The Breast 21 (2012) 20–26

Contents lists available at SciVerse ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Review

Triple negative breast cancer: Proposals for a pragmatic definition and implications for patient management and trial design

W. Eiermann[a,*], J. Bergh[b,c], F. Cardoso[d], P. Conte[e], J. Crown[f], N.J. Curtin[g], J. Gligorov[h], B. Gusterson[i], H. Joensuu[j], B.K. Linderholm[k], M. Martin[l], F. Penault-Llorca[m], B.C. Pestalozzi[n], E. Razis[o], C. Sotiriou[p], S. Tjulandin[q], G. Viale[r]

[a] Rotkreuzklinikum München GmbH, Frauenklinik, München, Germany
[b] Radiumhemmet, Karolinska Institutet and University Hospital, Stockholm, Sweden
[c] Manchester University, UK
[d] Champalimaud Cancer Center, Breast Cancer Unit, Lisbon, Portugal
[e] Division of Medical Oncology, Department of Oncology, Hematology and Respiratory Diseases, University Hospital, Modena, Italy
[f] St. Vincent's Hospital, Dublin, Ireland
[g] Newcastle University, Northern Institute for Cancer Research, Paul O'Gorman Building, Medical School, Newcastle upon Tyne, United Kingdom
[h] CancerEst, APHP Tenon, Medical Oncology, APREC, Paris, France
[i] Department of Pathology, Institute of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom
[j] Helsinki University Central Hospital, Helsinki, Finland
[k] Department of Oncology, Sahlgrenska Academy and University Hospital, Gothenburg, Sweden
[l] Hospital Gregorio Maranon, Universidad Complutense, Madrid, Spain
[m] Department of Pathology, Centre Jean Perrin, Clermont-Ferrand, France
[n] Department of Oncology, UniversitätsSpital Zürich, Zürich, Switzerland
[o] Hygeia hospital, 1st Dept of Medical Oncology, Marousi, Greece
[p] Jules Bordet Institute, Brussels, Belgium
[q] Russian Cancer Research Center, Clinical Pharmacology and Chemotherapy, Moscow, Russia
[r] Department of Pathology and Laboratory Medicine, European Institute of Oncology and University of Milan School of Medicine, Milan, Italy

ARTICLE INFO

Article history:
Received 4 July 2011
Received in revised form
2 September 2011
Accepted 4 September 2011

Keywords:
Basal-like
Breast cancer
Her2
Histopathology
Oestrogen receptor
Triple negative

ABSTRACT

In trials in triple negative breast cancer (TNBC), oestrogen and progesterone receptor negativity should be defined as < 1% positive cells. Negativity is a ratio of <2 between Her2 gene copy number and centromere of chromosome 17 or a copy number of 4 or less. In routine practice, immunohistochemistry is acceptable given stringent quality assurance. Triple negativity emerging after neoadjuvant treatment differs from primary TN and such patients should not enter TNBC trials. Patients relapsing with TN metastases should be eligible even if their primary was positive. Rare TN subtypes such as apocrine, adenoid-cystic and low-grade metaplastic tumours should be excluded. TN and basal-like (BL) signatures overlap but are not equivalent. Since the significance of basal cytokeratin or EGFR overexpression is not known and we lack validated assays, these features should not be used to subclassify TN tumours. Tissue collection in trials is mandatory so the effect on outcome of different tumour phenotypes and BRCA mutation can be explored. No prospective studies have established that TN tumours have particular sensitivity or resistance to any specific chemotherapy agent or radiation. TNBC patients should be treated according to tumour and clinical characteristics.

Introduction

The nature and implications of a triple negative (TN) phenotype – ie minimal or low expression of both oestrogen and progesterone receptors (ER and PgR) and the lack of type-2 human epidermal growth factor receptor (Her2) overexpression or gene amplification – is one of the most active areas of research and debate in breast cancer.[1–6] Triple negativity is associated with younger age at diagnosis and occurs with greater frequency in non-Caucasian, premenopausal women and those who are overweight (particularly with abdominal obesity).[7] TN cancers are more likely than other kinds of breast tumour to occur in the intervals between mammographic screening.[7] Triple negative breast cancer (TNBC) is aggressive, showing a tendency towards early metastasis and

* Corresponding author. Tel.: +49 8915706620; fax: +49 8915706623.
E-mail address: w.eiermann@gmx.net (W. Eiermann).

0960-9776/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.breast.2011.09.006

having a poor overall outcome despite being highly responsive to conventional chemotherapy. TN tumours have a greater tendency to metastasise to lung and brain[8] and (when compared with luminal tumours) relatively little propensity to metastasise to bone.[9] Recurrence within two-three years is relatively common, and absence of recurrence of TN tumours within five years suggests a low risk of subsequent distant metastasis.

Given the lack of hormone and growth factor receptor drug targets, non-surgical treatment options for patients with TNBC have until recently been confined to chemotherapy and radiation. Today, increased understanding of the molecular biology of TN tumours is generating a wealth of clinical trial activity. Around twenty current trials are specifically accruing TN patients for studies in the adjuvant, neoadjuvant and advanced or metastatic settings.[1]

Despite its attraction to triallists, the concept of triple negativity is not without problems. Firstly, receptor expression is not all-or-nothing; and there is no uniformly accepted cut-off point that defines its absence. Current trials therefore differ in the receptor expression thresholds below which patients must fall to qualify for entry. Secondly, as with any phenomenon categorised by exclusion, we can be reasonably sure of what TNBC is not, but not necessarily of what it is. Having excluded patients whose tumours express hormone receptors and overexpress Her2, we are left with a heterogeneous collection of cases. High grade predominates, as does an invasive ductal (not otherwise specified) origin. However, TN tumours display many different morphologies and molecular characteristics. The complexity of the field is illustrated by the relationship between the concepts of triple negativity and of basal-like (BL) breast cancer: certain authors consider these terms as virtually synonymous while others emphasise their differences.[10–14] As in many complex situations, there is a tension between seeing the picture clearly and seeing it whole. In respect of the sensitivity of TNBCs to particular classes of cytotoxics, for example, the more closely the literature is scrutinised, the less clear are the conclusions that can be drawn.

Both the recognition that hormone receptor-positive breast cancer was sensitive to endocrine treatment and the demonstration that Her 2 positive tumours responded to drugs directed at this growth factor brought major advances in care. A pressing question now is whether definition of a TN phenotype, and the development of treatments tailored to it, can bring similar progress.

Under the auspices of Eticho (European Training in Clinical Hematology and Oncology) an ad hoc but expert group of clinicians and pathologists convened in Milan in October 2010. The meeting discussed first how TNBC might best be defined pragmatically (taking into account not only the classical markers of hormone and growth factor receptor status but also possible additional molecular and clinical characteristics) and, secondly, how to apply this definition in selecting patients for clinical trials and, in the everyday clinical setting, as a guide to management. This report presents a series of proposals for further discussion.

Defining triple negativity

Hormone receptor status

There are considerable practical limitations to relevant assays. Using IHC to determine hormone receptor status, the rate of false negative or positive findings may be as high as 20%.[15] There is lack of reproducibility and variability between institutions. In the individual patient, the results obtained may reflect intratumoural heterogeneity in the expression of relevant markers; primary and metastatic deposits can differ appreciably in their receptor expression; and receptor positivity/negativity may change during tumour progression and in response to systemic therapy.

Two recent expert groups have recommended thresholds for the use of specific adjuvant therapies. The 2009 St Gallen consensus proposed endocrine therapy for patients whose tumours showed any ER staining, or “the presence of any detectable oestrogen receptor”.[16] The 2010 American Society of Clinical Oncology-College of American Pathologists (ASCO-CAP) recommendations suggest 1% as the threshold for an ER positive tumour, so as not to deny any patient a potentially helpful treatment.[15]

However, having a single threshold for hormone receptor positivity is arbitrary and confusing to clinicians and patients who cannot understand why a difference that in itself is small – but sufficient to place a patient one side of the cut-off rather than the other – should have such profound implications for treatment. The suggestion is that there be two cut-offs – a higher value above which almost everyone agrees the patient is positive, and a lower one which almost everyone agrees means negativity, leaving a grey zone in between.

We propose that – when designing clinical trials of novel agents in TNBC – ER negativity should be defined strictly as the lack of or presence of fewer than 1% positive cells, irrespective of staining intensity. When developing novel agents for TNBC, there is virtue in working with a clearly defined biological entity. In everyday clinical practice, however, a less stringent cut-off may be adopted. In the zone between 1% and 10% we look for guidance to other features of the tumour and the circumstances of the patient when deciding on the appropriateness of endocrine therapy since there is evidence of some response to endocrine manipulation in this grey zone.[17,18] In patients whose tumours have more than 10% of cells expressing ER, endocrine therapy should be offered.

The presence of PgR negativity in the definition of the triple negative patient has been questioned. Although PgR status is of independent prognostic significance in meta-analyses,[19] its lack of predictive value in the individual patient has led some to reject its inclusion, favouring instead the concept of “dual negativity”. Furthermore, perhaps because PgR expression is downstream of ER, it is rare for a particular patient to be ER negative but PgR positive. Indeed, the St Gallen group argue that such findings are largely or wholly artefactual.[16] An ER-negative but PgR positive result strongly suggests the need for repeat ER assay. For this reason alone (and despite its cost), there is merit in the simultaneous determination of ER, PgR and Her2.

For the purposes of clinical trial eligibility, PgR negativity should be defined (as for ER) as fewer than 1% positive cells, irrespective of staining intensity. In routine clinical practice, up to 10% positive cells may also be considered PgR negative. Tumours with more than 10% of cells positive for PgR should be considered positive.

Some studies have suggested that triple negative but androgen receptor-positive tumours (around 30% of TN cases) may have a better prognosis than TN androgen-negative tumours.[20,21] Androgen receptors may in the future help constitute a clinically relevant subgroup. However, current data do not justify assay of androgen receptors in clinical practice, and indeed no well-validated diagnostic antibody is available. Nevertheless, we strongly recommend that androgen receptor status should be investigated in TNBC trials, particularly since new agents targeting this receptor are being developed.

Her2 status

In a study of 24 Swedish pathology departments, each laboratory was sent a tissue microarray including eleven primary breast cancer samples for Her2 analysis. IHC showed reasonable reproducibility: for six of the eleven samples, all laboratories reported the same findings (0/1 + vs 2 + vs 3+; mean kappa value 0.77). However, reproducibility across centres was considerably higher

when using the FISH technique: all laboratories agreed on the result (normal vs amplified) for ten of the eleven samples (mean kappa value 0.96).[22] In a series of two thousand Spanish patients, 5% of tumours reported by IHC as 0 or 1 + proved to have gene amplification on FISH while 10% of IHC 3 + cases were false positives (M Martin, personal communication). A figure of >10% false positives is suggested by other analyses (G Viale, personal communication). The rate of false negativity with IHC (as determined by confirmatory ISH) is 10% in patients being assessed for entry into two multicentre French studies of anti-EGFR agents in TNBC (F Penault-Llorca, personal communication).

FISH is more accurate and reproducible than IHC, partly because the methodology is less influenced by variability in tissue fixation methods and timing. Data obtained by FISH are also more closely correlated than IHC status with the outcome of anti-Her2 therapies.[23,24]

Since amplification of the Her2 gene is directly related to protein overexpression in tumour and since the method is more accurate and reproducible, we strongly recommend that − for clinical trial purposes – Her2 status is determined in a central laboratory by ISH.[25] Her2 negativity should be defined as a ratio of less than two between Her2 gene copy number and centromere of chromosome 17 or a gene copy number of 4 or less. Three major trials in adjuvant breast cancer (Breast Cancer International Research Group (BCIRG) 005, 006 and BETH) specify FISH as the means of establishing Her2 status. The BCIRG has now randomised more than ten thousand patients across fifty countries. Large scale use of the technology is therefore entirely feasible.

The recommendation that all future TN trials confirm Her2 status by FISH is not without potential disadvantage. There is at least the theoretical possibility that regulatory authorities might require negativity to be demonstrated by this assay before the treatment can be used in routine practice; in many countries, the expense would have to be carefully considered; and it may also seem perverse to argue that IHC, a validated method routinely used to assess patients for trastuzumab, is not adequate for clinical trials. However, our conclusion remains that the need to establish uniformity across institutions makes centralised FISH assay the gold standard.

In general clinical practice, assay by IHC is acceptable if quality assurance is stringent. Nevertheless, while a finding of 0 or +1 by IHC is clearly Her2 negative, in cases where the tumour is 2 + by IHC, its Her2 status should be determined by ISH. An IHC score of 3 + defines Her2 positive tumours.

Focal Her2 positivity

A small number of patients with Her2 negative breast cancer on the above criteria have clones within their tumours which result in focal amplification of the Her2 gene.[26] Data from the Curie Institute on 30,000 patients indicate that 3.5% of the 5% of cases which were 2 + or equivocal showed Her2 heterogeneity, and such heterogeneity was associated with a poor prognosis similar to that in patients with "real" 3 + tumours (Frederique Penault-Llorca, personal communication; and [27]). In such cases, the testing of additional blocks from the primary tumour or − where there is metastasis − from regional lymph nodes may provide further information.

In routine practice, there is a case for considering patients with focal amplification as weakly Her2 positive: they may benefit from trastuzumab, although this has not yet been demonstrated. Focal Her2 positivity may be at least partly responsible for the unexpected data from clinical trials showing response to trastuzumab in patients classified as having Her2 negative tumours.[28] However, such patients should ideally not be entered into clinical trials in TN disease since there is merit in defining the population as stringently as possible.

Triple negativity and the basal-like phenotype

The initial subclassification of breast cancers by gene expression profiling has led to considerable interest in the concept of basal-like (BL) tumours as a subgroup of biological and prognostic significance.[10,29–34] Although there is overlap between the clinical and molecular characteristics of TN and BL tumours (two thirds of TN tumours have BL features on microarray[33,35]), these two categories are not equivalent.Within BL tumours there is heterogeneity: a proportion expresses Her2 and ER receptors, and hence is not triple negative by IHC/FISH criteria.[35]

Moreover, triple negativity is essentially a pragmatic concept. Defining it requires only three assays, all routinely used to determine eligibility for treatments proven to benefit women whose tumours express the relevant drug target. In contrast, the BL concept was initially based on expression profiling involving almost 500 genes.[10] It has been suggested that TN tumours should be subclassified according to additional IHC markers relating to basal cytokeratins 5 and 6 and EGFR. Triple negativity along with absence of cytokeratin and EGFR expression would constitute a new "five negative" tumour category.[36,37] However, hormone receptor and Her2 status apart, the characteristics supposed to define basal-like (although arguably related to poor prognosis) are not clinically proven drug targets. Therefore, identification of these basal-like features within TN tumours does not at present have implications for management.

Given current limitations in our understanding of their significance and in the availability of validated assays, the routine subclassification of TN patients according to expression of EGFR and basal cytokeratins cannot be endorsed. If the target population for a trial is TN cancers, subclassification of tumours into basal-like and non basal-like should not form part of the entry criteria.

However, there is a need within the TN category to identify specific and molecularly distinct subgroups with different responsiveness to standard cytotoxics as well as potential sensitivity to new biological agents so that they can avoid highly aggressive adjuvant chemotherapy. It would also be helpful to have ways of assessing biological differences that distinguish subgroups likely to respond or be resistant to standard chemotherapy in the neoadjuvant setting.

Claudin low tumours

There is considerable interest in the subtype (found within both TN and BL tumours) characterised by low expression of claudin 3 and e-cadherin proteins and proliferation genes.[38] The claudin low subtype is said to account for 7–10% of breast cancers and around 30% of those that are TN. Around 60% are invasive ductal carcinomas; some are medullary or metaplastic.

The data derive from only one research group and need independent confirmation by others (and using different platforms) but may have clinical implications. Claudin low status may indicate tumours with a high content of stem cell-like or tumour-initiating cells perhaps responding less well to chemotherapy than BL tumours but better than luminal subtypes. Perou's group has reported that the response rate to neoadjuvant chemotherapy in BL tumours excluding those that are claudin low is 70% but in the claudin low subtype less than 40%, and in luminal tumours 7–10%.[38] However, ultimate outcome may be better.

Implications of the TN definition for trial design

The definition outlined above brings together a large and heterogeneous group of tumours which have triple negativity in common but which differ from each other in a range of histopathological and clinical features. Among the most relevant

differences are tumour type and grade, proliferation rate, association with BRCA mutation and the presence of markers suggestive of basal cell origin.

The largest single group is made up of invasive ductal carcinomas not otherwise specified (NOS). The remainder are a heterogeneous mixture of tumour types. The triple negative concept defines tumours which are unlikely to respond to endocrine or anti-Her2 therapy and which – overall – have a poor prognosis. However, we cannot say that all TN tumours should be treated in the same way in everyday practice since not all subtypes are high risk. Nor are all TN tumours equally appropriate for accrual to trials of investigational agents in this setting.

Non-ductal NOS subtypes

Among tumours which are frequently triple negative but which have additional special characteristics are pleomorphic lobular, medullary, myoepthelial, apocrine, adenoid-cystic, metaplastic and neuroendocrine carcinomas.[39] Although rare individually, when taken together the above subtypes represent about 10% of all TN tumours, and about 1% of the entire population of breast cancers.

We propose that high grade invasive lobular, myoepithelial, apocrine, adenoid-cystic and metaplastic TN tumours should be eligible for trials in TNBC. However, low-grade tumours and the remaining subtypes should be excluded since they have a good prognosis even if triple negative.[6] TN cases included in studies should be stratified according to whether they are invasive ductal in nature or of other histological types. Attempts can then be made to determine whether the response of high grade non-ductal tumours differs from that of the majority group making up the TN phenotype.

Our suggestion conforms broadly with the St Gallen consensus that – in the absence of factors indicating increased metastatic risk – patients with medullary, low-grade apocrine and adenoid-cystic carcinomas may in most cases not require adjuvant chemotherapy.[15,40,41]

Triple negativity emerging during or after systemic treatment

Residual tumour after neoadjuvant therapy

Among patients in whom neoadjuvant therapy does not achieve a pathological complete response, residual tumour is TN in up to 20% of cases which did not originally fall into this category.[9] A proportion of this discordance may be due to false positive findings prior to therapy or false negative findings afterwards. However, there may be triple negativity resulting from treatment, in which case the number of such cases is likely to increase as we deploy more effective anti-tumour agents which become the dominant determinants of growth and clonal selection. We do not know whether such tumours should be treated according to the original phenotype or the one that has emerged. For the purposes of clinical trials in TNBC, such emergent triple negativity should be considered different from that evident in the primary and hence should be investigated in separate studies.

Relapsed disease

In patients who relapse with distant metastases there may also be discordance in hormone receptor and Her2 expression between the primary tumour and secondary disease.[42–44] In a recent retrospectively analysed series, a third of patients whose primary tumours were ER or PgR positive were found on relapse to have receptor-negative metastases.[45] This may be due to technical factors but probably reflects heterogeneity within the original tumour and the selection of negative clones during disease progression. Such changes will be increasingly observed as oncologists are encouraged to biopsy recurrent tumour. The expectation when carrying out such biopsies is that their results will influence management. This consideration is reinforced by the fact that it is metastatic disease rather than the primary which will become life threatening.

Although there is a risk that such tumours represent a somewhat different biology, patients whose metastatic disease is TN should be eligible for trials in this setting even when their primary tumour expressed hormone receptors and/or Her2.

TNBC in carriers of BRCA1 mutations

TN tumours in BRCA1 carriers may be different from TN tumours in non-carriers and should perhaps be considered a distinct subgroup. The specific alteration present in the genome is likely to translate into different sensitivities to various forms of treatment. One suggestion is that there may be greater sensitivity to platinum salts.[46] In one retrospective series of 102 women with BRCA1 mutations (which generated the hypothesis) the pCR rate was substantially greater in those who were given neoadjuvant cisplatin than in those treated with doxorubicin plus either cyclophosphamide or docetaxel.[47] However, there are potentially confounding factors in this study and the current recommendation is that BRCA-related tumours should be treated in the same way as non-BRCA associated cancers.

Since BRCA mutation reflects a specific aetiology for triple negativity and may have implications for response to treatment (especially with investigational PARP inhibitors), patients included in trials in TNBC should be stratified according to BRCA status.

However, this is not a simple step. Currently, we do not have an antibody that enables BRCA1 to be reliably identified in tissue sections by IHC. Hence mutational analysis is required, possibly with the addition of assays for promoter methylation since this gene silencing event seems also of importance.

Tissue collection

In all trials in TNBC – as in all breast cancer trials – collection of tissue must be mandatory in all patients to help resolve issues such as the relationship of basal-like phenotype, claudin low subtype and BRCA mutation to outcome and to allow translational studies to be undertaken to identify novel prognostic or predictive parameters. It is also helpful to have central pathological confirmation of TN phenotype.

Implications of triple negativity for patient management

Screening of TN patients for BRCA mutations

BRCA1 mutation (and also that of BRCA2, though less commonly) is associated with the TN breast cancer phenotype.[48] In a recent series of 36 women with no relevant family history whose TNBC was diagnosed before the age of forty years, 47% were found to have a BRCA1 mutation.[49]Triple negativity defines a group of patients in whom it is important to identify BRCA mutation since this has profound implications for the management of the breast cancer itself (relating to the extent of surgery in the affected breast and the possibility of prophylactic mastectomy), for the prevention of ovarian cancer, and for the counselling of family members.

However, the need for BRCA screening is determined not by triple negativity per se but triple negativity in the context of the patient's family history of breast and ovarian cancer and her age at diagnosis. The age at which screening would be undertaken *in the absence of a positive family history* varies between countries and institutions from 35 years to 50 years. BRCA screening is relatively expensive and should be undertaken in accordance with the agreed practice in individual countries.[50]

Radiosensitivity

The relative radiosensitivity of TNBC is an important question with implications for the recommendation of mastectomy, especially in young patients who are over-represented among those with TN tumours and who in themselves have a higher incidence of local relapse. Retrospective data provide a hint that TN tumours are more likely than non-TN tumours to recur locally.[51–54] However, there have been no prospective studies. Carriers of the BRCA mutation will be more prone than others to the occurrence of a second primary, and it may be that this plays a part in the higher rates of local "recurrence" in studies which have not controlled for this possibility.

On the basis of current data, it is not possible to say TN tumours are relatively unresponsive to radiation and so recommend mastectomy as a means of preventing locoregional recurrence. TN pts should receive state of the art radiotherapy, should not be treated differently from women with other forms of breast cancer, and remain appropriate candidates for breast conservation.[55] The radiation sensitivity of TN tumours is an important priority for research.

Is responsiveness to chemotherapy different in TN breast cancer?

The literature contains many assertions about cytotoxic agents – relating both to those that are likely to be particularly effective and those that are likely to be relatively ineffective – in TNBC.[56] Thus one school of thought holds that cyclophosphamide is a necessary component of any adjuvant regimen and advocates an approach such as dose-dense CMF or six courses of FEC for highly proliferative tumours.[57] Another suggests that TN tumours should not be treated with anthracyclines because they are topoisomerase2 as well as Her2 negative.[58] In the adjuvant setting, the ongoing "TIC TAC" trial of TAC vs TC should provide an authoritative answer to the question of whether doxorubicin can be avoided in certain TN patients.

When given as neoadjuvant therapy, data from a German series suggest that an anthracycline/taxane regimen, just as many other chemotherapeutic approaches, can produce a high rate of pCR in TN disease.[59] It has been argued that TN tumours are less sensitive than others to conventional chemotherapy. However, both the Finnish data with capecitabine and the docetaxel overview show benefit is similar in TN and non-TN tumours.[60]

Particular attention has been given to the idea that platinums are likely to be more effective in TNBC, especially those associated with BRCA mutations. Thus Chang et al have recently reported that docetaxel and carboplatin are promising in the neoadjuvant treatment of TNBC, albeit in a small series.[61] However, the literature is conflicting and encouraging results have also been reported with, for example, dose-dense epirubicin plus cyclophosphamide followed by docetaxel.[62]

The suggestions considered above are based almost invariably on retrospective analyses, with all the potential for bias that such analysis brings. They should be regarded as hypotheses for testing in prospective controlled trials. In the absence of data from such randomised studies, no conclusions can be drawn about the responsiveness of TN tumours to specific chemotherapy agents, classes of agent or regimens in the adjuvant, neoadjuvant or metastatic settings. Indeed, currently, we have no prospectively validated biomarkers that predict responsiveness to any form of chemotherapy in any group of breast cancer patients. In these circumstances, it is not surprising that studies of novel agents such as PARP inhibitors have added them to a variety of chemotherapy "backbones". There is no consensus on what this backbone should be in TNBC, nor on whether it should include anthracyclines.

Novel targets in TNBC

Among the targets under investigation in TNBC are the process of angiogenesis, the EGFR, src kinase, IGFR-1, mTOR and PARP.[63] Evidence to date suggests that TN tumours respond no better than non-TN tumours to the anti-angiogenic approach.[64] Conflicting data on the EGFR strategy mean that this approach has not come to the forefront.[65–67] Studies with src kinase and mTOR inhibitors are at a very early stage.

Compared with other breast cancers, those that are TN more frequently have reduced BRCA 1 expression.[68] PARP activity is higher in cells with defective homologous recombination (including those that are BRCA defective), which may confer sensitivity to PARP inhibitors.[69–72]

Following evidence of improved overall survival in a randomised phase II trial of adding the PARP inhibitor BSI201 (iniparib) to the combination of gemcitabine and carboplatin in metastatic TNBC,[70] a pivotal phase III study was undertaken in 519 patients. This study failed to show significant benefit on the co-primary endpoint of OS and PFS.[73] Analyses aimed at further elucidating these findings are ongoing. It may prove the case that the most appropriate drug target in triple negative breast tumours remains to be discovered.

Discussion

In prospectively screened populations, the prevalence of TN tumours as we have defined them may be around 10%. Efforts to identify and characterise tumours which are unlikely to respond to endocrine or anti-Her2 agents – and then to find appropriate treatments – are at the forefront of current attempts to improve and individualise the management of breast cancer.

We regard the concept of triple negativity (which can be established by well-validated and routine assays) as pragmatically useful. Nevertheless, there is a challenging heterogeneity within triple negative tumours. Even among those that are high grade and associated with poor prognosis, our current state of knowledge does not allow us to use triple negativity to make decisions about the relative effectiveness of different classes of cytotoxic agent, the role of radiotherapy, or the likely future impact of investigational agents. Greater understanding of relevant targets is needed as a prelude to the further development of improved treatments.

A high priority should be given to collecting and analysing tissue from patients enrolled in randomised clinical trials so that we can better understand the prognostic value and implications for treatment of markers such as basal cytokeratins, proliferation index and claudin low subtype. In doing so, we should bear in mind the criteria for true prognostic factors that were established some years ago by McGuire but continue to be highly relevant.[74]

Acknowledgement

This Expert Forum was arranged by Eticho (European Training in Clinical Hematology and Oncology), the Hague, the Netherlands. Rob Stepney (medical writer, Charlbury, UK) acted as rapporteur. Support for this manuscript, which is based on material from the Expert Forum, was obtained through an unrestricted educational grant from sanofi-aventis.

References

1. Carey L, Winer E, Viale G, Cameron D, Gianni L. Triple-negative breast cancer: disease entity or title of convenience. *Nat Rev Clin Oncol* 2010. Published on doi:10.1038/nrclinonc.2010.154.
2. Berrada N, Delaloge S, Andre F. Treatment of triple negative metastatic breast cancer: toward individualized targeted treatments or chemosensitisation? *Ann Oncol* 2010;**21**(Suppl 7):vii30–35.

3. Santana-Davila R, Perez EA. Treatment options for patients with triple negative breast cancer. *J Hematol Oncol 2010 Published* October 2010;**27**. doi:10.1186/1756-8722-3-42.
4. Badve S, Dabbs DJ, Schnitt SJ, Baehne FL, Decker T, Eusebi V, et al. Basal like and triple negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. *Mod Pathol* 2011;**24**:157–67.
5. Rastelli F, Biancanelli S, Falzetta A, Martignetti A, Casic C, Bascioni R, et al. Triple-negative breast cancer: current state of the art. *Tumori* 2010;**96**:875–88.
6. Hudis CA, Gianni L. Triple-negative breast cancer: an unmet medical need. *Oncologist* 2011;**16**(Suppl 1):1–11.
7. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 2007;**13**:4429–34.
8. Heitz F, Harter P, Lueck HJ, Fissler-Eckhoff A, Lorenz-Salehi F, Scheij-Bertram S, et al. Triple negative and Her2-overexpressing breast cancers exhibit an elevated risk and an earlier occurrence of cerebral metastases. *Eur J Cancer* 2009;**45**:2792–8.
9. Liedtke C, Mazouni C, Hess KR, Andre F, Tordai A, Mejia JA, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. *J Clin Oncol* 2008;**26**:1275–81.
10. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001;**98**:10869–74.
11. Seal MD, Chia SK. What is the difference between triple-negative and basal breast cancers? *Cancer J* 2010;**16**:12–6.
12. Reis-Filho JS, Tutt AN. Triple-negative tumours: a critical review. *Histopathology* 2008;**52**:108–18.
13. Rakha EA, Elsheikh SE, Aleskandarany MA, Habashi HO, Green AR, Powe DG, et al. Triple-negative breast cancer: distinguishing between basal and nonbasal subtypes. *Clin Cancer Res* 2009;**15**:2302–10.
14. Foulkes WD, Smith IE, Reis-Filho JS. Triple negative breast cancer. *N Engl J Med* 2010:1938–48.
15. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *Arch Path Lab Med* 2010;**134**:907–32.
16. Goldhirsch A, Ingle JN, Gelber RD, Coates AS, Thurlimann S, Senn HJ, et al. Thresholds for therapies: highlights of the St Gallen international expert consensus on the primary therapy of early breast cancer. *Ann Oncol* 2009;**2009**(20):1319–29.
17. Viale G, Regan MM, Maiorano E, Mastropasqua MG, Dell'Orto P, Rasmussen BB, et al. Prognostic and predictive value of centrally reviewed expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer: BIG 1-98. *J Clin Oncol* 2007;**25**:3846–52.
18. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). 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**(77):1–84.
19. Dowsett M, Dunbier AK. Emerging biomarkers and new understanding of predictive markers in personalized therapy for breast cancer. *Clin Cancer Res* 2008;**14**:8019–26.
20. Park S, Koo J, Park HS, Kim JH, Choi SY, Lee JH, et al. Expression of androgen receptors in primary breast cancer. *Ann Oncol* 2010;**21**:488–92.
21. Peters AA, Buchanan G, Ricciardelli C, Bianco-Miotto T, Centenera MM, Harris JM, et al. Androgen receptor inhibits estrogen receptor-alpha activity and is prognostic in breast cancer. *Cancer Res* 2009;**69**:6131–40.
22. Ryden L, Haglund M, Bendahl PO, Hatschek T, Kolaric A, Kovacs A, et al. Reproducibility of human epidermal growth factor receptor 2 analysis in primary breast cancer – a national survey performed at pathology departments in Sweden. *Acta Oncologica* 2009;**48**:860–6.
23. Sauter G, Lee J, Bartlett JM, Slamon DJ, Press MF. Guidelines for human epidermal growth factor receptor 2 testing: biologic and methodologic considerations. *J Clin Oncol* 2009;**27**:1323–33.
24. Pauletti G, Dandekar S, Rong H, Ramos L, Peng H, Seshadri R, et al. Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemistry. *J Clin Oncol* 2000;**18**:3651–64.
25. Zujewski JA. Build quality in" – HER2 testing in the real world. *J Natnl Cancer Inst* 2002;**94**:788–9.
26. Vance GH, Barry TS, Bloom KJ, Fitzgibbons PL, Hicks DG, Jenkins RB, et al. Genetic heterogeneity in HER2 testing in breast cancer: panel summary and guidelines. *Arch Pathol Lab Med* 2009;**133**:611–2.
27. Iurisci I, Cottu P, Ngo C, Lae M, Pierga J-Y, Dieras V, et al. Heterogeneous amplification of HER2 is rare but clinically significant event in invasive ductal carcinoma. *Cancer Res* 2009;**59**(24 suppl). Abs 6034.
28. Paik S, Kim C, Wolmark N. HER2 status and benefit from adjuvant trastuzumab in breast cancer. *N Engl J Med* 2008;**358**:1409–11.
29. Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res* 2004;**10**:5367–74.
30. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature* 2000;**406**:747–52.
31. Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol* 2009;**27**:1160–7.
32. Laakso M, Tanner M, Nilsson J, Wiklund T, Erikstein B, Kellokumpu-Lehtinen P, et al. Basoluminal carcinoma: a new biologically and prognostically distinct entity between basal and luminal breast cancer. *Clin Cancer Res* 2006;**12**:4185–91.
33. Gusterson B. Do "basal-like" breast cancers really exist? *Nat Rev Cancer* 2009;**9**:128–34.
34. Collins LC, Martyniak A, Kandel MJ, Stadler ZK, Masciari S, Miron A, et al. Basal cytokeratin and epidermal growth factor receptor expression are not predictive of BRCA1 mutation status in women with triple negative breast cancers. *Am J Surg Pathol* 2009;**33**:1093–7.
35. Rakha E, Ellis I, Reis-Filho J. Are triple-negative and basal-like breast cancer synonymous? *Clin Cancer Res* 2008;**14**. 6-8-619.
36. Cheang MC, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res* 2008;**14**:1368–76.
37. Choi YL, Oh E, Park S, Kim Y, Park YH, Song K, et al. Triple-negative, basal-like and quintuple negative breast cancers: better prediction model for survival. *BMC Cancer* 2010;**10**:507. doi:10.1186/1471.2407-10-507.
38. Prat A, Parker SJ, Karginova O, Fan C, Livasy C, Herschkowitz JI, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. *Breast Cancer Res* 2010;**12**:R68.
39. Constantinidou A, Jones RL, Reis-Filho JS. Beyond triple negative breast cancer: the need to define new subtypes. *Expert Rev Anticancer Ther* 2010;**10**:1197–213.
40. Marchio C, Weigelt B, Reis-Filho JS. Adenoid cystic carcinomas of the breast and salivary glands (or "The strange case of Dr Jekyll and Mr Hyde" of exocrine gland carcinomas). *J Clin Pathol* 2010;**63**:220–8.
41. Wells CA, El-Ayat GA. Non-operative breast pathology: apocrine lesions. *J Clin Pathol* 2007;**60**:1313–20.
42. Ellis IO, Lee AH. The prognostic significance of inflammation and medullary histological subtype in invasive carcinoma of the breast. *Eur J Cancer* 2009;**45**:1780–7.
43. Pusztai L, Viale G, Kelly CM, Hudis CA. Estrogen and HER-2 receptor discordance between primary breast cancer and metastasis. *Oncologist* 2010;**15**:1164–8.
44. Simmons C, Miller N, Geddie W, Gianfelice D, Oldfield M, Dranitsaris G, et al. Does confirmatory tumor biopsy alter the management of breast cancer patients with distant metastases? *Ann Oncol* 2009;**20**:1499–504.
45. Lindstrom LS, Karlsson E, Wilking U, Bergh J. *Discordance in hormone receptor and HER2 status in breast cancer during tumor progression*. San Antonio Breast Cancer Symposium 2010. Abstract S3–S5.
46. Carey L. Targeted chemotherapy? Platinum in BRCA1-dysfunctional breast cancer. *J Clin Oncol* 2010;**28**:361–3.
47. Byrski T, Gronwald J, Huzarski T, Grzybowska E, Budryk M, Stawicka M, et al. Pathologic complete response rates in young women with BRCA-1 positive breast cancers after neoadjuvant chemotherapy. *J Clin Oncol* 2010;**28**:375–9.
48. Somer R, Farengo-Clark D, Patel A, Schwarting R, Grana G, Rodier E, et al. Screening for BRCA1 mutations in patients with triple negative breast cancer and basal phenotype. *Cancer Res* 2009;**69**. Abs 4075.
49. Fostira F, Tsitiadou M, Gogas H, Pertesi M, Yannoukakos D, Bournakis E, et al. Prevalence of BRCA1 mutations among 284 women with triple-negative breast cancer. *J Clin Oncol* 2010;**28**. Abs 1511.
50. Kwon JS, Gutierrez-Barrera AM, Young D, Sun CC, Daniels MS, Lu KH, et al. Expanding the criteria for BRCA mutation testing in breast cancer survivors. *J Clin Oncol* 2010;**28**:4214–20.
51. Dragun AE, Pan J, Rai SN, Kruse B, Jain D. Locoregional recurrence in patients with triple-negative breast cancer: preliminary results of a single institution study. *Am J Clin Oncol* 2010.
52. Voduc KD, Cheang MC, Tyldesley S, Gelmon K, Nielsen TO, Kennecke H, et al. Breast cancer subtypes and the risk of local and regional relapse. *J Clin Oncol* 2010;**28**:1684–9.
53. Parikh RR, Housman D, Yang Q, Toppmeyer D, Wilson LD, Haffty BG, et al. Prognostic value of triple-negative phenotype at the time of locally recurrent, conservatively treated breast cancer. *Int J Radiaion Oncol Biol Phys* 2008;**72**:1056–63.
54. Haffty BG, Yang Q, Reiss M, Kearney T, Higgins SA, Weidhaas J, et al. Locoregional relapse and distant metastasis in conservatively managed triple negative early-stage breast cancer. *J Clin Oncol* 2006;**24**:5652–7.
55. Freedman GM, Anderson PR, Li T, Nicolaou N. Locoregional recurrence of triple negative breast cancer after breast-conserving surgery and radiation. *Cancer* 2009;**115**:946–51.
56. Isakoff SJ. Triple negative breast cancer: role of specific chemotherapy agents. *Cancer J* 2010;**16**:53–61.
57. Colleoni M, Cole BF, Viale G, Regan MM, Price KN, Maiorano E, et al. Classical cyclophosphamide, methotrexate, and fluorouracil chemotherapy is more effective in triple-negative, node-negative breast cancer: results from two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer. *J Clin Oncol* 2010;**28**:2966–73.
58. Weigelt B, Kreike B, Reis-Filho JS. Metaplastic breast carcinomas are basal-like breast cancers: a genomic profiling analysis. *Breast Cancer Res Treat* 2009;**117**:273–80.
59. Von Minckwitz G, Untch M, Nuesch E, Loibl S, Kaufmann M, Kummel S, et al. Impact of treatment characteristics on response of different breast cancer phenotypes: pooled analysis of the German neoadjuvant chemotherapy trials. *Breast Cancer Res Treat* 2011;**125**:145–56.
60. Laporte S, Jones S, Chapelle C, Jaquin J-P, Martin M. *Consistency of effect of docetaxel-containing adjuvant chemotherapy in patients with early stage breast cancer independent of nodal status: meta-analysis of 12 randomized clinical trials*. San Antonio Breast Cancer Symposium 2009. abstract 605.

61. Chang HR, Glaspy J, Allison MA, Kass FC, Elashoff R, Chung DU, Gornbein J, et al. Differential response of triple-negative breast cancer to a docetaxel and carboplatin-based neoadjuvant treatment. *Cancer* 2010;**116**:4227–37.
62. Warm M, Kates R, Grosse-Onnebrink E, Stoff-Khalili M, Hoopmann M, Mallmann P, et al. Impact of tumor biology, particularly triple negative status, on response to pre-operative, sequential, dose-dense epirubicin, cyclophosphamide followed by docetaxel in breast cancer. *Anticancer Res* 2010;**30**:4251–9.
63. Amir E, Seruga B, Serrano R, Ocana A. Targeting DNA repair in breast cancer: a clinical and translational update. *Cancer Treat Rev* 2010;**36**:557–65.
64. O'Shaughnessy J, Miles D, Gray RJ, Dieras V, Perez EA, Zon R, et al. A meta-analysis of overall survival data from three randomized trials of bevacizumab and first line chemotherapy as treatment for patients with metastatic breast cancer. *J Clin Oncol* 2010;**28**. 15s abstract 1005.
65. Carey LA, Rugo HS, Marcom K, Irvin W, Ferraro M, Burrows E, et al. TBCR:001 EGFR inhibition with cetuximab added to carboplatin in metastatic triple negative (basal like) breast cancer. *J Clin Oncol* 2008; May 20. suppl abstract 1009.
66. O'Shaughnessy J, Weckstein D, Vukelja SJ, McIntyre K, Krekow L, Holmes FA, et al. Preliminary results of a randomised phase II study of weekly irinotecan/carboplatin with or without cetuximab in patients with metastatic breast cancer. *Breast Cancer Res Treat* 2007;**106**(suppl S32). abstract 308.
67. Baselga J, Gomez P, Awada A, Greil R, Braga S, Climent MA, et al. The addition of cetuximab to cisplatin increases overall response rate in metastatic triple negative breast cancer: results of a randomized phase II study (BALI-1). *ESMO* 2010. abstract 2740.
68. Cleator S, Heller W, Coombes RC. Triple-negative breast cancer: therapeutic options. *Lancet Oncol* 2007;**8**:235–44.
69. Gottipati P, Vischioni B, Schultz N, Solomons J, Bryant HE, Djureinovic T, et al. Poly(ADP-ribose) polymerase is hyperactivated in homologous recombination-defective cells. *Cancer Res* 2010;**70**:5389–98.
70. O'Shaughnessy J, Osborne C, Pippen JE, Yoffe M, Patt D, Rocha C, et al. Iniparib plus chemotherapy in metastatic triple negative breast cancer. *N Engl J Med* 2011;**364**:205–14.
71. Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. *Lancet* 2010;**376**:235–44.
72. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose)polymerase in tumors from BRCA mutation carriers. *N Engl J Med* 2009;**361**:123–34.
73. O'Shaugnessy J, Schwartzberg LS, Danso MA, Rugo H, Miller K, Yardley DA, et al. A randomized phase III study of iniparib (BSI-201) in combination with gemcitabine/carboplatin in metastatic triple negative breast cancer. *J Clin Oncol* 2011;**29**. suppl; Abs 1007.
74. McGuire WL. Breast cancer prognostic factors: evaluation guidelines. *J Natl Cancer Inst* 1991;**83**:154–5.