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Concordance of HER2 Central Assessment by Two International Central Laboratories: A Ring Study within the Framework of the Adjuvant HER2-Positive ALTTO Trial (BIG2-06/N063D/EGF106708).

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In the Breast International Group (BIG) and North Central Cancer Treatment Group (NCCTG) co-led phase III adjuvant breast cancer trial, ALTTO (Adjuvant Lapatinib and/or Trastuzumab Treatment Optimisation), two central laboratories, the European Institute of Oncology (IEO; Milan, Italy) and Mayo Clinic (Rochester, MN), are responsible for confirming the human epidermal growth factor receptor-2 (HER2), estrogen receptor α (ER), and progesterone receptor status of the primary breast tumors for the Rest of World (excluding China, which conducts a separate central review) and North American patients, respectively, prior to patient study entry. As of December 2009, discordance in HER2 and ER testing between local and central laboratories was observed by both central laboratories. For IEO, 14.5% of 8,037 HER2 cases locally positive [by either immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH)] were not able to be confirmed as centrally positive; whereas for Mayo, 5.8% of 412 HER2 cases locally positive were not able to be confirmed as centrally positive (Table 1). Central and local IHC ER discordance was 12.1% and 11.7% for 9,021 IEO screened cases and 419 Mayo screened cases, respectively (Table 2). In particular, IEO observed more false-positive (locally positive/centrally negative) HER2 findings than Mayo and Mayo observed more false-positive ER findings than IEO. **Purpose:** Motivated by the above findings, we launched a ring study to assess whether the central lab results of a subset of local/central discordant ALTTO cases could be confirmed in the other central lab. **Methods:** IEO and Mayo exchanged and retested a subset of FFPE breast tumors collected in ALTTO. IEO sent 20 HER2 false-positive, 5 ER false-positive, and 5 ER false-negative (locally negative/centrally positive) ALTTO breast tumors to Mayo. Mayo sent 5 HER2 false-positive, 20 ER false-positive, and 5 ER false-negative ALTTO breast tumors to IEO. IEO and Mayo performed IHC for ER according to their own methodology: DAKO cocktail of ER 1D5 and 2.123 monoclonal antibodies and monoclonal ER 1D5 antibody, respectively. The two laboratories performed IHC for HER2 according to the HerceptTest® manufacturers' instructions (Dako, Carpenteria, CA) and FISH for HER2 using the PathVysion HER2 DNA probe kit and the HER2/centromere 17 probe mixture (Abbott Molecular, Des Plaines, IL). **Results:** IEO and Mayo confirmed the central HER2-negative result in 100% of 25 cases. Analyses of ER are ongoing and ER results will be presented. **Conclusions:** In this subset of patients, enrollment ineligibility did not change when HER2 testing was performed by either IEO or Mayo Clinic central laboratories.

Table 1. HER2 Local/Central Lab Results

HER2		Central		
Local		HER2 Eligible	HER2 Not Eligible	Total
Positive	IEO	6871	1166 (14.5%)	8037
	Mayo	388	24 (5.8%)	412
Equivocal	IEO	647	394	1041
	Mayo	6	7	13

Table 2. ER Local/Central Lab Results

ER		Central			
Local		Positive ≥10%	Positive ≥1% and <10%	Negative	Total
Positive	IEO	4550	101	288 (6.2%)	4939
	Mayo	224	3	44 (16.2%)	272
Negative	IEO	665 (16.1%)	217 (5.3%)	3240	4122
	Mayo	5 (3.4%)	0 (0%)	142	147

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Molecular Analysis of Single Circulating Tumor Cells for Characterization of the Targets of Systemic Breast Cancer Therapy as Chance to Individualize Therapy.

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Background: In breast cancer therapy predictive and prognostic markers are derived from the primary tumor but systemic therapy aims at eliminating the remnant tumor cells left in the body after surgery which the cells circulating in peripheral blood are part of. For individualized therapy, therefore, it would be advantageous to better characterize this remnant disease for more targeted therapeutic approaches.

Materials and Methods: 10 to 20 single live epithelial antigen positive cells were isolated from peripheral blood of 50 newly diagnosed breast cancer patients using the automated capillary cell isolation system of the MMI CellEctor and individually deposited under visual control onto Ampli Grid slides (Advalytics). The mRNA was the reverse transcribed and amplified using primers for 6 different tumor associated targets among them EpCAM and Her2/neu.

Results: Expression profiling could be successfully performed from more than 80% of all individually deposited isolated cells demonstrating the epithelial nature of these cells but also heterogeneity among the cells of in individual patients with respect to other genes. Thus we show that circulating epithelial cells from breast cancer patients can be individually deposited and these single celsl can subsequently be subject to expression profiling.

Discussion: Further analysis by high through-put profiling of the remnant tumor burden in breast cancer patients after surgery comprising the cells circulating in peripheral blood may contribute to better characterize the targets of systemic therapy in order to individualize cancer therapy.

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Histopathological Subclassification of Triple Negative Breast Carcinoma Using Prognostic Scoring System.

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Purpose: Triple negative breast carcinoma (TNBC) is currently being required to be classified pluralistically in order to provide the most appropriate therapy to the patients. We attempted to subclassify TNBC cases into subgroups based on clinical outcome or prognosis of the patients with TNBC using archival specimens. Materials and Methods: We analyzed 102 Japanese cases of invasive TNBC who underwent surgery between January 1998 and December 2007. The clinicopathological factors and clinical information of these patients were retrospectively retrieved from charts of the patients. Immunohistochemical staining was performed for estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor 1 (EGFR1), CK5/6, Ki-67 and CD31 for microvessel density (MVD). Results: Median follow-up time of the patients was 68.5 months. Multivariable analysis demonstrated that the pathologic node status was the most significantly associated with relapse-free survival (RFS) and breast cancer-specific survival (BCSS) of the patients. Pathological tumor size, basal-like type, Ki-67 labeling index (LI) and MVD were also independently associated with RFS and BCSS. Based on these results, we devised the Risk Score system reflecting Hazard ratios (HRs) of these prognostic factors above.

Multivariate analysis and the Risk Score for TNBC patients.

	RFS			BCSS		
Variables	HR	P value	Score	HR	P value	Score
Pathological tumor size; >20mm	2.23	0.035	2	2.63	0.025	3
Pathological node status; positive	2.99	0.003	3	2.71	0.019	3
Basal-like type	2.76	0.024	3	3.02	0.040	3
Ki-67 labeling index; >40%	2.44	0.014	2	2.68	0.023	3
Microvessel density; >20	2.32	0.029	2	2.44	0.046	2