Clinical Investigation

Disseminated Tumor Cells Predict Efficacy of Regional Nodal Irradiation in Early Stage Breast Cancer

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Summary

This long-term analysis (median follow-up, 11.7 years) confirms the predictive impact of disseminated tumor cell (DTC) status on the efficacy of regional radiation therapy for locoregional relapse in early breast cancer. This study also confirms that DTC is an independent prognostic factor for locoregional relapse. These data can be used to

Purpose: Disseminated tumor cells (DTCs) collect in the bone marrow and indicate micrometastatic spread. We previously reported that DTCs could be a predictive factor for the efficacy of regional node irradiation (internal mammary nodes [IMNs]/supra- and infraclavicular nodes [SCNs]). In this article, we report the long-term results (>10 years) on the impact of DTC status in early stage breast cancer.

Methods and Materials: Patients with localized breast cancer were eligible for inclusion in this prospective cohort. DTCs were obtained from a medullary iliac crest sample performed before any primary therapy. DTC status was prospectively assessed by pathologists. Irradiation volumes were defined per standard of care. Cumulative incidence rates and hazard ratios were obtained using both Cox and Fine-Gray models. Interaction tests were performed to confirm the predictive value of DTC status in a multivariate analysis.

Results: Six hundred twenty patients with localized breast cancer were included. Overall, 94 patients (15.2%) were DTC-positive. After a median follow-up of

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conduct a prospective comparative study based on DTC or circulating tumor cells detection to determine whether it could constitute a predictive marker, allowing better selection of patients for regional irradiation.

11.7 years, 47 patients (7.6%) experienced locoregional relapse. DTC detection was associated with a higher risk of locoregional relapse in univariate and multivariate analyses (Cox hazard ratio, 3.26; 95% confidence interval, 1.6-5.7; $P = .001$). In the multivariate subgroup analysis, IMN/SCN irradiation significantly reduced locoregional relapse among DTC-positive patients compared with DTC-negative patients (interaction test: hazard ratio, 0.3; 95% confidence interval, 0.1-0.9; $P = .02$). IMN/SCN was the only irradiation volume with an impact on locoregional relapse in patients according to DTC status, and the predictive value of DTC status for the benefit of locoregional irradiation was independent of locoregional nodal status.

Conclusions: This long-term analysis confirms the predictive impact of DTC status on the efficacy of regional radiation therapy for locoregional relapse in early breast cancer. After further studies, DTC status could be used as a decision tool to better tailor adjuvant radiation therapy in patients with early stage breast cancer. © 2018 Elsevier Inc. All rights reserved.

Introduction

Disseminated tumor cells (DTCs) and circulating tumor cells (CTCs) are isolated cancer cells detected in bone marrow and blood, respectively. In patients with breast cancer without distant metastasis, the DTC detection rate (by immunocytology-based methods) is around 10% to 30%.¹⁻⁴ Although these tumor cells do not systematically develop into distant metastases, their detection has been repeatedly associated with a higher risk of distant relapse and poorer patient outcomes.^{1-3,5-8} To acknowledge this prognostic impact, the 7th TNM classification of breast cancer introduced the cM0(i+) category, defined by the presence of DTCs or CTCs detected in a patient with no overt distant metastasis.⁹

Recent discussion has focused on the benefits of regional nodal irradiation (supra- and infraclavicular nodes [SCNs] and internal mammary node [IMNs]) irradiation in early stage breast cancer. Large-scale phase 3 randomized studies have demonstrated that SCN/IMN irradiation decreased the risk of locoregional and distant relapse. Distant disease-free survival and disease-free survival were also improved in the regional nodal irradiation group, but this irradiation increased the risk of pulmonary and cardiac fibrosis, as well as long-term skin toxicity.^{10,11} More reliable factors for the selection of patients who should receive regional irradiation must be identified, and the decision to perform SCN/IMN radiation remains debated in current practice.

In a first analysis published in 2009, with a short median follow-up of 56 months, we observed that DTC-positive (DTC+) patients were at higher risk of locoregional relapse, confirming that DTC status was an independent prognostic factor in early stage breast cancer.¹² Similar findings have been more recently reported in CTC-positive patients.¹³ More importantly, we observed that DTC+ patients with no SCN/IMN irradiation had a high locoregional relapse rate, whereas DTC+ patients with SCN/IMN irradiation had a low risk of locoregional

relapse, similar to that observed in DTC-negative (DTC-) patients.¹²

In this article, we report the long-term results on the impact of DTC status as a predictive factor for the efficacy of regional nodal irradiation.

Methods and Materials

Study population

This prospective cohort was approved by the regional ethics committee. Eligibility criteria were women with histologically proven nonmetastatic unilateral breast adenocarcinoma (stage I-III; American Joint Committee on Cancer 5th edition breast cancer staging)¹⁴; age >18 years; treatment at Institut Curie (Paris, France); no history of previous malignancy (except in situ carcinoma of the cervix or nonmelanoma skin cancer); and signed informed consent. Patient and tumor characteristics were recorded prospectively in the patients' medical charts, except for HER2 status, which was assessed retrospectively.

Bone marrow status

Bone marrow sampling by anterior iliac crest aspiration for DTC detection was performed during breast surgery or before initiation of neoadjuvant chemotherapy. Samples were processed, and mononuclear cells were collected and stained using the pancytokeratin mAb A45-B/B3 (Micro-met, Munich, Germany; Chromavision, San Juan, CA), as previously described.^{2,15}

DTC status was assessed prospectively by experienced pathologists after immunocytochemical staining. Only cytokeratin-positive cells with atypical cytologic features were classified as DTC+ by experienced pathologists according to the European Working Group for standardization of tumor cell detection.¹⁶ Patients and clinicians both remained blinded to the results of bone marrow analysis.

Treatment regimens and follow-up

Treatment decisions were not based on DTC status. All patients underwent breast surgery (either breast-conserving surgery or mastectomy); axillary dissection was performed in the presence of lymph node involvement or positive sentinel lymph node biopsy. Patients at high risk of relapse received anthracycline- and docetaxel-based neoadjuvant and/or adjuvant chemotherapy, according to institutional guidelines. Hormone therapy was delivered to patients with estrogen receptor–positive tumors.

Radiation therapy was performed after surgery or adjuvant chemotherapy to a total dose of 50 Gy in 25 fractions to the whole breast or chest wall, with or without lymph nodes. A boost dose of 16 Gy could be delivered to tumor bed in women under the age of 60 years or with grade 3 tumor, high proliferation index, or lymphovascular invasion (LVI).

SCN/IMN irradiation was indicated in the case of lymph node involvement or, for internal or central tumors, when at least 2 of the following factors were present: age <40 years, presence of LVI, or tumor size >2 cm. The low axilla (level I) was irradiated only in the presence of massive lymph node involvement (>50% after axillary lymph node dissection). The IMN region was treated by a mixed photon–electron technique. Whole breast or chest wall and SCN/axillary regions were irradiated, using previously described techniques set up at Institut Curie.¹⁷⁻¹⁹

Follow-up was performed at Institut Curie for the first 5 years after treatment and included clinical examination every 6 months and annual bilateral mammography. Breast cancer relapses were prospectively recorded. Annual follow-up was ensured after the first 5 years.

Statistical analysis

The first objective of this prospective study was to assess the impact of DTC detection on disease-free survival; the impact on locoregional relapse–free interval (LRFI) was an exploratory endpoint with no prespecified statistical power. Locoregional relapses were defined as documented invasive breast cancer recurrence in ipsilateral breast, chest wall, or regional lymph nodes (axillary nodes, SCNs, or IMNs) before any distant metastatic relapse. Overall survival (OS) was defined as the time from surgery to death from any cause. Breast cancer–specific survival (BCSS) was calculated by only taking into account deaths from breast cancer (patients who died from other causes were censored at the date of death). LRFI was defined as the time interval between surgery and any locoregional relapse; patients experiencing distant metastatic relapse were censored at the time of metastatic relapse. Patients still alive with no breast cancer relapse were censored at the date of last news.

Quantitative data were expressed as the mean and range, and qualitative data were expressed as numbers and proportions; missing data were not taken into account. LRFI,

OS, and BCSS were estimated according to the Kaplan-Meier method and compared using the log-rank test. Variables significant at the 10% level on univariate analysis were included in a multivariate Cox proportional hazard model to determine the clinical variables associated with OS and BCSS.

Because death is a competing risk for locoregional relapse, assessment of the risk of locoregional relapse based on survival analysis was performed according to methodologic recommendations for competing risks frameworks, using cumulative incidence functions, models for cause-specific hazards ratio, and subdistribution hazards ratio.²⁰ A Cox model was used for regression on cause-specific hazards ratios, whereas a Fine-Gray model was used for regression on sub-distribution hazards ratios.^{21,22} The cumulative incidence of LR was estimated and displayed in 2 different ways: by using the complement of the Kaplan-Meier product estimator (1-KM) and by using the Fine-Gray method for competing risks. All statistical analyses were performed with R software (version 3.22; <https://cran.r-project.org/>). The limit of significance was defined as $P < .05$.

Results

Six hundred twenty women with early stage invasive breast cancer were included in this cohort. In this series, 93% of patients ($n = 575$) received adjuvant radiation therapy (Table 1), 65% received SCN/IMN irradiation, and 21% received axillary irradiation. An additional boost to the tumor bed was delivered to 280 patients (45%). Pathologic positive lymph nodes (pN+) were observed in 323 patients (52%); as expected, positive lymph node status influenced the SCN/IMN irradiation rate: 41% and 87% of pN0 and pN+ patients received SCN/IMN irradiation, respectively ($P < .001$). Likewise, adjuvant chemotherapy was administered to 26% and 82% of pN0 and pN+ patients, respectively ($P < .001$). The DTC detection rate was 15.2% ($n = 94$), and no significant correlation was observed among patient characteristics, treatments, and DTC status (Table 1). No correlation was observed between irradiation volumes and DTC status (Table 2).

With a median follow-up of 11.7 years (range, 0.1-16.7), 47 patients (7.6%) experienced locoregional relapse as first event. Most relapses occurred in the breast or chest wall (72.3% of locoregional relapses). Details on the site of relapse are shown in Table E1 (available online at <https://doi.org/10.1016/j.ijrobp.2018.09.033>). Long-term analysis confirmed the role of DTC status as an independent prognostic factor for locoregional relapse. LRFI was significantly shorter in DTC+ patients compared with DTC– patients in both univariate (hazard ratio [HR], 2.8; 95% confidence interval [CI], 1.5-5.3; $P = .001$) and multivariate analyses (HR, 3.26; 95% CI, 1.6-5.7; $P = .001$) (Table 3). The 10-year locoregional relapse was 10% in DTC– patients versus 20% in DTC+ patients (Fig. 1).

Table 1 Patient characteristics, treatments, and DTC detection rate

Patient characteristics	No. of patients	DTC detection rate	P value
Age (y)			.5
<45	103	17.5%	
≥45	517	14.7%	
cT			.2
1	325	17.5%	
2	172	12.2%	
3/4	123	13%	
pN			.8
Negative	295	15.6%	
Positive	323	14.9%	
Histology			.3
Ductal	513	14.5%	
Lobular	75	16%	
Tumor grade			.3
1	203	14.8%	
2	235	14.9%	
3	163	15.3%	
HR status			.4
Positive	496	14.5%	
Negative	115	17.4%	
LVI			.3
No	411	13.6%	
Yes	189	18%	
Surgery			.6
Lumpectomy	448	15%	
Mastectomy	165	15.2%	
RT (all modalities)			.3
Yes	575	14.7%	
No	42	20.9%	
Breast/chest wall RT			.5
Yes	572	14.8%	
No	45	20.9%	
Boost RT			1
Yes	280	15.2%	
No	337	15.1%	
SCN/IMN RT			1
Yes	375	15.1%	
No	242	15.2%	
Axillary RT			.8
Yes	119	15.1%	
No	498	15.2%	
Chemotherapy			.7
Yes	346	15.7%	
No	273	14.5%	
Hormone therapy			1
Yes	401	14.9%	
No	219	16.2%	

Abbreviations: DTC = disseminated tumor cell; HR = hormone receptors; LVI = lymphovascular invasion; RT = radiation therapy; SCN/IMN = supra- and infraclavicular and internal mammary nodes.

We then examined the interaction between SCN/IMN irradiation and DTC status in terms of LRFI. We found that SCN/IMN irradiation was associated with significant risk reduction in DTC+ patients (estimated by the Fine and

Gray Model: HR, 0.29; 95% CI, 0.1-0.9; $P = .02$), but had no obvious effect in DTC− patients (Fig. 2). Similar results were observed when using the complement of the Kaplan-Meier estimator (ie, without considering death relapses as competitive events) and a Cox model. Interestingly, analysis limited to the subgroup of pN0 patients demonstrated a similar amplitude of risk reduction (Fine and Gray model: HR, 0.14; 95% CI, 0.02-1.18; $P = .07$; Fig. E1A, available online at <https://doi.org/10.1016/j.ijrobp.2018.09.033>). In contrast, for pN+ patients, DTC status had no significant impact on SCN/IMN irradiation efficiency (HR, 0.48; 95% CI, 0.09-2.45; $P = .38$; Fig. E1B, available online at <https://doi.org/10.1016/j.ijrobp.2018.09.033>). This effect was not observed with any of the other radiation fields or any of the other treatment modalities (chemotherapy, hormone therapy) (Table E2; available online at <https://doi.org/10.1016/j.ijrobp.2018.09.033>).

In terms of OS and BCSS, 152 patients (24.5%) had died at the time of analysis, and 121 of these deaths (79.6%) were from breast cancer. Univariate analysis showed that DTC status was a prognostic factor for BCSS ($P = .04$; Fig. E2, available online at <https://doi.org/10.1016/j.ijrobp.2018.09.033>), but not for OS ($P = .09$; Fig. E3, available online at <https://doi.org/10.1016/j.ijrobp.2018.09.033>). On multivariate analysis, DTC positivity at baseline was associated with significantly shorter BCSS (HR, 1.9; 95% CI, 1.2-3.0; $P = .01$), together with large tumor size ($P < .001$), grade 2/3 ($P < .001$), negative hormone receptor status ($P < .001$) and axillary lymph node involvement ($P < .001$; Table 4).

Discussion

To our knowledge, no biologic or genomic criteria are available to predict the efficacy of irradiation fields in early breast cancer. In this cohort, the DTC detection rate was in the range of that in other recent reports.²³ We have previously reported that DTC+ patients derived benefit from regional irradiation, with a short median follow-up of 56 months ($P = .028$).¹² The present study confirms these results, with a median long-term follow-up of 11.7 years. We also report a nonstatistically significant trend toward a predictive impact of DTC status in pN0 patients. This study also confirms that DTC is an independent prognostic factor for locoregional relapse. Hartkopf et al recently reported an increased risk of locoregional relapse in DTC+ patients (multivariate analysis: HR, 1.65; 95% CI, 1.13-2.40; $P = .009$) in a series of 3072 patients with a median follow-up of 44 months.²⁴ The prognostic impact of DTC on locoregional relapse observed in our study remained significant in the long term and is consistent with the literature.

A potential limitation of our findings concerns the heterogeneous treatments used during cohort inclusion, particularly in relation to anti-HER2 therapies, considering most patients were treated in the pre-trastuzumab era.

Table 2 Treatments according to pN status and DTC status

	pN0 (n = 295)	pN+ (n = 323)	P value	DTC- (n = 526)	DTC+ (n = 94)	P value
Surgery						
Lumpectomy	252 (85.4%)	195 (60.4%)	.006	381 (72.4%)	67 (71.3%)	1
Mastectomy	41 (13.9%)	123 (38.1%)	<.001	141 (26.8%)	25 (26.6%)	1
RT (all modalities)	272 (92.2%)	304 (94.1%)	.8	492 (93.5%)	85 (90.4%)	.9
Breast/chest wall RT	271 (99.6%)	300 (93.9%)	.9	487 (92.6%)	85 (100%)	.9
Boost RT	146 (53.7%)	134 (41.5%)	.3	237 (45.1%)	43 (50.6%)	.9
SCN/IMN RT	112 (41.2%)	262 (81.1%)	<.001	318 (60.5%)	57 (67.1%)	1
Axillary RT	9 (3.3%)	109 (35.9%)	<.001	101 (20.5)	18 (21.2%)	1
Chemotherapy	79 (26.8%)	266 (87.2%)	<.001	293 (55.7%)	54 (63.5%)	.9
Hormone therapy	171 (58.0%)	230 (71.2%)	.1	343 (65.2%)	60 (63.8%)	.9

Abbreviations: DTC = disseminated tumor cell; RT = radiation therapy; SCN/IMN = supra- and infraclavicular and internal mammary nodes.

Table 3 Multivariate analysis on locoregional relapse-free interval

Patient characteristics	HR (95% CI)	P value
DTC status		
Positive	3.26 (1.6-5.7)	.001
Negative	1	
Age (y)		
≥45	0.73 (0.28-1.88)	.51
<45	1	
cT		
1	1	
2	1.08 (0.49-2.36)	.85
3/4	1.27 (0.35-4.60)	.71
pN		
Positive	1.11 (0.59-2.07)	.74
Negative	1	
Histology		
Ductal	1	
Lobular	1.08 (0.40-2.93)	.88
Tumor grade		
1	1	
2	1.76 (0.83-3.69)	.14
3	1.70 (0.62-4.71)	.30
HR status		
Positive	2.52 (0.82-7.79)	.11
Negative	1	
LVI		
No	1	
Yes	1.58 (0.75-3.36)	.23
RT (all modalities)		
Yes	0.50 (0.14-1.83)	.29
No	1	
Chemotherapy		
Yes	0.78 (0.31-2.01)	.61
No	1	
Hormone therapy		
Yes	0.45 (0.22-0.94)	.03
No	1	

Abbreviations: CI = confidence interval; DTC = disseminated tumor cell; HR = hormone receptors; LVI = lymphovascular invasion; RT = radiation therapy.

However, radiation therapy (radiation technique and dose) was homogeneous over time. In addition, DTC status was unknown to clinicians for chemotherapy and radiation volume decision making.

The benefits of regional nodal irradiation (SCNs/IMNs) have recently been questioned. According to the St Gallen international breast cancer guidelines, regional irradiation is indicated in patients with pN1 cancers (1-3 positive nodes) and adverse clinical features, including young age (≤ 40 years), adverse biology such as low or negative estrogen receptor expression, high grade, extensive LVI, and all patients with 4 or more positive lymph nodes.²⁵ Two recent phase 3 randomized studies have provided concrete evidence in support of these guidelines. Whelan et al demonstrated that SCN/IMN irradiation decreased the risk of locoregional and distant relapse but did not affect BCSS and OS.¹⁰ The European Organisation for Research and

Fig. 1. Locoregional relapse-free interval according to disseminated tumor cell status estimated by the Kaplan-Meier method.

Fig. 2. Cumulative incidence of locoregional relapses according to DTC status and SCN/IMN irradiation based on the sub-distribution hazard and considering death as competitive events. *Abbreviations:* DTC = disseminated tumor cell; DTC- = DTC negative; DTC+ = DTC positive; IMN = supra- and infraclavicular nodes/internal mammary node; SCN/IMN+ = Radiation therapy to SCNs/IMNs; SCN/IMN- = No radiation therapy to SCNs/IMNs.

Treatment of Cancer trial conducted in 4004 patients demonstrated a significant improvement of BCSS and a decreased risk of relapse.¹¹ In these 2 trials, SCN/IMN irradiation had no impact on OS, but a significant increase

in pulmonary and cardiac toxicity was observed. In a Danish cohort of 3089 patients with early node-positive breast cancer, IMN irradiation (for right breast cancer) improved survival compared to no IMN irradiation (for left breast cancer) with no increased cardiac toxicity.²⁶ A French randomized trial did not demonstrate any significant survival benefit in IMN-irradiated patients, but had some methodologic limitations.²⁷ Regional irradiation decisions are, therefore, based on clinical and pathologic criteria and remain a subject of debate, particularly in pN0 patients. Better patient selection in order to personalize irradiation volumes therefore constitutes a major challenge.

The biologic link between DTC detection in bone marrow and locoregional relapse is unknown. DTC appears as a marker of higher disease burden within the locoregional volumes that hormone therapy or chemotherapy could not adequately manage. Tumor “self-seeding” could also be a further explanation. In this model, some cancer cells from the primary tumor acquire the ability to intravasate into the circulation, stop in a sanctuary organ such as bone marrow or lymph nodes, and enter into quiescence.²⁸⁻³⁰ These cells can subsequently reseed the initial tumor site.³¹⁻³³ Cancer cells can therefore escape local treatment by surgery and radiation therapy and subsequently reinitiate tumor growth at the initial tumor site, possibly guided by locally released chemokines and growth factors during the wound healing process after breast surgery. SCN/IMN irradiation would kill quiescent DTCs located in the sternal bone marrow and lymph node sanctuary areas. Regional irradiation would therefore provide a benefit in DTC+ patients, in contrast with DTC- patients. Interestingly, in addition to our study based on DTC detection (in bone marrow), data reported by Goodman et al have

Table 4 Multivariate analysis on overall survival and breast cancer-specific survival estimated by Cox regression model for cause-specific HRs

Patient characteristics	Overall survival		Breast cancer-specific survival	
	HR (95% CI)	P value	HR (95% CI)	P value
DTC status				
Positive	1		1	
Negative	1.43 (1-2.2)	.09	1.85 (1.2-3)	.01
cT				
1	1		1	
2	3.1 (1.8-5.2)	<.001	2.1 (0.9-4.8)	.07
3/4	7.64 (4.5-12.9)	<.001	3.9 (1.7-9)	.002
pN				
Negative	1		1	
Positive	3.42 (2.3-5)	<.001	2.2 (1.3-3.8)	.002
Tumor grade				
1	1		1	
2	3.24 (1.9-5.6)	<.001	4.41 (1.9-10.4)	<.001
3	6.04 (3.6-10.3)	<.001	5.2 (2.1-12.7)	<.001
HR status				
Negative	1		1	
Positive	0.34 (0.2-0.5)	<.001	0.46 (0.3-0.7)	<.001

Abbreviations: CI = confidence interval; HR = hazard ratio; HR status = hormone receptor status.

suggested a benefit of radiation therapy in patients with CTC detected in blood.³⁴ In a cohort of 1407 patients, CTC-positive patients not receiving radiation therapy had an increased risk of mortality compared with irradiated CTC-positive patients (HR, 4.82; 95% CI, 2.62-8.85; $P < .001$). The authors concluded that CTC status, which is a known prognostic factor in early breast cancer, could be a useful predictive factor for the benefit of radiation therapy in early breast cancer, but irradiation fields were not specified in this CTC-based report. Although DTC and CTC do not completely overlap,⁷ a practical advantage of CTC detection over DTC detection is that the latter requires trained pathologists. Combining DTC and CTC detection might also lower the risk of false negative, but its clinical validity has not been properly assessed.⁷

Conclusions

This long-term analysis confirms the predictive impact of DTC status on the efficacy of regional radiation therapy for locoregional relapse in early breast cancer. These data can be used to conduct a prospective comparative study based on DTC or CTC detection to determine whether it could constitute a predictive marker allowing better selection of patients for regional irradiation.

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