DTCs in Breast cancer

Background

Despite major improvements in the diagnosis and treatment of breast cancer (**BC**), about **30%** of primary BC patients show a **relapse** of the disease years after first diagnosis which is explained by micrometastatic spread to the bone marrow **(BM**) by disseminated tumor cells (**DTCs**) being present in up to **40%** of the patients (Wölfle et al., 2006). Their presence as well as persistence after therapy is associated with a **reduced** progression free survival (**PFS**) and overall survival (**OS**) and our recently published **meta-analysis** of more than **10.000 patients** nicely confirmed these findings **(Hartkopf et al., 2021)**. The lack of therapeutic approaches to eliminate these cells constitutes major obstacles to the successful treatment of this disease. Due to their nature and rarity, only a few studies have already unraveled **DTC characteristics**. Most of the DTCs are **non-proliferating** and have the ability to survive in a **dormant state** and stay unrecognized for many years before re-appearing as local or distant **recurrences 10** or **20 years later** (Banys-Paluchowski et al., 2020; Risson et al., 2020). This also explains why chemotherapy is not able to eliminate them. Besides dormancy, a **discordant receptor status** with regard to the so-called predictive markers, **HER2** as well as the **hormonal receptors** with treatment options available, on the **primary tumor** and corresponding **DTCs** has been well described. In addition, some of the DTCs have been identified as cancer stem cells (**CSCs**) that might have the ability to renew themselves (Balic et al., 2006; Reuben et al., 2011; Krawczyk et al., 2021).

Methods for detection

The most frequently used method for their detection is **immunocytochemistry** using cytokeratins (**CKs**), abundantly expressed as stable proteins in the majority of epithelial tumors to facilitate the detection of isolated carcinoma cells in mesenchymal organs like the BM (**Figure 1**). To compare the results for DTCs, a **consensus concept** for the standardized detection and enrichment of DTCs was proposed and several criteria were defined to evaluate morphology and staining results after automated microscopic screening (**Fehm et al., 2006; Borgen et al., 2006**).

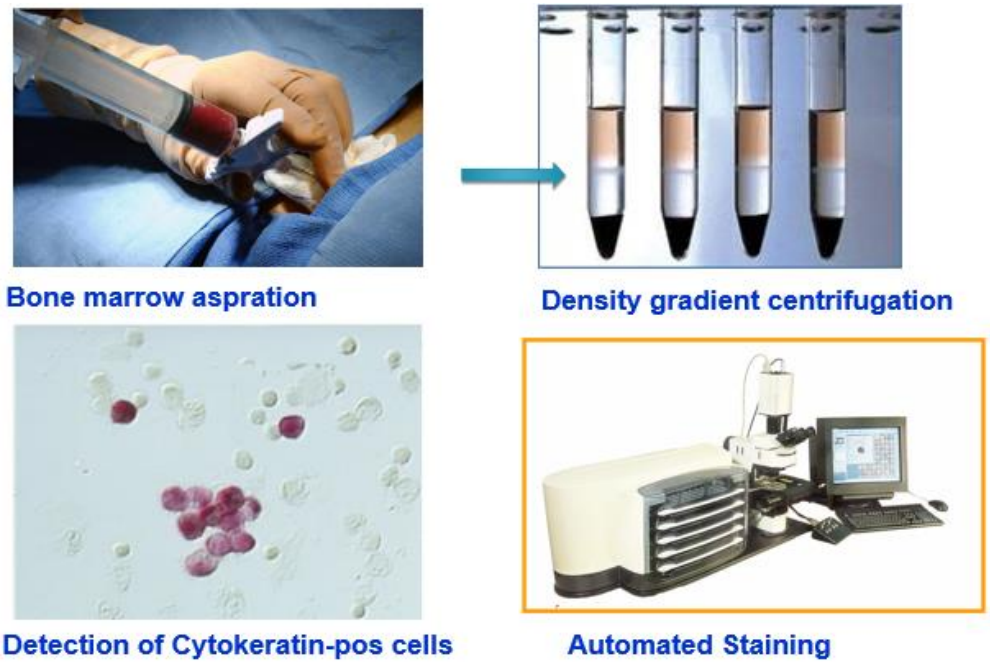

Figure 1: Detection of CK-pos DTCs. BM is aspirated from the iliac crest and separated using density gradient centrifugation. For staining of DTC in BM samples, a panCK antibody (A45-B/B3; Fab fragment) complexed with alkaline phosphatase anti-alkaline phosphatase (APAAP) is used. Microscopic evaluation of the slides is carried out using the ARIOL system (Applied Imaging).

Own work in the field

The intake of bisphosphonates

DTCs in primary BC have been studied in **our laboratory** since 1998. Contradictory to previous findings of other groups, we demonstrated **no prognostic value of DTCs** with regard to **PFS** and **OS**. We explain our results with **early bisphosphonate intake** for a duration of at least two years, which we recommend in case of DTC-positivity. This recommendation is based upon the results of Diel et al. 1998 who were able to show the effect of **clodronate** intake upon DTCs. Briefly, bisphosphonates are potent inhibitors of bone resorption, they inhibit bone loss and reduce the risk of skeletal-related events in patients with bone metastases. Besides inhibiting the activity of osteoclasts, there is growing evidence that bisphosphonates have antitumor and antimetastatic properties, including the inhibition of angiogenesis, tumor cell invasion, adhesion in bone, the induction of apoptosis, anti-tumor synergy with cytotoxic chemotherapy and immunomodulatory effects through induction of γ/δ T-cells (EBCTCG 2015; Alasmari et al., 2016).

In a **small pilot study** enrolling 54 patients, we demonstrated **a positive effect of ibandronate** for **eradication of DTCs** 2-10 years after primary diagnosis. Patients with DTC persistence received oral ibandronate treatment (50 mg per day) for up to twelve months resulting in **DTC elimination in all patients** (**Hoffmann et al., 2011**). We confirmed these results in two follow-up studies including **more than 500 primary BC** patients **(Figure 2; Hoffmann et al., 2015; Kasimir-Bauer et al., 2016a)**. In addition, we also offered bisphosphonates to BC patients in case of DTC-positivity **after neoadjuvant chemotherapy** and did not see any negative prognostic effect of DTCs before and/or after therapy with regard to PFS and OS after a median follow-up of nearly five years (**Kasimir-Bauer et al., 2016b**).

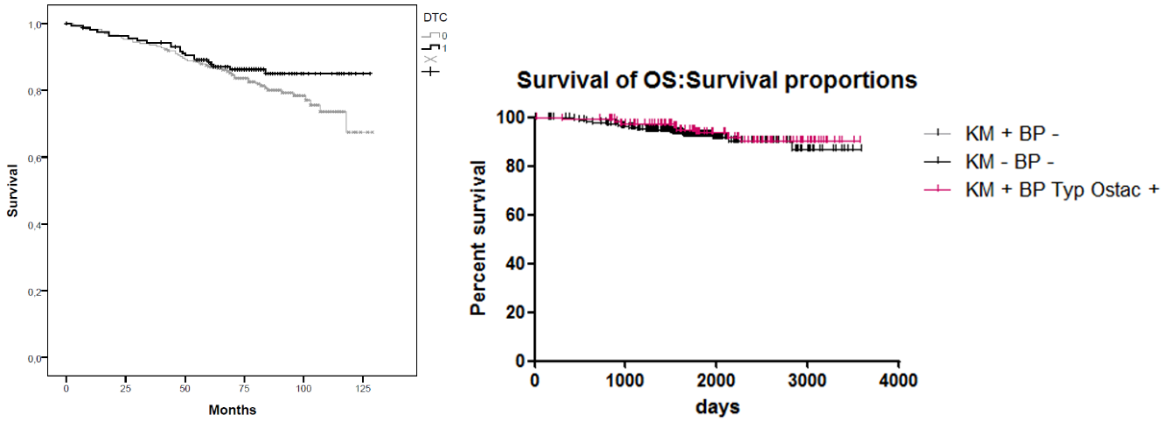

Figure 2: The presence of DTCs was not associated with diminished OS (p=0.156), left Figure (***Hoffmann et al., 2015***). DTC-positive patients who took oral clodronate for at least two years did not differ in OS from patients where no DTCs were detected (***Kasimir-Bauer et al., 2016a; modified***).

We also correlated results of the **Oncotype DX**, a validated genomic test using tumor tissue that predicts the likelihood of BC recurrence, with the presence of **DTCs** in 68 patients with HER2-negative early BC but did **not** find any **correlations** **(Aktas et al., 2013)**.

We also explored whether **DTCs** could be a suitable **biomarker** in **male BC**. DTC were found in **3/5 patients** with two DTCs detected in one patient and one DTC detected in each of the other two patients. After a median follow-up time of three years (range **1–10 years**), **all patients** were **still alive** and **free of relapse** **(Tewes et al., 2013)**.

Characterization of DTCs

In collaboration with **Prof. Dr. Galatia Kallergi** from Greece, we demonstrated for the first time that **DTCs** in the BM of primary, non-metastatic BC patients **frequently harbour (CXCR4+JUNB+CK+) cells** and that the presence of these DTCs was associated with **unfavourable clinical outcome** (Kallergi et al., 2020). While this phenotype was exclusively observed **in about 41%** of the patients, none of the patients showed up with exclusively (CXCR4-JUNB-CK+)-expressing DTCs. However, there was a **phenotypic heterogeneity of DTCs** since most patients harboured both, (CXCR4+JUNB+CK+) and (CXCR4-JUNB+CK+) cells. Furthermore, **DTC clusters** which were observed in one patient, presented **negative for CXCR4 and positive for JUNB**, whereas **single DTCs** in the same aspiration expressed **higher levels of CXCR4**. Finally, based on their cyto-morphological characteristics (nuclear/cytoplasmic ratio, number of nucleus etc), **(CXCR4+JUNB+CK-) cells**, detected in some cases could potentially represent tumor cells **undergoing EMT**.

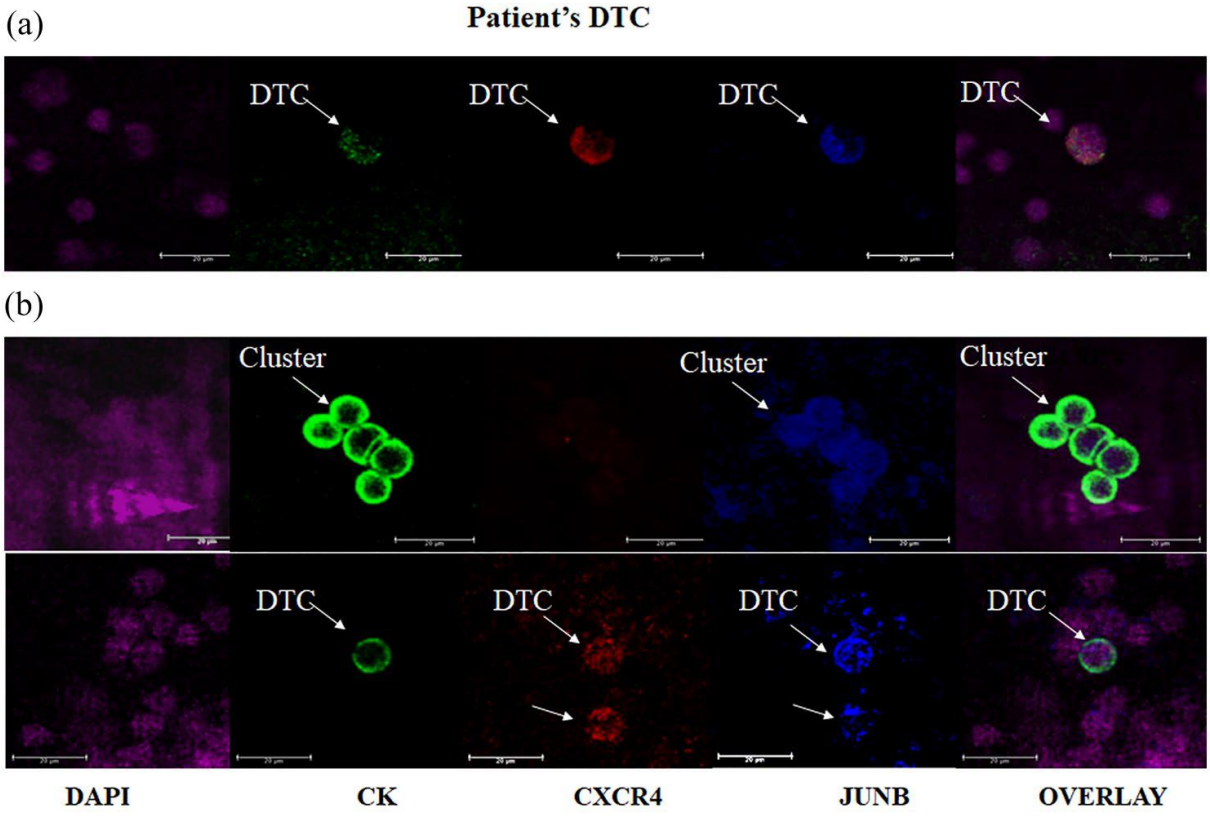

Figure 3: Characterization of DTCs in early BC. Expression of cytokeratin (green), CXCR4 (red), JUNB (blue), and DAPI (purple) in DTCs isolated from BC patients. **(a)** Representative confocal laser scanning images of a patient's DTC expressing CK, JUNB, and CXCR4. Samples were triple stained with pan-cytokeratin A45-B/B3-zenon-conjugated antibody (green) along with CXCR4 anti-rabbit, JUNB anti-mouse antibodies, and DAPI. **(b)** Representative confocal laser scanning images of two different phenotypes observed in the same patient. The first panel shows a DTC cluster with the (CXCR4–JUNB+CK+) phenotype, while the second panel shows a single DTC expressing the (CK+CXCR4+JUNB+) phenotype. BC, breast cancer; BM, bone marrow; CK, cytokeratin; DAPI, 4′,6-diamidino-2-phenylindole; DTC, disseminated tumor cell (***Kallergi et al., 2020***).

References

Wölfle U, Müller V, Pantel K. Disseminated tumor cells in breast cancer: detection, characterization and clinical relevance. Future Oncol. 2006 Aug;2(4):553-61.

Hartkopf AD, Brucker SY, Taran FA, Harbeck N, von Au A, Naume B, Pierga JY, Hoffmann O, Beckmann MW, Rydén L, Fehm T, Aft R, Solà M, Walter V, Rack B, Schuetz F, Borgen E, Ta MH, Bittner AK, Fasching PA, Fernö M, Krawczyk N, Weilbaecher K, Margelí M, Hahn M, Jueckstock J, Domschke C, Bidard FC, Kasimir-Bauer S, Schoenfisch B, Kurt AG, Wallwiener M, Gebauer G, Klein CA, Wallwiener D, Janni W, Pantel K.Hartkopf AD, et al. Disseminated tumour cells from the bone marrow of early breast cancer patients: Results from an international pooled analysis. Eur J Cancer. 2021 Jul 12;154:128-137.

Banys-Paluchowski M, Reinhardt F, Fehm T. Disseminated Tumor Cells and Dormancy in Breast Cancer Progression. Adv Exp Med Biol. 2020;1220:35-43. Risson E, Nobre AR, Maguer-Satta V, Aguirre-Ghiso JA. The current paradigm and challenges ahead for the dormancy of disseminated tumor cells. Nat Cancer. 2020 Jul;1(7):672-680.

Balic M, Lin H, Young L et al. Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. Clin Cancer Res 2006;12:5615-5621.

Reuben JM, Lee BN, Gao H et al. Primary breast cancer patients with high risk clinicopathologic features have high percentages of bone marrow epithelial cells with ALDH activity and CD44(+)CD24lo cancer stem cell phenotype. Eur J Cancer 2011;47:1527-1536.

Krawczyk N, Janowski K, Banys-Paluchowski M, Staebler A, Neubauer H, Meisner C, Hartkopf A, Brucker S, Wallwiener D, Fehm T. The SOX2 Status of Disseminated Tumor Cells in Breast Cancer Patients Treated With Neoadjuvant Chemotherapy. Anticancer Res. 2021 Jun;41(6):2849-2858.

Fehm T, Braun S, Muller V, Janni W, Gebauer G, Marth C, Schindlbeck C, Wallwiener D, Borgen E, Naume B, Pantel K, Solomayer E. A concept for the standardized detection of disseminated tumor cells in bone marrow from patients with primary breast cancer and its clinical implementation. Cancer. 2006 Sep 1;107(5):885-92.

Borgen E, Pantel K, Schlimok G, Müller P, Otte M, Renolen A, Ehnle S, Coith C, Nesland JM, Naume B. A European interlaboratory testing of three well-known procedures for immunocytochemical detection of epithelial cells in bone marrow. Results from analysis of normal bone marrow. Cytometry B Clin Cytom. 2006 Nov 15;70(6):400-9.

Diel IJ, Solomayer EF, Costa SD, Gollan C, Goerner R, Wallwiener D, Kaufmann M, Bastert G. Reduction in new metastases in breast cancer with adjuvant clodronate treatment.N Engl J Med. 1998 Aug 6;339(6):357-63.

Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. Lancet 2015 Oct 3;386(10001):1353-1361.

Alasmari A, Lin SC, Dibart S, Salih E. Bone microenvironment-mediated resistance of cancer cells to bisphosphonates and impact on bone osteocytes/stem cells. Clin Exp Metastasis. 2016 Aug;33(6):563-88.

Hoffmann O, Aktas B, Goldnau C, Heubner M, Oberhoff C, Kimmig R, Kasimir-Bauer S. Effect of ibandronate on disseminated tumor cells in the bone marrow of patients with primary breast cancer: a pilot study. Anticancer Res. 2011 Oct;31(10):3623-8.

Hoffmann O, Schroer-Zuendorf IA, Kasimir-Bauer S, Oberhoff C, Kimmig R, Heubner M. Evaluation of the prognostic significance of disseminated tumor cells in the bone marrow of primary, non-metastatic breast cancer patients after a 7-year follow-up. Arch Gynecol Obstet. 2015 Nov;292(5):1117-25.

Kasimir-Bauer S, Bittner AK, König L, Reiter K, Keller T, Kimmig R, Hoffmann O. Does primary neoadjuvant systemic therapy eradicate minimal residual disease? Analysis of disseminated and circulating tumor cells before and after therapy. Breast Cancer Res. 2016 Feb 12;18(1):20.

Kasimir-Bauer S, Reiter K, Aktas B, Bittner AK, Weber S, Keller T, Kimmig R, Hoffmann O. Different prognostic value of circulating and disseminated tumor cells in primary breast cancer: Influence of bisphosphonate intake? Sci Rep. 2016 May 23;6:26355.

Aktas B, Bankfalvi A, Heubner M, Kimmig R, Kasimir-Bauer S. Evaluation and correlation of risk recurrence in early breast cancer assessed by Oncotype DX ®, clinicopathological markers and tumor cell dissemination in the blood and bone marrow. Mol Clin Oncol. 2013 Nov;1(6):1049-1054.

Tewes M, Kasimir-Bauer S, Welt A, Schuler M, Kimmig R, Aktas B. Detection of disseminated tumor cells in bone marrow and circulating tumor cells in blood of patients with early-stage male breast cancer. J Cancer Res Clin Oncol. 2015 Jan;141(1):87-92.

Kallergi G, Hoffmann O, Bittner AK, Papadimitriou L, Katsarou SD, Zacharopoulou N, Zervakis M, Sfakianakis S, Stournaras C, Georgoulias V, Kimmig R, Kasimir-Bauer S. CXCR4 and JUNB double-positive disseminated tumor cells are detected frequently in breast cancer patients at primary diagnosis. Ther Adv Med Oncol. 2020 Apr 28;12:1758835919895754.