4EVER: Assessment of circulating tumor cells with a novel, filtration-based method, in a phase IIIb multicenter study for postmenopausal, HER2- negative, estrogen receptor-positive, advanced breast cancer patients.

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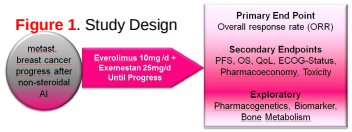
Background: The presence of circulating tumor cells (CTCs) has been shown to be of prognostic relevance for patients with early and advanced breast cancer (BC). The usefulness of CTC assessments depends on accurate cell counts and corresponding analysis of molecular targets. The aim of this sub study was to assess the feasibility of a novel, integrated CTC platform for automated cellular protein and nucleic acid analysis in a prospective multi-center study (**Fig.1**).

Figure 1. Study Design



Methods: The 4EVER study included patients with, metastastic postmenopausal, ER positive, HER2 negative BC, who progressed after therapy with a non-steroidal aromatase inhibitor and were treated with exemestane and the mTOR inhibitor everolimus. Baseline blood samples (TransFix BD) were used for CTC analysis and processed on the modified Versant kPCR Sample Prep system using 8µm pore size Whatman Nuclepore track-etched membranes (GE Healthcare Piscataway, NJ). After CTC capture, immunostaining was performed for Cytokeratin 8/18/19 and CD45. CTCs were detected by image analytics after fluorescence scanning microscopy using a dedicated software solution implemented by Siemens (**Fig. 2-6**). The data is summarized and correlated with baseline clinical characteristics

Results: A total of 287 patients were enrolled from May 2012 to October 2012. CTC samples were taken from 121 patients. CTC ounts and clinical data were available for 111 blood samples (91.7%). Patient characteristics are shown in **Table 1**. CTCs were found in 75 patients (67,6%), 13 patients having 1 CTC, 38 having 2-9 CTCs and 24 patients having 10 or more CTCs (**Figure 7**). status or tumor type (**Table 1**). In an exploratory analysis the presence of CTCs was correlated with baseline patient and tumor characteristics. There were no associations with primary TNM stage, hormone receptor status or tumor type.

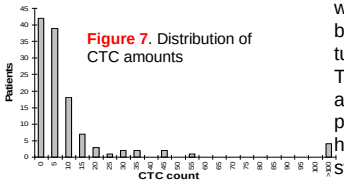


Figure 7. Distribution of CTC amounts

Table 1. Patient Characteristics. For univariate and multivariate analysis patients were categorized in “CTCs present” (CTC count>0) and “CTCs not present” (CTC count =0)

Characteristic		No CTC (=0)	CTCs>0
Time to metast.	Years	10.0 (7.2)	8.8 (6.2)
Tumor Stage	pT1	10 (29.4)	23 (33.3)
	pT2-pT4	24 (70.6)	44 (66.7)
Nodal status	pN+	22 (62.9)	47 (70.1)
	pN0	13 (37.1)	20 (29.9)
M status	Secondary	29 (82.9)	49 (75.4)
	Primary	6 (17.1)	16 (24.6)
Grading	G1	1 (3.2)	2 (2.9)
	G2	16 (51.6)	54 (77.1)
	G3	14 (45.2)	14 (20.0)
PR	Negative	6 (17.1)	17 (22.7)
	Positive	29 (82.9)	58 (77.3)
Histology	ductal	23 (67.6)	52 (74.3)
	lobular	8 (23.5)	16 (22.9)
	Other	3 (8.8)	2 (2.9)

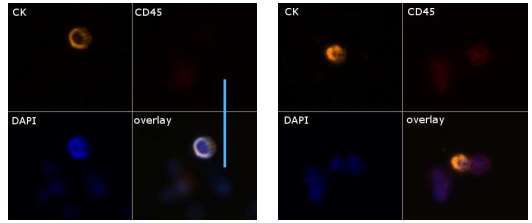


Figure 8. Examples of CTCs found in the 4ever study

Conclusions: CTC assessment with this novel filtration based method was feasible in a multi-center study setting. The CTC positivity rate was within the expected range. The follow-up of this study will give first insights, how the CTC measures of this platform can be used as a prognostic tool. As this CTC assessment platform was developed to perform additional automated cellular protein and nucleic acid analysis, the usefulness might derive from these analytic tools as well. Clinical trial information: NCT01626222.

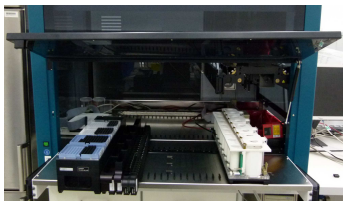


Figure 2. Overview of CTC isolation, autostaining and analysis workflow. The instrument platform is based on the VERSANT® SP platform with the addition of cell capture and autostaining components which may be utilized with both CTC and FFPE slides for either protein or nucleic acid analysis.

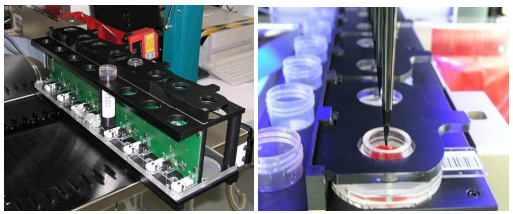


Figure 2. 8-channel CTC processing unit with filtration-pressure control

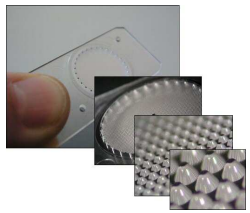


Figure 3. Filter-slide with micro-structure membrane support (membrane not shown)

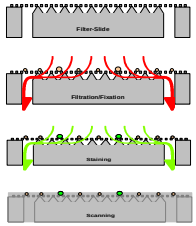


Figure 4. Schematic drawing of filtration- and immunostaining workflow

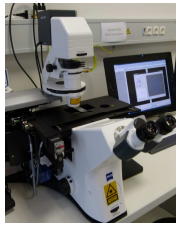


Figure 5. Detection Microscope

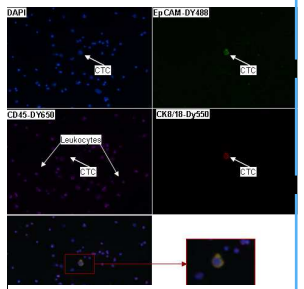


Figure 6. Staining for DAPI, EPCAM, CD45, and CD8/18

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