France) confirmed the clinical value of CTCs in a prospective, specifically designed, statistically powered cohort of patients treated with first-line chemotherapy (10). These results show that CTCs are a level-of-evidence-1 prognostic marker in MBCs. However, CTC enumeration is not the sole prognostic factor in MBCs; prognostic evaluations of patients with MBCs should also consider the above-mentioned clinical–pathologic characteristics.

The aim of this retrospective study was to combine CTCs and other outcome predictors in a nomogram that would enable clinicians to assess the prognosis of patients starting a first-line chemotherapy for MBCs. For this purpose, we used a training dataset from The University of Texas MD Anderson Cancer Center (Houston, TX) to establish nomograms to calculate the predicted probability of OS and progression-free survival (PFS). The nomograms were validated using an independent dataset from the Institut Curie.

Authors' Affiliations: Departments of [1]Hematopathology and [2]Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas; [3]Biostatistics and Bioinformatics Facility, [4]Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania; and Departments of [5]Biostatistics and [6]Medical Oncology, Institut Curie, Paris, France

Corresponding Author: François-Clement Bidard, Department of Medical Oncology, Institut Curie, 26 rue d'Ulm, Paris 75005, France. Phone: 33-144324672; Fax: 33-153104041; E-mail: fcbidard@curie.fr

Translational Relevance

In the past 50 years, several prognostic clinical and pathologic characteristics have been reported to be independently associated with overall survival and different prognostic indexes that combine these parameters have been proposed to predict outcomes of patients with metastatic breast cancer. Over the past 8 years, we have shown the independent prognostic value of circulating tumor cells (CTC) in patients with advanced breast cancer. These data have been confirmed by independent groups and strengthened the evidence for the clinical value of measuring CTCs as standard of care for these patients. In this study, we combined CTCs and other outcome predictors in a nomogram that would empower clinicians to assess the prognosis of patients starting first-line chemotherapy for metastatic breast cancer. The dedicated web interface (CTConline) is an easy-to-use open web platform that clinicians, researchers, and patients can use to predict overall survival and progression-free survival for patients with metastatic breast cancer receiving first-line therapies.

Patients and Methods

The Institutional Review Board at MD Anderson approved the study (DR10-0227) and granted a waiver of informed consent, considering the retrospective nature of the analysis.

Training set

We searched the prospectively maintained laboratory database at MD Anderson to identify all MBC women who had undergone standard baseline CTC evaluations at the start of first-line treatment (with or without targeted therapy) between September 2002 and November 2009. Moreover, to be included in our study, patients were required to have measurable or evaluable disease. Specific details of the MD Anderson patient population have been reported in previous articles (13–15).

Validation set

The validation set was obtained from the prospective IC 2006-04 study (NCT00898014) conducted from June 2007 to September 2009 in France. Two hundred sixty-seven patients with MBCs with measurable or evaluable disease, all undergoing chemotherapy-based first-line treatment (with or without targeted therapy), were included; all underwent baseline CTC evaluations using CellSearch. The patient, tumor, and treatment characteristics, together with the main results, were published after a median follow-up of 15 months (10, 16). For the current report, we used an actualized database, with a median follow-up of 29 months.

CTC count

CTCs were isolated and counted from 7.5 mL of peripheral blood, using the U.S. Food and Drug Administration–cleared CellSearch system (Veridex, LLC), as previously reported (17, 18). All CTC assessments were conducted centrally, in the Clinical Laboratory Improvement Amendments–certified MD Anderson laboratory and Institut Curie laboratory, by experienced investigators.

Statistical analysis

OS duration was defined as the time of basal blood draw for CTCs to the date of death. PFS duration was defined as the time of baseline blood draw to documentation of disease progression [according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1]. For PFS and OS, patients were censored at the last follow-up date if they were still progression-free and alive, respectively.

We used Cox proportional hazards regressions analyses and Breslow's estimator of the baseline survivor function to predict OS probabilities at 1, 2, and 5 years and PFS at 6 months and 1 and 2 years, respectively. We used estimated model coefficients to assign points to each characteristic and predictions from the model to map cumulative point totals for each outcome to estimated probabilities. For continuous variables, we used restricted cubic splines with 3 knots at the 10%, 50%, and 90% empirical quantiles, respectively, to model continuous variables (19, 20). On the basis of findings from previous analyses of these 2 databases (10, 13), we included the following covariates in the model, according to a previously reported multivariable analysis conducted for OS in the training set (13): age, disease subtype, visceral metastasis, Eastern Cooperative Oncology Group performance status, and CTCs as a continuous number (15). These covariates were mandatory in this analysis.

The discriminatory abilities of the models were assessed using Harrell c-statistic (19), evaluating the training sample (MD Anderson data) and validation sample (Institut Curie data). The accuracy of the nomogram was assessed using both samples via calibration plots. For the calibration plots, we averaged Cox predictions at 2 years within the quintiles of the ordered predictions. Within each quintile, we also estimated the unadjusted probability of death using Kaplan–Meier survival estimators. We then plotted unadjusted versus model average predictions. We used the model coefficients, as estimated in the training set, for the discrimination and calibration analyses in the test set. All statistical analyses were conducted at Fox Chase Cancer Center (Philadelphia, PA).

Results

Patients

We identified 236 patients in the MD Anderson database who met the study's inclusion criteria and for whom the mandatory covariates were known. Among the 267 patients included in the IC 2006-04 study, mandatory covariates were available in the database for 210 patients. Table 1 shows MD Anderson and Institut Curie patients' pathologic and clinical prognostic factors. Patients in the MD Anderson dataset were younger (median, 49.6 vs. 54.5 years, $P =$

Table 1. Patient and tumor characteristics

Characteristics	MD Anderson N (%)	Institut Curie N (%)	P
Total patients	236 (100)	210 (100)	—
Age, y			
Median	50	54	0.0004
Mean	50	58	
Range	23–82	28–83	
Receptor status			
HR+, HER2 normal	130 (55)	125 (60)	0.629
HER2-amplified	43 (18)	36 (17)	
HR− and HER2 normal	63 (27)	49 (23)	
Visceral metastasis			
No	92 (39)	41 (20)	<0.0001
Yes	144 (61)	169 (80)	
Bone metastasis			
No	97 (41)	95 (45)	0.390
Yes	139 (59)	115 (55)	
No. of metastatic site			
<3	156 (66)	151 (58)	0.096
≥3	81 (34)	108 (42)	
ECOG performance status at CTC test			
0 or 1	225 (95)	180 (86)	<0.0001
>1	11 (5)	30 (14)	
CTCs per 7.5 mL			
0–4	141 (60)	136 (65)	0.739
5–20	39 (16)	31 (15)	
21–40	21 (9)	15 (7)	
>40	35 (15)	28 (13)	

Abbreviation: HR, hormone receptor.

0.0004) and also had a better performance status (95.3% vs. 85.7% with performance status = 0 or 1, $P < 0.0001$), which was consistent with a lower incidence of visceral metastases (61% vs. 80.5%, $P < 0.0001$). Other covariates were well-balanced, including the CTC count distribution. Table 2 shows the first-line therapies; the IC 2006-04 dataset did not include patients treated with endocrine therapy and was

Table 2. Treatments received

Treatment	MD Anderson N (%)	Institut Curie N (%)
Endocrine therapy	47 (20)	0 (0)
Chemotherapy	110 (47)	79 (38)
Chemotherapy + bevacizumab	38 (16)	95 (45)
Chemotherapy + trastuzumab	32 (13)	36 (17)
Chemotherapy + lapatinib	9 (4)	0 (0)

conducted later than the MD Anderson study, with a more frequent use of bevacizumab.

Clinical outcome

After median follow-up periods of 23 and 29 months, 110 (46.6%) and 93 (44.3%) patients had died, respectively, in the MD Anderson and Institut Curie datasets. As shown in Fig. 1A, OS was not different ($P = 0.54$) in the MD Anderson group ($n = 236$) and in the Institut Curie group ($n = 210$). The survival analysis and independent prognostic factors had been previously reported for the training cohort (13) and with a shorter follow-up for the validation cohort (10). In the whole study population, we observed that the CTC count, if considered a continuous variable, was correlated with the HR of death (Fig. 2). We found no significant difference between CTC-related hazard ratio of death between the MD Anderson and Institut Curie cohorts ($P = 0.85$). The CTC count as a continuous variable provided a better estimate of the OS than did the CTC count as a dichotomized variable, using a threshold of 5 CTCs/7.5 mL of blood, with Harell c-statistic of 0.663 and 0.629, respectively (P value for difference = 0.001).

In addition, 191 (80.9%) and 163 (77.6%) patients had experienced progression, respectively, in the MD Anderson and Institut Curie datasets; PFS turned out to be longer in the French cohort, in line with the differences in treatment modalities between the 2 groups (Fig. 1B, $P = 0.03$).

Outcome prediction nomogram

Nomograms were obtained for OS at 1, 2, and 5 years and PFS at 6 months and 1 and 2 years. Figure 3 shows the full nomograms for OS and PFS predictions at different time points. For example, a 45-year-old woman with hormone receptor–positive and HER2-negative breast cancer, presenting with no visceral metastasis, a performance status of 1, and a CTC count of 12 CTCs/7.5 mL, would have total scores of 107 and 90 points, according to the OS and PFS nomograms, respectively. Her probability of survival (i.e., OS) was 90% at 1 year, 71% at 2 years, and 38% at 5 years. Her probabilities of experiencing no disease progression after first-line treatment (i.e., PFS) were 73% at 6 months, 50% at 1 year, and 24% at 2 years. OS and PFS predictions can be calculated online for any patient via the Fox Chase Web site (CTConline; ref. 21).

The discriminatory ability of the model was evaluated in the training (MD Anderson) and validation (Institut Curie) samples. The Harrell c-indexes for OS were 0.737 in the training set and 0.726 in the validation set; for PFS, they were 0.651 in both sets. Not taking into account the CTCs in our models would have led to significantly worse prediction performances for both OS (c-index without CTCs in MD Anderson sample = 0.653, in Institut Curie sample = 0.699; P for CTC effect in model < 0.0001) and PFS (c-index without CTCs in MD Anderson sample = 0.615, in Institut Curie sample = 0.65; P for CTC effect in model < 0.0001).

To assess the nomograms' accuracy, we plotted actual OS and PFS probabilities against the calculated predicted probabilities of recurrence for each patient in the training and
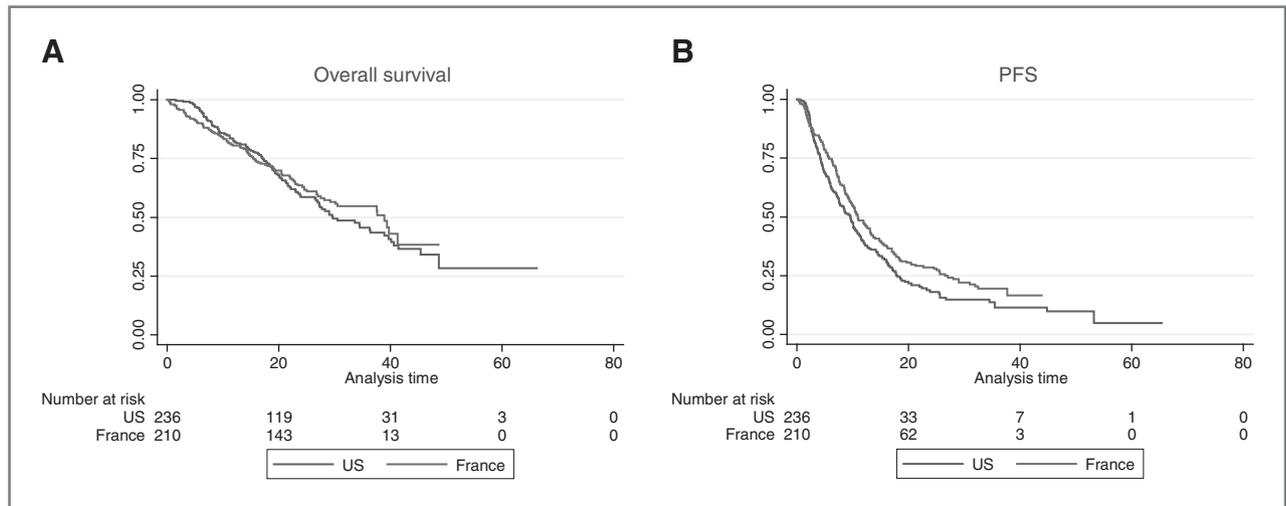
Figure 1. Survival in U.S. and French cohorts. (A) OS and (B) PFS Kaplan–Meier curves for patients included in the nomogram training set (The University of Texas MD Anderson Cancer Center) and validation set (Institut Curie).

validation sets at different time points. Figure 4 shows good OS calibrations at 2 years and PFS at 1 year. Calibrations at all other time points are shown in Supplementary Fig. S1 (online).

Discussion

In this study, we established nomograms to predict clinical outcomes in a large cohort of patients undergoing first-line treatment for MBCs at 2 different institutions. To our knowledge, our model is the first to consider the CTC count as a continuous variable, in combination with other well-established clinical–pathologic characteristics; these variables were derived from a previously reported multi-variable analysis of the training cohort (13). Our use of CTC count as a continuous prognostic factor was justified by our findings that a continuous count leads to a significantly more powerful prediction of OS than a dichotomized count. Moreover, among the other well-established covari-

Figure 2. HRs for OS according to continuous CTC count. This figure was obtained in the whole population ($N = 446$ patients).

ates included in the nomograms, it is interesting that the prognostic value of HER2-positive MBCs was even better than that of hormone receptor–positive cancers; this may be possibly explained by the fact that, at time of MD Anderson and Curie studies, patients with metastatic HER2+ breast cancer received trastuzumab or lapatinib as part of their first-line treatment but were likely to have no prior exposure to these targeted therapies in the adjuvant setting, in contrast to hormone receptor–positive patients with cancer, who have relapsed despite adjuvant hormone therapy.

The validation step revealed that our model had good predictive value for OS, with a Harell c-index of 0.74 in the MD Anderson cohort and of 0.73 in the Institut Curie cohort. Furthermore, nomograms for OS predictions at 1, 2, and 5 years revealed good calibration plots in both cohorts and can be considered validated. In contrast, the model for PFS prediction was less predictive, with a Harell c-index of 0.65 in both cohorts. However, good calibration plots were also obtained at all time points tested for PFS (6 months and 1 and 2 years). The difference between OS and PFS prediction performances can be explained considering that OS globally relies on tumor biology and tumor burden, both of which were taken into account in our model, whereas PFS also depends on numerous predictive factors of response to treatment, which are treatment-specific and were not considered in our model. We further hypothesize that taking into account early CTC changes under treatment would be necessary to get a better estimate of the PFS. Including treatment type (e.g., hormone therapy or bevacizumab) in our model did not further improve its performance (data not shown); therefore, it was not included in the reported nomograms. Of note, the validation cohort received exclusively chemotherapy regimens. Therefore, the nomograms presented here cannot be reasonably applied to patients for whom first-line endocrine therapy appears to be the best treatment choice. However, for those treated with chemotherapy, these nomograms showed high performances.
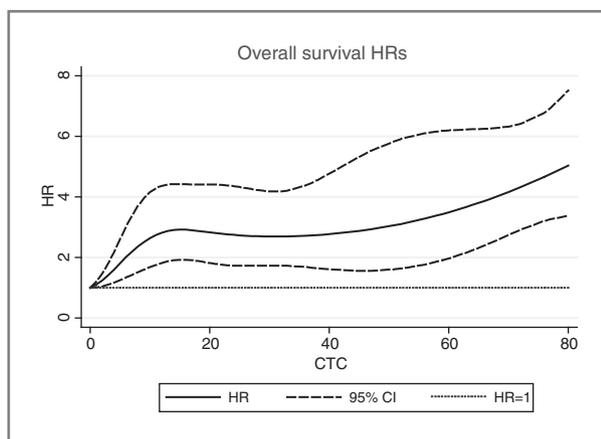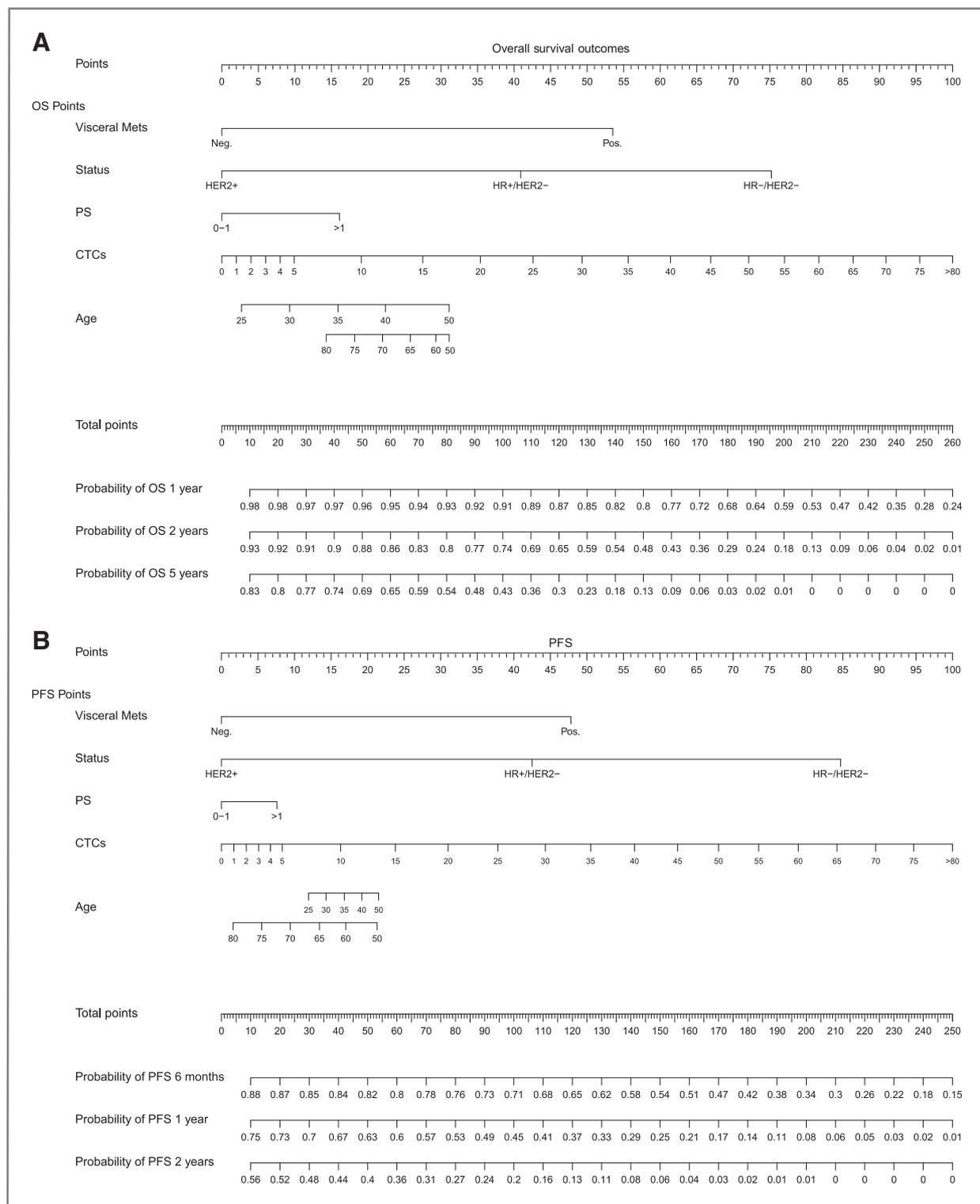
Figure 3. Nomograms for survival prediction. The nomograms allow calculating OS (A) and PFS (B) probabilities at different time points.

The dedicated web interface (CTConline; ref. 21) is an easy-to-use web platform that clinicians, researchers, and patients can use to predict OS and PFS for first-line patients with MBCs, using the validated nomograms obtained in this study. CTCs as a decision tool to choose between hormone therapy or chemotherapy as first-line treatment is currently investigated by the STIC CTC randomized phase III trial (22). Similar trial should be set up to estimate whether these
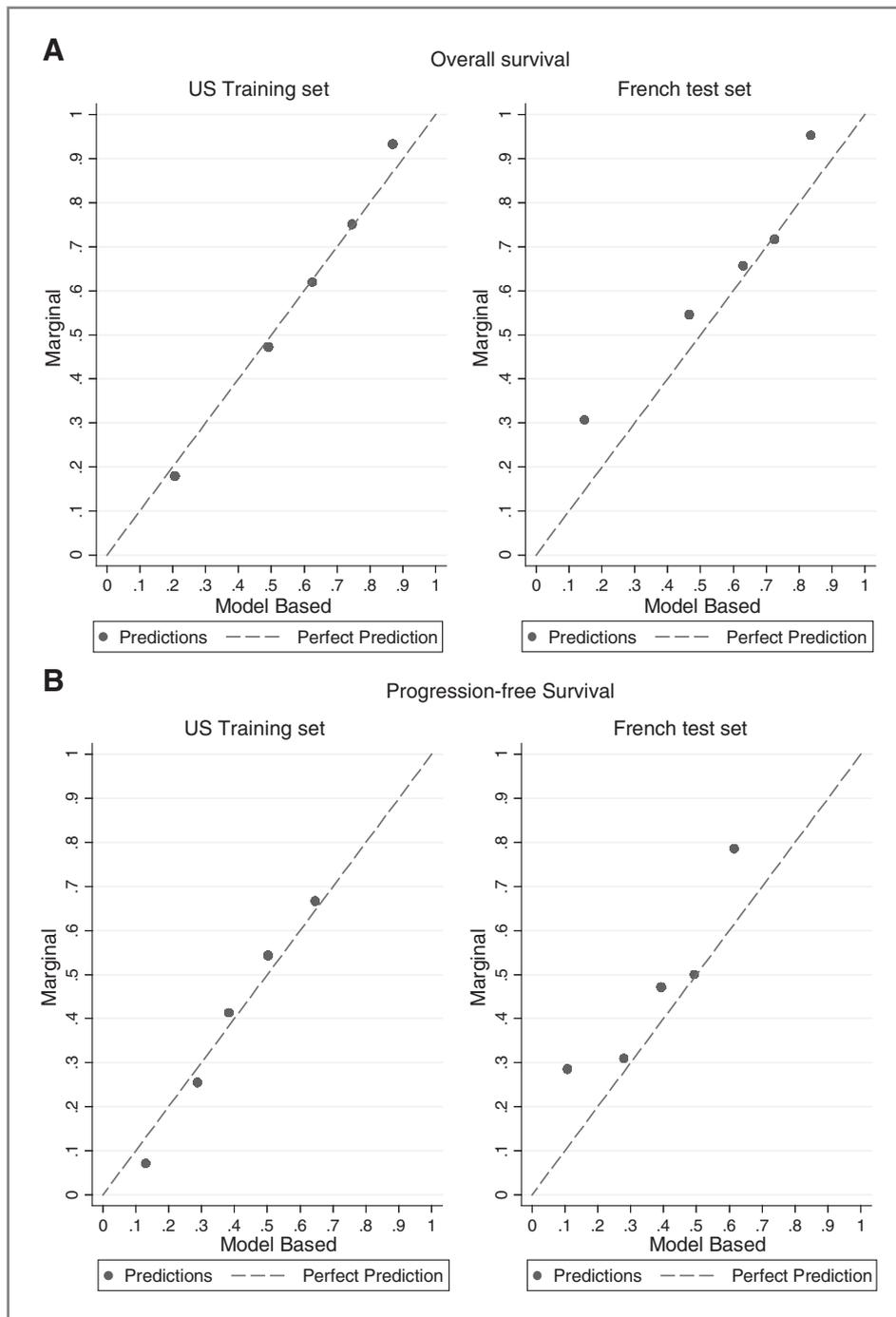
Figure 4. Calibration plots. Calibration plots of (A) OS at 2 years and (B) PFS at 1 year in both cohorts. Cox predictions were averaged at 2 years within the quintiles of the ordered predictions. Within each quintile, the unadjusted probability of death using Kaplan–Meier survival estimators was estimated. We then plotted unadjusted versus model average predictions. The red line corresponds to the perfect prediction.

nomograms help clinician to choose the right treatment intensity, using or not polychemotherapy and/or combined targeted therapy.

Disclosure of Potential Conflicts of Interest

J.-Y. Pierga has a commercial research grant and honoraria from speakers' bureau from Veridex. M. Cristofanilli is a consultant/advisory board member of Alere. F.-C. Bidard has honoraria from speakers' bureau from Veridex. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: A. Giordano, B.L. Egleston, J.M. Reuben, J.-Y. Pierga, M. Cristofanilli, F.-C. Bidard
Development of methodology: A. Giordano, B.L. Egleston, J.M. Reuben, F.-C. Bidard
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Giordano, J.M. Reuben, J.-Y. Pierga, M. Cristofanilli, F.-C. Bidard
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Giordano, B.L. Egleston, D. Hajage, G.N. Hortobagyi, J.M. Reuben, J.-Y. Pierga, M. Cristofanilli, F.-C. Bidard

References

1. American Cancer Society. Breast cancer facts & figures 2011-2012. 2012 [cited 2012 Jan 20]; Available from: URL: www.cancer.gov.
2. Burstein HJ, Harris JR, Morrow M. Malignant tumors of the breast. In: DeVita VT, Lawrence TS, Rosenberg SA, editors. Cancer principles & practice of oncology. 9th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2011. p. 1645–54.
3. Yamamoto N, Watanabe T, Katsumata N, Omuro Y, Ando M, Fukuda H, et al. Construction and validation of a practical prognostic index for patients with metastatic breast cancer. J Clin Oncol 1998;16:2401–8.
4. Puente J, Lopez-Tarruella S, Ruiz A, Lluch A, Pastor M, Alba E, et al. Practical prognostic index for patients with metastatic recurrent breast cancer: retrospective analysis of 2,322 patients from the GEICAM Spanish El Alamo Register. Breast Cancer Res Treat 2010;122:591–600.
5. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004;351:781–91.
6. Cristofanilli M, Hayes DF, Budd GT, Ellis MJ, Stopeck A, Reuben JM, et al. Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. J Clin Oncol 2005;23:1420–30.
7. Nole F, Munzone E, Zorzino L, Minchella I, Salvatici M, Botteri E, et al. Variation of circulating tumor cell levels during treatment of metastatic breast cancer: prognostic and therapeutic implications. Ann Oncol 2008;19:891–7.
8. Maestro LM, Sastre J, Rafael SB, Veganzones SB, Vidaurreta M, Martin M, et al. Circulating tumor cells in solid tumor in metastatic and localized stages. Anticancer Res 2009;29:4839–43.
9. Bidard FC, Mathiot C, Degeorges A, Etienne-Grimaldi MC, Delva R, Pivot X, et al. Clinical value of circulating endothelial cells and circulating tumor cells in metastatic breast cancer patients treated first line with bevacizumab and chemotherapy. Ann Oncol 2010;21:1765–71.
10. Pierga JY, Hajage D, Bachelot T, Delaloge S, Brain E, Campone M, et al. High independent prognostic and predictive value of circulating tumor cells compared with serum tumor markers in a large prospective trial in first-line chemotherapy for metastatic breast cancer patients. Ann Oncol 2012;23:618–24.
11. Liu MC, Shields PG, Warren RD, Cohen P, Wilkinson M, Ottaviano YL, et al. Circulating tumor cells: a useful predictor of treatment efficacy in metastatic breast cancer. J Clin Oncol 2009;27:5153–9.
12. Nakamura S, Yagata H, Ohno S, Yamaguchi H, Iwata H, Tsunoda N, et al. Multi-center study evaluating circulating tumor cells as a surrogate for response to treatment and overall survival in metastatic breast cancer. Breast Cancer 2010;17:199–204.
13. Giordano A, Giuliano M, De LM, Arpino G, Jackson S, Handy BC, et al. Circulating tumor cells in immunohistochemical subtypes of metastatic breast cancer: lack of prediction in HER2-positive disease treated with targeted therapy. Ann Oncol 2012;23:1144–50.
14. Giuliano M, Giordano A, Jackson S, Hess KR, De GU, Mego M, et al. Circulating tumor cells as prognostic and predictive markers in metastatic breast cancer patients receiving first-line systemic treatment. Breast Cancer Res 2011;13:R67.
15. Giordano A, Giuliano M, De LM, Eleuteri A, Iorio F, Tagliaferri R, et al. Artificial neural network analysis of circulating tumor cells in metastatic breast cancer patients. Breast Cancer Res Treat 2011;129:451–8.
16. Bidard FC, Hajage D, Bachelot T, Delaloge S, Brain E, Campone M, et al. Assessment of circulating tumor cells and serum markers for progression-free survival prediction in metastatic breast cancer: a prospective observational study. Breast Cancer Res 2012;14:R29.
17. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res 2004;10:6897–904.
18. Riethdorf S, Fritsche H, Muller V, Rau T, Schindlbeck C, Rack B, et al. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. Clin Cancer Res 2007;13:920–8.
19. Harrell FE Jr. General aspects of fitting regression models. Regression modeling strategies. Springer; 2001. p. 17–60.
20. Kattan MW, Heller G, Brennan MF. A competing-risks nomogram for sarcoma-specific death following local recurrence. Stat Med 2003;22:3515–25.
21. CTConline [cited 2012 Dec 1]. Available from: http://cancernomo-grams.com/CTCOnline.html.
22. Bidard FC, Fehm T, Ignatiadis M, Smerage JB, Alix-Panabieres C, Janni W, et al. Clinical application of circulating tumor cells in breast cancer: overview of the current interventional trials. Cancer Metastasis Rev. 2012 Nov 6. [Epub ahead of print].

Clinical Cancer Research

Establishment and Validation of Circulating Tumor Cell–Based Prognostic Nomograms in First-Line Metastatic Breast Cancer Patients

Antonio Giordano, Brian L. Egleston, David Hajage, et al.

Updated version	Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-12-3137
Supplementary Material	Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2013/01/22/1078-0432.CCR-12-3137.DC1

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/early/2013/02/28/1078-0432.CCR-12-3137. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.