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Disseminated tumor cells (DTCs) in primary and recurrent breast cancer patients with various molecular subtypes

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Background

Disseminated tumor cells (DTCs) in the bone marrow (BM) aspirates of patients with primary breast cancer may serve as independent prognostic markers associated with impaired survival. Despite successful treatment of the primary tumor, recurrence occurs in about 30% of breast cancer patients. Little is known concerning the occurrence and properties of DTCs in recurrent or progressive patients. The adjuvant intake of bisphosphonates was shown to have an apoptotic effect on DTCs and to decrease the risk of bone metastases thus, providing an overall survival benefit [1–3]. An alternative therapy choice might be Densusomab, a human antibody against the receptor activator of nuclear factor-kappaB (RANK) ligand, a protein essential for osteoclast differentiation, activity and survival which is still under investigation in clinical trials such as the GeparX trial. Breast cancer is a very heterogeneous disease and can be classified into distinct molecular subtypes. Based on immunohistochemical definition of estrogen receptor (ER) and progesterone receptor (PR), the detection of the human epidermal growth factor receptor 2 (HER2), and the cell proliferation marker Ki-67 [4] tumors are categorized into luminal A, luminal B, HER2 enriched or triple-negative tumors. At the time being there is no clinical test to stratify patients at elevated risk for recurrence based on their DTC profile at primary diagnosis and therapeutic strategies are still controversially discussed.

Methods

Between February 2019 and July 2021 BM aspirates from 206 primary and 27 recurrent breast cancer patients were collected. Per patient about 4 million BM cells were analyzed.

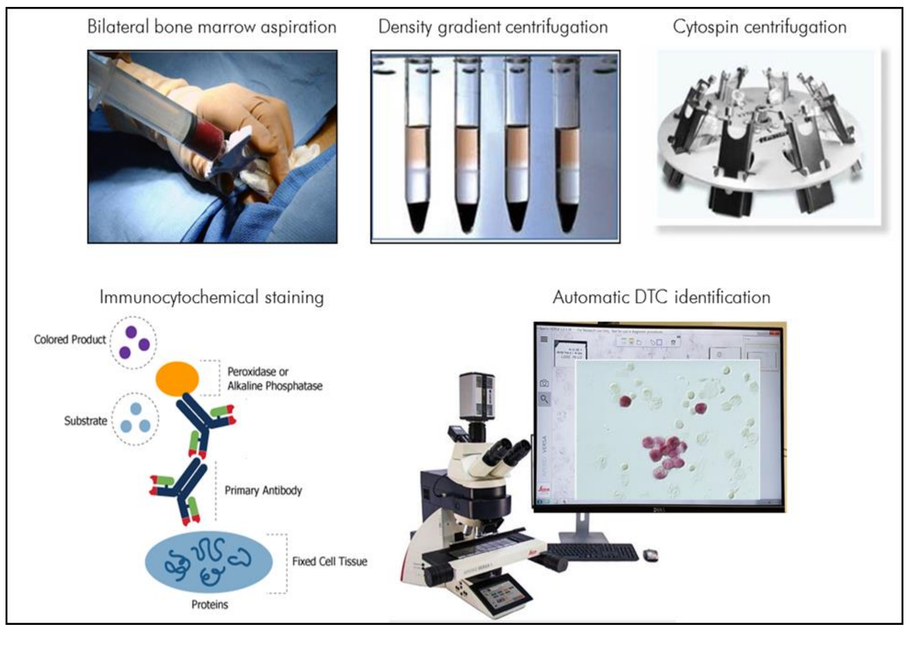

Fig. 1: BM aspirates were collected from the anterior iliac crest of patients with primary or recurrent mamma carcinoma during surgery. After density gradient centrifugation, cell suspensions were transferred onto glass slides and subjected to immunocytochemical staining against pan-cytokeratin. DTCs were visualized in pink using alkaline phosphatase and short counterstaining with hematoxylin which colored the nuclei light blue. DTCs were semi-automatically detected and enumerated using the Aperio Versa microscope based scanning system with a rare events algorithm that was trained to identify DTC candidates according to color, shape, intensity and size. As a positive control with each run, we used reference slides with a mix of bone marrow cells and a defined number of HCT116 cells.

Results

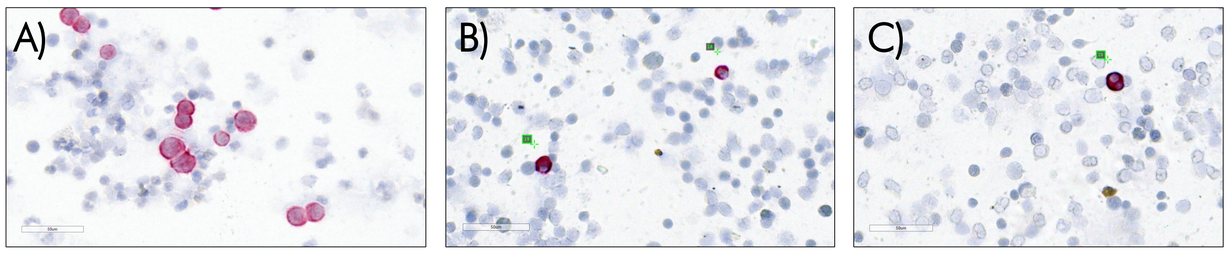

Fig. 2: Detection of Pan-CK positive cells in bone marrow. A) Positive control consisting of bone marrow cells mixed with HCT116 colon cancer cells. B) and C) DTCs in bone marrow sample. Two independet investigators, a certified cytologist and a trained pathologist, individually evaluated cell morphology and cytological staining patterns of the selected DTC candidates.

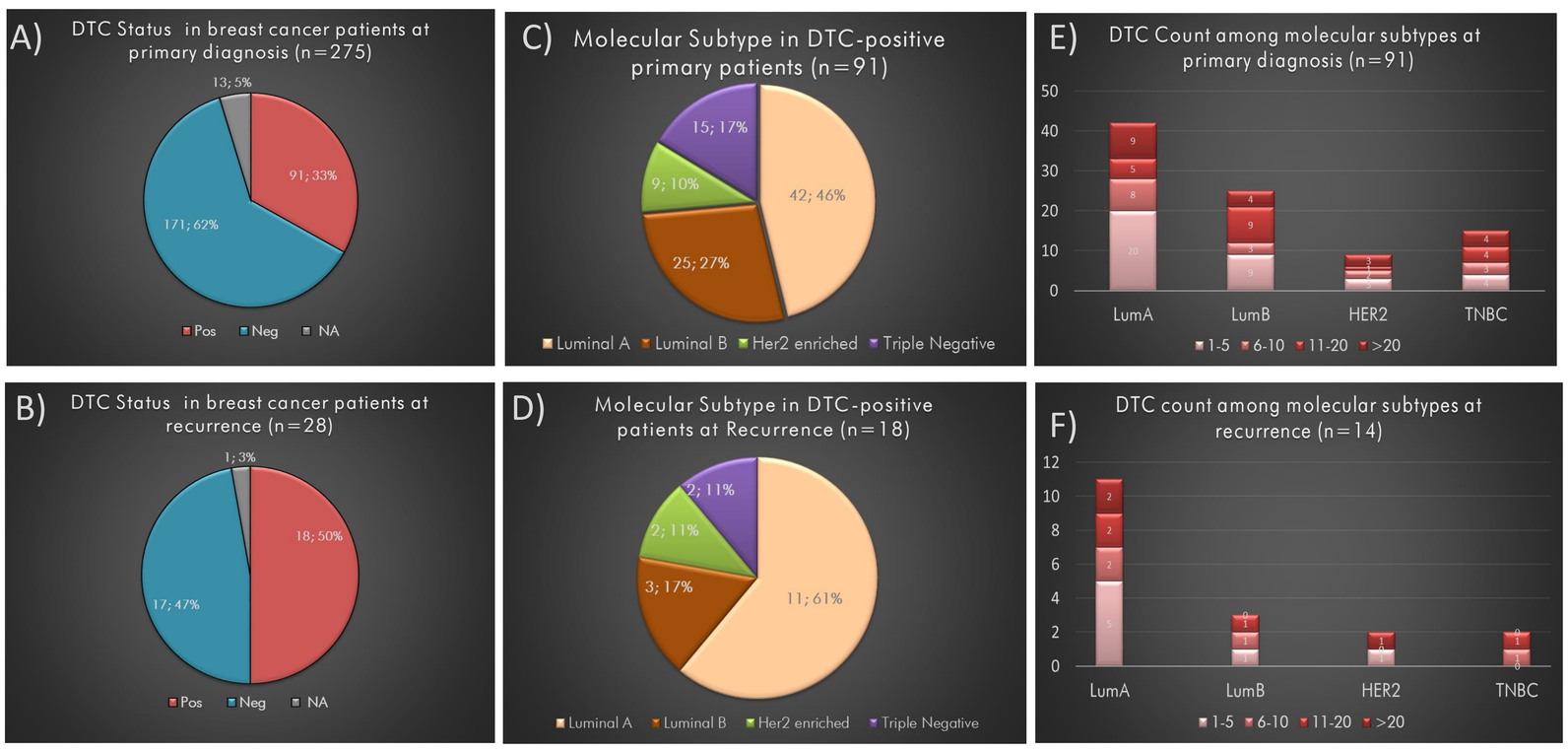

Fig. 3. DTC detection revealed a positivity rate of 33% (91 patients) among primary (A) and 50% (18 patients) among recurrent cases (B). Molecular subtype analysis of DTC positive patients showed that 46% of the primary tumors (42 patients; C) and 61% (11 patients) of the recurrent tumors (D) were luminal A. Further, 27% (25 patients) of primary vs. 17% (3 patients) of recurrent DTC-positive cases were luminal B. Tumors were HER2 enriched in 10% (9 patients) vs. 11% (2 patients) of primary (C) vs. recurrent (D) cases. Of the primarily diagnosed, DTC-positive cases 17% (15 patients; C) were triple negative vs. 11% (2 patients) in the recurrent setting (D). Further, 14 of 18 DTC-positive patients, nearly 80%, had hormone receptor positive tumors (HR+) at recurrence. E) and F) are showing the DTC count among the subtypes at primary diagnoses vs. recurrence.

Setting			DTC Status					
	n	%	Pos	%	Neg	%	NA	%
Primary	**275**		91	33,1	171	62,18	13	4,7
Subtype								
Luminal A	141	51,3	42	29,8	91	64,5	8	5,7
Luminal B	66	24,0	25	37,9	39	59,1	2	3,0
HER2 enriched	31	11,3	9	29,0	20	64,5	2	6,5
Triple Negative	37	13,5	15	40,5	21	56,8	1	2,7
			DTC Status					
	n	%	Pos	%	Neg	%	NA	%
Recurrent	**36**		18	50	17	47,2	1	2,8
Subtype								
Luminal A	19	52,8	11	57,9	8	42,1	0	0
Luminal B	7	19,4	3	42,9	4	57,1	0	0
HER2 enriched	3	8,3	2	66,7	1	33,3	0	0
Triple Negative	7	19,4	2	28,6	4	57,1	1	14,3

Table 1. DTC status was assessed in primary (n=275) and recurrent/progressive (n=36) breast cancer patients. Patients were divided into subgroups based on the molecular subtype of the tumor. Of the primary breast cancer patients, 33% were DTC-positive (n=91). Subtype analysis revealed that 38% of primary luminal B patients (n=66) were DTC positive (n=25). Further, only 30% of primary luminal A patients (42/141) were DTC-positive. Among patients with recurrent/progressive breast cancer, the positivity rate was 50%. Remarkably, about 60% of recurrent patients with luminal A (11/19) or HER2-enriched tumors (2/3) were DTC-positive. Sub-cohorts are still very small though.

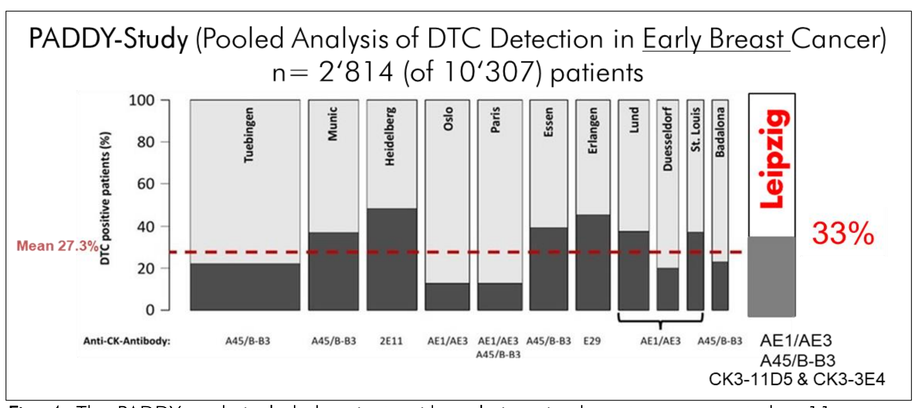

Fig. 4. The PADDY study included patients with early invasive breast cancer treated at 11 centers between 1986 and 2017. Of the 10'307 patients, 27.3% were DTC-positive. A positive DTC status was associated with the luminal B subtype and decreased survival (Hartkopf et al, 2021). The study did not consider the number of DTCs and their relative prognostic value, though.

Conclusion

- Patients with primary luminal B tumors appeared to have an increased DTC-positivity rate compared to luminal A tumors. In the recurrent setting, luminal A tumors revealed an increased DTC-rate, though (very small sub-cohort).
- Follow-up data might reveal whether DTC quantification and molecular subtypes at primary diagnosis can be used to stratify patients at elevated risk for recurrence.

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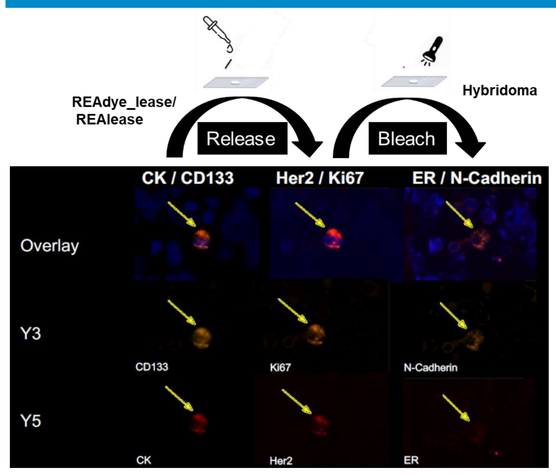
Outlook



- Detection of DTC-subtypes using multi-parameter imaging might play a more important role rather than DTC quantity.

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