Neoadjuvant Systemic Therapy for ER + Breast Cancer:

should the immunohistochemical surrogates of molecular sub-types be really used to assess therapy responses?

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Neo-adjuvant chemotherapy (NACT) has become a standard treatment for locally advanced breast cancers; it helps to down stage the disease and facilitate breast conserving surgery (BCS) in patients who would have otherwise needed mastectomy. However, compared to adjuvant treatment, it offers no added survival benefits [1]. Multiple chemotherapeutic regimens have been studied in combination for the neo-adjuvant setting; however, desired clinical benefits from a particular specific 'tailored' regimen is yet to be established [2]. Clinical decisions to give NACT are usually based on the local advancement of breast cancer, tumour size (large operable breast cancer) and/or proven axillary lymph node metastases [3]. Compared to adjuvant setting, patients receiving NACT benefit from the ability to monitor therapy responses on treatment (e.g. US and/or MRI) thereby allowing adjustments to chemotherapy regimen and/or drug dosages according to therapy responses [4]. Using the same approach, non-responders to therapy can also be identified early in the course of treatment thereby sparing them from the unnecessary side effects of chemotherapeutic drugs for no obvious clinical gains. Further, breast tumours that show a complete pathological response (pCR) to neo-adjuvant treatment usually tends to show a better clinical prognosis than to those with the residual disease [5]. Currently, a commonly practiced NACT regimen worldwide includes anthracyclines with cyclophosphomide and 5-fluorouracil [6]. Taxanes are added to anthracycline–based regimens in neoadjuvant setting to improve their clinical effectiveness and achieve pathological complete response [7].

Clinically, responses to NACT are assessed using radiological imaging such as ultrasound (US) and/or magnetic resonance imaging (MRI). Several guidelines to define tumour response on radiological imaging have been proposed. Among these, the criteria validated by the Response Evaluation Criteria in Solid Tumours (RECIST) group [8] and the UICC (International Union Against Cancer) are widely accepted and frequently used [9]. Using the commonly used RECIST guidelines, responses can be classified as a complete or partial, stable disease and progressive disease (Table 1). In contrast, definition of pathological complete response (pCR) can be absence of invasive and non-invasive disease from breast or from both breast and axillary lymph nodes [10]. However, the above definition of pCR, varies between studies, and therefore, to attain an uniformity in reporting pCR an international expert panel (IEP) re-defined pCR as the absence of invasive and non-invasive disease from both breast and axillary tissues at the time of surgery [11]. A more recent definition of pCR given by the US Food and Drug Administration (FDA) agency for evaluation of NACT trials [12] differs from the IEP's definition in that, it considers absence of only invasive disease from breast and ipsilateral axilla ignoring the presence of any in-situ cancer.

Breast cancers on their cell surface express hormone receptors; these can be used to determine cancer prognosis and to formulate adjuvant hormonal treatment strategies. In routine clinical practice, immunohistochemical (IHC) analysis is used as a primary screening tool to evaluate oestrogen (ER), progesterone (PR), and human epidermal growth factor receptor 2 (HER2) status in breast cancers [13,14]. Traditionally, IHC determined breast cancer hormonal (ER, PR and HER2) status is considered as a surrogate to molecular intrinsic subtypes due to the difficulties at employing gene expression profiling (GEP) more routinely [15]. Earlier studies reported a higher degree of concordance between GEP and IHC defined breast cancer taxonomies. However, with more recent evidence, a significant variability has been reported between IHC and GEP based breast cancer classifications [16-18]. Therefore, in light of the new evidence, concordance between the clinical sub-typing based on the IHC and the molecular sub-typing can be questioned.

Aim

The aim of our study was to assess anthracycline-taxane based neoadjuvant breast chemotherapy pCR and clinical response rates of the breast intrinsic molecular sub-types based on the immunohistochemical (IHC) determined ER, PR and HER2 surrogates.

Patients and methods

A retrospective clinical and pathological data analysis for 58 patients treated with Epirubicin Cyclophosphamide-Docetaxel NACT regimen from January 2009 to December 2011 was performed. NACT consisted of 4 cycles of Epirubicin, Cyclophosphamide (EC) and 4 cycles of Docetaxel. The pre-treatment baseline tumour measurements were established on breast MR scan and compared with the breast histological invasive cancer component using RECIST criteria to assess therapy responses. Assessing response this way, eliminated any inaccuracies in tumour measurements as a result of tumour fragmentation following taxanes. The pathological complete response in our study was determined according to the FDA's definition of absence of invasive carcinoma from breast on histology. Statistically significant correlations were determined on two-sided chi-square test; a probability value (p) of less than 0.05 was considered significant. Using the immunohistochemical

Table 1: RECIST CRITERIA (Adapted European Journal of Cancer 45 (2009) 228-247)

Response	Definition
Complete Response (CR)	Disappearance of all target lesions
Partial Response (PR)	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters
Progressive Disease (PD)	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study) In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5mm OR Appearance of one or more new lesions
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters

Table 2: Data from the Complete Study Population

	IDC	%	ILC	%
No. of Patients	52	89.6	6	10.3
Pre-operative Tumour Grades				
1	4	7.6	0	0
2	23	44.2	5	83.3
3	25	48	1	16.6
Tumour size				
T1	0	0	0	0
T2	25	48	1	16.6
T3	27	52	5	83.3
Molecular Sub-types				
Luminal A	36	69.2	3	50
erbB2-overexpressives	6	11.5	2	33.3
Basal-like (Triple negatives)	10	19.2	1	16.6
Response to NACT				
pCR	5	9.6	0	0
Non-pCR	28	53.8	2	33.3
Non-Responder	19	36.5	4	66.6
Surgical Treatment				
WLE	20	38.4	2	33.3
Mastectomy	32	61.5	4	66.6
ANC	17	32.6	3	50

Table 3: Therapy Responses Based on IHC Determined Receptor Status

Receptor Status	Number	Therapy Responses			
		pCR	PR	SD	PD
ER+	43	3 (6.9%)	26 (60.4%)	13 (30.2%)	1 (2.3%)
ER-	15	1 (6.6%)	10 (66.6%)	0 (0%)	4 (26.6%)

profiling, molecular surrogates (luminal-A, luminal-B, Erbb2 overexpressive and basal phenotype) were defined from the ER, PR and HER2 receptor status of the resection specimens for all 58 breast cancer patients. The above method to define molecular surrogates' remains valid and currently accepted in routine clinical practice.

Results

A total of 58 breast tumours were analysed. 52/58 (89.6%) were invasive ductal non-specific type and 6/58 (10.3%) invasive lobular type. The median age of the patient groups was 55 years (IQR 48-72). Majority of the ductal tumours were grade 3 (25/52), 23/52 of grade 2 and 4/52 of grade 1. In contrast, lobular cancers were majority of grade 2 (5/6) with only one patient showing a grade 3 disease. A total of 32/58 (55.1%) tumours were T3 and 26/58 (44.8%) tumours were T2. Molecular classification of 58 tumours into intrinsic sub-types based on IHC surrogates showed, 39/58 (67.2%) were luminal A sub-type; 11/58 (18.9%) basal-like triple negatives and 8/58 (13.7%) Erbb2 overexpressives sub-type. Of the 52 ductal tumours, 63.4% were found to show partial clinical response with a recorded pCR rate of 9.6%. In contrast, 33.3% lobular subtypes were found to show partial clinical response with none of the patients in the lobular group having a pCR (Table 2). A further subgroup analysis of the data based on ER+ vs. ER- tumours showed pCR rates of 6.9% vs 6.6% and partial clinical response rates of 60.4% vs 66.6% (Table 3).

Evaluation of therapy responses based on the molecular subtypes showed: 7.6% of luminal A had pCR, 51.2% had partial clinical response and 41% stable disease. Similarly for the Erbb2 sub-type, 12.5% of patients had a pCR, 50% had partial clinical response and 37.5% stable disease. Findings from the basal like triple negatives showed a pCR rate of 9%, partial clinical response rate of 54.5% and stable disease rate in 36.3% (Table 4).

Discussion

For many years, histological classification of breast cancers along with the immunohistochemical assessment of predictive biomarkers such as oestrogen (ER), progesterone (PR) and human epidermal growth factor-2 (HER2) has remained gold standard and clinically useful [19-21]. Over the past decade, using microarray based-gene expression profiling, four classes of intrinsic breast cancer molecular subtypes (luminals, basal-like, Erbb2 over-expressive and normal-like) have been discovered with distinct tumour biologies and varying clinical outcomes [22]. As believed, the differences in tumour biology determine not just the tumour prognosis but also sensitivities to primary systemic chemotherapy [23]. Rouzier et al. evaluated the gene expression profiles of 82 breast cancer patients treated with anthracycline-taxane NACT and reported pCR rates of 45% for the basal-like and HER2-positive sub-types and 6% for the luminal sub-types [24]. Kim et al. from their series of 257 patients treated with adriamycin-taxane NACT showed similar findings of a higher pCR rates 21.1% vs 10.5% vs 8.9% $p = 0.001$ for basal-like, HER2+ and luminal sub-type [23]. Carey et al. from their 107 patients series showed 36% of HER2+ and 27% of basal-like sub-types acheived pCR ($p = 0.01$) compared to only 7% of luminal tumours following anthracycline only NACT [25]. Lv et al.from their analysis of 102 tumours treated with anthraccyline-taxane-carboplatin NACT regimens found pCR rates of 24.4% ,22.2% and 8.7% ($p = 0.041$) for basl-like, HER2+ and luminal sub-types respectively [26]. Furthermore, the effects of anthracycline-taxane based NACT in different biological breast cancer phenotypes were examined in the GeparTRio study involving 2,072 locally

Table 4: Patients therapy response summary based on molecular sub-types

Characteristics	Responders		Stable Disease
	pCR	Partial	
Total Number of patients 58			
Molecular Sub-types Luminal (39/58) (A) erbB2-overexpressive (8/58) Basal-like (11/58) (Triple negatives)	 3 (7.6%) 1 (12.5%) 1 (9%)	 20 (51.2%) 4 (50%) 6 (54.5%)	 16 (41%) 3 (37.5%) 4 (36.3%)
Pre-operative Tumour Grades Grade 1 Grade 2 Grade 3	 0 2 3	 2 12 16	 2 14 7
Tumour size T1 T2 T3	 0 3 2	 0 12 18	 0 11 12
Surgical Treatment WLE Mastectomy ANC	 2 3 1	 13 17 11	 7 16

advanced breast cancers [27]. Findings from this study showed a highest pCR rate of 57% in patients age < 40 years with triple negative (basal-like) status comapared to 35% pCR rate for luminal sub-types (Table 5). Therefore, a summary all the above studies suggest luminal sub-type compared to basal-like and HER2+ molecular sub-types generally have a pCR rate with different chemotherapy regimens adopting different pCR definitions. One possible explantation for the low pCR rates observed with the luminal sub-type is a low expression of proliferation cluster genes that determine chemo-sensitivity at the molecular level [22,28].

Our study, showed pCR and partial clinical response rates statistically significantly correlated (pCR, $p = 0.03$ vs. partial clinical response, $p = 0.04$) with luminal and Erbb2 subtypes respectively [26-30]. Compared to other larger studies, the findings of low pCR rates with basal-like triple negatives as observed in our study may be related to the smaller study size. Therefore, this finding does not aim to contradict the results from larger studies or carry a clinical significance. However, within the limitations of a smaller study and a retrospective analysis, results from our study highlighted an interesting finding of discordance in the pCR rates between luminal subtypes as determined on IHC and ER+ tumours. For all practical purposes, in our clinical practice, IHC determined ER, PR and HER2 status were considered as surrogates of molecular intrinsic sub-types and used to assess the therapy response.

Recently, an initially thought higher degree of concordance between the gene expression profiling and IHC defined breast cancer taxonomies have been challenged with a more recent version of classifications [16-18]. A limited concordance has now emerged between the taxonomies challenging the accuracy and validity of IHC defined biomarkers surrogates of molecular status. The 12th St Gallen International Breast Cancer Conference (2011) Expert Panel adopted a new approach to the classification of patients for therapeutic purposes based on the recognition of intrinsic biological subtypes within the breast cancer spectrum. [29]. In this consensus, sub-types defined by GEP were included after an approximation based on IHC surrogates. Variation in concordance between IHC and GEP defined ER+ tumours ranged between 73%-100% for luminal A and B categories; 41%-69% for HER2-enriched tumours and 80% between basal-like. In contrast, a recent study, the 2012 IMPAKT task force investigated the medical usefulness of current methods for the classification of breast cancer into the 'intrinsic' molecular subtypes using the prediction analysis of microarray (PAM) 50 assay and an immunohistochemical surrogate panel including oestrogen receptor (ER), HER2 and Ki67 [15]. Findings from the study showed the available evidence on the analytical validity, clinical validity and clinical utility of ER/HER2 to be convincing and concluded classifying breast cancers into molecular subtypes based on the IHC assessment of ER, HER2 and Ki67 with a 14% cut-off.

Furthermore, responses to NACT and the prognosis can also vary for based on the histological type; therefore, defining the relationship between each histological type and the clinico-pathological response to NACT is essential to optimise individualised treatments. In a recently published Japanese study by Nagao et al. marked variations in therapy responses to anthracycline-taxane based chemotherapy were noted within the same histological subtype [30]. Gonzalez-Angulo et al. compared expression of established histopathologic and biologic markers of proliferation, apoptosis, and angiogenesis in invasive lobular carcinoma and invasive ductal carcinoma (no special type) and concluded that both ductal and lobular pathologies are different biologic entities and therefore should require tailored options for systemic treatment [31]. The low pCR rate seen with lobular subtypes in our study strengthens the above argument for a need for individualised treatment approach. Goldstein et al. evaluated correlation between response to neoadjuvant chemotherapy and the molecular subtypes in a cohort of patients previously treated with neoadjuvant chemotherapy. Overall, pCR was observed in 28 of 68 cancers (41.2%). Although the numbers classified as invasive lobular carcinoma studied were small (11 of the 68

Table 5: Multivariate analysis for pCR within different molecular groups (Adapted from GeparTrio Study; Huober von Minckwitz et al. 2010)

Factor	Luminal A, pCR at surgery (n=562)		Luminal B, pCR at surgery (n=462)		HER2 like, pCR at surgery (n=193)		Triple negative, pCR at surgery (n=351)	
	pCR (%)	P value	pCR (%)	P value	pCR (%)	P value	pCR (%)	P value
Age (years) <40 >40	 10.1 6.6	 0.256	 24.1 17.8	 0.163	 33.3 28.2	 0.441	 57.0 34.1	 0.01
Tumour Grade III I+II	 16.2 3.8	 <0.0001	 24.3 17.8	 0.018	 31.3 25.3	 0.164	 39.5 30.5	 0.137
Histological Type Ductal / others Lobular	 7.8 4.3	 0.376	 20.0 11.9	 0.058	 31.0 7.7	 0.164	 38.9 39.1	 0.702

tumours), they observed that none of the morphologically classical lobular carcinomas, classified as luminal-A neoplasms (n = 3), showed a pCR [32, 33]. Results from our study mirrored the above findings, reiterating that the poor therapy responses in luminal subtypes as determined on IHC could be related to the poor histological subtypes rather than a faulty intrinsic biology. However, in our study, the mitotic rate or Ki67 index was not analysed in lobular cancers to see if this could have contributed to the poor pCR rate observed with this histological subtype. In the light of all this evidence, and in the era of tailored therapy for individual patients, neoadjuvant chemotherapy in patients with ER+, HER2-, classical type invasive lobular carcinoma is no longer recommended [33].

A superior NACT response rate does not always translate into improved clinical benefit as seen with the triple negatives (basal-like) tumours. Dent et al. from the study of 1,061 patients showed triple negative (basal-like) tumours generally have a more aggressive clinical behaviour with a poor recurrence free and a 5 year overall survival (HR 2.6 vs 3.2, $p < 0.001$) [34]. This paradox of high sensitivity to NACT and poor clinical outcome can be explained by studies looking at the ability of tumours to achieve a pCR following therapy rather than the molecular characteristics itself. As shown by Carey et al. and Kim et al., presence of residual disease and not the molecular sub-type that determines the 5 year survival [23, 25]. In both these studies, the overall survival was found to be uniformly low across different subtypes if the tumours failed to acheive a pathological complete response. Therefore, failure to acheive pCR may be considered as a more acurate determinant of cancer prognosis rather than the a particular molecular subtype itself.

Conclusion

In summary, our study showed pCR and partial clinical response rates were mainly associated with the luminal and Erbb2 intrinsic subtypes. Luminal molecular subtype showed a relatively good concordance with their IHC surrogates (ER+). With the recent evidence suggesting a high variation in the concordance rates between the IHC/GEP based classifications; an accurate molecular diagnosis and an appreciation of the individual subtype sensitivity and responsiveness to NACT is necessary to plan treatment and assess therapy responses in clinical trials. ■

References

1. Steger GG and Bartsch R. *Trends and Novel Approaches in Neoadjuvant Treatment of Breast Cancer. Breast Care (Basel)*. 6(6):427-33.
2. Hudis C and Modi S. *Preoperative chemotherapy for breast cancer: miracle or mirage?* JAMA, 2007;298(22): 2665-7.
3. Fisher B, et al. *Effect of preoperative chemotherapy on the outcome of women with operable breast cancer.* J Clin Oncol, 1998;16(8):2672-85.
4. Wolmark N, et al. *Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18.* J Natl Cancer Inst Monogr, 2001(30):96-102.
5. Loo CE, et al. *Dynamic contrast-enhanced MRI for prediction of breast cancer response to neoadjuvant chemotherapy: initial results.* AJR Am J Roentgenol, 2008;191(5):1331-8.
6. van der Hage JA, et al. *Preoperative chemotherapy in primary operable breast cancer: results from the European Organization for Research and Treatment of Cancer trial 10902.* J Clin Oncol, 2001;19(22):4224-37.
7. Bonneterre J, et al. *Phase II multicentre randomised study of docetaxel plus epirubicin vs 5-fluorouracil plus epirubicin and cyclophosphamide in metastatic breast cancer.* Br J Cancer, 2004;91(8):1466-71.
8. Therasse P, et al. *New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada.* J Natl Cancer Inst, 2000;92(3):205-16.
9. Hayward JL, et al. *Assessment of response to therapy in advanced breast cancer: a project of the Programme on Clinical Oncology of the International Union Against Cancer, Geneva, Switzerland.* Cancer, 1977;39(3):1289-94.
10. Jones RL and Smith IE. *Neoadjuvant treatment for early-stage breast cancer: opportunities to assess tumour response.* Lancet Oncol, 2006;7(10):869-74.
11. Kaufmann M, et al. *Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: an update.* J Clin Oncol, 2006;24(12):1940-9.
12. Services, U.S.D.o.H.a.H., F.a.D. Administration, and C.f.D.E.a.R. (CDER), Guidance for Industry Pathologic Complete Response in Neoadjuvant Treatment of High-Risk Early-Stage Breast Cancer: Use as an Endpoint to Support Accelerated Approval DRAFT GUIDANCE, FDA, Editor 2012: Rockville.
13. Yaziji H and Gown AM. *Accuracy and precision in HER2/neu testing in breast cancer: are we there yet?* Hum Pathol, 2004;35(2):143-6.
14. Hanley KZ, et al. *Immunohistochemical detection of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 expression in breast carcinomas: comparison on cell block, needle-core, and tissue block preparations.* Cancer, 2009;117(4):279-88.
15. Guiu S, et al. *Molecular subclasses of breast cancer: how do we define them? The IMPAKT 2012 Working Group Statement.* Ann Oncol, 2012;23(12):2997-3006.
16. Prat A, et al. *Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer.* Breast Cancer Res, 2010;12(5):R68.
17. de Ronde JJ, et al. *Concordance of clinical and molecular breast cancer subtyping in the context of preoperative chemotherapy response.* Breast Cancer Res Treat, 2010;119(1):119-26.
18. Sorlie T, et al. *Repeated observation of breast tumor subtypes in independent gene expression data sets.* Proc Natl Acad Sci USA, 2003;100(14):8418-23.
19. Davies C, et al. *Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials.* Lancet, 2011;378(9793):771-84.
20. Hammond ME, et al. *American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version).* Arch Pathol Lab Med, 2010;134(7):e48-72.
21. Wolff AC, et al. *American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer.* Arch Pathol Lab Med, 2007;131(1):18-43.
22. Perou CM, et al. *Molecular portraits of human breast tumours.* Nature, 2000;406(6797):747-52.
23. Kim SI, et al. *Molecular subtypes and tumor response to neoadjuvant chemotherapy in patients with locally advanced breast cancer.* Oncology, 2010;79(5-6):324-30.
24. Rouzier R, et al. *Breast cancer molecular subtypes respond differently to preoperative chemotherapy.* Clin Cancer Res, 2005;11(16):5678-85.
25. Carey LA, et al. *The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes.* Clin Cancer Res, 2007;13(8):2329-34.
26. Lv M. et al. *Predictive role of molecular subtypes in response to neoadjuvant chemotherapy in breast cancer patients in Northeast China.* Asian Pac J Cancer Prev, 2011;12(9):2411-7.
27. Huober J, et al. *Effect of neoadjuvant anthracycline-taxane-based chemotherapy in different biological breast cancer phenotypes: overall results from the GeparTrio study.* Breast Cancer Res Treat, 2010;124(1):133-40.
28. Sorlie, T, et al. *Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications.* Proc Natl Acad Sci USA, 2001;98(19):10869-74.
29. Goldhirsch A, et al. *Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011.* Ann Oncol, 2011;22(8):1736-47.
30. Nagao T, et al. *The differences in the histological types of breast cancer and the response to neoadjuvant chemotherapy: the relationship between the outcome and the clinicopathological characteristics.* Breast, 2012;21(3):289-95.
31. Gonzalez-Angulo AM, et al. *Biologic markers in axillary node-negative breast cancer: differential expression in invasive ductal carcinoma versus invasive lobular carcinoma.* Clin Breast Cancer, 2006;7(5):396-400.
32. Goldstein NS, et al. *Molecular classification system identifies invasive breast carcinoma patients who are most likely and those who are least likely to achieve a complete pathologic response after neoadjuvant chemotherapy.* Cancer, 2007;110(8):1687-96.
33. Purushotham A, et al. *Neoadjuvant chemotherapy: not the best option in estrogen receptor-positive, HER2-negative, invasive classical lobular carcinoma of the breast?* J Clin Oncol, 2010;28(22):3552-4.
34. Dent R, et al. *Triple-negative breast cancer: clinical features and patterns of recurrence.* Clin Cancer Res, 2007;13(15 Pt 1):4429-34.

With variation in concordance between IHC and GEP defined ER+ tumours, the time has come for the routine use of molecular sub-typing in the neoadjuvant treatment setting of breast cancer