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Management of Human Epidermal Growth Factor Receptor-2(HER2) Positive Breast Cancer Patients at University Hospitals of Leicester

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Background: The HER2 protein is overexpressed in upto 30% of breast cancers. This is correlated with more aggressive breast cancer and poor prognosis. Trastuzumab (Herceptin; Genentech) is a recombinant humanized monoclonal antibody against the HER2 receptor and is the only FDA-approved targeted agent for treatment of HER2 over-expressing breast cancer. Phase II and III clinical trials performed in women with metastatic breast cancers that overexpress HER2 have shown trastuzumab to have clinical activity when used as monotherapy, while also improving survival when used as a first line therapy in combination with chemotherapy. NICE guidelines recommends use of trastuzumab in combination with chemotherapy. However, evidence is lacking to guide use of this as single agent therapy in adjuvant setting in breast cancer patients having significant medical comorbidities. The aim of our retrospective audit was to study the management of patients who were HER2 positive and to identify patients who could have benefited with trastuzumab as single agent.

Methods: The data was collected on patients who presented with invasive breast cancer between January 2007 and April 2010. Information was obtained using electronic databases and case notes. Patients with HER2 + receptor status were identified and analysed. Number of patients who received trastuzumab and patients who did not receive trastuzumab were identified and assessed.

Results: A total of 2124 female patients with invasive breast carcinoma were identified. 234 patients (11.01%) were HER2 positive out of which 162 (69.2%) received chemotherapy and trastuzumab, 7 (0.02%) patients had chemotherapy but did not receive trastuzumab (4 with advanced metastatic cancer, 3 had cardiac toxicity). 65 (27.8%) patients did not get chemotherapy because of comorbidities. Out of these however, 3 patients did receive trastuzumab as single agent therapy. Out of the 65 patients who did not receive chemotherapy, 59 patients had ductal cancer, 3 had lobular cancer and 3 had mixed ductal and lobular cancer features. 27 (41.5%) of these 65 patients had grade 3, 22 (33.8%) had grade 2, and 2 (0.03%) had grade 1 cancer. The mean Nottingham Prognostic Index in this group was 4.12. 35 (53.8%) of these patients were estrogen receptor (ER) +ve, while 30 (46.1%) were ER −ve. 9 (13.8%) of these patients had vascular invasion.

Discussion: In our study, we identified that about 29% of our HER2 +ve patients have not received trastuzumab as they were not 'fit' to have chemotherapy. These patients are known to have aggressive tumours and have a poor prognosis. Evidence based guidelines are lacking to manage this unique subset of patients who are HER2 positive but cannot have chemotherapy due to medical comorbidities or significant side effects. Further studies regarding the efficacy and toxicity of single agent trastuzumab in the adjuvant setting are needed.

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Is Angiogenesis in Inflammatory Breast Cancer a Prognostic Finding?

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Background: With combined modality treatment, five-year overall survival rates for inflammatory breast cancer range from about 30 to 70 percent. Emerging data shows that inflammatory breast cancer (IBC) is angiogenic, thereby making angiogenesis a potential attractive therapeutic target. By elucidating the biologic characteristics of IBC, new targeted treatment options may become available**.** The aim of this study is to confirm the angiogenic phenotype of inflammatory breast cancer compared to non-inflammatory primary resectable breast cancer and to investigate the response to neoadjuvant chemotherapy in patients with inflammatory breast cancer.

Material and Methods: Core needle biopsies from the primary tumor and samples obtained from the surgical specimen will be analyzed in 25 patients with IBC and compared to 25 patients with primary resectable non-IBC. Microvessel density and angiogenic factors such as vascular endothelial growth factor (VEGF)-A, VEGF-C, VEGF-D, fibroblast growth factor-2 (FGF-2), will be assessed as well as CD31 and CD34 mediated endothelial cell-cell interactions. Moreover, derivatives of hypoxia (e.g. carbonic anhydrase IX, hypoxia inducible factor alpha) will be analyzed. Results will be correlated with response to neo-adjuvant chemotherapy and clinical outcome.

Conclusions. We will investigate whether inflammatory breast cancer has a higher expression of angiogenic factors, thereby facilitating further research into drugs that potentially target angiogenesis and may inhibit tumor growth in inflammatory breast cancer.

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Developing New Generation of Effective Anti-breast Cancer Drugs Based on the Anti-HIF-1a Mechanism − A Novel High-throughput Screening System for Rapidly Selecting Potent Anti-breast Cancer Compounds

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Background: One characteristic of fast-growing breast cancer (BCa) is the development of intratumoral hypoxia, whose existence correlates to a more malignant phenotype and worse diagnosis. Adaptation to the hypoxic environment is critical for BCa cell survival and growth. The hypoxic BCa cells in tumors modify gene expression in order to obtain a blood supply and prevent cellular damage; the main mediator of the hypoxia response is hypoxia-inducible factor-1 or HIF-1. HIF-1 activity is mainly dependent on the level of HIF-1a protein, the inducible and regulatory subunit of the HIF-1 dimer complex. The activation of HIF-1a stimulates a group of downstream genes including VEGF that are responsible for tumor angiogenesis and malignant progression; therefore, targeting and inhibiting the activity of HIF-1a should provide an effective strategy to suppress BCa growth, angiogenesis, and metastasis.

Material and Methods: To screen for effective anti-HIF-1a compounds, human BCa cells that have been stably transfected with VEGF-Luc (a chimeric construct containing a VEGF promoter fused to a reporter gene luciferase) were treated with or without candidate compounds, followed by incubation in the hypoxic conditions. As the HIF-1a-mediated VEGF transactivation is reflected in the increased luciferase activity, the potential anti-HIF-1a effect of the compound is identified by its significant reduction of luciferase activity compared to the untreated control group (which will result in high luciferase activity due to the HIF-1a-mediated VEGF transactivation). The screen procedure has been developed as a 96-well platform as a high-throughput screening system.

Results: In our pilot screening of 120 plus compounds, more than a dozen of the positive anti-HIF-1a compounds have been identified by this screening system. The positive compounds were validated and confirmed by Western blot and VEGF ELISA assay for anti-HIF-1a abilities. One such lead compound (PG-928310) and its synthetic derivative (CJ-III-60), demonstrated 70% and 82% inhibition, respectively, on BCa-induced angiogenesis compared to the control, as well as their powerful abilities of anti-breast tumor growth.

Conclusions: This technology has the potential to make a significant impact in the translational drug discovery and development. This novel high-throughput screening system can rapidly identify anti-HIF-1a compounds and lead to clinical application of new drugs for treatment of breast cancer patients.

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Efficacy and Safety of Lapatinib Treatment in Trastuzumab Pretreated Patients with HER2 Positive Metastatic Breast Cancer − An Analysis of IntERB Registry in the Czech Republic

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Background: Lapatinib is an oral dual tyrosin kinase inhibitor of EGFRand HER2. In the Czech Republic is currently available for treatment for patients with HER2 positive metastatic breast cancer that progressed during trastuzumabtreatment. We evaluated effectiveness, safety and tolerability of lapatinib treatment using data from IntERB registry that has been initiated and run by Czech Society for Oncology and Institute of Biostatistics and Analyses at Masaryk University, Brno, Czech Republic.

Materials and Methods: An analysis included 213 patients with HER2 positive metastatic breast cancer treated from January 2007 to September. Median age was 56 years (range 23−78). Lapatinib was mostly administered orally 1250 mg/day with capecitabine (2000 mg/m^2 D1−14), 16 patients received lapatinib in monotherapy. All patients had experienced progression during prior trastuzumab based therapy. Median ejection fraction of LV before lapatinib treatment was 60% (range 48−90%, evaluated in 172 patients).

Results: Lapatinib was used as a 2nd line treatment in 77 patients, as 3rd line in 65 and as 4th or further line in 53, 14 patients received lapatinib after failure of adjuvant trastuzumab therapy. Median duration of lapatinib therapy was 20.6 weeks (range 1–146 w). Complete response was achieved in 13 patients(6.1%), partial response in 31 (14.6%), stable disease in 118 (55.4%), in 26 disease progressed (12.2%) and in 25 the response could not be assessed (11.7%). Most common toxicities were diarrhea in 11.7%, rash/skin toxicity in 10.8%, nausea/vomitus in 5.2% and hepatotoxicity in 2.3%. No cardiac toxicity was reported. Therapy was discontinued due toxicity in 9.0%. PFS (95% CI) for whole group was 7.1 months (range 5.9−8.5). Overall survival (95% CI) was 17.2 months (range 15.8–18.6), probability of 1-year OS was 64%. An analysis of lapatinib efficacyfor different lines of treatment revealed overall response rate 24.7% in 2nd line, 10.8% in 3rd, and 22.7% in 4th or further line. PFS was 7.6 months in 2nd line, 7.0 in 3rd, and 6.3 in 4th or further line. OS was 16.8 m in 2nd line, 18.8 in 3rd, and 15.7 in 4th or further lines.

Conclusion: Lapatinib in combination with capecitabine proved its efficacy in trastuzumab pretreated metastatic breast cancer. Therapy was well tolerated. Diagnosis and treatment specific registry is a useful and feasible method for cancer treatment evaluation in standard clinical practice.

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Antitumor Effect of the MTor Inhibitor Everolimus in Combination with Trastuzumab On Human Breast Cancer Stem Cells in Vitro and in Vivo

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Background: Recent evidence has suggested that breast cancer contains a rare population of cells called cancer stem cells (CSCs), which have the ability of extensive self-renewal and contribute to metastasis and therapeutic resistance. This study evaluated the effects of an mTOR inhibitior everolimus alone or in combination with trastuzumab on stem cells sorted from HER2-overexpressing primary breast cancer cells and breast cancer cell lines (BT474) in vitro and in vivo.

Material and Methods: *In vitro* studies, we sorted $ESA^+CD44^+CD24^{-/low}$ cells as stem cells using flow cytometry from primary breast cancer cells and BT474 cell lines. MTT assays were used to quantify the inhibitory effect of the drugs on total cells and stem cells. Apoptosis and the cell cycle distributions of stem cells were examined by flow cytometry. The tumorigenicity of stem cells after treatment was investigated by soft agar colony formation assays. In vivo studies, the BALB/c mice were injected with BT474 stem cells and the different treatments were administered. After necropsy, the expression of Ki67, CD31, AKT1, and phospho-AKT (Thr308) was analyzed by immunohistochemistry.

Results: In vitro studies, compared with their total cells, there were greater resistance to the standard treatment doses of trastuzumab in cancer stem cells sorted from primary breast cancer and BT474 cells (16- and 19-fold, respectively).Treatment with everolimus resulted in growth inhibition of stem cells in a dose-dependent manner. Compared with single-agent therapy, the combination of everolimus with Trastuzumab was more effective in the inhibition of cell growth($P < 0.001$) and tumorigenicity($P < 0.001$).In addition, an increase in G1 cell cycle arrest and an increased population of cells in early apoptosis were seen in the combination treatment group compared with either single-agent group ($P < 0.01$).In vivo, the volumes of the xenograft tumors significantly decreased in everolimus alone group compared to untreated group ($P = 0.007$), and everolimus plus trastuzumab therapy was much more effective at reducing tumor volume in mice compared with either single-agent alone ($P < 0.05$). Compared with everolimus alone, the combination of everolimus and trastuzumab reduced the expression of KI-67, AKT1 and phospho-AKT (Thr308) ($P < 0.05$).

Conclusion: Everolimus has effective inhibitory effects on HER2-overexpressing stem cell in vitro and vivo. Combination treatment of everolimus and trastuzumab could inhibit the growth of HER2-overexpressing stem cells in vitro and in vivo, in addition its effect was more effective than either drug alone. Everolimus plus trastuzumab were considered to be rational combination treatments and valuable to test in human clinical trials.

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An Audit of the Impact of New Cardiac Guidelines On Adjuvant Transtuzumab Therapy in the Northern Cancer Network

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Background: In March 2009 Jones et al published updated cardiac guidelines for the management of adverse cardiac events following the use of transtuzumab in HER-2-positive early breast cancer (EBC). These guidelines aimed to encourage a proactive and practical approach to the management of transtuzumab cardiotoxicity. These guidelines were rapidly adopted within the Northern Cancer Network in the UK. We performed a retrospective audit to assess whether the new guidelines altered the number of patients receiving and completing adjuvant transtuzumab for EBC.

Methods: Patients who had received transtuzumab for HER−2-positive EBC at Newcastle Hospitals and Wansbeck hospital in the North of England between June 2006 and June 2010 were identified from pharmacy records. Patients were split into 2 groups: those who commenced treatment before March 2009 and those after March 2009. Information was collected from patient records (electronic and paper) for demographics, changes in ejection fraction, number of cycles of transtuzumab received, delays in treatment and cardiology interventions.

Results: A total of 163 patients were identified. 108 before and 55 after March 2009. The average age of the patients and the average initial cardiac ejection fraction were similar. 93% (51) completed the full course of transtuzumab post the guidelines changing and 81% (88) prior to March 2009 ($p = 0.06$). There were less delays in treatment 5.5% versus 10%, 3 and 11 patients respectively; and fewer referrals for cardiology review 30% vs 20%, 32 and 11 patients respectively.

Conclusions: Adopting the new guidelines has allowed more women to complete their planned transtuzumab treatment and with fewer delays. Further follow-up is needed to assess whether this will have an impact on long term survival and future cardiac complications.

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DETECT III − a Multicenter, Randomized, Phase III Study to Compare Standard Therapy Alone Versus Standard Therapy Plus Lapatinib in Patients with Initially HER2-negative Metastatic Breast Cancer and HER2-positive Circulating Tumor Cells

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Background: In breast cancer patients, HER2 status may change over the course of the disease. Approximately 20−30 % of initially HER2-negative patients have HER2-positive metastasis (Zidan 2005, Tewes 2009). Re-evaluation of HER2 status on metastatic tissue is warranted, but not always possible, especially during the course of therapy. Determining HER2 status on circulating tumor cells is one option for re-evaluating HER2 status at the time metastasis is diagnosed as described in our previous study DETECT I (Fehm 2010). However, at present it is unclear if therapy based on the HER2 status of CTC offers a clinical benefit for patients. Therefore, the study DETECT III aims to assess whether lapatinib, as one of the HER2-targeted therapies, in initially HER2-negative breast cancer patients with HER2-positive CTC is effective at the time of distant disease.

Trial Design: DETECT III is a prospective, multicenter, randomized, open-label, two arm phase III study. As only half of the patients with HER2-negative MBC will be CTC-positive and approx. 32% will exhibit HER2-positive CTCs, a screening of about 1420 patients will be needed. Approx. 228 patients will be enrolled in the study and randomized 1:1 to one of the following regimens Arm A ($n = 114$): Standard Treatment, Arm B ($n = 114$): Standard Treatment plus Lapatinib.