in MBC for the last fifteen years and second to explore its association to prognostic factors affecting outcome including therapeutic regimen.
Material and Methods: This meta-analysis uses individual patient data collected from all ten trials on MBC (6 non randomized, 4 randomized) conducted by HeCOG from 1991 through 2006. Four 4-year time periods (1991–1994, 1995–1998, 1999–2002 and 2003–2006) were constructed for exploration of time trends in survival according to the patient's date of metastatic diagnosis. Different first line regimens in the 10 trials include anthracycline monotherapy (epirubicin, in the early 90s) and taxane-containing regimens either as monotherapy or in different combinations with anthracyclines or other drugs. In two phase II studies and the last randomized study, trastuzumab was administered in all patients with HER2 overexpressing tumors.
Results: Information is based on a total of 1365 patients with a median follow up of 3.7 years and median survival of 1.9 years (median survival 1.3, 1.7, 2.2 and 2.6 years for 1991–1994, 1995–1998, 1999–2002, and 2003–2006, respectively). Survival improved significantly across diagnosis time periods, by 26%, 44% and 52% respectively in each time period as compared to the first (1991–1994), (1995–1998: HR = 0.74, p = 0.002; 1999–2002: HR = 0.56, p < 0.001; 2003–2006: HR = 0.48, p < 0.001). The effect of metastatic diagnosis time period remains almost unchanged in the presence of the following significant prognostic factors: performance status, hormonal receptor status, previous adjuvant treatment, visceral metastasis at entry and number of metastatic sites. When exploring the effect of new treatment introduction, taking into account the same significant prognostic factors, the effect of time period disappears and the same effect magnitude is explained directly by the introduction of taxanes or trastuzumab (taxanes at 1st line: yes vs. no: HR = 0.73, p = 0.004; trastuzumab at 1st line: yes vs. no: HR = 0.64, p < 0.001).
Conclusions: The results provide significant evidence of improvement in prognosis of MBC patients within the last 15 years, taking into account all important significant prognostic factors, and this improvement could be explained almost fully by the use of new agents in the management of the disease.

5007 ORAL

MicroRNA profiling of circulating tumor cells (CTC) present in large quantities of leukocytes

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Background: The CellSearch Circulating Tumor Cell Test (Veridex) is the only FDA approved diagnostic test for the detection and enumeration of CTCs. CTC enumeration by this technique has proven clinical relevance in metastatic prostate, colorectal and breast cancer. Next to enumeration, there is great interest in the molecular characterization of CTCs, which may yield better prognostic and predictive factors and models. Although this system allows capture of CTCs in blood of cancer patients by selectively isolating EpCAM-positive cells followed by visual quantification of DAPI- and CK-8/18/19-positive cells [1], there are still considerable quantities of contaminating leukocytes (DAPI+/CD45+) present after enrichment. Previously, we optimized a method to determine mRNA expression of up to 96 genes in as little as a single breast cancer cell [2]. By using a set of genes with no or minor expression by leukocytes, we succeeded to specifically determine gene expression profiling in a small number (frequently less than 5) of CTCs present in a CTC-enriched blood sample typically containing over 800 contaminating leukocytes. In this study we set out to similarly characterize these CTCs at the miRNA level. MiRNAs are naturally occurring non-coding RNAs that play a role in gene regulation. Expression of various miRNAs have been associated with outcome in breast cancer.
Methods: We screened healthy blood donors (HBDs), breast cancer tissues, breast cancer cell lines spiked in blood from HBDs, and breast cancer patients for miRNA expression specific for breast cancer tumor cells with the TaqMan human MicroRNA assay v1 set (Applied Biosystems) containing 446 miRNAs.
Results: Of the 446 miRNAs, ~300 could be measured reliable in human breast cancer tissue specimens. Out of these, 60 appeared to be specific for the breast cancer tumor cells, i.e expression was over 10-fold higher when compared with the levels measured in the healthy blood donors. Next, the potential clinical applicability of these 60 differentially expressed miRNAs was validated on CTCs from a cohort of breast cancer patients with metastatic disease as detected by the CellSearch CTC test.
Conclusion: We consider our approach of great interest for the further characterization of CTCs, thereby improving insight into biological processes involved in cancer progression and ultimately patient management.

References

[1] Sieuwerts, A.M., et al. J Natl Cancer Inst, 2009.
[2] Sieuwerts, A.M., et al. Breast Cancer Res Treat, 2008.

Oral presentations (Tue, 22 Sep, 09:00−11:00)
Breast cancer II − Early disease

5008 ORAL

Minimal axillary lymph node involvement in breast cancer has different prognostic implications according to the staging procedure

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Purpose: It is still controversial whether the identification of micrometastases and isolated tumor cells in the axillary lymph nodes of patients with breast cancer has any prognostic value.
Patients and Methods: We evaluated the prognostic role of isolated tumor cells and micrometastases in the axillary lymph nodes in 3,158 consecutive patients (pT1−2 pN0-N1mi (with a single involved lymph node) and M0, referred to the Division of Medical Oncology after surgery performed at the European Institute of Oncology from April 1997 to December 2002. Median follow-up was 6.3 years (range 0.1−11 years).
Results: Sentinel lymph node biopsy (SLNB) and axillary lymph node dissection (ALND) were performed in 2,087 and 1,071 patients respectively. A worse metastasis-free survival (MFS) was observed for patients with micrometastatic disease compared to node negative patients, if staged with ALND (log-rank p < 0.0001; HR 3.17; 95% CI: 1.72−5.83 at multivariate analysis), but not for patients who underwent SLNB (log-rank p = 0.36).
Conclusion: The presence of a single micrometastatic lymph node is associated with a higher risk of distant recurrence as compared to node negative disease only for patients undergoing ALND for staging purposes. Treatment recommendations for systemic therapy should not take into account the presence of a single micrometastatic lymph node identified during complete serial sectioning of sentinel node(s).

5009 ORAL

Influence of isolated tumor cells in sentinel nodes on outcome in early pT1N0M0 breast cancer

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Aim: The aim of the study was to evaluate the prognostic significance of isolated tumor cells found in a sentinel node biopsy.
Patients and Methods: The study is based on a prospectively followed-up cohort of 1,865 consecutive patients diagnosed with invasive pT1 (tumor size $\leqslant$20 mm) breast cancer in one university breast unit between February 2001 and August 2005. Of the 1,390 patients who had received no neoadjuvant therapy and who underwent a sentinel node biopsy, 63 had isolated tumor cells in the sentinel nodes (stage pT1N0i+M0, verified by axillary node dissection), and 868 had not (pT1N0i-M0). The median follow-up time was 55 months.
Results: Patients with pN0i+ disease were treated more often with systemic adjuvant therapy than those with pN0i-disease (87% vs. 51%; $P < 0.0001$). There was no significant difference between the groups in 5-year recurrence-free survival (90.3% vs. 93.2%, respectively; $P = 0.32$) or overall survival, but patients with pN0i+ cancer had less favorable 5-year breast cancer-specific survival (95.2% vs. 98.4%; $P = 0.035$), and they were more frequently diagnosed with distant metastases from breast cancer (8.1% vs. 1.9%) during the first 5 years of follow-up ($P = 0.001$). Several