Breast Cancer

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Abstract: Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, cancers can cause serious illness and death. Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researches on the breast cancer.
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1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death.

The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

Literatures

Alpert, L. C., R. L. Schecter, et al. (1997). "Relation of glutathione S-transferase alpha and mu isoforms to response to therapy in human breast cancer." Clin Cancer Res **3**(5): 661-7.

Glutathione S-transferase (GST) represents a multifunctional enzyme family consisting of four known cytosolic isoforms (alpha, mu, pi, and Phi) that detoxify a variety of xenobiotic chemicals and may confer resistance to both chemotherapeutic drugs and carcinogens in various experimental models. GST-pi has already been extensively studied in clinical specimens, including breast cancer. We studied the immuno-histochemical distribution and relative immunopositivity of GST-alpha and GST-mu, based on a grading system for immunointensity, in samples of 51 neoplastic and 46 normal breast samples and 12 lymph node metastases from patients treated with intensive chemotherapy and bone marrow transplant. In normal breast tissue, GST-alpha localized predominantly to the cytoplasm of scattered cells lining the luminal aspects of the ducts. Occasional cells showed both cytoplasmic and nuclear GST-alpha immunoreactivity. GST-mu was stained in myoepithelial cells preferentially as well as in occasional ductal cells (including apocrine epithelium), vascular smooth muscle, and plasma cells. GST-alpha and GST-mu were detected in 22 of 51 (43%) and 24 of 48 (50%) invasive cancers, respectively. In paired samples of normal and malignant tissue from the same patient, GST-alpha immunostaining in cancers was significantly less intense compared to that of normal breast tissue in 13 of 41 (32%) cases. No such trend was found for GST-mu in paired samples. Neither GST-alpha nor GST-mu immunopositivity in tumor or nonneoplastic breast was found to correlate with relapse-free or overall survival in this clinical context; however, the apparent decreased expression of GST-alpha in malignant versus normal breast epithelial cells could have important implications in breast carcinogenesis.

Bachier, C. R., E. Gokmen, et al. (1999). "Ex-vivo expansion of bone marrow progenitor cells for hematopoietic reconstitution following high-dose chemotherapy for breast cancer." Exp Hematol **27**(4): 615-23.

The use of hematopoietic growth factors, stromal monolayers, and frequent medium exchange allows the expansion of hematopoietic progenitors ex-vivo. We evaluated the use of ex-vivo expanded progenitor cells for hematopoietic reconstitution following high dose chemotherapy (HDC) in breast cancer patients. Patients with high-risk Stage II or metastatic breast carcinoma underwent bone marrow aspirations using general anesthesia. A total of 675-1125 x 10(6) mononuclear cells (MNC) were seeded for ex-vivo expansion for 12 days in controlled perfusion bioreactors (Aastrom Biosciences, Inc.). The bone marrow cultures, which included the stromal cells collected with the aspirate, were supplemented with erythropoietin, granulocyte-macrophage-colony stimulating factor (GM-CSF)/IL-3 fusion protein (PIXY 321), and flt3 ligand. Stem cell transplant was performed with expanded cells after HDC. A median bone marrow volume of 52.9 mL (range 42-187 mL) was needed to inoculate the bioreactors. Median fold expansion of nucleated cells (NC) and colony forming

unit granulocyte-macrophage (CFU-GM) was 4.9 and 9.5, respectively. The median fold expansion of CD34+lin- and long-term culture-initiating culture (LTC-IC) was 0.42 and 0.32, respectively. Five patients were transplanted with ex-vivo expanded NC. Median days to an absolute neutrophil count > 500/microL was 18 (range 15-22). Median days to a platelet count > 20,000/microl was 23 (range 19-39). All patients had sustained engraftment of both neutrophils and platelets. Immune reconstitution was similar to that seen after HDC and conventional stem cell transplantation. We conclude that ex-vivo expansion of progenitor cells from perfusion cultures of small volume bone marrow aspirates, allows hematopoietic reconstitution after HDC.

Balic, M., H. Lin, et al. (2006). "Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype." Clin Cancer Res **12**(19): 5615-21.

The presence of disseminated tumor cells (DTC) in the bone marrow of breast cancer patients is an acknowledged independent prognostic factor. The biological metastatic potential of these cells has not yet been shown. The presence of putative breast cancer stem cells is shown both in primary tumors and distant metastases. These cells with a CD44(+)CD24(-/low) phenotype represent a minor population in primary breast cancer and are associated with self-renewal and tumorigenic potential. Recognizing the potential effect of prevalence of putative stem cells among DTC, we evaluated the bone marrow DTC. We employed the double/triple-staining immunohistochemistry protocol and modified the established bone marrow cytokeratin (CK) staining protocol by adding steps for additional antigens, CD44 and/or CD24. We evaluated 50 bone marrow specimens, previously categorized as CK(+) from early breast cancer patients. CK(+) cells were examined for CD44 and CD24 expression by light microscopy, fluorescence microscopy, and spectral imaging. We detected the putative stem cell-like phenotype in all CK(+) specimens. The mean prevalence of putative stem/progenitor cells was 72% and median prevalence was 65% (range, 33-100%) among the overall DTC per patient, compared with primary tumors where this phenotype is reported in <10% of cells. CONCLUSIONS: This is the first evidence of the existence of the putative stem-like phenotype within the DTC in bone marrow in early breast cancer patients. All patients had a putative stem cell phenotype among the DTC and most individual DTC showed such phenotype. Future molecular characterization of these cells is warranted.

Bentires-Alj, M., R. B. Clarke, et al. (2009). "It's all in the details: methods in breast development and cancer." Breast Cancer Res **11**(4): 305.

The inaugural European Network for Breast Development and Cancer (ENBDC) meeting on 'Methods in Mammary Gland Development and Cancer' was held in Weggis, Switzerland last April. The goal was to discuss the details of techniques used to study mammary gland biology and tumourigenesis. Highlights of this meeting included the use of four-colour fluorescence for protein co-localisation in tissue microarrays, genome analysis at single cell resolution, technical issues in the isolation of normal and tumour stem cells, and the use of mouse models and mammary gland transplantations to elucidate gene function in mammary development and to study drug resistance in breast cancer.

Bertucci, F., C. Tarpin, et al. (2004). "Multivariate analysis of survival in inflammatory breast cancer: impact of intensity of chemotherapy in multimodality treatment." Bone Marrow Transplant **33**(9): 913-20.

The prognosis of inflammatory breast cancer (IBC) is poor. We evaluated clinical and biopathological characteristics that could affect survival in 74 women with nonmetastatic IBC consecutively treated in our institution between 1976 and 2000. Patients received primary anthracycline-based chemotherapy at conventional doses (n=20) or high-dose chemotherapy (HDC) with haematopoietic stem cell support (HSCS) (n=54). After chemotherapy, 84% of patients underwent mastectomy, 95% were given radiotherapy and 55% tamoxifen. Immunohistochemistry data (ER, PR, ERBB2, P53) on pre-chemotherapy specimens suggested strong differences between IBC and non-IBC. The rate of pathological complete response to chemotherapy was 26% (27% with HDC and 17% with conventional doses, not significant). No single factor was found predictive of response. With a median follow-up of 48 months after diagnosis, the 5-year projected disease-free survival (DFS) was 24% and overall survival (OS) 41%. In multivariate analysis, the strongest independent prognostic factor was the delivery of HDC. The 5-year DFS and OS of patients were respectively 28 and 50% with HDC and 15 and 18% with conventional chemotherapy. These results and comparisons with other series of patients suggest a role for HDC with HSCS as part of the therapeutic approach in IBC. Further prospective studies are required to confirm it.

Brozova, M., Z. Kleibl, et al. (2007). "Establishment, growth and in vivo differentiation of a new clonal human cell line, EM-G3, derived from breast cancer progenitors." Breast Cancer Res Treat **103**(2): 247-57.

A new clonal cell line, EM-G3, was derived from a primary lesion of human infiltrating ductal breast carcinoma. The line consisted of cuboidal cells with occasional appearance of more differentiated branched cells apparently involved in cell-to-cell communication. The EM-G3 cells, population doubling time 34 h, are dependent on the epidermal growth factor. Multicolor fluorescence in situ hybridization (mFISH) analysis demonstrated a stable diploid genome with several genetic changes. Immunocytochemical analysis of EM-G3 in vitro revealed positivity for keratins (K) K5, K14, K18, nuclear protein p63, epithelial membrane antigen (EMA) and other proteins indicative of a pattern of mammary epithelium bipotent progenitors. Detection of integrins alpha-6, beta-1, and protein CD44 by cDNA array also pointed to the character of basal/stem cells. In contrast, dominant cells in the human original tumor showed the luminal character (K18+, K19+, K5-, K14-, and p63-). However, cells with the immunocytochemical profile similar to that of cultured EM-G3 cells were found in minor clusters in the patient's tumor sections. The EM-G3 cells formed limited tumors in nu/nu mice. The cells in mouse tumors were organized in primitive ductal-like structures consisting of 1-3 large central luminal-like cells (EMA+) surrounded by peripheral myoepithelial-like cells (p63+/EMA-). The large central cells gradually disintegrated, forming a pseudolumen. Apparently, EM-G3 cells are able to partially differentiate in vivo as well as in vitro. Our results indicate that EM-G3 cells were derived from a premalignant population of common progenitors of luminal and myoepithelial cells that were immortalized in an early stage of tumorigenesis.

Cooper, B. W., T. J. Moss, et al. (1998). "Occult tumor contamination of hematopoietic stem-cell products does not affect clinical outcome of autologous transplantation in patients with metastatic breast cancer." J Clin Oncol **16**(11): 3509-17.
　　　To determine whether occult tumor contamination of autologous bone marrow or peripheral-blood progenitor cells (PBPC) influences clinical outcome after high-dose chemotherapy in patients with stage IV breast cancer. We used an immunocytochemical assay capable of detecting one tumor cell in 5 x 10(5) hematopoietic cells to analyze bone marrow and/or PBPC collections obtained from 57 consecutive women with chemotherapy-sensitive metastatic breast cancer who received high-dose chemotherapy. The influence of occult tumor on time to progression, overall survival, and first site of recurrence (old or new) was studied. Twenty-three of 57 (40%) patients received bone marrow (n=6) or peripheral-blood progenitor collections (n=17) that

contained microscopic cancer. Median time to progression and overall survival were 9 and 22 months in patients who did not receive infused tumor cells, compared with 10 and 24 months, respectively, in those who received occult tumor (P=not significant [NS]). Worse survival, but not time to progression, was observed in six patients who received > or = 2/100,000 tumor cells. Regardless of whether occult tumor was infused, the majority of relapses occurred in prior, rather than new sites of disease. Three patients who received stem-cell products contaminated by microscopic breast cancer remain free from progression at 21+, 47+, and 52+ months. Microscopic tumor was frequently detected by immunocytochemistry in hematopoietic stem-cell products, but did not predict for inferior treatment outcome in this cohort of patients with metastatic breast cancer. Quantitative information regarding infused tumor burden may have prognostic significance.

Dal Cortivo, L., P. H. Cottu, et al. (2001). "Residual tumor cell contamination in peripheral blood stem cells collections of 117 breast cancer patients evaluated by immunocytochemical technique." J Hematother Stem Cell Res **10**(6): 855-62.
　　　During the last years, high-dose chemotherapy and hematopoietic stem cell support have been thought to improve the treatment of poor-prognosis breast cancer. Nevertheless, the question remained as to whether the reinfusion of contaminating residual malignant cells could contribute to relapse. By using an immunocytochemical method, we have analyzed the tumor cell contamination of peripheral blood stem cells (PBSC) collected from advanced breast cancer patients. We studied 153 PBSC samples from 117 stage III and IV breast cancer patients and compared two screening methods-the usual microscopic observation and the automated cellular image analysis system (ACIS-assisted) screening. With manual observation, we found that 7 of 117 patients (5.9%) presented circulating epithelial tumor cells in 9 of 153 (5.8%) PBSC analyzed, whereas automated screening allowed positive detection in 15 of the same 117 patients (12.8%) and in 18 of the 153 PBSC (11.7%). No difference was found between presence or absence of circulating tumor cells and previous chemotherapy treatment (p = 0.5) or stage TNM (p = 0.13) in this group of poor-prognosis breast cancer. We did not find incidence of infusion of contaminated PBSC on overall survival or time to progression.

Diallo, R., E. Ting, et al. (2006). "C-kit expression in high-risk breast cancer subgroup treated with high-

dose or conventional dose-dense chemotherapy." Verh Dtsch Ges Pathol **90**: 177-85.

The proto-oncogene c-kit is known to be expressed in poorly differentiated breast cancer. In this study, we retrospectively evaluated the prognostic and predictive impact of c-kit in a high risk subgroup of breast cancer patients (>9 axillary node metastases) who received high-dose (HDCT) or dose-dense (DDCT) conventional chemotherapy and correlated these findings with the expression of the basal-type markers CK5 and CK 17, estrogen (ER) and progesterone (PR) receptor, Her-2/neu and MIB 1. C-kit, CK5, CK17, ER, PR, Her-2/neu and MIBI expression was evaluated immunohistochemically using tissue microarrays containing breast cancer samples from 236 patients who were randomized to the WSG AM01 trial (median follow-up of 60 months). There was a significant overall survival (OS) benefit for patients receiving HDCT compared to DDCT (p = 0.027). C-KIT expression was found in 12 % of all breast cancers and correlated with a poorer OS in multivariate analysis (p = 0.051). Furthermore, c-kit correlated with high grade (p = 0.019), CK5- and CK17-positivity (p <0.0001 and p = 0.001, respectively) and ER- and PR-negativity (p = 0.04 and p = 0.008, respectively). In contrast to CK5 and CK17, patients with c-kit positive breast cancers revealed no benefit from high-dose chemotherapy. These findings underline that c-kit expression represents an independent negative prognostic marker in high-risk breast cancer. Correlation with CK5 +/CK17+ and ER-/PR-suggests that c-kit positive carcinomas are at least partly of basal-type.

Dickson, B. C., A. M. Mulligan, et al. (2007). "High-level JAG1 mRNA and protein predict poor outcome in breast cancer." Mod Pathol **20**(6): 685-93.

Notch receptors regulate cell fate determination, stem cell self-renewal, proliferation and apoptosis. We previously reported that elevated mRNA expression of the Notch ligand JAG1 identifies breast cancer patients with a poor prognosis. Here we show through immunohistochemical analysis of the same breast cancer cases (N=127) that patients with tumors expressing high levels of JAG1 protein had a worse outcome than those with tumors expressing low levels (10-year survival 26 vs 48%, and median survival 63 vs 108 months, respectively; P=0.03). We also describe the novel application of the Allred score to quantify JAG1 mRNA and protein expression levels. Using the Allred score, patients with tumors expressing high levels of JAG1 mRNA had a worse outcome than those with tumors expressing low levels (10-year survival 16 vs 47%, and median survival 43 months vs 100 months, respectively; P<0.001). Interestingly, when tumors

were classified as either high or low for JAG1 mRNA or protein expression, there was only 65% agreement (kappa=0.08) between the two methods of expression analysis. When JAG1 mRNA and protein data were combined, patients with tumors expressing low levels of both had a 10-year survival of 53% and median survival of 131 months. In comparison, patients with tumors expressing either high levels of JAG1 protein, mRNA or both had reduced 10-year survival and median survival (31%, 19%, 11% and 77, 43, 23 months respectively; P<0.0001). There was marginal evidence of an interaction effect (P=0.055), which indicated that the prognostic value of JAG1 protein was limited to the JAG1 mRNA-low subgroup. These data show that the Allred score can be used to rapidly quantify JAG1 mRNA and protein levels in breast cancer to identify patients who have a significant survival disadvantage and who may benefit from therapies (such as gamma-secretase inhibitors) that target signaling through the Notch pathway.

Dowsett, M., A. Goldhirsch, et al. (2007). "International Web-based consultation on priorities for translational breast cancer research." Breast Cancer Res **9**(6): R81.

BACKGROUND: Large numbers of translational breast cancer research topics have been completed or are underway, but they differ widely in their immediate and/or future importance to clinical management. We therefore conducted an international Web-based consultation of breast cancer professionals to identify the topics most widely considered to be of highest priority. Potential participants were contacted via two large e-mail databases and asked to register, at a Web site, the issues that they felt to be of highest priority. Four hundred nine questions were reduced by a steering committee to 70 unique issues, and registrants were asked to select the 6 questions they considered to be the most important. Votes were recorded from 420 voters (2,520 votes) from 48 countries, with 48% of voters coming from North America. Half of the voters identified themselves as clinicians, with the remainder being academics, research scientists, or pathologists. The highest priority was to identify molecular signatures to select patients who could be spared chemotherapy, which gained about 50% more votes than the second topic and was consistently voted top by voters in North America, Europe, and the rest of the world. Research scientists voted the determination of the role of stem cells in breast cancer development, progression, and treatment sensitivity as the most important issue, but this was considered the sixth priority for clinicians and fourth overall. This exercise may bring a greater focus of research resources onto issues voted as top priorities.

Fehm, T., S. Braun, et al. (2006). "A concept for the standardized detection of disseminated tumor cells in bone marrow from patients with primary breast cancer and its clinical implementation." Cancer **107**(5): 885-92.

Numerous single-institutional studies and a large pooled analysis have demonstrated that the presence of disseminated tumor cells (DTCs) in the bone marrow (BM) from patients with primary, nonmetastatic breast cancer (Stages I-III) is associated with impaired prognosis. To date, sampling of BM and assessment of DTCs is not considered a routine procedure in the clinical management of breast cancer patients; however, emerging data suggest a future role for risk stratification and monitoring of therapeutic efficacy. Because these clinical options need to be evaluated in trials to verify the principle of this concept in the clinical setting, agreement on the standardized detection of DTCs is necessary. Consequently, the German, Austrian, and Swiss Societies for Senology recently formed a panel 1) to review and discuss the existing methodologies, 2) to find a consensus for a standardized detection of DTCs, and 3) to explore the options for its clinical implementation.

Glinsky, G. V., J. E. Price, et al. (1996). "Inhibition of human breast cancer metastasis in nude mice by synthetic glycoamines." Cancer Res **56**(23): 5319-24.

We have examined the effect of synthetic low molecular weight glycoamine analogues on the metastasis of MDA-MB-435 human breast carcinoma xenografts growing in the mammary fat pads of nude mice. Initial in vitro screening of a panel of synthetic glycoamines was performed using a clonogenic growth assay in 0.9% agarose. Eight of nine compounds manifested a significant dose-dependent inhibition of colony formation by MDA-MB-435 cells in 0.9% agarose. The relative activity ranks of the compounds, based on ID50S independently determined for each synthetic glycoamine analogue, identified N-(1-deoxy-D-lactulos-1-yl)-L-leucine (Lac-L-Leu), N-(1-deoxy-D-fructos-1-yl)-D-leucine (Fru-D-Leu), N-(1-deoxy-D-fructos-1-yl)-L-phenylalanine, and N-(1-deoxy-D-fructos-1-yl)-L-leucine as the most effective inhibitors of colony formation. Two separate experimental treatment protocols were used to examine the effect of selected synthetic glycoamines on human breast cancer growth and metastasis in athymic nude mice. Group A mice were treated intraperitoneally daily from day 2 after injection of the breast cancer cells until the end of the experiment (17 weeks). In group B, the mice were untreated until the mean tumor diameter was 10 mm, at which time daily i.p. treatment began. After 7 days,

the primary tumors were resected, and the mice were treated for an additional 4 weeks (a total of 5 weeks of treatment). The synthetic glycoamines did not have significant antitumor effects, and there was no difference in the tumor incidence or tumor growth rates in mice treated continuously with synthetic glycoamines or PBS. The significant antimetastatic activity of synthetic glycoamines was detected in both experimental treatment protocols. In mice continuously treated with synthetic glycoamines according to protocol A, the incidence of metastasis was decreased 4.6-fold (P = 0.014) and 2.7-fold (P = 0.031) in mice treated with Fru-D-Leu and Lac-L-Leu, respectively. In mice in protocol B, the incidence of pulmonary metastasis was decreased 1.9-fold (P = 0.069) and 2.5-fold (P = 0.042) in mice treated with Fru-D-Leu and Lac-L-Leu, respectively. Correspondingly, the average number of spontaneous pulmonary metastases was reduced from 37 in control mice to 0.2 (P = 0.005) and 0.9 (P < 0.02) in mice treated according to the protocol A with Fru-D-Leu and Lac-L-Leu, respectively. Treatment of mice with N-(1-deoxy-D-fructos-1-yl)-L-leucine did not have significant antimetastatic effects, and no reduction in metastasis incidence or number was noted in mice treated with this synthetic glycoamine analogue. The treated animals had no apparent toxicity from chronic daily injection (up to 17 weeks of treatment) of synthetic glycoamines, and no obvious pathology was noted in the histological slides of the livers, kidneys, or spleens of the treated mice. Therefore, we have identified two synthetic glycoamines (Fru-D-Leu and Lac-L-Leu) that were the effective inhibitors of spontaneous human breast cancer metastasis in nude mice. Potential mechanisms for antimetastatic activity of synthetic glycoamines may include the inhibition of beta-galectin-mediated homotypic cancer cell aggregation and induction of apoptosis in target cells.

Goeminne, J. C., T. Guillaume, et al. (1999). "Unreliability of carcinoembryonic antigen (CEA) reverse transcriptase-polymerase chain reaction (RT-PCR) in detecting contaminating breast cancer cells in peripheral blood stem cells due to induction of CEA by growth factors." Bone Marrow Transplant **24**(7): 769-75.

RT-PCR is increasingly used for the detection of minimal residual disease in solid tumors. Carcinoembryonic antigen (CEA) RT-PCR seemed to be highly specific for detection of tumor cells when tested on PBMC. A very high frequency of RT-PCR amplification product for CEA in PBSC from breast cancer patients mobilized with G-CSF was found. However, this result contrasted with tumor cell detection by immunocytochemistry (ICC) which showed no correlation with RT-PCR results. In

addition, CEA mRNA was amplified in most G-CSF-mobilized PBSC samples derived from patients with hematological malignances and from healthy donors of allogeneic stem cells, although no circulating epithelial cells could be demonstrated by ICC. CEA RT-PCR expression was observed in PBMC from healthy individuals incubated in vitro with G-CSF. These data suggest that CEA transcription can be induced by G-CSF, resulting in a loss of specificity of CEA RT-PCR for tumor cell detection in PBMC. We conclude, CEA RT-PCR may not be recommended to detect tumor cell contamination in peripheral blood from patients treated with G-CSF. This may have implications on tumor cell detection by RT-PCR in tissues where endogenous or exogenous growth factors may induce the transcription of CEA or other genes.

Hawes, D., A. M. Neville, et al. (2001). "Detection of occult metastasis in patients with breast cancer." Semin Surg Oncol **20**(4): 312-8.
        The most important factor affecting the outcome of patients with invasive cancer is whether the tumor has spread, either regionally (to regional lymph nodes) or systemically. However, a proportion of patients with no evidence of systemic dissemination will develop recurrent disease after primary "curative" therapy. Clearly, these patients had occult systemic spread of disease that was undetectable by routinely employed methods (careful pathological, clinical, biochemical, and radiological evaluation). In addition, the success of adjuvant therapy is assumed to stem from its ability to eradicate occult metastases before they become clinically evident. Therefore, methods for the detection of occult metastases in patients with the earliest stage of cancer, i.e., prior to detection of metastases by any other clinical or pathological analysis, have received a great deal of attention.

Hays, A. P., A. Naini, et al. (2006). "Sporadic amyotrophic lateral sclerosis and breast cancer: Hyaline conglomerate inclusions lead to identification of SOD1 mutation." J Neurol Sci **242**(1-2): 67-9.
        Autopsy of patients with sporadic amyotrophic lateral sclerosis (ALS) rarely provides clues to a genetic etiology. We studied a 66-year-old woman who developed progressive weakness, fasciculations and upper motor neuron signs 1 year after mastectomy and chemotherapy for a breast carcinoma. She died 14 months after the onset of neurological symptoms. Autopsy showed characteristic features of ALS but also with posterior column degeneration and conglomerate hyaline inclusions. These features suggested a mutation of SOD1 mutation although no other family members were affected. DNA analysis of autopsy tissue indicated an I113T SOD1 mutation.

Hensel, M., A. D. Ho, et al. (2003). "Prognostic markers for survival after high-dose chemotherapy with autologous stem-cell transplantation for breast cancer." Ann Oncol **14**(2): 341.

Hensel, M., A. Schneeweiss, et al. (2002). "Stem cell dose and tumorbiologic parameters as prognostic markers for patients with metastatic breast cancer undergoing high-dose chemotherapy with autologous blood stem cell support." Stem Cells **20**(1): 32-40.
        We report on the prognostic significance of tumorbiologic parameters and CD34(+) cell dose in 120 patients with metastatic breast cancer (MBC) who received high-dose chemotherapy (HDCT) with autologous blood stem cell transplantation as first-line treatment. Her2/neu, p53, Ki67, and bcl-2 protein expression were studied using immunohistochemical staining on formalin-fixed, paraffin-embedded primary tumor sections. DNA content of tumor cells (DNA-index) and tumor cell proliferation (S-phase fraction) were measured by DNA flow cytometry. The relationship between these parameters and the CD34(+) cell dose and progression free (PFS) and overall survival (OS) was analyzed. With a median follow-up period of 40 months (range, 7-89 months), no more than two metastatic sites (relative risk [RR] = 3.84 [95% confidence interval (CI) 1.49-10]; p =.005) and hyperploidy (RR = 2.58 [95% CI 1.26-5.26]; p =.009) were independent predictors of longer PFS according to multivariate analysis. Independent prognostic factors of longer OS included one or two metastatic sites (RR = 4.16 [95% CI 1.96-4.16]; p <.001), a positive combined hormone receptor status (RR = 2.45 [95% CI 1.45-4.14]; p =.001) and a high number of infused stem cells (>7.8 x 10(6) CD34(+) cells per kg body weight) (RR = 2.0 [95% CI 1.17-3.42]; p =.01). In conclusion, positive hormone receptors, < or =2 metastatic sites, high DNA-index and high CD34(+) cell dose (>7.8 x 10(6) CD34(+) cells per kg) are predictors for a favorable outcome after autotransplantation for MBC. Our observation might indicate a favorable effect of HDCT in MBC patients with overexpression of Her2/neu who might have a worse prognosis when treated with conventional chemotherapy.

Hensel, M., A. Schneeweiss, et al. (2002). "P53 is the strongest predictor of survival in high-risk primary breast cancer patients undergoing high-dose chemotherapy with autologous blood stem cell support." Int J Cancer **100**(3): 290-6.
        Our purpose was to determine the predictive value of tumor biologic parameters in patients with

HRPBC who received HDCT with ASCT as first-line treatment. From September 1992 to May 2000, 149 stage II or III HRPBC patients were enrolled in a single-arm trial using a tandem HDCT regimen followed by ASCT. Her2/neu, p53, Ki67 and bcl-2 protein expression was studied using immunohistochemic staining on formalin-fixed, paraffin-embedded primary tumor sections. DNA content of tumor cells (DNA index) and tumor cell proliferation (SPF) were measured by DNA flow cytometry. The relationship between these tumor biologic parameters, on the one hand, and DFS, DDFS and OS, on the other, was analyzed. With a median follow-up of 43 months (range 7-106), p53 protein accumulation (p = 0.000004), negative combined hormone receptor status (p = 0.003) and Her2/neu overexpression (p = 0.02) were significant negative predictors of OS in univariate analysis. A poorer DFS was associated with p53 positivity (p = 0.04) and nodal ratio > or = 0.8 (p = 0.008). Poorer DDFS was associated with p53 positivity (p = 0.03). In multivariate analysis, Her2/neu overexpression (RR = 3.86, 95% CI 1.48-10.1, p = 0.006) and p53 overexpression (RR = 6.06, 95% CI 2.22-16.52, p < 0.001) proved to be independent predictors of adverse OS. p53 overexpression was the only independent predictor of DFS (RR = 2.21, 95% CI 1.07-4.57, p = 0.03). p53 overexpression and Her2/neu overexpression are independent negative predictors of survival in HRPBC treated with HDCT. The adverse impact of these biologic features was probably not altered by HDCT. For HRPBC patients with tumors not overexpressing Her2/neu or p53, HDCT may be an appropriate approach to achieve long-term survival and tumor control.

Hergueta-Redondo, M., J. Palacios, et al. (2008). ""New" molecular taxonomy in breast cancer." Clin Transl Oncol **10**(12): 777-85.

Advances in the analysis of expression profiles, using genomic techniques, have revealed the high heterogeneity present in breast cancers. These approaches have served to identify different breast cancer subgroups with specific molecular characteristics that could sub-classify these tumours as carcinomas expressing hormone receptors, denominated Luminal subtype, and tumours with negative expression of hormone receptors, the Basal and HER2+ phenotypes. Therefore, during recent years, identification of markers characteristic of each subtype has been the focus of many research groups. All of these breast tumour subtypes probably have specific clinical and morphological features; however, this hypothesis needs to be confirmed by analysing more homogenous series. Although this "new" classification has limitations, it could be useful in the clinical practice, allowing not only a more accurate prognosis in breast cancer patients but also a selective treatment for each predefined subtype.

Hsieh, T. C., E. K. Wijeratne, et al. (2005). "Differential control of growth, cell cycle progression, and expression of NF-kappaB in human breast cancer cells MCF-7, MCF-10A, and MDA-MB-231 by ponicidin and oridonin, diterpenoids from the chinese herb Rabdosia rubescens." Biochem Biophys Res Commun **337**(1): 224-31.

Ponicidin and oridonin are novel diterpenoids isolated from Rabdosia rubescens. We tested their effects in MCF-7 and MDA-MB-231 cells, as representing low and high invasive breast carcinoma, with normal MCF-10A cells. Clonogenicity and proliferation in MCF-7 cells were inhibited more significantly by ponicidin than oridonin, while the reverse was observed in MCF-10A cells. Ponicidin and oridonin induced S/G2M arrest and G1/S block in MCF-7 cells. In MCF-10A cells treated with either diterpenoid, induction of apoptosis was observed. Moreover, oridonin almost completely blocked MCF-10A progression from S to G2/M phase; in contrast, ponicidin-treated MCF-10A cells showed no discernable changes in cell cycle phase distribution. Neither diterpenoid affected growth of MDA-MB-231 cells, at the dose range effective for MCF-7 or MCF-10A cells. Ponicidin-treated MCF-7 cells expressed reduced levels of cyclin B1, cdc2, transcription factor E2F, and Rb including phosphorylation at S780. Less pronounced effects were found in cells treated with oridonin. Neither compound altered cyclin D1 and cdk4 in MCF-7 cells. In MCF-10A cells, oridonin was more active than ponicidin in inhibiting the expression of cyclin B1, cdc2, S780-phosphorylated Rb, and E2F. To further investigate induction of apoptosis in MCF-10A cells, we measured changes in NF-kappaB. Decreases in p65 or p50 forms of NF-kappaB and its upstream regulator I-kappaB were found in oridonin-treated MCF-10A and not MCF-7 cells. Taken together, these results provide a mechanistic framework for the cellular effects of ponicidin and oridonin in different stage breast cancer cells.

Huang, G. L., X. H. Zhang, et al. (2009). "Clinical significance of miR-21 expression in breast cancer: SYBR-Green I-based real-time RT-PCR study of invasive ductal carcinoma." Oncol Rep **21**(3): 673-9.

Growing evidence suggests microRNAs (miRNAs) have an important role in tumorigenesis. MicroRNA-21 (miR-21) is up-regulated in many malignant tumors, including breast cancer. Its association with clinicopathologic features and expression of PTEN (phosphatase and tensin homolog deleted on chromosome 10), one of its target genes, in

breast cancer has not been reported systematically. To further determine the potential involvement of miR-21 in breast cancer, we have evaluated the expression level of miR-21 by stem-loop real-time RT-PCR based on SYBR-Green I in human invasive ductal carcinoma of the breast, and we have correlated the results with clinicopathologic features and PTEN protein expression. Matched non-tumor and tumor tissues of 40 human invasive ductal carcinoma of the breast were analyzed for miR-21 expression by stem-loop real-time RT-PCR based on SYBR-Green I. Immunohistochemistry (IHC) was used to estimate PTEN expression in tumor tissue. The expression levels of miR-21 were correlated with PTEN and commonly used clinicopathologic features of breast cancer. The stem-loop real-time RT-PCR based on SYBR-Green I was sensitive and specific enough to detect miR-21. Expression levels of miR-21 were significantly higher in tumor tissues than the levels in matched non-tumor tissues (P=0.000). Expression of miR-21 was negatively correlated with expression of PTEN (P=0.013). Up-regulated miR-21 expression was associated with lymph node positivity (P=0.01), higher proliferation index (ki67>10%) (P=0.03) and advanced breast cancer TNM clinical stage (P=0.021). These findings suggest that PTEN is possibly one of the targets of miR-21 in breast cancer and high expression of mir-21 indicates a more aggressive phenotype.

Kao, J., K. Salari, et al. (2009). "Molecular profiling of breast cancer cell lines defines relevant tumor models and provides a resource for cancer gene discovery." PLoS One **4**(7): e6146.

BACKGROUND: Breast cancer cell lines have been used widely to investigate breast cancer pathobiology and new therapies. Breast cancer is a molecularly heterogeneous disease, and it is important to understand how well and which cell lines best model that diversity. In particular, microarray studies have identified molecular subtypes-luminal A, luminal B, ERBB2-associated, basal-like and normal-like-with characteristic gene-expression patterns and underlying DNA copy number alterations (CNAs). Here, we studied a collection of breast cancer cell lines to catalog molecular profiles and to assess their relation to breast cancer subtypes. Whole-genome DNA microarrays were used to profile gene expression and CNAs in a collection of 52 widely-used breast cancer cell lines, and comparisons were made to existing profiles of primary breast tumors. Hierarchical clustering was used to identify gene-expression subtypes, and Gene Set Enrichment Analysis (GSEA) to discover biological features of those subtypes. Genomic and transcriptional profiles were integrated to discover within high-amplitude CNAs candidate cancer genes with coordinately altered gene copy number and expression. FINDINGS: Transcriptional profiling of breast cancer cell lines identified one luminal and two basal-like (A and B) subtypes. Luminal lines displayed an estrogen receptor (ER) signature and resembled luminal-A/B tumors, basal-A lines were associated with ETS-pathway and BRCA1 signatures and resembled basal-like tumors, and basal-B lines displayed mesenchymal and stem/progenitor-cell characteristics. Compared to tumors, cell lines exhibited similar patterns of CNA, but an overall higher complexity of CNA (genetically simple luminal-A tumors were not represented), and only partial conservation of subtype-specific CNAs. We identified 80 high-level DNA amplifications and 13 multi-copy deletions, and the resident genes with concomitantly altered gene-expression, highlighting known and novel candidate breast cancer genes. CONCLUSIONS: Overall, breast cancer cell lines were genetically more complex than tumors, but retained expression patterns with relevance to the luminal-basal subtype distinction. The compendium of molecular profiles defines cell lines suitable for investigations of subtype-specific pathobiology, cancer stem cell biology, biomarkers and therapies, and provides a resource for discovery of new breast cancer genes.

Kasimir-Bauer, S., S. Mayer, et al. (2001). "Survival of tumor cells in stem cell preparations and bone marrow of patients with high-risk or metastatic breast cancer after receiving dose-intensive or high-dose chemotherapy." Clin Cancer Res **7**(6): 1582-9.

We evaluated whether dose-intensive or high-dose chemotherapy can eliminate micrometastases in high-risk breast cancer patients. We monitored cytokeratin (CK)/17-1A positive cells in the bone marrow (BM) and peripheral blood stem cells (PBSC) and studied Her-2/neu serum levels of patients with locally advanced (n = 13; group 1) and metastatic breast cancer (n = 30; group 2) using immunomagnetic separation, immunocytochemistry, and ELISA. CK+ cells were found in the BM of 3 of 13 (23%) group 1 patients before but not after chemotherapy, resulting in an overall survival (OS) of 92% after a median follow-up of 33 months. Contamination of PBSC in 2 of 9 (22%) patients was not associated with decreased survival. In group 2 patients, the CK+ rate was 60% (18 of 30 patients) before and 40% (4 of 10 patients) after therapy with an OS rate of 43% after 29 months. PBSC samples were positive in 7 of 24 (29%) patients. CK+ BM and PBSC led to a rapid progress and short OS, whereas tumor cell-free BM and PBSC resulted in a mean OS of 30 months. The antigen 17-1A was detected on most CK+ cells in both patient groups before therapy,

on all of CK+ PBSC, and on CK+ cells in group 2 patients after therapy. Increased Her-2/neu levels were found in group 2 patients before chemotherapy. Micrometastatic cells are present in PBSC grafts and can survive even high-dose chemotherapy. The presence of immunotherapeutic target antigens supports the idea that a combined chemoimmunotherapy might be successful in eliminating minimal residual disease.

Keimling, M., J. Kaur, et al. (2008). "A sensitive test for the detection of specific DSB repair defects in primary cells from breast cancer specimens." Int J Cancer **123**(3): 730-6.

Increasing evidence indicates that breast cancer pathogenesis is linked with DNA double-strand break (DSB) repair dysfunction. This conclusion is based on advances in the study of functions of breast cancer susceptibility genes such as BRCA1 and BRCA2, on the identification of breast cancer-associated changes regarding the genetics, expression, and localization of multiple DSB repair factors, and on observations indicating enhanced radiation-induced chromosomal damage in cells from predisposed individuals and sporadic breast cancer patients. In this pilot study, we describe a sensitive method for the analysis of DSB repair functions in mammary carcinomas. Using this method we firstly document alterations in pathway-specific DSB repair activities in primary cells originating from familial as well as sporadic breast cancer. In particular, we identified increases in the mutagenic nonhomologous end joining and single-strand annealing mechanisms in sporadic breast cancers with wild-type BRCA1 and BRCA2, and, thus, similar phenotypes to tumors with mutant alleles of BRCA1 and BRCA2. This suggests that detection of error-prone DSB repair activities may be useful to extend the limits of genotypic characterization of high-risk susceptibility genes. This method may, therefore, serve as a marker for breast cancer risk assessment and, even more importantly, for the prediction of responsiveness to targeted therapies such as to inhibitors of poly(ADP-ribose)polymerase (PARP1).

Kim, Y. S., S. N. Konoplev, et al. (2001). "HER-2/neu overexpression as a poor prognostic factor for patients with metastatic breast cancer undergoing high-dose chemotherapy with autologous stem cell transplantation." Clin Cancer Res **7**(12): 4008-12.

High-dose chemotherapy with autologous stem cell transplantation (HDCT) produces a high tumor response rate for patients with metastatic breast cancer and have 20% long-term progression-free survival. Overexpression of HER-2/neu oncoprotein predicts outcome in patients with breast cancer given standard-dose chemotherapy. Therefore, we evaluated whether the HER-2/neu overexpression in the primary tumor predicts clinical outcome in patients with metastatic breast cancer given HDCT. A total of 236 patients were given standard-dose induction chemotherapy followed by stem cell collection; high-dose chemotherapy with cyclophosphamide, thiotepa, and carmustine; and stem cell infusion. HER-2/neu expression was assessed by immunostaining with anti-HER-2/neu e2-4001 monoclonal antibody in 63 patients. Clinical characteristics and survival were similar for patients with known and unknown HER-2/neu status. HER-2/neu was overexpressed in 22 of 63 tumors (35%). There was some tendency for HER-2/neu overexpression to be associated with the absence of estrogen or progesterone receptors. In considering the association of HER-2/neu expression with patient outcomes, HER-2/neu overexpression was associated with generally shorter overall survival (P = 0.02) and progression-free survival (P < 0.01), and this association persisted to a lesser extent after adjustment for differences in important prognostic factors between the two groups. We conclude that HER-2/neu overexpression may represent an additional prognostic factor for patients with metastatic breast cancer who undergo HDCT.

Klein, J. L., C. Hamm, et al. (2001). "High-dose chemotherapy and CD34-selected peripheral blood progenitor cell transplantation for patients with breast cancer metastatic to bone and/or bone marrow." Bone Marrow Transplant **28**(11): 1023-9.

Fifty women with breast cancer metastatic to bone or bone marrow involvement on light microscopy at the time of initial evaluation were treated with high-dose chemotherapy (HDC) and peripheral blood progenitor cell (PBPC) transplantation with CD34(+) cell selection using the Isolex 300i system. All patients received induction chemotherapy. PBPC were mobilized with chemotherapy and granulocyte colony-stimulating factor. The median CD34(+) progenitor purity was 94.7% (range 72-98.7%) and recovery 38.4% (range 21-60%). Forty-eight hours after HDC with cyclophosphamide, cisplatin and carmustine, PBPC were reinfused. Median time to neutrophil count >0.5 x 10(9)/l was 9 (range 9-12) days and to platelet transfusion independence 11 (4-30) days. These data demonstrate that selected CD34(+) PBPCs allow rapid hematologic reconstitution after HDC. During follow-up, 23% of patients developed herpes zoster. Two patients developed cytomegalovirus infections. Three patients developed fungal infections. The development of these infections was not associated with steroid use but appeared more frequently in patients with diabetes mellitus. Seventy-four per cent of patients received

steroids for pulmonary toxicity. Treatment-related mortality was 4%. Progression-free survival and overall survival at 35 months was 22.4% and 40.5%, with a median of 11.4 months and 15.4 months, respectively.

Kleinman, M. B., E. L. Wiley, et al. (1999). "Immunohistochemical detection of breast cancer cells in paired peripheral blood progenitor cell specimens collected after cytokine or cytokine and myelosuppressive chemotherapy." Bone Marrow Transplant **23**(12): 1297-301.

Mobilized peripheral blood progenitor cells (PBPC) from 30 patients with advanced breast cancer were studied for the presence of tumor cell contamination using a highly sensitive immunohistochemical technique with the capacity to detect one tumor cell in one million mononuclear cells. Aliquots of PBPC were obtained after 4 days of G-CSF and/or GM-CSF and again during G-CSF-stimulated recovery from myelosuppressive doses of cyclophosphamide. The overall incidence of tumor cell contamination was 23%, occurring in PBPC specimens from seven of 30 patients. All four cases in which tumor cells were detected after mobilization with cytokine alone also had tumor cells detected in PBPCs collected following chemotherapy and G-CSF. There were three cases in which malignant contamination was detected only in the specimens collected after cyclophosphamide. There was a greater frequency of tumor cell contamination in aphereses performed during G-CSF-stimulated recovery from cyclophosphamide than in collections primed by cytokine alone (13% vs 23%; P = 0.08), although this did not reach statistical significance. This trend suggests that collection of PBPC during cytokine-stimulated recovery from myelosuppressive chemotherapy may be associated with a greater risk of contamination with malignant cells than apheresis during mobilization with cytokines in the steady state.

Korsching, E., J. Packeisen, et al. (2005). "The origin of vimentin expression in invasive breast cancer: epithelial-mesenchymal transition, myoepithelial histogenesis or histogenesis from progenitor cells with bilinear differentiation potential?" J Pathol **206**(4): 451-7.

Vimentin expression is a rather rare finding in invasive breast cancer, and is associated with high tumour invasiveness and chemoresistance. It is currently explained by two different biological theories: direct histogenetic derivation from myoepithelial cells, and epithelial-mesenchymal transition (EMT) reflecting the end-stage of breast cancer dedifferentiation. In this study we aimed to obtain further insights into the biological hallmarks of

these vimentin-expressing breast cancers. We applied immunohistochemistry for vimentin and 15 other differentiation markers to a series of 364 invasive breast cancer cases, using tissue microarray technology. 7.7% of all tumours expressed vimentin. Almost all of these cases (19/21) were Grade 3 invasive ductal carcinomas, and the majority (13/21) of these were associated with a ductal in situ component. Vimentin expression was also seen in the respective in situ components and correlated positively with the expression of SMA, CD10, CK 5, p53, Mib-1 and EGFR. A negative correlation was seen for the expression of CK 8/18 and the oestrogen receptor. Vimentin-expressing carcinomas revealed a significantly higher average absolute number of cytogenetic alterations per case, but a significantly lower frequency of chromosome 16q losses compared to vimentin-negative cases. Our present results demonstrate that, despite analogies between vimentin-positive breast cancers and myoepithelial cells in their expression of differentiation-related proteins, neither myoepithelial histogenesis nor EMT can exclusively explain the biology of these distinct tumours. This is mainly supported by the significantly higher incidence of vimentin-expressing breast cancers compared to any other myoepithelial breast tumours and the fact that vimentin is already observed in ductal in situ components. We therefore propose the alternative hypothesis that vimentin-expressing breast carcinomas may derive from breast progenitor cells with bilinear (glandular and myoepithelial) differentiation potential.

Kouros-Mehr, H., S. K. Bechis, et al. (2008). "GATA-3 links tumor differentiation and dissemination in a luminal breast cancer model." Cancer Cell **13**(2): 141-52.

How breast cancers are able to disseminate and metastasize is poorly understood. Using a hyperplasia transplant system, we show that tumor dissemination and metastasis occur in discrete steps during tumor progression. Bioinformatic analysis revealed that loss of the transcription factor GATA-3 marked progression from adenoma to early carcinoma and onset of tumor dissemination. Restoration of GATA-3 in late carcinomas induced tumor differentiation and suppressed tumor dissemination. Targeted deletion of GATA-3 in early tumors led to apoptosis of differentiated cells, indicating that its loss is not sufficient for malignant conversion. Rather, malignant progression occurred with an expanding GATA-3-negative tumor cell population. These data indicate that GATA-3 regulates tumor differentiation and suppresses tumor dissemination in breast cancer.

Kruger, W., F. Togel, et al. (1999). "Tumour cell detection in G-CSF mobilised stem cell harvests of patients with breast cancer." Med Oncol **16**(1): 17-22.

Peripheral blood stem cells were mobilised with G-CSF from steady-state haemopoiesis after previous anthracyclin-containing standard dose chemotherapy in patients with high-risk breast cancer. 48 samples were obtained from patients with stage II-III breast cancer and > or = 10 lymph nodes, 15 samples from patients with chemotherapy sensitive metastatic disease, and 13 samples from women with inflammatory breast cancer. 44 samples were first or single leukaphereses and 32 samples were second or third harvests. Aliquots were searched for contaminating tumour cells by immunocytochemistry (IC) and cytokeratin-19 reverse transcriptase polymerase chain reaction rtPCR). The median count of MNCs examined by IC was 2 x 10(6); cDNA prepared from 2 x 10(7) cells was subjected to PCR. Fifty-nine samples were examined by immunocytochemistry, 36 samples by rtPCR, and 19 samples by both techniques. Samples investigated by IC and rtPCR were judged as positive if there was at least one positive test. On the whole, 42/79 (55.3%) of the samples were positive with an insignificant trend to a higher positivity rate in second or subsequent leukaphereses (52.3% vs 59.3%). The median tumour cell load per 10(6) MNCs was low with 0.5 (0-7) cells in all, and a total of 2.2 (0.5-7) cells in positive specimen. Differences in the cancer cell load of first and subsequent leukaphereses and between subgroups of patients were not found. PCR and IC gave consistent results in 63.2%. This phenomenon can be explained by the greater sensitivity of the molecular method and by a Poisson distribution of coharvested tumour cells in samples. Tumour cell contamination in G-CSF mobilised stem cells from patients with breast cancer from steady state haemopoiesis after preceding anthacyclin-containing chemotherapy is frequent, but the tumour cell load is low. To allow a comparison of different studies dealing with cancer cell contamination in stem cells, standardisation of assays is necessary.

Kruger, W., F. Togel, et al. (1999). "Improvement of breast cancer cell detection by immunomagnetic enrichment." Cytotherapy **1**(2): 135-9.

BACKGROUND: Contaminating breast cancer cells in leukapheresis harvested for reinfusion to rebuild hemopoiesis after high-dose therapy have been described by several investigators. Methods for tumor cell detection are conventional immunocytochemistry, culture techniques and reverse transcriptase PCR. The percentage of tumor cell positive leukaphereses shows a wide variation. An approach to clarify if these cells can induce systemic relapse is to characterize them molecular-genetically and immunologically, but these techniques require a sufficient cell count. We compared conventional immunocytochemistry with immunocytochemistry after immunomagnetic enrichment of cancer cells by HEA-125 magnetic microbeads for the detection of micrometastatic tumor cells. A total of 25 samples, consisting of 16 samples from G-CSF-mobilized peripheral stem cell harvests, eight BM aspirations and one peripheral blood sample were investigated without [median 2, range 1-3 x 10(6) MNCs] and after [median 5, range 1-10 x 10(7) MNCs] HEA-microbead selection. Additionally 10 buffy coat samples from healthy subjects were investigated. Using conventional immunocytochemistry, tumor cells could be detected in nine stem cell samples. Two BM samples and the blood sample (48%) were positive, with a median tumor cell load of 0 (0-12) cells per sample (mean: 2.4). By HEA-bead selection the rate of positivity could be increased to 88% (13 stem cell samples, eight marrow samples and one blood sample) with a median load of 6 (0-47) (mean 10.6) suspected cells (p < 0.007). However, calculation of recovery revealed tumor cell losses by immunobead selection. False positive results were not seen. DISCUSSION: We conclude first that immunomagnetic selection is an excellent and highly sensitive tool to enrich contaminating cancer cells from marrow and stem cell samples; second that the existence of real tumor cell negative stem cell harvests is doubtful; and third that immunobead selection delivers sufficient tumor cell counts for their further characterization by molecular and immunological methods.

Kruger, W. H., N. Kroger, et al. (2001). "Disseminated breast cancer cells prior to and after high-dose therapy." J Hematother Stem Cell Res **10**(5): 681-9.

Women with breast cancer in a distinct stage of disease can benefit from high-dose therapy (HDT) with autologous stem cell support; however, a significant number of these patients relapse despite this intensive treatment. This study investigates the persistence of malignancy on the single-cell level. A total of 194 data sets consisting of bone marrow and blood samples obtained prior to and after HDT and of aliquots of apheresis products were searched with immunocytochemistry and reverse transcriptase polymerase chain reaction (RT-PCR) for disseminated cancer cells. Presence of cancer cells in the marrow is frequent prior to and after HDT, but HDT reduces the amount of malignant cells in marrow significantly. In contrast, there was no effect on the number of circulating cancer cells. Reinfusion of contaminated apheresis products was surprisingly associated with a

low number of malignant cells in bone marrow after HDT and vice versa. The impact of disseminated tumor cells in bone marrow, apheresis, and peripheral blood on disease-free survival after HDT could be investigated in a total of 165 samples. Surprisingly, neither the presence of tumor cells in marrow or blood nor in apheresis was associated with a bad prognosis in Kaplan-Meyer survival analysis. These results suggest that apheresis products and bone marrow should be regarded as different biological compartments for epithelial cancer cells. It can be concluded that complete elimination of disseminated cancer cells by HDT is not always possible. The theory of reinduction of metastatic breast cancer by accidentally reinfused contaminants is not supported by this study so far. However, further research is necessary to identify distinct cell populations with the potentially capacity to metastasize.

Kruger, W. H., M. Stockschlader, et al. (1996). "Detection of cancer cells in peripheral blood stem cells of women with breast cancer by RT-PCR and cell culture." Bone Marrow Transplant **18 Suppl 1**: S18-20.

The benefit of high-dose therapy and blood stem cell reinfusion for women with high-risk breast cancer is currently under investigation. Contaminations of autologous blood stem cells with cancer cells have been described. Cancer micrometastases may be detected by immunocytochemistry, culture techniques and cytokeratin-19 mRNA reverse transcriptase PCR. Women with breast cancer received adjuvant HD-CTM with peripheral blood stem cell (PBSC) support after surgical therapy and 4 cycles conventional chemotherapy. Peripheral blood stem cells were mobilised by G-CsF and harvested after the third or fourth cycle of standard therapy. Aliquots of PBSC-collections $(10(7)-2*10(7)$ cells) were subjected to CK19-mRNA reverse transcriptase PCR. RNA was extracted by standard methods and reverse transcription was performed with MMV-RT. Integrity of RNA was checked by coamplification of housekeeping sequences. Aliquots of the RT-mix were subjected to PCR-amplification with outer and inner primer pairs, subsequently. A second aliquot of $2*10(7)$ cells was cultured over 42 days in liquid culture. Cytospins were prepared weekly from cultured cells and evaluated by light microscopy with or without prior immunocytochemistry. Ten leukaphereses from 6 women were available for PCR-analysis and cell culture. Six leukaphereses were negative for CK19-mRNA and for detection of cancer cells by culture technique, two samples were positive for CK19-mRNA and culturally enriched cells and two samples were positive for CK19-mRNA and

negative for cultured cancer cells. No sample was positive for cultured cells and negative for CK19-mRNA. Overall, the results corresponded in 80%. Two sensitive techniques for the detection of cancer micrometastases were applied to aliquots from 10 leukaphereses of six breast cancer patients with corresponding results in 80%. PCR-mediated detection of cancer cells was confirmed by culture technique and light microscopy, however, further comparison of CK19-PCR with standard techniques like cell culture and immunocytochemistry is still necessary.

Lalle, M., L. De Rosa, et al. (2000). "Detection of breast cancer cells in the bone marrow or peripheral blood: methods and prognostic significance." Tumori **86**(3): 183-90.

Tumor cells can reach every anatomic district, organ and tissue through the peripheral blood circulation. Tumor cell shedding is considered an early event in the multi-phase process of metastasis, and the possibility of detecting tumor cells in the bloodstream and/or bone marrow before clinical evidence of distant metastases needs to be explored. The use of new sophisticated diagnostic and investigative techniques has boosted the study of tumor cell contamination of bone marrow and peripheral blood. Molecular techniques, such as reverse-transcriptase polymerase chain reaction, may be useful tools to reach this target, but, today, immunocytochemistry is still considered the gold standard to assess new techniques to detect isolated tumor cells in hematopoietic tissue. Little is known about the biology of isolated tumor cells in the peripheral blood or bone marrow. Two crucial points need to be evaluated: the identification of specific markers of breast cancer cells with clonogenic potential and their biologic properties, and the prognostic impact of the detection of isolated tumor cells in the bone marrow or peripheral blood stem cell collections.

Lawson, J. C., G. L. Blatch, et al. (2009). "Cancer stem cells in breast cancer and metastasis." Breast Cancer Res Treat **118**(2): 241-54.

The cancer stem cell theory poses that cancers develop from a subset of malignant cells that possess stem cell characteristics and has been proposed to account for the development of a variety of malignancies, including breast cancer. These cancer stem cells (CSC) possess characteristics of both stem cells and cancer cells, in that they have the properties of self-renewal, asymmetric cell division, resistance to apoptosis, independent growth, tumourigenicity and metastatic potential. A CSC origin for breast cancer can neatly explain both the heterogeneity of breast

cancers and the relapse of the tumours after treatment. However, many reports on CSC in the breast are contradictory. There is variation with respect to how breast cancer stem cells should be identified, their characteristics and a possible lack of correlation between clinical outcome and breast cancer stem cell status of a tumour. These combined factors have made breast cancer stem cells a highly contentious issue. In this review, we highlight the progress in the analysis of cancer stem cells, with an emphasis on breast cancer.

Lee, J. E., H. J. Kim, et al. (2005). "Neogenin expression may be inversely correlated to the tumorigenicity of human breast cancer." BMC Cancer **5**: 154.

BACKGROUND: Neogenin is expressed in cap cells that have been suggested to be mammary stem or precursor cells. Neogenin is known to play an important role in mammary morphogenesis; however its relationship to tumorigenesis remains to be elucidated. To compare the expression levels of neogenin in cells with different tumorigenicity, the expression levels in M13SV1, M13SV1R2 and M13SV1R2N1 cells, which are immortalized derivatives of type I human breast epithelial cells, were evaluated. Then we measured the expression level of neogenin in paired normal and cancer tissues from eight breast cancer patients. Tissue array analysis was performed for 54 human breast tissue samples with different histology, and the results were divided into four categories (none, weak, moderate, strong) by a single well-trained blinded pathologist and statistically analyzed. The nontumorigenic M13SV1 cells and normal tissues showed stronger expression of neogenin than the M13SV1R2N1 cells and the paired cancer tissues. In the tissue array, all (8/8) of the normal breast tissues showed strong neogenin expression, while 93.5% (43/46) of breast cancer tissues had either no expression or only moderate levels of neogenin expression. There was a significant difference, in the expression level of neogenin, in comparisons between normal and infiltrating ductal carcinoma (p < 0.001). Neogenin may play a role in mammary carcinogenesis as well as morphogenesis, and the expression may be inversely correlated with mammary carcinogenicity. The value of neogenin as a potential prognostic factor needs further evaluation.

Leinung, S., P. Wurl, et al. (2000). "Detection of cytokeratin-positive cells in bone marrow in breast cancer and colorectal carcinoma in comparison with other factors of prognosis." J Hematother Stem Cell Res **9**(6): 905-11.

A prospective study is presented in which 293 patients suffering from breast cancer and colorectal carcinoma were analyzed for prognostic relevance of detected isolated disseminated tumor cells in bone marrow (IDTBM). The patients underwent surgery in the period from 1995 to 1997 and remained under observation until 1999. The monoclonal antibody A 45-B/B3 was used in the standard immuno-cytochemical method for detecting IDTBM, which represented an independent prognostic factor for survival time in patients with breast cancer or colorectal cancer. In breast cancer, when IDTBM were detected, the survival period was reduced by at least half. When disseminated tumor cells containing the A45-B/B3 antibody were detected in bone marrow, the risk of an earlier relapse of the tumor increased at least fourfold. In colorectal cancer, detection of IDTBM reduced survival time by a factor of 1.2-4.3. The risk of earlier relapse increased when disseminated tumor cells containing the A45-B/B3 antibody were detected in bone marrow by 2.8-8.1. Therefore, the use of IDTBM as an independent prognostic factor would provide an important method for determining the pathological stage of various cancers. Standardization of this technique into a generally accepted method would be especially desirable in treatment of patients with breast or colorectal cancer.

Ling, L. J., S. Wang, et al. (2008). "A novel mouse model of human breast cancer stem-like cells with high CD44+CD24-/lower phenotype metastasis to human bone." Chin Med J (Engl) **121**(20): 1980-6.

BACKGROUND: A satisfactory animal model of breast cancer metastasizing to bone is unavailable. In this study, we used human breast cancer stem-like cells and human bone to build a novel "human-source" model of human breast cancer skeletal metastasis. Human breast cancer stem-like cells, the CD44+/CD24-/lower subpopulation, was separated and cultured. Before injection with the stem-like cells, mice were implanted with human bone in the right or left dorsal flanks. Animals in Groups A, B, and C were injected with 1 x 10(5), 1 x 10(6) human breast cancer stem-like cells, and 1 x 10(6) parental MDA-MB-231 cells, respectively. A positive control group (D) without implantation of human bone was also injected with 1 x 10(6) MDA-MB-231 cells. Immunohistochemistry was performed for determination of CD34, CD105, smooth muscle antibody, CD44, CD24, cytokine, CXC chemokine receptor-4 (CXCR4), and osteopontin (OPN). mRNA levels of CD44, CD24, CXCR4, and OPN in bone metastasis tissues were analyzed by real-time quantitative polymerase chain reaction (PCR). Our results demonstrated that cells in implanted human bones of group B, which received 1 x 10(6) cancer stem-like cells, stained strongly positive for CD44,

CXCR4, and OPN, whereas those of other groups showed no or minimum staining. Moreover, group B had the highest incidence of human bone metastasis (77.8%, P = 0.0230) and no accompaniment of other tissue metastasis. The real-time PCR showed an increase of CD44, CXCR4, and OPN mRNA in metastatic bone tissues in group B compared with those of groups C and D, however the expression of CD24 mRNA in group B were the lowest. CONCLUSIONS: In the novel "human source" model of breast cancer, breast cancer stem-like cells demonstrated a higher human bone-seeking ability. Its mechanism might be related to the higher expressions of CD44, CXCR4, and OPN, and the lower expression of CD24 in breast cancer stem-like cells.

Liu, M., M. C. Casimiro, et al. (2009). "p21CIP1 attenuates Ras- and c-Myc-dependent breast tumor epithelial mesenchymal transition and cancer stem cell-like gene expression in vivo." Proc Natl Acad Sci U S A **106**(45): 19035-9.

p21(CIP1/WAF1) is a downstream effector of tumor suppressors and functions as a cyclin-dependent kinase inhibitor to block cellular proliferation. Breast tumors may derive from self-renewing tumor-initiating cells (BT-ICs), which contribute to tumor progression, recurrence, and therapy resistance. The role of p21(CIP1) in regulating features of tumor stem cells in vivo is unknown. Herein, deletion of p21(CIP1), which enhanced the rate of tumorigenesis induced by mammary-targeted Ha-Ras or c-Myc, enhanced gene expression profiles and immunohistochemical features of epithelial mesenchymal transition (EMT) and putative cancer stem cells in vivo. Silencing of p21(CIP1) enhanced, and expression of p21(CIP1) repressed, features of EMT in transformed immortal human MEC lines. p21(CIP1) attenuated oncogene-induced BT-IC and mammosphere formation. Thus, the in vitro cell culture assays reflect the changes observed in vivo in transgenic mice. These findings establish a link between the loss of p21(CIP1) and the acquisition of breast cancer EMT and stem cell properties in vivo.

Malki, A., M. El-Saadani, et al. (2009). "Garlic constituent diallyl trisulfide induced apoptosis in MCF7 human breast cancer cells." Cancer Biol Ther **8**(22): 2175-85.

Identification of agents that are nontoxic but can delay onset and/or progression of breast cancer, which is the main leading cause of cancer-related deaths among women, is highly desirable. Garlic-derived organosulfur compounds (OSCs) have highly effective antitumor effects, but the mechanism has yet to be investigated. The aim of the present study was undertaken to examine the effect of diallyl trisulfide (DATS), a promising cancer chemopreventive constituent of garlic, on growth of two cell lines respectively, MCF-7 human breast cancer cells and nontumorigenic MCF-12a mammary epithelial cells. The effects of DATS were examined by MTT assay, clonogenic survival assay, ELISA based apoptotic assay, TUNEL assay, immunofluoresence staining, flow Cytometry, RT-PCR and western blot analysis. Garlic constituent diallyl trisulfide (DATS) suppresses viability of cultured MCF-7 and MCF-12a cells respectively by decreasing the percent of cells in G(2)/M and inducing apoptotic cell death. DATS-induced apoptosis was markedly elevated in MCF-7 cells compared with MCF-12a cells and this was correlated with elevated levels of cyclin B1. The results from semi-quantitative and real-time RT-PCR indicated that DATS-enhanced the expression levels of FAS and cyclin D1, but in contrast, downregulated the expression levels of Akt and Bcl-2. Furthermore, the DATS-induced apoptosis was correlated with induction of pro-apoptotic Bax protein and p53 protein expression was upregulated and translocation to nucleus in MCF-7 cells. Together, the results of the present study show, for the first time, that DATS administration might offer a novel strategy for the treatment of human breast cancer.

Mapara, M. Y., I. J. Korner, et al. (1999). "Combined positive/negative purging and transplantation of peripheral blood progenitor cell autografts in breast cancer patients: a pilot study." Exp Hematol **27**(1): 169-75.

This trial studied the feasibility and efficiency of a novel procedure of double purging to eliminate tumor cells from leukapheresis products of stage IV breast cancer patients. After induction and mobilization therapy, 35 leukapheresis products from 16 breast cancer patients were subjected to CD34+ enrichment (i.e., positive selection) with the Isolex 300 device and subsequent immunomagnetic depletion of tumor cells (i.e., negative selection) using a cocktail of three monoclonal antibodies directed against epithelial antigens. Patients with clinical response to induction chemotherapy proceeded to tandem high-dose chemotherapy, which consisted of melphalan (140 mg/m2) followed by retransfusion of the purged graft. After hematologic recovery, patients received ifosfamide 14 g/m2, carboplatin 1.5 g/m2, and etoposide 1.5g/m2 (ICE), again followed by autografting. After positive selection, a median purity of 96.6% CD34+ cells (range 48.4-99.2%) and a recovery of 56.8% (range 25.8-92.6%) were achieved. Subsequent negative purging resulted in a median CD34+ purity of 97.2%. Overall CD34+ recovery after both purging procedures was 51.1% (range 18.5-82.4%). Tumor cells were detectable in 8 of 16 (50%)

starting fractions before purging. After both purging cycles, only 1 of 16 autografts remained positive for tumor cells compared to 3 of 16 after CD34+ selection. A calculated purging efficiency of 2 to >4 log was achieved. Engraftment was rapid, reaching > or =500/microL neutrophils on day +10 after melphalan and on day +9 after ICE. A platelet count of > or =20.000/microL was reached on day +12 after melphalan and on day +11 after ICE. Thus, combining positive and negative purging is feasible, further enhances purging efficiency, and does not compromise the quality of the graft, leading to rapid engraftment after high-dose chemotherapy.

Martin, N. L., M. K. Saba-El-Leil, et al. (2005). "EN2 is a candidate oncogene in human breast cancer." Oncogene **24**(46): 6890-901.

Only a few critical oncogenes have been identified in the more commonly occurring cases of sporadic breast cancer. We provide evidence that EN2 is ectopically expressed in a subset of human breast cancer and may have a causal role in mammary tumorigenesis. Nontumorigenic mammary cell lines engineered to ectopically express En-2 have a marked reduction in their cycling time, lose cell contact inhibition, become sensitive to 17-AAG treatment, fail to differentiate when exposed to lactogenic hormones and induce mammary tumors when transplanted into cleared mammary glands of syngeneic hosts. RNA interference studies suggest that EN2 expression is required for the maintenance of the transformed phenotype of a human breast tumor cell line.

Mozziconacci, M. J., C. Arnoulet, et al. (1996). "[Contamination of cytapheresis by tumor cells: apropos of 39 breast cancer cases]." Bull Cancer **83**(8): 649-53.

The purpose of the present study was the detection of tumor cells in aphereses after mobilization of peripheral blood stem cells (PBSCs) with G-CSF from 39 breast cancer patients. Circulating tumor cells were searched using sensitive immunocytochemical technique (APAAP) with three anticytokeratin monoclonal antibodies. Counting of mononuclear cells and CD34 progenitor cells was also performed. Circulating tumor cells were detected in 35% of the patients. Cytokeratin-positive cells were detected in 45% of the patients of the metastatic group compared with 20% of the non metastatic one. Aphereses contamination was not correlated with lymph node involvement. Numbers of mononuclear cells and CD34 cells were not significantly different in positive and negative PBSCs collections. In our study, presence of tumor cells was associated with advanced clinical stage and could not be related to a higher CD34 cells mobilization by G-CSF.

Naume, B., E. Borgen, et al. (1998). "Increased sensitivity for detection of micrometastases in bone-marrow/peripheral-blood stem-cell products from breast-cancer patients by negative immunomagnetic separation." Int J Cancer **78**(5): 556-60.

Immunocytochemical detection (ICC) of isolated tumor cells in bone marrow (BM) is currently the most established method for monitoring early dissemination in epithelial cancer. However, the low sample size that can practically be analyzed restricts the sensitivity and reliability of the ICC method. To be able to analyze larger samples, a negative immunomagnetic separation (IMS) technique, utilising anti-CD45-conjugated Dynabeads, has been developed. Tumor-cell enrichment by depletion of CD45-expressing mononuclear cells (MNC) is followed by ICC for detection of the cytokeratin (CK)-positive (+) epithelial cells. In this study, bone-marrow samples (n = 165) and peripheral-blood-progenitor-cell (PBPC) apheresis products (n = 22) from breast-cancer patients were analyzed. The negative IMS analysis of 1 to 2 x 10(7) MNC was compared with ICC analysis of 2 x 10(6) unseparated MNC. Negative IMS resulted in 85% mean depletion of MNC. The results showed that 11.7% of the samples were positive by ICC analysis of unseparated MNC, as compared with 23.5% after negative IMS. In samples presenting > 10 CK+ cells, a 4-fold higher number of positive cells was detected by the negative IMS technique. Moreover, there was no evidence for general enrichment of false-positive cells. Altogether our results show that negative IMS is an efficient enrichment method for sensitive detection of CK+ cells in BM/PBPC products from breast-cancer patients. This opens the possibility for further characterization of micrometastatic tumor cells.

Nieto, Y., P. J. Cagnoni, et al. (2000). "Evaluation of the predictive value of Her-2/neu overexpression and p53 mutations in high-risk primary breast cancer patients treated with high-dose chemotherapy and autologous stem-cell transplantation." J Clin Oncol **18**(10): 2070-80.

To ascertain the predictive value of Her-2/neu overexpression and p53 mutations, assessed by immunohistochemistry, in high-risk primary breast cancer (HRPBC) treated with high-dose chemotherapy (HDCT). We obtained paraffin-embedded tumor blocks from 146 HRPBC patients previously enrolled at our program onto clinical trials of HDCT for four to nine involved axillary lymph nodes, > or = 10 involved axillary nodes, or inflammatory carcinoma. All patients received the same HDCT regimen, with cyclophosphamide, cisplatin, and carmustine (STAMP-I), followed by autologous stem-cell

transplantation. Median follow-up was 42 months (range, 5 to 90 months). The same pathologist, blinded to clinical outcome, reviewed all immunostained slides. Positive results for Her-2/neu and p53 were found in 44.5% and 34% of the patients, respectively. Positivity for Her-2/neu was significantly associated with increased risk of relapse and death. No correlation was found between p53 mutations and relapse-free survival (RFS) or overall survival (OS). Multivariate analyses included Her-2/neu overexpression and the following variables previously identified as independent predictors of outcome in this population: tumor size, nodal ratio (number of involved nodes/number of dissected nodes), and hormone receptor status. All four variables had independent value. Her-2/neu overexpression is an independent negative predictor of RFS and OS in HRPBC treated with HDCT. Its inclusion in our previously described predictive model increases the predictive capacity of this model for the low-risk subgroup. In contrast, p53 mutations lack predictive value in this setting.

Nieto, Y., W. A. Franklin, et al. (2004). "Prognostic significance of occult tumor cells in the apheresis products of patients with advanced breast cancer receiving high-dose chemotherapy and autologous hematopoietic progenitor cell support." Biol Blood Marrow Transplant **10**(6): 415-25.

We prospectively evaluated the prognostic significance of occult tumor cells (OTCs) contaminating the peripheral blood progenitor cell apheresis products of patients with advanced breast cancer receiving high-dose chemotherapy. Immunocytochemistry of peripheral blood progenitor cells was performed in 242 patients with high-risk primary breast cancer (HRPBC) and in 111 patients with metastatic breast cancer (MBC). OTCs were detected in 6.6% of HRPBC patients and in 16.2% of MBC patients (P = .005). In HRPBC, OTCs correlated with worse prognostic scores and larger tumor sizes, but not with axillary nodal status, hormone receptors, or HER2. In the MBC group, OTCs correlated with bone marrow involvement and with disease status at transplantation. The number of apheresis procedures was not associated with the risk of contamination. In HRPBC patients, at a median follow-up of 7 years (range, 1.5-11 years), the presence of OTCs correlated with worse event-free survival (P = .007) and overall survival (P = .002). In the MBC group, OTCs correlated with worse event-free survival (P = .04), but not overall survival (P = .2). In multivariate analyses, the presence of OTCs had an independent adverse effect on outcome in HRPBC, but not MBC. Our observations imply a direct role of OTCs in posttransplantation relapse in HRPBC.

Nieto, Y., J. Woods, et al. (2007). "Prognostic analysis of tumour angiogenesis, determined by microvessel density and expression of vascular endothelial growth factor, in high-risk primary breast cancer patients treated with high-dose chemotherapy." Br J Cancer **97**(3): 391-7.

In contrast to early breast cancer, the prognostic effect of tumour angiogenesis in tumours with advanced axillary spread has been less studied. We retrospectively analysed the effect of microvessel density (MVD) and vascular endothelial growth factor (VEGF) by immunohistochemistry on the outcome of 215 patients treated uniformly within prospective trials of high-dose chemotherapy for 4-9 and >/=10 positive nodes, and followed for a median of 9 (range 3-13) years. Microvessel density was associated with epidermal growth factor receptor (EGFR) expression (P<0.001) and tumour size (P=0.001). Vascular endothelial growth factor overexpression (51% of patients) was associated with overexpression of EGFR (P=0.01) and HER2 (P<0.05), but not with MVD (P=0.3). High MVD was associated with worse relapse-free survival (74 vs 44%, P<0.001) and overall survival (76 vs 44%, P<0.001). Vascular endothelial growth factor overexpression had no effect on outcome. Multivariate analyses showed a prognostic effect of MVD independently of other known prognostic factors in this patient population. In conclusion, tumour angiogenesis, expressed as MVD, is a major independent prognostic factor in breast cancer patients with extensive axillary involvement.

Oktem, G., A. Bilir, et al. (2006). "Role of intercellular communications in breast cancer multicellular tumor spheroids after chemotherapy." Oncol Res **16**(5): 225-33.

Tumor heterogeneity is an important feature that is especially involved in tumor aggressiveness. Multicellular tumor spheroids (MTS) may provide some benefits in different steps for investigation of the aggregation, organization, differentiation, and network formation of tumor cells in 3D space. This model offers a unique opportunity for improvements in the capability of a current strategy to detect the effect of an appropriate anticancer agent. The aim of this study was to investigate the cellular interactions and morphological changes following chemotherapy in a 3D breast cancer spheroid model. Distribution of the gap junction protein "connexin-43" and the tight junction protein "occludin" was investigated by immunohistochemistry. Cellular interactions were examined by using transmission and scanning electron microscopies as well as light microscopy with Giemsa staining after treating cells with doxorubicin, docetaxel, and doxorubicin/docetaxel combination.

Statistical analyses showed significant changes and various alterations that were observed in all groups; however, the most prominent effect was detected in the doxorubicin/docetaxel combination group. Distinct composition as a vessel-like structure and a pseudoglandular pattern of control spheroids were detected in drug-administered groups. Immunohistochemical results were consistent with the ultrastructural changes. In conclusion, doxorubicin/docetaxel combination may be more effective than the single drug usage as shown in a 3D model. The MTS model has been found to be an appropriate and reliable method for the detection of the changes in the expression of cellular junction proteins as well as other cellular proteins occurring after chemotherapy. The MTS model can be used to validate the effects of various combinations or new chemotherapeutic agents as well as documentation of possible mechanisms of new drugs.

Okumura, A., Y. Tokuda, et al. (1998). "Immunohistochemical detection of tumor cells in the bone marrow of breast cancer patients." Jpn J Clin Oncol **28**(8): 480-5.

BACKGROUND: Contamination of bone marrow and peripheral blood stem cells with tumor cells is a problem that may be encountered when autologous hematopoietic stem cell transplantation is conducted concurrently with high-dose chemotherapy. Using monoclonal antibodies to a variety of tumors, the detection of tumor cells in the bone marrow of breast cancer patients was studied by immunohistochemistry. KL-1 and CAM5.2 were strongly reactive with breast cancer cells, but not with normal bone marrow cells. The reactivity of the tumor cells with EMA was not strong, and DF-3 and 115D8 yielded only slightly positive reactions. These latter antibodies also exhibited some reactivity to normal bone marrow cells. When tumor cells were admixed with normal cells, the sensitivity of CAM5.2 and EMA permitted the detection of one cell in 10(4), but with KL-1, the detection of one in 10(5) cells was possible. When immunohistochemical staining was used in testing 40 patients with advanced or recurrent breast cancer, positive reactions were obtained in four of 27 patients (14.8%) with KL-1, four of 26 (15.4%) with CAM5.2, and nine of 37 (23.7%) with KL-1 + CAM5.2, figures similar to those reported by others who studied stage IV patients. CONCLUSIONS: Immunohistochemical staining with KL-1 and CAM5.2 is therefore considered to be a useful technique for detecting contamination by tumor cells.

Passos-Coelho, J. L., A. A. Ross, et al. (1996). "Similar breast cancer cell contamination of single-day peripheral-blood progenitor-cell collections obtained after priming with hematopoietic growth factor alone or after cyclophosphamide followed by growth factor." J Clin Oncol **14**(9): 2569-75.

To evaluate tumor-cell contamination of peripheral-blood progenitor-cell (PBPC) collections obtained after priming with granulocyte colony-stimulating factor (G-CSF). Immunocytochemical (ICC) and tumor clonogenic (TCA) assays were used to analyze tumor-cell contamination of pretreatment peripheral-blood (PB) and bone marrow (BM) samples, and of PBPC collection samples obtained after priming with G-CSF 5 micrograms/kg/d for 5 or 7 days in 38 women with advanced breast cancer undergoing high-dose chemotherapy (HDC). Results were compared with 37 historical control patients who underwent PBPC mobilization with cyclophosphamide (4 g/m2) followed by granulocyte-macrophage colony-stimulating factor (GM-CSF) 5 micrograms/kg/d for 14 days. Before PBPC priming with G-CSF, only one of 37 (3%) PB and four of 36 (11%) BM samples had tumor cells detected by ICC. Tumor-cell contamination of PBPC collections obtained after 5 or 7 days of G-CSF priming was observed in only three of 38 patients (8%). All patients with tumor cells detected in the PBPC collection had stage IV disease. Cells with in vitro clonogenic potential were detected only in the pretreatment BM sample in one patient, and another two patients had ICC- and TCA-positive PBPC samples despite tumor-negative PB and BM before priming. These results are similar to those previously reported for PBPC primed with cyclophosphamide and GM-CSF. In patients with advanced breast cancer responsive to cytotoxic chemotherapy, tumor-cell contamination is not increased in PBPC collected after 5 or 7 days priming with G-CSF and appears similar to that seen when PBPC are primed with cyclophosphamide followed by GM-CSF.

Pontier, S. M. and W. J. Muller (2008). "Integrins in breast cancer dormancy." Apmis **116**(7-8): 677-84.

Among breast cancer patients, 20% to 45% develop malignant lesions following their initial treatment. This relapse may occur after an apparent remission period that can range from years to several decades. Clinical observations suggest that breast-derived malignant cells have the ability to survive subclinically for a very long period of time before eventually resuming proliferation and forming detectable lesions. While the precise molecular events that correspond to this dormant phenotype remain poorly understood, data published during the last 10 years have underlined an important role of integrin proteins in the regulation of this phenomenon.

Qiao, L., Z. L. Xu, et al. (2008). "Dkk-1 secreted by mesenchymal stem cells inhibits growth of breast cancer cells via depression of Wnt signalling." Cancer Lett **269**(1): 67-77.

We determined the molecular mechanism of inhibitory effect of human mesenchymal stem cells (hMSCs) on the growth of human MCF-7 breast cancer cells. Our finding showed that beta-catenin was down-regulated in MCF-7 cells by conditioned media from Z3 hMSCs, and the expression level of dickkopf-1 (Dkk-1) was higher in Z3 cells than that in MCF-7 cells. Neutralization of Dkk-1 and small interference RNA targeting Dkk-1 mRNA in Z3 cells attenuated the inhibitory effect of Z3 cells on MCF-7 cells. Overexpression of Dkk-1 in Z3 cells enhanced the inhibition. Therefore, Dkk-1 secreted by Z3 cells involves the inhibition via the Wnt pathway.

Rahim, R. and J. S. Strobl (2009). "Hydroxychloroquine, chloroquine, and all-trans retinoic acid regulate growth, survival, and histone acetylation in breast cancer cells." Anticancer Drugs **20**(8): 736-45.

The antimalarial drugs chloroquine (CQ) and hydroxychloroquine (HCQ) have potential applications in cancer treatment. The growth of MCF-7 and MDA-MB-231 human breast cancer cells in vitro was inhibited by CQ and HCQ and these cells were more sensitive than nontumorigenic MCF-10A breast epithelial cells. Furthermore, all-trans retinoic acid (ATRA) augmented the anticancer effects of CQ and HCQ as evidenced by significant reductions in Ki67-positive cancer cells and clonogenicity compared with cells treated with CQ or HCQ in the absence of ATRA. As an earlier study suggested that CQ, HCQ, and ATRA are breast cancer cell differentiation agents, these agents were screened in cell-free histone deacetylase (HDAC) and histone acetyltransferase (HAT) assays. ATRA, but not CQ or HCQ, inhibited HDAC activity in HeLa nuclear extracts. Growth inhibitory concentrations of HCQ and ATRA stimulated purified p300/CBP-associated factor, where CBP is the cAMP-response element binding protein, HAT activity. To investigate whether growth inhibitory concentrations of these agents influenced protein acetylation in cells, gel-purified histone H3 and histone H4 were analyzed using mass spectrometry. HCQ alone and HCQ+ATRA treatments altered the acetylation status in the N-terminal lysines of histones H3 and H4 compared with dimethyl sulfoxide (DMSO) controls. The results indicated that HCQ and ATRA regulate protein acetylation events in MCF-7 breast cancer cells, and identify a potential mechanism for their effects on breast cancer cell growth and differentiation.

Rakha, E. A., M. E. El-Sayed, et al. (2009). "Patho-biological aspects of basal-like breast cancer." Breast Cancer Res Treat **113**(3): 411-22.

Breast cancer comprises a remarkably diverse group of diseases in terms of presentation, morphology, molecular profile and response to therapy. Recent gene expression profiling of breast cancer has identified specific molecular subtypes of clinical significance. Basal-like cancers (BLC) comprise a group of tumours that are characterised by an expression signature similar to that of the basal/myoepithelial cells of the breast and cluster together with BRCA1 associated tumours. Although BLC has fascinated oncologists and scientists alike due to its enigmatic clinical and pathological parameters, there is no consensus about the definition and method of identification in routine practice of this rather heterogeneous group of cancers. Furthermore, the prognostic significance of BLCs and response to specific chemotherapy regimens are still a matter debate. In this review, we discuss the molecular and morphological features, prognostic significance of BLC, and explore its impact on the concept of the breast cancer stem cell.

Ribeiro-Silva, A., L. N. Ramalho, et al. (2005). "p63 correlates with both BRCA1 and cytokeratin 5 in invasive breast carcinomas: further evidence for the pathogenesis of the basal phenotype of breast cancer." Histopathology **47**(5): 458-66.

AIMS: To study the expression of p63, cytokeratin (CK) 5 and CK8/18 in invasive ductal carcinomas and their relationship with BRCA1 and other pathological and immunohistochemical features of clinical significance. Immunohistochemistry with the antibodies p63, CK5, CK8/18, BRCA1, oestrogen receptor, progesterone receptor, p53, c-erbB-2 and Ki67 was performed in 102 formalin-fixed paraffin-embedded samples of invasive ductal carcinomas. The CK5+ cases were submitted to a double-immunolabelling study with p63. There was a strong relationship between CK5 and p63 expression and both markers were associated with hormonal receptor-negative high-grade carcinomas with high proliferative rate. Furthermore, there was coexpression of CK5 and p63 in neoplastic cells, indicating that p63, like CK5, is a marker of the basal phenotype of breast cancer. There was a strong relationship between reduced expression of BRCA1 with both p63 and CK5 expression as well as an inverse correlation between p63 and CK8/18 expression, suggesting that loss of p63 expression is required for the transition between a basal to a luminal phenotype of breast carcinoma. CONCLUSIONS: Since p63 is thought to be a marker of stem cells and

may act as an oncogene, our data support the idea that BRCA1 acts as stem cell regulator.

Ritter, E., A. Perry, et al. (2008). "Breast cancer cell-derived fibroblast growth factor 2 and vascular endothelial growth factor are chemoattractants for bone marrow stromal stem cells." Ann Surg **247**(2): 310-4.

OBJECTIVE: Recent efforts by the scientific community to characterize the complex interplay between different cell types involved in the development of tumors have led us to investigate the roles of vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2) in the development of breast cancer. Using modified Boyden chamber assays, we measured the in vitro migration effect on murine mesenchymal stem cells (MSCs). Additionally, we assayed for the presence of receptors for these growth factors on MSCs, and for the presence of VEGF and FGF2 in breast cancer-conditioned media. We measured the change in migration of MSCs toward breast cancer when we depleted these growth factors from breast cancer-conditioned media. Further, we conducted a series of standard curve migration assays for basal media supplemented with physiologic concentrations of VEGF and FGF2. Analysis of gene expression and protein analysis demonstrated the expression of FGF2 and VEGF by the breast cancer cells, and the presence of VEGF (FLK1) and FGF2 receptors on the MSCs. We also demonstrated a reduction in migration when we antibody-depleted VEGF and FGF2 from breast cancer-conditioned media. Additionally, we found the physiologic concentrations of VEGF and FGF2 at 12 and 15 ng/mL, respectively. CONCLUSIONS: We demonstrate that VEGF and FGF2 induce migration of MSCs are secreted by breast cancer cells, their receptors are present on MSCs, and depletion of these growth factors reduces migration, and are therefore 2 relevant growth factors for MSC migration toward breast cancer cells.

Rocha, A., I. Azevedo, et al. (2008). "Progesterone sensitizes breast cancer MCF7 cells to imatinib inhibitory effects." J Cell Biochem **103**(2): 607-14.

In previous studies, we found that progesterone was able to induce the expression of platelet-derived growth factor (PDGF) in human breast cancer MCF7 cells. Knowing that imatinib mesylate targets PDGF receptor tyrosine kinase activity, the aim of the present study was to examine the effects of imatinib on progesterone-treated MCF7 cells. Expression of phosphorylated (activated) platelet-derived growth factor receptor-alpha (PDGFRalpha) was detected in MCF7 cells. Interestingly, phosphorylated-PDGFRalpha expression was significantly downregulated by imatinib. The effects of imatinib on cell growth, apoptosis and migration were then analyzed. Imatinib effectively inhibited anchorage-dependent colony formation, and cell viability as evaluated by MTT assay. Corroborating these findings, a significant increase in the percentage of apoptotic cells was also observed when cells were treated with imatinib. Surprisingly, these inhibitory effects were all enhanced by the presence of progesterone. Cell migration assays did also show a reduction in the migratory capacity after incubation with imatinib. These findings reveal that imatinib acts by decreasing MCF7 cell viability, growth and migration, with concomitant increase in apoptosis. Furthermore, incubation with progesterone seems to prompt cells to the inhibitory action of imatinib, probably by sustaining PDGFRalpha activity. The current study points out imatinib as a possible therapeutic strategy in progesterone-dependent breast cancer.

Rodriguez-Pinilla, S. M., D. Sarrio, et al. (2007). "Sox2: a possible driver of the basal-like phenotype in sporadic breast cancer." Mod Pathol **20**(4): 474-81.

Tumours arising in BRCA1 mutation carriers and sporadic basal-like breast carcinomas have similar phenotypic, immunohistochemical and clinical characteristics. SOX2 is an embryonic transcription factor located at chromosome 3q, a region frequently gained in sporadic basal-like and BRCA1 germline mutated tumours. The aim of the study was to establish whether sox2 expression was related to basal-like sporadic breast tumours. Two hundred and twenty-six sporadic node-negative invasive breast carcinomas were immunohistochemically analysed for oestrogen receptor (ER), progesterone receptor (PR), CK5/6, EGFR, vimentin, HER2, ki67, p53 and sox2 using tissue microarrays. Tumours were considered to have basal-like phenotype if they were ER/HER2-negative and CK5/6 and/or EGFR-positive. Thirty cases of this series (13.7%) displayed a basal-like phenotype. Sox2 expression was observed in 16.7% of cases and was significantly more frequently expressed in basal-like breast carcinomas (43.3% in basal-like, 10.6% in luminal and 13.3% in HER2+ tumours, P<0.001). Moreover, Sox2 showed a statistically significant inverse association with ER and PR (P=0.001 and 0.017, respectively) and direct association with CK5/6, EGFR and vimentin (P=0.022, 0.005 and <0.001, respectively). Sox2 is preferentially expressed in tumours with basal-like phenotype and may play a role in defining their less differentiated/'stem cell' phenotypic characteristics.

Rogelsperger, O., C. Ekmekcioglu, et al. (2009). "Coexpression of the melatonin receptor 1 and nestin

in human breast cancer specimens." J Pineal Res **46**(4): 422-32.

Activation of the G-protein-coupled receptor (GPCR) for melatonin (MT1) suppresses breast cancer cell growth in experimental models. To elucidate whether MT1 might play a role in cancer cells positive for the stem cell marker nestin, we assessed paired carcinomatous (Ca) and adjacent noncancerous (NCa) samples from 42 patients with primary breast cancer for MT1 and nestin by double immunofluorescence staining and quantitative image analysis with Tissue-Quest software. MT1 was located in luminal and myoepithelial cells in milk ducts and in tumor cells in 40/42 and 39/42 of NCa and Ca specimens, respectively, independent of hormone receptor and HER-2 status. Nestin was located together with MT1 in myoepithelial cells in 38 NCa specimens (total n = 42) and in 18 Ca specimens with intact milk ducts. Quantitative evaluation of selected 16 NCa and Ca samples revealed that MT1 levels were higher in invasive Ca sections than in NCa specimens in eight and lower in six cases. Specimens from higher tumor stages (TII/III) with a higher risk of relapse were associated with MT1/nestin co-staining in more than 10% of tumor cells, whereas a lack of co-staining correlated with lower tumor stages. Abundant expression of MT1 and, particularly, coexpression of MT1 with nestin in invading tumor cells in more advanced tumors suggest an important role for this GPCR in the pathogenesis of breast cancer.

Schneeweiss, A., I. Diel, et al. (2004). "Micrometastatic bone marrow cells at diagnosis have no impact on survival of primary breast cancer patients with extensive axillary lymph node involvement treated with stem cell-supported high-dose chemotherapy." Ann Oncol **15**(11): 1627-32.

BACKGROUND: To determine the impact of micrometastatic bone marrow cells (MMC) on survival in high-risk primary breast cancer (HRPBC) patients treated with high-dose chemotherapy (HDCT) and autologous stem cell transplantation (ASCT). Ninety-one HRPBC patients (73 patients with > or =10 involved axillary lymph nodes (ALN), 18 premenopausal women with > or =4 involved ALN) received one cycle (eight patients) or two cycles of HDCT and ASCT. Bone marrow aspiration was performed before systemic treatment to search for MMC using a cocktail of four monoclonal epithelial-specific antibodies (5D3, HEA125, BM7 and BM8). The influence of MMC and other prognostic factors on disease-free survival (DFS), distant DFS (DDFS), and overall survival (OS) was analysed. In 23 of 91 patients (25%) we detected a median of three MMC (range, 1-43) among 10(6) mononuclear cells. With a median follow-up of 62 months (range, 10-117), the detection of MMC was not associated with DFS (P=0.929), DDFS (P=0.664) or OS (P=0.642). In multivariate analysis the strongest predictor was nodal ratio for DFS (P=0.012) and expression of p53 for OS (P <0.001). The detection of MMC at diagnosis has no impact on survival in HRPBC patients treated with HDCT and ASCT.

Schrama, J. G., I. F. Faneyte, et al. (2002). "Randomized trial of high-dose chemotherapy and hematopoietic progenitor-cell support in operable breast cancer with extensive lymph node involvement: final analysis with 7 years of follow-up." Ann Oncol **13**(5): 689-98.

BACKGROUND: The aim of this study was to present an update of overall (OS) and disease-free survival (DFS) and to evaluate the correlation between outcome and pathological findings at surgery in a randomized trial of high-dose chemotherapy following neoadjuvant chemotherapy and surgery in high-risk breast cancer patients. Ninety-seven women <60 years of age with breast cancer and extensive axillary lymph node involvement received three courses of FE120C (5-fluorouracil 500 mg/m2, epirubicin 120 mg/m2, cyclophosphamide 500 mg/m2) followed by surgery. Eighty-one patients were randomized to receive either a fourth FE120C course alone or a fourth FE120C course followed by high-dose chemotherapy (cyclophosphamide 6 g/m2, thiotepa 480 mg/m2, carboplatin 1600 mg/m2). We performed a univariate analysis on possible prognostic factors and analyzed the sites of relapse. After a median follow-up of 6.9 years, 47 (48%) patients were alive, of whom 36 (38%) were without disease. Sixty patients relapsed after treatment. One patient died of myelodysplastic syndrome, without a relapse. In intention-to-treat analysis, the 5-year DFS rates were 47.5% in the conventional treatment arm and 49% in the high-dose arm, and the 5-year OS rates were 62.5% and 61%, respectively. In the univariate analysis, the clinical T-stage before chemotherapy and the number of tumor-positive axillary lymph nodes after induction chemotherapy (P = 0.027) were significant prognostic factors for OS. The same factors (both P = 0.06) plus the estrogen receptor (P = 0.08) were borderline significant factors for DFS. CONCLUSIONS: After a median follow-up of 6.9 years there was no difference in OS or DFS rates between the two treatment groups. The number of tumor-positive axillary lymph nodes after induction chemotherapy and the clinical T-stage before chemotherapy were significant factors for OS.

Selicharova, I., K. Smutna, et al. (2007). "2-DE analysis of a new human cell line EM-G3 derived from breast cancer progenitor cells and comparison

with normal mammary epithelial cells." Proteomics **7**(9): 1549-59.

We performed a 2-DE analysis of proteins of the newly established spontaneously immortalized clonal cell line EM-G3 derived from a primary lesion of infiltrating ductal breast carcinoma. EM-G3 cells may represent progenitors of the mammary epithelial cells spontaneously immortalized in early phase of cancerogenesis. We compared the protein profile of EM-G3 line with proteins from populations of normal mammary epithelial cells (NME), and determined the phenotype of both types of cells. NME cells are a mixture of both main cell types in breast epithelia, myoepithelial and luminal cells. The EM-G3 breast cancer cell line has a unique basal-like phenotype. We identified proteins that are differently expressed in these cells. Cytokeratin 16, cytokeratin 19, squamous cell carcinoma antigen 1, caphepsin B and caspase 14 were predominantly expressed by NME cells. Cytokeratin 13, isoelectric variant of annexin 5, isoelectric variant of chloride intracellular channel protein 1, glyoxalase 1 and glutamine synthetase were predominantly expressed by EM-G3 cells. The proteins up-regulated in EM-G3 cells may represent potential protein markers of mammary epithelial cells progenitors and may be important in early phase of carcinogenesis.

Shammo, J. M., S. L. Smith, et al. (1999). "Use of a tumor-cell enrichment column for the enhanced detection of minimal residual disease in the BM or apheresis peripheral blood transplant products of breast-cancer patients." Cytotherapy **1**(5): 367-76.

BACKGROUND: Contaminating tumor cells present in the BM or apheresis peripheral blood (APB) autologous transplant products have been shown to contribute to relapse following high-dose chemotherapy and stem-cell rescue (HDC/ASCR). Enhanced methods for tumor detection in BM or APB products for breast-cancer patients are required. We evaluated a laboratory-scale tumor-cell enrichment column (TEC) as an enhanced method of detecting tumor cells in APB or BM of breast-cancer patients. Seventeen women with breast cancer (14 Stage IV and three Stage III) were evaluated using the TEC for residual tumor cells present in 20 samples of APB or BM biopsies following HDC/ASCR. Using conventional histological staining methods (without TEC), only one patient had evidence of tumor cells present in the BM biopsy, while 16 patients had negative biopsies. Using the TEC for tumor cell capture and immunocytochemical (ICC) staining with anti-cytokeratin MAb (CAM 5.2) for tumor detection, we were able to positively identify tumor cells in 20 samples (14 BM aspirates and six APB products). In 15 samples (nine BM and six APB), we used CAM

5.2 to positively identify cytokeratin(+) cells prior to using the TEC. However, positive cells were detected only after using the TEC in the remaining five samples. The level of sensitivity was significantly enhanced (p < or = 0.05) by 100-400 fold in the post-TEC (absorbed) fraction compared with the pre-TEC (post-Ficoll) fraction. DISCUSSION: We conclude from this study that the use of TEC improves our ability to detect residual breast-cancer cells in the APB or BM and could be potentially utilized to purge contaminating tumor cells from the stem-cell transplant.

Solano, C., B. Badia, et al. (2001). "Prognostic significance of the immunocytochemical detection of contaminating tumor cells (CTC) in apheresis products of patients with high-risk breast cancer treated with high-dose chemotherapy and stem cell transplantation." Bone Marrow Transplant **27**(3): 287-93.

The aim of this study was to determine whether the detection of CTC in the apheresis product contribute significantly to treatment failure of patients with high-risk breast carcinoma treated with high-dose chemotherapy (HDC) and stem cell transplantation (SCT). Patients were with stage II and III adenocarcinoma of the breast with > or = 10 axillary lymph nodes affected after primary surgery (> or = 10 N+) who had received HDC with SCT. We analyzed retrospectively the presence of CTC as assessed by immunocytochemistry (ICC) in the apheresis products obtained after standard adjuvant chemotherapy. We compared the clinical outcome of patients who received HDC and SCT with or without CTC-positive apheresis. One hundred and twenty-seven apheresis products samples were obtained from 51 patients. Fourteen (27.4%) of these samples were CTC positive. After a median follow-up of 4.6 years, 20 patients have relapsed, 14 died from progression of their disease and 30 patients remain alive and free of progression. For the whole group of patients the 5 year probabilities of DFS and OS were 60% (IC 95%, 47-75%) and 71% (IC 95%, 55-83%), respectively. However, the 5 year probabilities of DFS were 23% (IC 95%, 0-46) and 75% (IC 95%, 60-89) for patients with CTC positive and negative, respectively. The 5 year probabilities of OS were 42% (IC 95%, 15-68) and 83% (IC 95%, 70-95) for patients with CTC positive and negative, respectively. Both univariate and multivariate analysis showed that the presence of CTC in the apheresis product was the only prognostic factor associated with a higher incidence of clinically overt disease relapse (P = 0.002) and shorter survival (P = 0.003). The presence of cytokeratin-positive metastatic cells in the apheresis product increases the risk of relapse after HDC and SCT in patients with

stage II and III adenocarcinoma of the breast with > or = 10 N+.

Theocharous, P., M. W. Lowdell, et al. (1997). "Immunocytochemical detection of breast cancer cells: a comparison of three attachment factors." J Hematother **6**(1): 21-9.
The evaluation of contaminating breast cancer cells in hematopoietic grafts is of considerable importance for monitoring the efficiency of purging procedures. The observed number of seeded cells showed a highly significant correlation with the number of cells seeded (p < 0.0001 in all cases). Finally, we used the Cell-Tak method to evaluate clinical material from various sources: from patients with primary carcinomas of the breast, prechemotherapy, and during various chemotherapeutic regimens, as well as from patients with metastatic disease. The system consistently detected tumor cells in bone marrow samples from these patients. All peripheral blood samples from patients with metastatic disease tested positive at incidences ranging from 5 to 19/10(6) peripheral blood mononuclear cells. This is a simple and reliable technique that allows rapid screening of large cell numbers with high resolution of positive cells.

Thirukkumaran, C. M., J. M. Luider, et al. (2005). "Biological purging of breast cancer cell lines using a replication-competent oncolytic virus in human stem cell autografts." Bone Marrow Transplant **35**(11): 1055-64.
Autologous hematological stem cell transplantation (ASCT) is used for the treatment of many hematological and several solid cancers. ASCT, however, has proven disappointing as a therapeutic strategy for breast cancer. Our group and others have previously shown that breast cancer micrometastases found in patients' apheresis products (APs) predict shorter progression-free and overall survival. The implications of this finding are twofold: (i) contaminating tumor cells (CTCs) in AP reflect a higher systemic disease burden and/or (ii) reinfused CTCs contribute to relapse/progressive disease. To date, purging strategies have been disappointing. We have previously demonstrated the oncolytic properties of reovirus in in vitro, in vivo and ex vivo systems. In the present study, we tested the hypothesis that reovirus purges CTCs in a breast cancer cell line purging model. Reovirus-infected human breast cancer cell lines (HTB 133, HTB 132, SKBR3 and MCF7) exhibited cell death within days. Admixtures of AP with cells from breast tumor cell lines, which were then exposed to reovirus, showed complete purging of CTCs (assessed via flow cytometry/tumor cell outgrowth analysis) without deleterious effect on

CD34+ cells. Our results provide preclinical support for the ex vivo use of reovirus as a purging modality for breast cancer during ASCT.

Ting, A. Y., B. F. Kimler, et al. (2008). "Tamoxifen prevents premalignant changes of breast, but not ovarian, cancer in rats at high risk for both diseases." Cancer Prev Res (Phila) **1**(7): 546-53.
Women at increased risk for breast cancer are at increased risk for ovarian cancer as well, reflecting common risk factors and intertwined etiology of the two diseases. We previously developed a rat model of elevated breast and ovarian cancer risk, allowing evaluation of dual-target cancer prevention strategies. Tamoxifen, a Food and Drug Administration-approved breast cancer chemoprevention drug, has been shown to promote ovarian cysts in premenopausal women; however, the effect of tamoxifen on ovarian cancer risk is still controversial. In the current experiment, Fischer 344 rats (n = 8 per treatment group) received tamoxifen (TAM) or vehicle (control) in factorial combination with combined breast and ovarian carcinogen (17beta-estradiol and 7,12 dimethylbenza[a]anthracene, respectively). Mammary and ovarian morphologies were normal in the control and TAM groups. Carcinogen (CARC) treatment induced mammary dysplasia with elevated cell proliferation and reduced estrogen receptor-alpha expression and promoted preneoplastic changes in the ovary. In the CARC + TAM group, tamoxifen reduced preneoplastic changes and proliferation rate in the mammary gland, but not in the ovary, compared with rats treated with carcinogen alone. Putative stem cell markers (Oct-4 and aldehyde dehydrogenase 1) were also elevated in the mammary tissue by carcinogen and this expansion of the stem cell population was not reversed by tamoxifen. Our study suggests that tamoxifen prevents early progression to mammary cancer but has no effect on ovarian cancer progression in this rat model.

Togel, F., C. Datta, et al. (2001). "Urokinase-like plasminogen activator receptor expression on disseminated breast cancer cells." J Hematother Stem Cell Res **10**(1): 141-5.
Disseminated tumor cells are detected frequently in bone marrow, peripheral blood, and cytokine-mobilized peripheral blood cell products of women undergoing high-dose therapy for breast cancer. Several attempts were made to purge autografts from contaminating cancer cells; however, the biological and clinical impact of these contaminations has not been clarified so far. Expression of distinct phenotypes is a surrogate marker for metastatic behavior of cancer cells. The expression of the urokinase-like plasminogen activator

receptor seems to be a factor of high importance. It is not expressed by normal mammary tissue. Disseminated cancer cells from marrow, blood, and stem cell products have been investigated by double-stain technique for urokinase-like plasminogen activator receptor (uPA-R) expressing cytokeratin-positive cells. uPA-R(+)/CK(+) cells could be found in all qualities of samples; however, significantly less in G-CSF-mobilized peripheral blood stem cells compared to samples of other provenance (p = 0.02). It can be concluded that epithelial cells of malignant phenotype occur in blood, marrow, and autografts of breast cancer patients. Populations of disseminated tumor cells are phenotypically heterogeneous. Reduced uPA-R expression on cancer cells from leukapheresis samples might suggest a less aggressive nature of these cells compared to disseminated cells found in bone marrow. Furthermore, the data suggest that the phenotype of tumor cell contamination in leukapheresis products differs significantly from those of disseminated cancer cells in bone marrow or blood.

Uno, H., M. Fujita, et al. (1999). "[High-dose chemotherapy with autologous peripheral blood stem cell transplantation support in a patient with breast cancer metastasis to bone marrow: immunocytochemical monitoring of cancer-cell contamination]." Rinsho Ketsueki **40**(7): 556-62.

A 32-year-old woman who 1 year earlier underwent a right mastectomy for stage II breast cancer with the histology of invasive ductal carcinoma (scirrhus type) was admitted due to recurrent, metastatic breast cancer in January 1997. She presented multiple metastatic lesions in the skin, lymph nodes, bone, lungs, liver, and spleen, and her bone marrow was replaced almost entirely by tumor cells. The patient was sequentially treated with 5 courses of cyclophosphamide (CPA) and adriamycin (ADM) (CA); 2 courses of CPA, ADM, and 5-fluorouracil; 5 caurses of docetaxel hydrate; and 1 course of CA. After recovery of the normal bone marrow by standard-dose chemotherapies, peripheral blood stem cells (PBSC) were then collected after mobilization with G-CSF. The number of breast cancer cells in bone marrow and PBSC samples was determined by immunocytochemical staining with an anti-cytokeratin monoclonal antibody. The number of tumor cells in PBSC sample was within the level for non-metastatic breast cancer. Complete remission was obtained with high-dose chemotherapy consisting of CPA and Thio-TEPA, and supported by autologous PBSC transplantation.

Unterholzner, S., M. J. Willhauck, et al. (2006). "Dexamethasone stimulation of retinoic Acid-induced sodium iodide symporter expression and cytotoxicity of 131-I in breast cancer cells." J Clin Endocrinol Metab **91**(1): 69-78.

CONTEXT: The sodium iodide symporter (NIS) mediates the active iodide uptake in the thyroid gland as well as lactating breast tissue. Recently induction of functional NIS expression was reported in the estrogen receptor-positive human breast cancer cell line MCF-7 by all-trans retinoic acid (atRA) treatment in vitro and in vivo, which might offer the potential to treat breast cancer with radioiodine. After incubation with Dex in the presence of atRA, NIS mRNA levels in MCF-7 cells were stimulated up to 11-fold in a concentration-dependent manner, whereas NIS protein levels increased up to 16-fold and iodide accumulation was stimulated up to 3- to 4-fold. Furthermore, iodide efflux was modestly decreased after stimulation with Dex in the presence of atRA. Furthermore, in the in vitro clonogenic assay, selective cytotoxicity of 131-I was significantly increased from approximately 17% in MCF-7 cells treated with atRA alone to 80% in MCF-7 cells treated with Dex in the presence of atRA. Treatment with Dex in the presence of atRA significantly increases functional NIS expression levels in addition to inhibiting iodide efflux, resulting in an enhanced selective killing effect of 131-I in MCF-7 breast cancer cells.

Vander Borght, S., L. Libbrecht, et al. (2006). "Breast cancer resistance protein (BCRP/ABCG2) is expressed by progenitor cells/reactive ductules and hepatocytes and its expression pattern is influenced by disease etiology and species type: possible functional consequences." J Histochem Cytochem **54**(9): 1051-9.

Breast cancer resistance protein (BCRP/ABCG2) is an ATP-binding cassette transport protein that is expressed in several organs including the liver. Previous studies have shown that ABC transport proteins play an important pathophysiological role in several liver diseases. Remarkably, there was also expression of BCRP at the basolateral pole of human hepatocytes, and this was most pronounced in chronic biliary diseases. In conclusion, BCRP positivity in the progenitor cells/reactive ductules could contribute to the resistance of these cells to cytotoxic agents and xenotoxins. Basolateral hepatocytic expression in chronic biliary diseases may be an adaptive mechanism to pump bile constituents back into the sinusoidal blood. Strong differences between human and rat liver must be taken into account in future studies with animal models.

Vani, G. and C. S. Devi (2005). "Effect of histone H1 on estrogen receptor status of human breast cancer MCF 7 cells." Mol Cell Biochem **272**(1-2): 151-5.

The effects of exogenous histone H1 on estrogen receptor status of human breast cancer MCF 7 cells were investigated in presence and absence of estrogen. Exogenous histone H1 was significantly cytotoxic in a dose- and time-dependent manner. Cell cycle analysis revealed a significant increase in the percentage of cell accumulation in G0/G1 phase. In histone H1-treated cells, a significant decrease in the estrogen receptor content and an increase in the dissociation constant (KD) of ER was observed compared to control.

Warri, A. M., N. M. Saarinen, et al. (2007). "Can modulation of mammary gland development by dietary factors support breast cancer prevention?" Horm Res **68**(5): 248-60.

Breast cancer continues to be a major challenge for public health, since it is the most common cancer of women in the Western world, and its prevalence is still increasing. In order to achieve better results in the prevention and treatment of breast cancer it is crucial to identify the mechanisms behind its initiation, i.e. the changes and deviations that have occurred in the mammary gland growth. It has long been known that a woman's reproductive history is the strongest breast cancer risk factor if genetic background and age are excluded. The reproductive hormones, and the timing of events leading to changes in these hormones, and consequently, in the mammary gland, are the most important players. However, it has become obvious that dietary components may also contribute to breast cancer risk through their effects on the mammary gland. The past few years have added important information to our knowledge of the mechanisms behind breast cancer initiation at the level of target cells (mammary stem cells) and gene expression (genetic 'fingerprint' associated with persistent pregnancy-induced protection against breast cancer), as well as of the effects of certain dietary factors (steroid action modulators). These results and their links to breast cancer initiation and progression will be discussed.

Watson, M. A., S. Dintzis, et al. (1999). "Mammaglobin expression in primary, metastatic, and occult breast cancer." Cancer Res **59**(13): 3028-31.

The mammaglobin gene encodes a novel, breast cancer-associated glycoprotein. In this study, we have evaluated the frequency with which mammaglobin expression can be detected in primary and metastatic breast tumors and in breast tumor cells present in the peripheral circulation. Of 100 primary human breast tumors examined, 81 were strongly immunopositive for mammaglobin protein. Staining was independent of tumor grade and histological type. Ten of 11 lymph nodes from patients with metastatic breast cancer contained detectable mammaglobin mRNA, whereas mammaglobin expression in uninvolved lymph nodes was undetectable. Using a nested reverse transcription-PCR assay, mammaglobin mRNA was also detected in 9 of 15 products (60%) used for autologous stem cell transplant. These results suggest that larger clinical studies are warranted to investigate the full clinical utility of mammaglobin as a tool for breast cancer patient management.

Weaver, C. H., T. Moss, et al. (1998). "High-dose chemotherapy in patients with breast cancer: evaluation of infusing peripheral blood stem cells containing occult tumor cells." Bone Marrow Transplant **21**(11): 1117-24.

The purpose of this study was to evaluate the frequency of detecting occult tumor cells in peripheral blood stem cell (PBSC) harvests and to determine the impact of infusing such cells on relapses after high-dose chemotherapy (HDC). Peripheral blood stem cell harvests from 223 patients with breast cancer were examined by an immunocytochemistry (ICC) method for detection of occult tumor cells, and infused after HDC without consideration of test results. Two hundred and four patients, 114 with stage II-III and 90 with stage IV disease who received only PBSC, that were tested by ICC were evaluated for time to relapse. Five hundred and eighty-one of 619 PBSC harvests (94%) from 223 patients were tested. Fifty-three of 581 harvests (9%), 8% from stage II-III and 10% from stage IV patients, were positive by ICC (P = 0.68). Forty-one of 223 patients (18%), 17/122 (14%) with stage II-III and 24/101 (24%) with stage IV disease, had positive harvests (P = 0.06). Eleven percent of patients who had 1-2 harvests tested were positive as compared to 32% of patients who had > or =3 PBSC harvests tested (P < 0.001). Nineteen patients who were infused with a mixture of ICC negative and untested PBSC harvests were excluded from analyses of relapse. The probabilities of relapse at 18 months for the 97 patients with stage II-III disease infused with ICC-negative and the 17 with ICC-positive PBSC were 0.19 and 0.13, respectively (P = 0.48). The probabilities of relapse at 18 months for patients achieving a CR or a CR in non-bone sites and improvement in bone lesions were 0.55 for the ICC-negative group (n = 30) and 0.45 for the ICC-positive group (n = 11) (P = 0.60). It was concluded that occult tumor cells were detected by ICC in PBSC harvests from a relatively small fraction of women with breast cancer, but were not associated with a significant increase in the probability of early relapse or progression when infused after HDC.

Wild, P. J., A. Reichle, et al. (2004). "Microsatellite instability predicts poor short-term survival in patients

with advanced breast cancer after high-dose chemotherapy and autologous stem-cell transplantation." Clin Cancer Res **10**(2): 556-64.

The purpose is to define molecular prognostic factors in patients with advanced breast cancer treated with high-dose chemotherapy (HDCT) and autologous stem cell transplantation (ASCT). Thirty-nine patients with breast cancer and extensive lymph node (level III) and/or systemic metastases from a prospective single-center study of sequential HDCT/ASCT were studied. Microsatellite analysis was performed after laser microdissection using 15 markers selected for sensitive detection of microsatellite instability (MSI) in breast cancer. Exons 5-9 of the P53 gene were directly sequenced. Expression of P53, HER-2/neu, and the mismatch repair proteins hMSH2 and hMLH1 was evaluated by immunohistochemistry. MSI of at least three markers was detected in 13 of 39 patients (33%) and was predominantly found at tetranucleotide markers. All MSI-positive tumors showed normal expression of hMSH2 and hMLH1. Complete sequence analysis of exons 5-9 of the P53 gene was successful in 34 cases; 18% (n = 6) revealed a mutation. Overexpression of HER-2/neu and P53 was observed in 7 (22%) and 12 (46%) of 26 evaluated cases, respectively. The presence of MSI strongly correlated with shorter overall survival (OS; P = 0.0004) and progression-free survival (PFS; P = 0.02). None of the other investigated clinical or molecular factors correlated with OS in univariate analyses, with the exception of menopausal status and previous adjuvant chemotherapy. Testing various multivariate Cox regression models, MSI remained a highly significant, independent, and adverse risk factor for OS. CONCLUSIONS: MSI is frequent in advanced breast cancer and could be an indicator of chemotherapy resistance and poor prognosis in breast cancer patients treated with HDCT/ASCT.

Williams, C., L. Helguero, et al. (2009). "Gene expression in murine mammary epithelial stem cell-like cells shows similarities to human breast cancer gene expression." Breast Cancer Res **11**(3): R26.

INTRODUCTION: Mammary stem cells are bipotential and suggested to be the origin of breast cancer development, but are elusive and vaguely characterized. Breast tumors can be divided into subgroups, each one requiring specific treatment. To determine a possible association between mammary stem cells and breast cancer, a detailed characterization of the transcriptome in mammary stem cells is essential. We have used a murine mammary epithelial stem-like cell line (HC11) and made a thorough investigation of global gene-expression changes during stepwise differentiation

using dual-color comparative microarray technique. Subsequently, we have performed a cross-species comparison to reveal conserved gene expression between stem cells and subtype-specific and prognosis gene signatures, and correlated gene expression to in vivo mammary gland development. Our analysis of mammary stem-like and stepwise cell differentiation, and an in-depth description of our findings in a breast cancer perspective provide a unique map of the transcriptomic changes and a number of novel mammary stem cell markers. We correlate the alterations to in vivo mammary gland differentiation, and describe novel changes in nuclear receptor gene expression. Interestingly, our comparisons show that specific subtypes of breast cancers with poor prognosis and metastasizing capabilities show resemblance to stem-like gene expression. CONCLUSIONS: The transcriptional characterization of these mammary stem-like cells and their differentiation-induced gene expression patterns is here made widely accessible and provides a basis for research on mammary stem-like cells. Our comparisons suggest that some tumors are more stem-like than others, with a corresponding worse prognosis. This information would, if established, be important for treatment decisions. We also suggest several marker candidates valuable to investigate further.

Witkiewicz, A. K., A. Dasgupta, et al. (2009). "Stromal caveolin-1 levels predict early DCIS progression to invasive breast cancer." Cancer Biol Ther **8**(11): 1071-9.

Here, we determined the possible association of stromal caveolin-1 (Cav-1) levels with DCIS recurrence and/or progression to invasive breast cancer. An initial cohort of 78 DCIS patients with follow-up data was examined. As ER-positivity was associated with recurrence, we focused our analysis on this subset of 56 patients. In this group, we observed that DCIS progressed to invasive breast cancer in approximately 14% of the patient population (8/56), in accordance with an expected progression rate of 12-15%. Nearly ninety percent of DCIS patients (7/8) that underwent recurrence to invasive breast cancer had reduced or absent levels of stromal Cav-1. Remarkably, an absence of stromal Cav-1 (score = 0) was specifically associated with early disease progression to invasive breast cancer, with reduced time to recurrence and higher recurrence rate. All DCIS patients with an absence of stromal Cav-1 underwent some form of recurrence (5/5) and the majority (4/5) underwent progression to invasive breast cancer. This represents an overall cumulative incidence rate of 100% for recurrence and 80% for progression. An absence of stromal Cav-1 in DCIS

lesions was also specifically associated with the presence of inflammatory cells. Conversely, ninety-seven percent of ER(+) DCIS patients (35/36) with high levels of stromal Cav-1 (score = 2) did not show any invasive recurrence over the duration of follow-up (4-208 mo), and 89% of such patients are estimated to remain free of invasive recurrence, even after 15 y. Thus, determination of stromal Cav-1 levels may be a useful new biomarker for guiding the treatment of ER(+) DCIS patients.

Zhou, C., A. M. Nitschke, et al. (2008). "Proteomic analysis of tumor necrosis factor-alpha resistant human breast cancer cells reveals a MEK5/Erk5-mediated epithelial-mesenchymal transition phenotype." Breast Cancer Res **10**(6): R105.

INTRODUCTION: Despite intensive study of the mechanisms of chemotherapeutic drug resistance in human breast cancer, few reports have systematically investigated the mechanisms that underlie resistance to the chemotherapy-sensitizing agent tumor necrosis factor (TNF)-alpha. Additionally, the relationship between TNF-alpha resistance mediated by MEK5/Erk5 signaling and epithelial-mesenchymal transition (EMT), a process associated with promotion of invasion, metastasis, and recurrence in breast cancer, has not previously been investigated. To compare differences in the proteome of the TNF-alpha resistant MCF-7 breast cancer cell line MCF-7-MEK5 (in which TNF-alpha resistance is mediated by MEK5/Erk5 signaling) and its parental TNF-a sensitive MCF-7 cell line MCF-7-VEC, two-dimensional gel electrophoresis and high performance capillary liquid chromatography coupled with tandem mass spectrometry approaches were used. Differential protein expression was verified at the transcriptional level using RT-PCR assays. An EMT phenotype was confirmed using immunofluorescence staining and gene expression analyses. A short hairpin RNA strategy targeting Erk5 was utilized to investigate the requirement for the MEK/Erk5 pathway in EMT. Proteomic analyses and PCR assays were used to identify and confirm differential expression of proteins. In MCF-7-MEK5 versus MCF-7-VEC cells, vimentin (VIM), glutathione-S-transferase P (GSTP1), and creatine kinase B-type (CKB) were upregulated, and keratin 8 (KRT8), keratin 19 (KRT19) and glutathione-S-transferase Mu 3 (GSTM3) were downregulated. Morphology and immunofluorescence staining for E-cadherin and vimentin revealed an EMT phenotype in the MCF-7-MEK5 cells. Furthermore, EMT regulatory genes SNAI2 (slug), ZEB1 (delta-EF1), and N-cadherin (CDH2) were upregulated, whereas E-cadherin (CDH1) was downregulated in MCF-7-MEK5 cells versus MCF-7-VEC cells. RNA interference targeting of Erk5 reversed MEK5-

mediated EMT gene expression. CONCLUSIONS: This study demonstrates that MEK5 over-expression promotes a TNF-alpha resistance phenotype associated with distinct proteomic changes (upregulation of VIM/vim, GSTP1/gstp1, and CKB/ckb; and downregulation of KRT8/krt8, KRT19/krt19, and GSTM3/gstm3). We further demonstrate that MEK5-mediated progression to an EMT phenotype is dependent upon intact Erk5 and associated with upregulation of SNAI2 and ZEB1 expression.

Zhou, X., J. Coad, et al. (2008). "SHP2 is up-regulated in breast cancer cells and in infiltrating ductal carcinoma of the breast, implying its involvement in breast oncogenesis." Histopathology **53**(4): 389-402.

AIMS: To determine whether Src homology phosphotyrosyl phosphatase 2 (SHP2) is up-regulated in breast cancer and, if so, to determine whether its up-regulation has any relationship with clinical variables of breast cancer. Immunoblotting, immunohistochemistry and immunofluorescence microscopy were used to assess the state of SHP2 expression in breast cancer cells and in infiltrating ductal carcinoma (IDC) of breast. The possible role of SHP2 in breast cancer cell transformation was determined by dominant-negative expression and anchorage-independent growth assays. All of the breast cancer cell lines tested and 72% of IDC breast tumours analysed had increased amounts of the SHP2 protein. In support of its positive role, dominant-negative SHP2 blocked anchorage-independent growth of breast cancer cells. Furthermore, overexpression of SHP2 seemed to have a positive relationship to HER2 overexpression, nuclear accumulation of hormone receptors, higher tumour grade and lymph node metastasis, but not to age of breast cancer patients. SHP2 is a widely overexpressed signalling protein in IDC breast tumours. Given SHP2's positive role in cell growth, transformation and stem cell survival, the positive relationship of its overexpression to lymph node metastasis, nuclear accumulation of hormone receptors and higher tumour grade suggests that SHP2 promotes breast oncogenesis.

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