

# Discordance of the ER- and HER2-Status on Disseminated Tumor Cells Compared to the Primary Tumor in Patients With Early Breast Cancer

Bernadette AS Jaeger<sup>1</sup>, Charlotte Finkenzeller<sup>2</sup>, Carolin Bock<sup>2</sup>, Leonie Majunke<sup>2</sup>, Julia Jueckstock<sup>2</sup>, Ulrich Andergassen<sup>2</sup>, Julia Neugebauer<sup>2</sup>, Aurelia Pestka<sup>2</sup>, Thomas Friedl<sup>1</sup>, Udo Jeschke<sup>2</sup>, Wolfgang Janni<sup>1</sup>, Sophie Doisneau-Sixou<sup>2,3</sup> and Brigitte Rack<sup>2</sup>

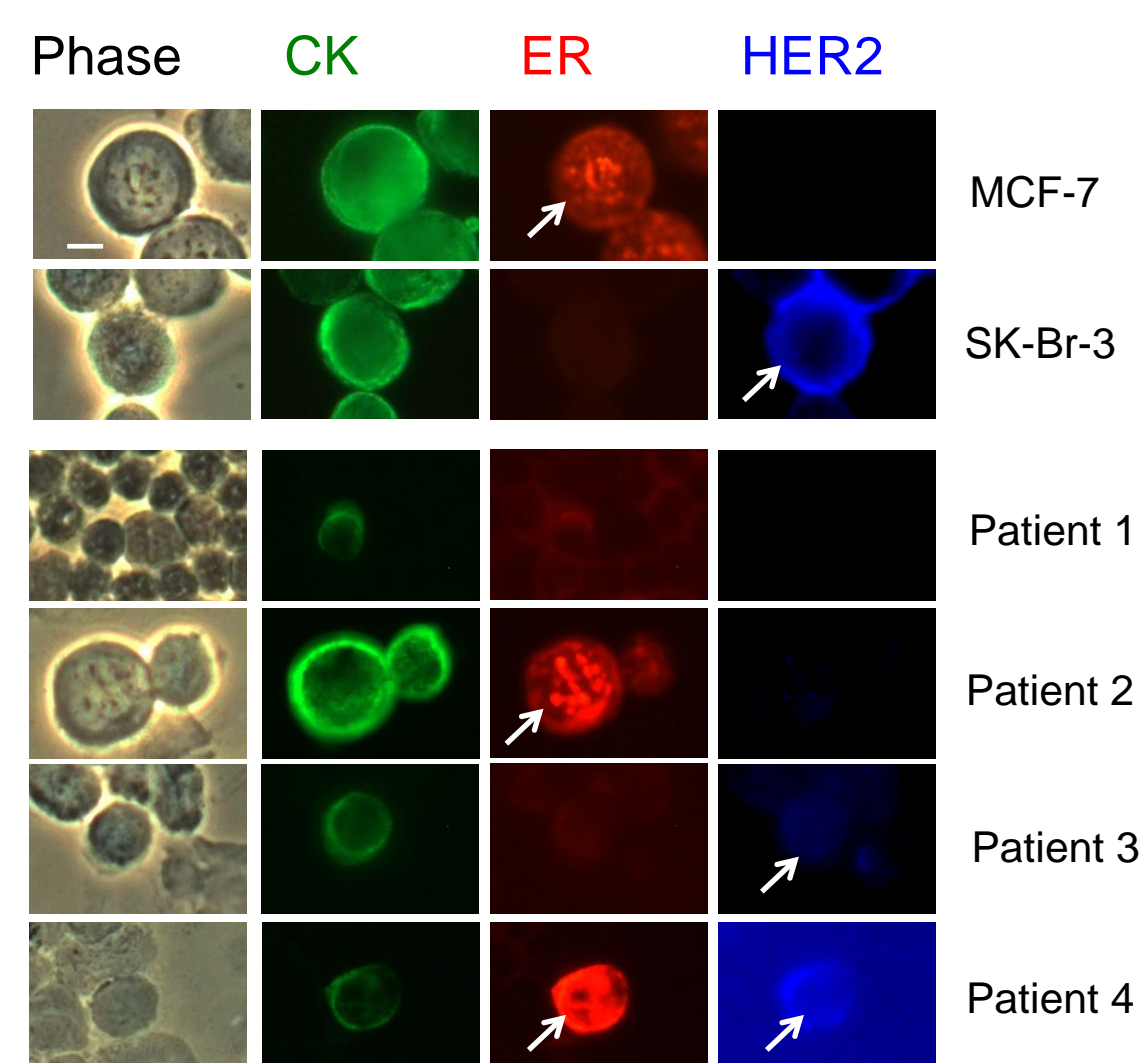
<sup>1</sup>Department of Gynecology and Obstetrics, University Hospital Ulm, Ulm, Germany; <sup>2</sup>Department of Gynecology and Obstetrics, Hospital of the Ludwig-Maximilians-University, Munich, Germany; <sup>3</sup>Université Paul Sabatier Toulouse III, Toulouse, France.

## Background

Differences in ER- and HER2-expression on metastases compared to the primary tumor (PT) are a known phenomenon and may have clinical implications in respect of targeted systemic treatment approaches. The aim of this study was to evaluate both ER- and HER2-status on disseminated tumor cells (DTCs) in the bone marrow (BM) of patients (pts) with early breast cancer (EBC; see table 1) and to compare these with the corresponding PT.

## Methods

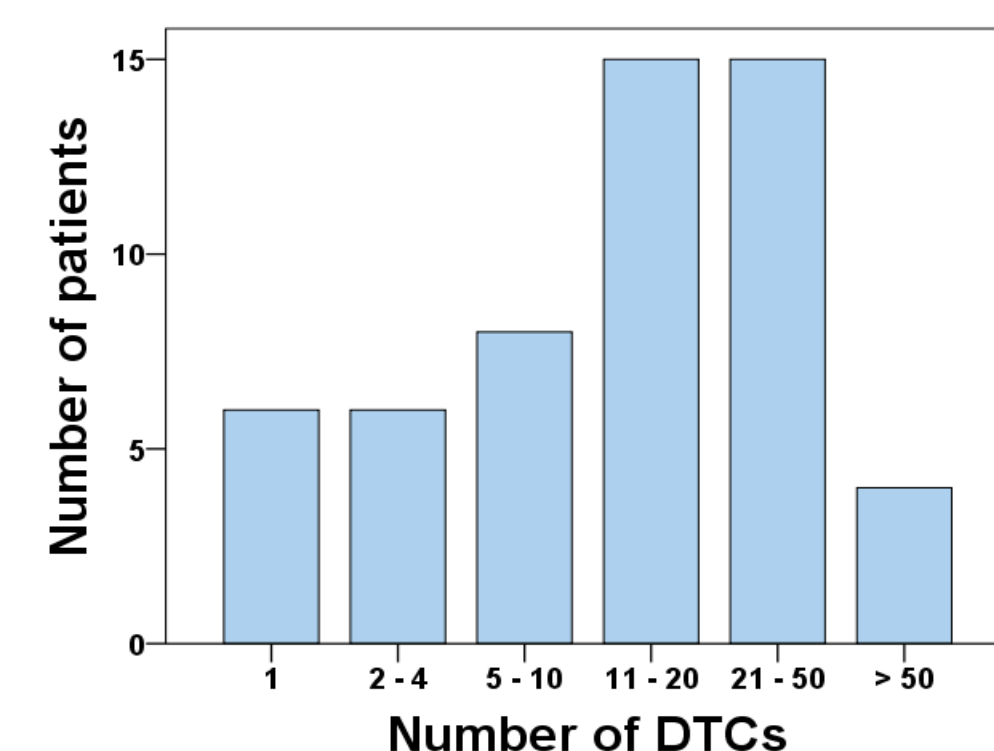
BM aspirates were obtained at the time of first surgery. After Ficoll enrichment for mononuclear cells two cytopspins with 10<sup>6</sup> BM cells were evaluated for ER-, HER2- and cytokeratin (CK) -expressions simultaneously by immunocytochemistry using a triple fluorescence staining method with antibodies directed against human ER (secondly labeled with Cy3, red), HER2 (Coumarin-AMCA, blue) and CK (DyLight488, green). The manual analysis was conducted using a computerized fluorescence microscope (Axioskop, Zeiss, Germany). Criteria for CK- and HER2-positivity were the ring-like appearance of the respective membrane stainings and for ER-expression a nuclear staining (see figure 1). Only pts with the detection of CK positive cells (DTC+) and known ER- and HER2-status of the PT (n= 54) were selected for this analysis.



**Figure 1:** Phase, CK, ER and HER2 staining for ER-positive cell line (MCF-7), HER2-positive cell line (SK-Br-3) and 4 patients with 4 different subtypes

Menopausal status	pre	15 (27.8%)
	post	39 (72.2%)
pT	pT1b	6 (11.1%)
	pT1c	23 (42.6%)
	pT2	18 (33.3%)
	pT3	7 (13.0%)
pN	pN0	34 (63.0%)
	pN1	10 (18.5%)
	pN2	3 (5.6%)
	pN3	5 (9.3%)
	n/a	2 (3.7%)
Histological grading	G1	5 (9.3%)
	G2	31 (57.4%)
	G3	18 (33.3%)
Histological type	ductal	40 (74.1%)
	lobular	10 (18.5%)
	other	4 (7.4%)
Estrogen receptor status	negative	12 (22.2%)
	positive	42 (77.8%)
Progesteron receptor status	negative	20 (37.0%)
	positive	34 (63.0%)
HER2 status	negative	48 (88.9%)
	positive	6 (11.1%)

**Table 1:** Patient characteristics.



**Figure 2:** Frequency distribution of the number of DTCs detected per patient (n = 54)

A	ER status	DTC		Total (%)
		only ER-negative DTCs (%)	at least one ER-positive DTC (%)	
Tumor	ER negative (%)	6 (11)	6 (11)	12 (22)
	ER positive (%)	8 (15)	34 (63)	42 (78)
	Total (%)	14 (26)	40 (74)	54 (100)*

B	HER2 status	DTC		Total (%)
		only HER2-negative DTCs (%)	at least one HER2-positive DTC (%)	
Tumor	HER2 negative (%)	26 (48)	22 (41)	48 (89)
	HER2 positive (%)	4 (7)	2 (4)	6 (11)
	Total (%)	30 (55)	24 (45)	54 (100)**

**Table 2:** Association between ER-(A) and HER2-(B) status of PT and DTC. \* p = 0.031 (Chi-Square-test). \*\* p = 0.56 (Chi-Square-test).

Primary tumor	DTC status	1 DTC profile		2 DTC profiles		3 DTC profiles		4 DTC profiles		
		+	-	+	-	+	-	+	-	
ER+/HER2-	n	2	1	5	13	1	2	2	4	8
	%	5.3	2.6	13.1	34.2	2.6	5.3	5.3	10.5	21.1
ER+/HER2+	n			1	2					1
	%			25.0	50.0					25.0
ER-/HER2-	n			3	1	3	1		1	1
	%			30.0	10.0	30.0	10.0		10.0	10.0
ER-/HER2+	n			1					1	
	%			50.0					50.0	
Total (n = 54)	n	2	1	10	1	18	1	3	2	6
	%	3.7	1.9	18.5	1.9	33.3	1.9	5.5	3.7	11.1

**Table 3:** Combined ER/HER2-status of DTCs and association with the ER/HER2-status of the PT.

## Results

The median number of DTCs was 13 (range 1-95; total number of DTCs detected: 1082; see figure 2). 40 (74%) of the pts had at least one ER-positive (pos) DTC, 24 (44%) at least one HER2-pos DTC, 14 (26%) at least one ER-pos/HER2-pos DTC, and 50 (93%) at least one ER-negative/HER2-negative (neg) DTC, while 10 (19%) pts had only ER-neg/HER2-neg DTCs.

The concordance rate between ER-status on DTCs and PT was 74%. Pts with an ER-pos PT were significantly more likely to have at least one ER-pos DTC (34 out of 42) than pts with an ER-neg PT (6 out of 12; Chi-square test,  $\chi^2 = 4.66$ ,  $p = 0.031$ ). 39 (93%) of the 42 pts with ER-pos PT had at least 1 ER-neg DTC (see table 2A).

The concordance rate between HER2-status on DTCs and PT was 52%. The probability of having at least one HER2-pos DTC was not related to the HER2-status of the PT (Chi-square test,  $\chi^2 = 0.34$ ,  $p = 0.56$ ). 22 (46%) of the 48 pts with a HER2-neg PT had at least one HER2-pos DTC. All of the 6 pts with a HER2-pos PT had at least one HER2-neg DTC (see table 2B).

7 out of 10 pts with a triple-neg PT had at least one DTC pos for ER, HER2 or both. Further the heterogeneity of the ER- and HER2-expression on DTCs compared to the PT for different DTC counts was evaluated. We detected all possible combinations of ER- and HER2-expression on DTCs regardless of the respective status of the PT (for details refer to table 3).

## Conclusions

Our study confirms that the ER- and/or HER2-status on DTCs may differ compared to the PT. This discordance could be especially important for pts with a triple-neg PT and ER-pos or HER2-pos DTCs, since they might respond favorably to an endocrine or HER2-targeted therapy. On the other hand, the presence of ER-neg or HER2-neg DTCs in pts with ER-pos or HER2-pos PT might explain some of the failures of adjuvant endocrine or HER2 targeted therapy.

## Acknowledgment

We would like to thank all patients for participating at this study and donating their BM samples for research purposes.

