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Tumour Review

Clinical implications of the non-luminal intrinsic subtypes in hormone receptor-positive breast cancer

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ABSTRACT

Gene expression profiling has had a considerable impact on our understanding of breast cancer biology. During the last decade, 4 intrinsic molecular subtypes of breast cancer (Luminal A, Luminal B, HER2-enriched [HER2-E] and Basal-like) have been identified and intensively studied. In this article, we review and discuss the clinical implications of the 2 non-luminal subtypes (i.e. HER2-E and Basal-like) identified within hormone receptor (HR)-positive disease. After reviewing 32 studies for a total of 13,091 samples, ~8% and ~ 15% of early and metastatic HR + /HER2-negative breast cancer, respectively, were found to be non-luminal. Clinically, HR + /HER2-negative/non-luminal subtypes have been associated with estrogen independence, chemo-sensitivity, resistance to CDK4/6 inhibition and poor outcome. Interestingly, EGFR/HER2 tyrosine kinase inhibition might be of value in the HR + /HER2-negative/HER2-E subtype. Finally, the HER2-E subtype within HR + /HER2 + disease represents ~ 30% and has been associated with anti-HER2 sensitivity, chemo-sensitivity and resistance to CDK4/6 inhibition. In the upcoming years, retrospective and prospective clinical trials evaluating both biomarkers should lead to improvements in patient outcomes.

Introduction

Breast cancer (BC) is a clinically and biologically heterogeneous disease [1,2] According to three pathology-based biomarkers (i.e. estrogen receptor [ER], progesterone receptor [PR] and HER2), BC can be classified today into 4 different groups: ER-positive and/or PR-positive and HER2-negative (HR + /HER2-negative), ER-positive and/or PR-positive and HER2-positive (HR + /HER2 +), ER-negative and PR-negative and HER2-positive (HR-negative/HER2 +) and triple-negative (TNBC). This pathology-based classification is routinely used in the clinic to select endocrine and anti-HER2 therapies, and include patients in clinical trials.

In the last decade, gene expression profiling has had a considerable impact on our understanding of BC biological heterogeneity [2,3]. For example, we and others have extensively characterized 4 main molecular subtypes of BC (Luminal A, Luminal B, HER-2 enriched [HER2-E], Basal-like) and a normal breast-like group [4–6]. Known as the "intrinsic subtypes of breast cancer", these entities have shown significant differences in terms of their incidence, risk factors, prognosis and treatment sensitivity. More importantly, accumulating evidence suggests that these molecular entities provide prognostic and/or predictive information beyond standard pathology-based classifications [7–9].

In this review article, we first describe the distribution of the intrinsic subtypes within HR + disease using publicly available data from 13,264 tumor samples and 39 studies, and then we discuss the potential current and future clinical implications of the 2 non-luminal subtypes identified within HR + BC.

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Subtype distribution within pathology-based groups

In 2009, Parker and colleagues introduced a clinically applicable gene expression-based test, known as PAM50, which identifies the main intrinsic molecular subtypes (i.e. Luminal A, Luminal B, HER2-E and Basal-like) in formalin-fixed paraffin embedded tumor tissues using 50 genes [10]. Since then, this assay has allowed the identification of these molecular entities across a large number of studies, including tumor samples from various phase III clinical trials [9,11–13].

To recapitulate all the data presented to date in HR+ disease, we searched for research articles published in English between January 2009 and December 2017 in PubMed, or in abstract from the San Antonio Breast Cancer Symposium (SABCS) and the American Society of Clinical Oncology (ASCO) annual meeting, using the search terms "PAM50", "HR-positive", "intrinsic subtype" and "breast cancer". A total of 39 studies were identified and the characteristics of each study can be found in Supplemental Data.

In the combined dataset (n = 13,264), most tumors were HR+/HER2-negative (n = 10,755; 81.08%), followed by HR+/HER2+ (n = 2509; 18.91%). Regarding intrinsic subtypes, most tumors were Luminal A (n = 6810; 51.34%) followed by Luminal B (n = 4301; 32.42%), HER2-E (n = 1424; 10.74%), Basal-like (n = 290; 2.19%) and Normal-like (n = 439; 3.31%) (Fig. 1A).

All the intrinsic molecular subtypes were identified in each HR+ group, albeit with different proportions. In HR+/HER2-negative early BC, 5.8% and 2.16% of tumors were identified as HER2-E and Basal-like, respectively (Fig. 1B). In HR+/HER2+ early BC, 28.28% and 2.22% of the tumors were HER2-E and Basal-like, respectively (Fig. 1C). While in HR+ advanced or metastatic breast cancer (note that in these studies, primary tumor tissue [archival or recent] could have been profiled instead of the metastatic tumor sample), 13.59% were HER2-E and 2.28% were Basal-like (Fig. 1D). Overall, these data confirm that intrinsic biology is not fully recapitulated by the current pathology-based classification and that substantial discrepancies exist that warrant further investigation.

IHC-based features of HR+/non-luminal tumors

The PAM50 intrinsic molecular subtypes are defined by an entire gene expression profile based on 50 genes [10]. Thus, four IHC-based biomarkers (ER, PR, Ki67 and HER2) alone, or in combination, are not going to allow us to identify these entities. Nonetheless, some of the features of HR+/non-luminal tumors might be captured by IHC. For example, in a previous study, we evaluated the percentage of positive cells for ER, PR and Ki67 across the PAM50 subtypes in 517 patients with HR+/HER2-negative disease [14]. The results revealed that non-luminal tumors tend to have lower positivity for ER and PR, and higher positivity for Ki67. However, no single biomarker, or cutoff, was enough by itself to identify these entities. Nonetheless, interesting observations were made: (1) non-luminal tumors can be identified within ER > 10%, (2) no HER2-E tumor was identified with a PR above 10% and (3) no non-luminal tumor was identified within Ki67 below 10% (Fig. 2). Further IHC-based studies are needed to help us better screen for non-luminal subtypes within HR+/HER2-negative and HR+/HER2+ diseases; however, as we learned in the past trying to identify an IHC-based definition of Luminal A versus B subtypes, this could be somewhat unreliable [7,15] and challenging.

Implications within HR+/HER2-negative disease

Over the years, multiple studies have consistently shown the prognostic differences in early disease of the two major molecular subtypes

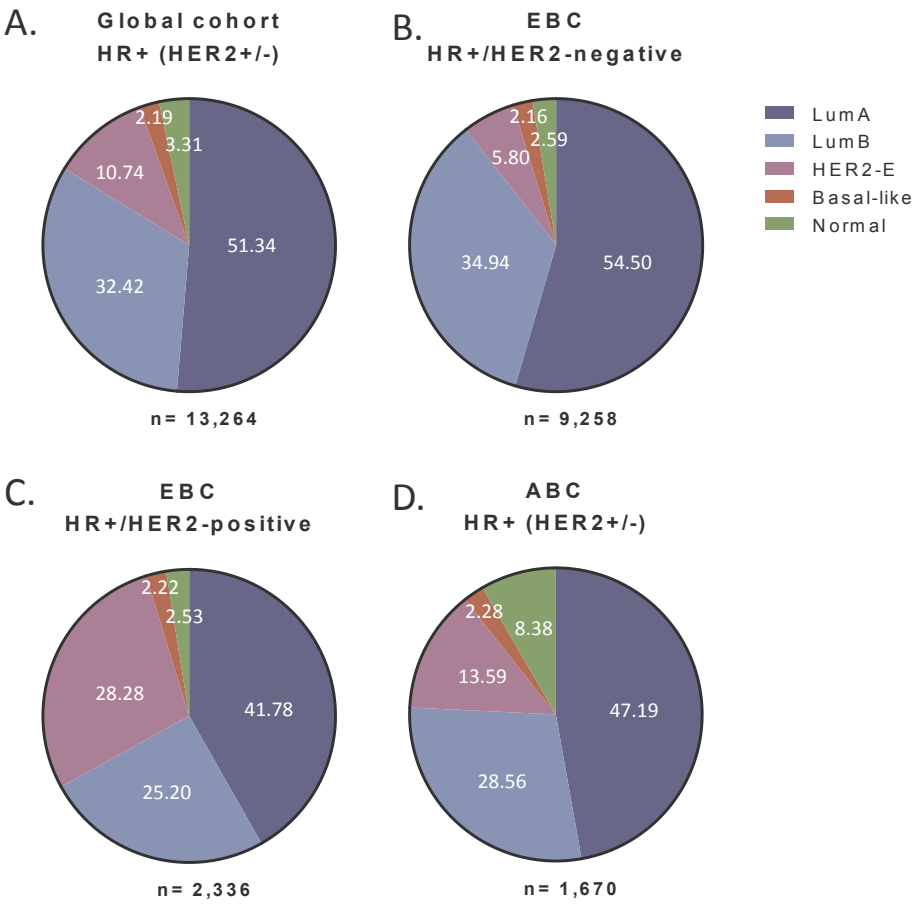

Fig. 1. Molecular heterogeneity of HR+ breast cancer. Intrinsic subtype distribution (A) in the combined dataset; (B) in HR+/HER2-negative early breast cancer; (C) in HR+/HER2-positive early breast cancer; (D) in HR+ advanced or metastatic breast cancer (note that in these studies, primary tumor tissue [archival or recent] could have been profiled instead of the metastatic tumor sample).

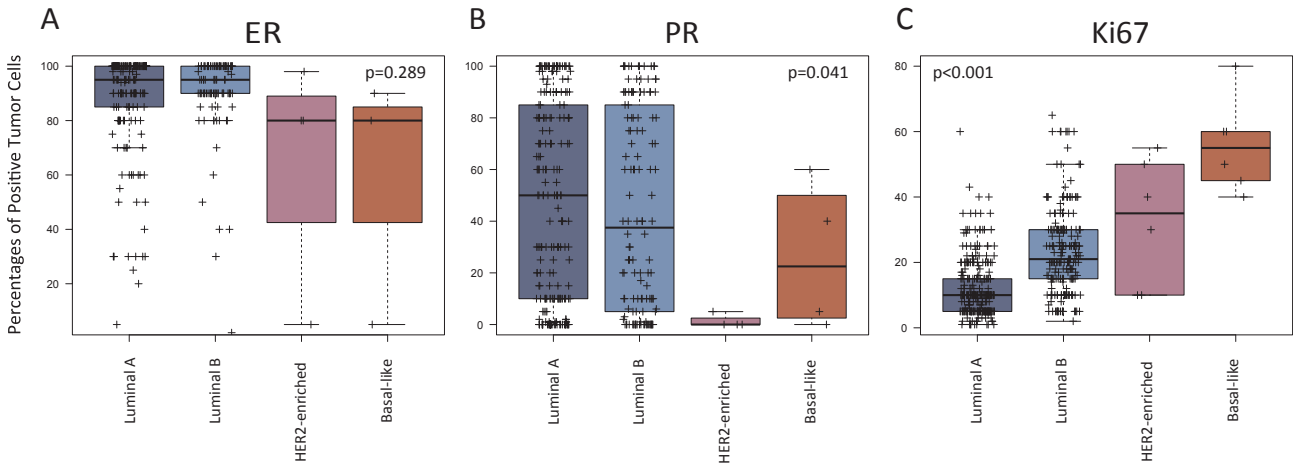

Fig. 2. Levels of estrogen receptor (ER), progesterone receptor (PR) and Ki67-positive cells across the intrinsic subtypes within 517 HR + /HER2-negative breast cancer. (A) ER; (B) PR; (C) Ki67. P-values were calculated by comparing mean values across all groups.

of HR + /HER2-negative disease (i.e. Luminal A and B). Identification of a subset of Luminal A tumors using well-validated prognostic gene expression-based tests such as Prosigna [10], OncotypeDX [16], MammaPrint [17] and EndoPredict [18] is helping clinicians and patients make decisions regarding the necessity to indicate (neo)adjuvant chemotherapy [19]. With further validation, these tests have the potential to help dictate the necessity to extend endocrine therapy beyond 5 years [20,21].

At the same time, cumulative evidence is elucidating the clinical value of the two non-luminal subtypes (i.e. HER2-E and Basal-like) within HR + /HER2-negative disease. At first glance, the percentage of patients with non-luminal early BC within HR + /HER2-negative disease (i.e. 8%) might seem small. However, this percentage represents 18,500 new cases a year diagnosed in the US, much higher than the 8950 annual new cases of chronic myeloid leukemia [22]. Thus, this group of patients is considerable and deserves special attention.

One interesting aspect is that, compared to early HR + /HER2-negative BC, the proportion of HER2-E subtype seems increased from 5% to 10% in patients with advanced or metastatic HR + /HER2-negative BC (Fig. S1). This increase in the HER2-E subtype in the advanced setting can be due to patient selection, a true shift in tumor biology due to inherent tumor evolution or treatment effects, or a combination of everything. The current evidence does support this last possibility. On one hand, patients with HR + /HER2-negative/HER2-E early BC have a higher likelihood of relapsing than luminal disease (see below). Thus, a given population of relapsed patients is likely to be more enriched for the HER2-E subtype compared to patients with early BC. On the other hand, we have previously shown, using 123 paired primary and metastasis tumor samples, that 10–15% of primary Luminal A or B tumors acquire a HER2-E profile in the metastatic tumor sample at first recurrence, despite being HER2-negative [23]. Overall, the acquisition of a HER2-E profile might reflect the appearance of estrogen-independency in a tumor previously estrogen-dependent (i.e. luminal) (see below).

Prognostic value

Four large studies in HR + /HER2-negative disease have revealed decreased survival outcomes of the HER2-E and Basal-like subtypes compared to the Luminal A subtype when treated with endocrine therapy-only [12,24,25]. In the first study [24], a large dataset of 1380 patients with early BC treated with 5-years of adjuvant tamoxifen-only was evaluated. In node-negative disease (n = 610), the distant relapse-free survival (DRFS) rates at 8.5 years of the Luminal A, Luminal B, HER2-E and Basal-like subtypes were 90.9%, 75.3%, 73.7% and 66.2%, respectively. In node-positive disease (n = 699), the DRFS rates at 8.5 years of the Luminal A, Luminal B, HER2-E and Basal-like subtypes were 74.5%, 53.4%, 53.3% and 62.2%, respectively.

In the second study [12], 644 independent HR + /HER2-negative tumors from the EGF3008 phase III clinical trial were gene expression profiled. In this trial, patients with advanced or metastatic disease had been treated with first-line letrozole + / − lapatinib [26]. Of note, 80% of the tumor samples analyzed were from primary tumor tissues. In this PAM50 cohort, HER2-E and Basal-like subtypes represented 6% of all HR + /HER2-negative tumors. Compared with the Luminal A subtype, the other subtypes showed a significantly decreased progression-free survival (PFS) independently of other clinical-pathological variables. Patients with Luminal B, HER2-E, and Basal-like subtypes had a 1.46, 2.87, and 2.26 times higher risk of tumor progression, respectively. Median PFS differed across the intrinsic subtypes: Luminal A (16.9 months), Luminal B (11.0 months), HER2-E (4.7 months), and Basal-like (4.1 months). Intrinsic subtype added more prognostic information regarding PFS than any of the other clinical-pathological variables evaluated in the model.

Similar results were observed in overall survival (OS) despite only 242 (38%) patients with an event [12]. Compared with patients with a Luminal A subtype, patients with Luminal B, HER2-E, and Basal-like subtype had a 1.52, 2.53, and 2.34 times higher risk of death, respectively. Median OS differed across the intrinsic subtypes: Luminal A (45 months), Luminal B (37 months), HER2-E (16 months) and Basal-like (23 months). Intrinsic subtype added more prognostic information regarding OS when added to the other clinical-pathological variables than any other variable evaluated, except prior endocrine therapy.

In the third study, PAM50 was performed in 261 tumor samples of 724 patients from the BOLERO-2 phase III trial [27,28]. This study randomized (2:1) 724 postmenopausal women with HR + /HER2-negative advanced or metastatic breast cancer, previously treated with a non-steroidal AI, to exemestane + / − everolimus. The subtype distribution was: 46.7% Luminal A, 21.5% HER2-E, 15.7% Luminal B, 14.2% Normal-like and 1.9% Basal-like [29]. Of note, compared with primary tumors, metastatic tumors were enriched for the HER2-E subtype, 32% in 50 metastatic samples versus 19% in 201 primary samples. The non-luminal subtype was independently associated with poor PFS and OS compared to the rest of the subtypes. Similar to results from other trials, the difference in median PFS between the 2 groups was statically significant, 6.67 in luminal group versus 5.16 months in non-luminal group with and adjusted hazard ratio of 0.66. Regarding OS, the difference in median OS between the 2 groups was more pronounced, 33.08 vs. 19.65 months, with an overall adjusted hazard ratio of 0.52.

In the fourth study, PAM50 was applied in 455 patients of 666

patients recruited in the PALOMA-2 phase III clinical trial that led to the approval of palbociclib in the first-line advanced or metastatic setting in combination with letrozole [25,30]. Non-luminal subtypes were identified in 20% of the cases (19% HER2-E and 1% Basal-like). In the letrozole-only arm, median PFS of the Luminal A, Luminal B, HER2-E and Basal-like were 17, 11, 11 and 6.4 months. Overall, these four retrospective studies, three using samples from phase III clinical trials, clearly highlight the prognostic value of the 2 non-luminal intrinsic subtypes in HR + /HER2-negative disease.

Anti-estrogen dependency

The decreased survival of the non-luminal subtypes in the context of endocrine therapy suggests that these are aggressive tumors that do not benefit from endocrine therapy (i.e. estrogen-independency). Data in the neoadjuvant setting, using Ki67 as the predictive biomarker of endocrine responsiveness, supports this hypothesis. In the Z1031 [31] neoadjuvant trial, patients with ER + disease (Allred score 6–8) were treated for 4 months with either letrozole, anastrozole or exemestane. The single patient with Basal-like disease had high pre- and surgical Ki67 values (38% and 26.8%, respectively). In addition, all 5 patients with HER2-E disease had persistently high surgical Ki67 levels (i.e. > 20%). This is consistent with high level of estrogen-independent growth in non-luminal disease even if they are ER + by IHC.

In another study, 112 postmenopausal women with stages I-IIIB ER + early BC were treated with neoadjuvant anastrozole [32]. Ki67 was evaluated before and after 2-weeks of treatment. Luminal A and B tumors obtained similar benefit from treatment, as measured by the proportional fall in the proliferation marker Ki67 upon treatment. Tumors classified as Basal-like and HER2-E showed poor reductions in Ki67 upon treatment. Finally, in the recently published NeoPalAna trial [33], 50 postmenopausal women with stages I-IIIB ER + early BC were treated with neoadjuvant anastrozole-only for 28 days, followed by the addition of palbociclib, a CDK4/6 inhibitor, for 4 28-day cycles unless Ki67 > 10% at Day 15 of adding palbociclib, in which case patients went off study. Intrinsic subtyping was performed in 31 samples before starting treatment. One (3.2%) patient was identified as Basal-like and 1 (3.2%) as HER2-E. Interestingly, whereas 1 patient of 29 (3.5%) with luminal disease did not show a suppression of Ki67, none of the two patients with non-luminal disease had suppression of Ki67 neither after 28 days of anastrozole nor after 2 weeks of adding palbociclib. These intriguing data would also suggest that non-luminal tumors within HR + /HER2-negative disease do not benefit from CDK4/6 inhibition (see below).

Although further studies that confirm the lack of survival benefit from endocrine therapy in HR + /HER2-negative/non-luminal disease, we must be realistic and a phase III clinical trial that randomizes patients with HR + /HER2-negative/non-Luminal disease to endocrine therapy versus no endocrine therapy is unlikely to be done. Thus, based on the current evidence, are we ready to embrace the use of the identification of non-luminal subtypes in HR + /HER2-negative disease to identify patients that should not be treated with endocrine therapy? Probably not for the general population. However, within ER/PR-low expressing tumors (i.e. 1–9% positive tumor cells), where the evidence of survival benefit of endocrine therapy is not Level 1 [34], consensus guidelines such as St. Gallen 2019 could endorse the identification of intrinsic subtype to help decide which patients might be, or might not be, treated with adjuvant endocrine therapy. In this group of patients with ER/PR-low tumors, the distribution of the intrinsic subtypes is 44% Luminal A/B, 31% HER2-E and 18% Basal-like [8].

Sensitivity to CDK4/6 inhibition

Non-luminal BC cell lines (regardless of HR status) have shown to be less sensitive to palbociclib *in vitro* than luminal cell lines [35]. However, whether this hold true in patients with HR + disease was unknown until recently. As previously noted, the PALOMA-2 phase III trial that led to the approval of palbociclib in combination with letrozole, recently presented the exploratory results at the 2017 San Antonio Breast Cancer Symposium (SABCS) of PAM50 in 455 patients of the 666 (68.3%) recruited in the trial. With median follow-up of 38 months, median PFS was 27.6 months in the experimental arm vs 14.5 months in control arm [30]. As expected, Luminal A and Luminal B subtypes both benefited substantially from palbociclib plus letrozole vs letrozole while both non-luminal subtypes, which represented 20% of the entire population, had very small absolute benefits, if any, from palbociclib: Luminal A (30.4 months vs 17 months), Luminal B (19.6 months vs 11 months), HER2-E (13.8 months vs 11 months), and Basal-like (5.6 months vs 6.4 months) [25]. Although this result by itself do not provide Level 1 evidence for the biomarker and we await the full results of this sub-study, these data are encouraging and consistent with the underlying biology of both non-luminal subtypes. At the same time, we encourage others to perform PAM50 gene expression analysis in tumor samples from the various pivotal Phase III trials that led to the approval of CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib in HR + /HER2-negative disease.

Chemotherapy sensitivity

Non-luminal disease, especially Basal-like, has shown higher chemosensitivity than luminal disease [36–38]. However, whether this premise held true in patients with HR + /HER2-negative disease was unknown until recently. A retrospective study evaluated PAM50 in 451 patients with HR + /HER2-negative disease treated across several studies with neoadjuvant multi-agent chemotherapy. The endpoint evaluated was pathological complete response (pCR) rates (in the breast and axilla). The pCR rates of Luminal A, Luminal B, HER2-E and Basal-like were 5%, 15%, 16% and 36%, respectively [39]. This difference was found statistically significant and independent of known clinical-pathological variables. Non-luminal (Basal-like and HER2-E) tumors, as a group, showed higher pCR rates than luminal (Luminal A and B) tumors (30.0% vs. 8.9%, adjusted odds ratio = 4.20) [39]. Of note, the pCR rate of 36% in HR + /HER2-negative/Basal-like tumors is similar to the pCR rate expected in TNBC/Basal-like disease treated with multi-agent chemotherapy [40–42].

A second study [38] analyzed 180 core needle biopsies from HR + /HER2-negative patients who were treated with neoadjuvant chemotherapy and correlated PAM50 intrinsic subtype with pathologic response (i.e. residual cancer burden). In this dataset, non-luminal disease represented 11.6% of the samples (n = 7 HER2-E and n = 14 Basal-like). More importantly, the residual cancer burden 0/1 rates varied significantly based on intrinsic subtype and were 9.3%, 20.0%, 14.3% and 50.0% for Luminal A, Luminal B, HER2-E and Basal-like, respectively.

In the SOLTI-NEOERIBULIN phase II clinical trial, neoadjuvant eribulin monotherapy was evaluated for 4 cycles in 101 patients with HR + /HER2-negative BC and PAM50 was performed before starting treatment [37]. As expected, the overall pCR in the breast rate was low (6.17%) [37]. However, pCR rates differed by subtype: 33.3% in HER2-E, 12.5% in Luminal B, 0% in Basal-like and 0% in Luminal A [37]. In addition, 100% of HER2-E tumors converted to Normal-like in the residual tumor. Thus, both from a response and biological perspective, patients with HR + /HER2-negative/HER2-E BC may benefit the most from eribulin therapy.

Overall, these data suggest that non-Luminal disease within HR + /HER2-negative disease is chemo-sensitive, which is concordant with their low or lack of endocrine sensitivity [43].

Drug targets for non-luminal subtypes

Beyond chemotherapy, how could HR + /HER2-negative/non-Luminal disease be targeted? For HR + /HER2-negative/Basal-like disease, probably the same treatment strategies being evaluated for TNBC/Basal-like disease should be explored here such as platinums, PARP inhibitors and immune therapy [44]. For HR + /HER2-negative/HER2-E disease, less clear is the treatment approach today since these tumors do

not overexpress HER2 gene, protein or phospho-protein, despite having an overall profile that is practically undistinguishable from the profile of HER2+/HER2-E tumors [45]. Although no clear driver(s) of the HER2-E profile in HER2-negative disease has been identified to date, HER2-negative/HER2-E tumors maintain high expression of EGFR as in a HER2+/HER2-E disease. Interestingly, EGFR activation has been identified as a resistance mechanism to endocrine therapy [46]. Moreover, lapatinib, an EGFR/HER2 reversible tyrosine kinase inhibitor, added to letrozole showed a survival benefit in the EGF3008 clinical trial [12] in patients with HR+/HER2-negative/HER2-E metastatic disease (median PFS, 6.49 vs 2.60 months; progression-free survival hazard ratio, 0.238; interaction P = .02), although the number of patients in each arm was very low. Further evidence of the clinical value of targeting HER2/EGFR in HER2-negative/HER2-E disease is needed.

Another interesting observation is the link between the HER2-E subtype and two genetic or mutational DNA-based signatures related to APOBEC [47,48]. APOBEC is a cytidine deaminase which converts cytosine to uracil during RNA editing and retrovirus or retrotransposon restriction, and may induce mutation clusters in human tumors [47–49]. Several studies have now linked APOBEC genetic signatures (i.e. 2 and 13) with the HER2-E subtype in BC [50]. In addition, both APOBEC genetic signatures have been found enriched in relapsed/metastatic HR+/HER2-negative tumors [47,49], concordant with the observed increased incidence of the HER2-E subtype in resistant/relapsed HR+/HER2-negative disease [23]. Intriguingly, both the APOBEC signatures and the HER2-E profile have been associated with high mutational burden and high expression of immune genes and immune infiltration. Thus, the immune system might be a target to explore in HR+/HER2-negative/HER2-E tumors.

Clinical implications within HR+/HER2+ disease

HER2+ BC has been classically viewed as two distinct diseases based on the expression of HR. Gene expression analyses, however, have revealed that HR+/HER2+ is composed of all the 4 main intrinsic subtypes of BC, and non-luminal subtypes represent 30.50% (Fig. 1C) [13,51,52]. This finding has now been replicated across many HER2+ studies [11,53]. To date, however, intrinsic subtype classification within HR+/HER2+ disease has not had an impact on patient management.

Among the two non-luminal subtypes within HR+/HER2+ disease, the HER2-E clearly predominates. HER2-E tumors are characterized by high expression of ERBB2 and other genes of the 17q amplicon, such as GRB7, and low to intermediate expression of luminal genes such as ESR1 and PGR. HER2-E subtype presents high frequency of TP53 (72%) and PIK3CA (39%) mutations [4]. From a protein perspective, HER2+ and HER2-E tumors show the highest expression of HER2 and phosphor-HER2 compared to the other groups; thus, this subtype not only shows the highest expression of the target, but also shows high signaling activity of the EGFR/HER2 pathway.

Sensitivity to anti-HER2-based chemotherapy

Over the years, intrinsic subtyping has been explored in retrospective samples from prospective trials evaluating anti-HER2-based chemotherapy in the neoadjuvant (i.e. NeoALTTO [54], CALGB40601 [13], NOAH [55], KRISTINE [56], CHER-LOB [57], SOLTI-OPTIHER-HEART [58], BERENICE [52]) and adjuvant (i.e. NSABP-B31 [53] and N9831 [11]) settings. In the neoadjuvant setting, the HER2-E is associated with a higher pCR rate compared to non-HER2-E following either trastuzumab plus chemotherapy or dual HER2 blockade (i.e. trastuzumab + lapatinib or trastuzumab + pertuzumab) plus chemotherapy. Of note, dual HER2 blockade with chemotherapy in HER2-E disease (without taking into account HR status) achieves pCR rates of 70–80% (Fig. 3) [13]. Interestingly, this association has been found independently of HR status and other clinical-pathological variables. Thus, it is expected that the pCR rates of the HER2-E subtype do not differ based on HR status. Indeed, in the KRISTINE trial the pCR rates within HR+/HER2+/HER2-E disease were similar as in HR-negative/HER2+/HER2-E disease [56] (64.9% vs 75.%).

Although HR+/HER2+/HER2-E tumors benefit the most from chemotherapy + anti-HER2 therapy, the previous neoadjuvant studies do not exclude a benefit from trastuzumab in non-HER2-E tumors. Indeed, the pCR rates following anti-HER2-based chemotherapy in

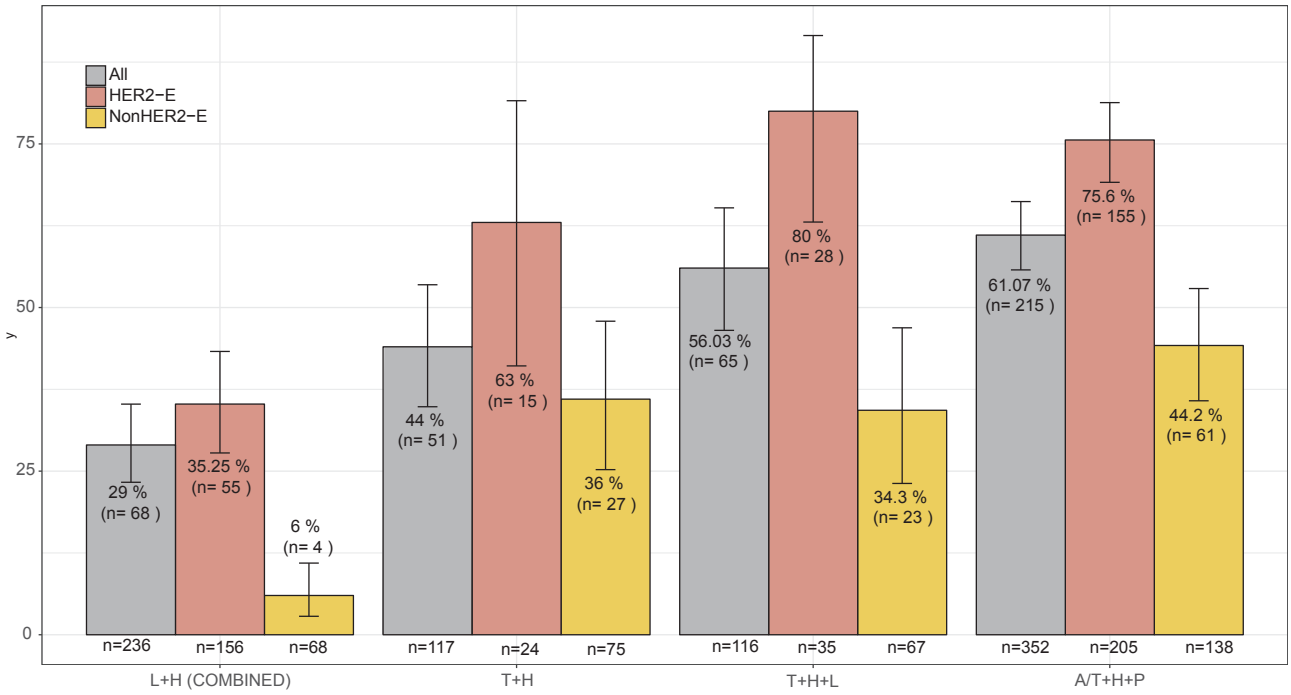

Fig. 3. Rates of pathological complete response (pCR) according to the type of chemotherapy and anti-HER2 therapy using data from 8 neoadjuvant clinical trials in HER2+ breast cancer. T, taxane; L, lapatinib; H, Herceptin (trastuzumab); A/T, anthracycline/taxane-based.

Luminal A disease are 10–45%, much higher than the expected pCR rates of 3–5% in Luminal A/HER2-negative disease following similar chemotherapy regimens. Thus, trastuzumab might still be effective in these tumors that have lower HER2 levels and low HER2 signaling activity. Indeed, intrinsic subtyping in samples from 2 large adjuvant trials (i.e. NSABP B31 [53] and N9831 [11]) reveal a survival benefit from trastuzumab in both luminal subtypes. Regarding HER2+/Basal-like disease, the NSABP B31 study showed a survival benefit from trastuzumab whereas N9831 did not. Thus, intrinsic subtyping cannot be used today to guide the use of (neo)adjuvant trastuzumab in HER2+ disease. However, this biomarker (HER2-E vs not) might help identify those patients that need (neo)adjuvant pertuzumab in combination with trastuzumab and chemotherapy. Analysis in tumor samples from the pivotal phase III trials that led to the approval of pertuzumab in the adjuvant (APHINITY) [59] and metastatic (CLEOPATRA) [60] settings seems warranted.

Sensitivity to dual HER2 blockade-only

Another area of great interest is to identify patients that might be cured with dual HER2 blockade-only in the absence of chemotherapy [61,62]. In this direction, the results of three chemotherapy-free neoadjuvant trials (i.e. TBCRC006 [63], SOLTI-PAMELA [51] and NeoSphere [64]) further support this hypothesis. In the TBCRC006 [63] and SOLTI-PAMELA [51] studies, 64 and 151 patients with primary HER2+ BC were treated with trastuzumab and lapatinib without chemotherapy for 12 and 18 weeks, respectively, in the neoadjuvant setting. Patients with HR+ disease also received endocrine therapy. The pCR rate in the breast was 27.0% (TBCRC006) and 30.2% (SOLTI-PAMELA). In the NeoSphere trial [64], the pCR rate in the breast was 16.8% after 12 weeks of trastuzumab and pertuzumab. Of note, no endocrine therapy was added in patients with HR+ disease in the NeoSphere trial. Overall, these results suggest that a subset of patients with HER2+ BC is sensitive to the dual HER2 blockade and potentially could be treated without cytotoxic therapy.

According to *in silico* and *omic* analyses from The Cancer Genome Atlas (TCGA) breast cancer project [4], HER2+ tumors of the HER2-E subtype are driven by HER2/EGFR signaling; thus, HER2-E tumors should benefit the most from a HER2 blockade. The previous studies showing higher response rates in the HER2-E subtype following anti-HER2-based chemotherapy could not distinguish anti-HER2 sensitivity versus chemotherapy-sensitivity. To test the pure anti-HER2 sensitivity of the HER2-E subtype, the SOLTI-PAMELA [5]1 phase II neoadjuvant clinical trial was undertaken. In this study, 151 patients with stage II-III HER2+ disease were treated for 18 weeks with neoadjuvant trastuzumab and lapatinib (and endocrine therapy if the tumor was HR+). The primary hypothesis was that the HER2-E subtype would obtain a higher pCR rate compared to non-HER2-E tumors. The overall pCR rate in the breast was 30.2%, and the primary objective was met. The pCR in the breast of the HER2-E subtype was 40.2% versus 10.0% in non-HER2-E tumors in all population, and 31.6% versus 5.1%, respectively, in HR+/HER2+ patients. Importantly, HR status lost its association with pCR once intrinsic subtype was taken into account in a multivariable model. A subsequent validation of the predictive value of the HER2-E subtype in 85 samples from the TBCRC023 [65] trial has recently been reported. Overall, these data suggest that HER2-E subtype is a predictor of anti-HER2 sensitivity, and could help identify in the future a group of patients with HER2+ early BC that might be cured with anti-HER2 treatment without chemotherapy, or a group of patients with HER2+ advanced or metastatic disease that can be treated with dual HER2 blockade-only.

Sensitivity to CDK4/6 inhibition

Although palbociclib and ribociclib are currently approved in HR+/HER2-negative advanced disease, these drugs are currently being evaluated in HR+/HER2+ advanced disease (NCT03054363, NCT02947685, NCT02448420 and NCT02657343). However, the distribution of the intrinsic subtypes in both subgroups is largely different. Indeed, whereas the vast majority (~80%) of HR+/HER2-negative advanced tumors fall into the Luminal A or B subtypes, only ~60% of all HR+/HER2+ tumors are luminal. Thus, the relevant question is how non-luminal subtypes within HR+/HER2+ disease benefit or respond to CDK4/6 inhibition. From a preclinical point of view, *in vitro* data evaluating palbociclib in a panel of 15 HER2+ BC cell lines already exist [66]. Subtype distribution in these HER2+ cell lines is 60% HER2-E, 26.7% Luminal B and 13.3% Basal-like. Using the reported IC50 data by Finn and colleagues, the median IC50s of Luminal B, HER2-E and Basal-like were 47.5, 179 and 546 [66]. Overall, these reclinical data suggest, similarly as in HR+/HER2-negative disease, that non-luminal subtypes within HER2+ disease might not benefit much from CDK4/6 inhibition.

Recent interim results from SOLTI 13-03 PATRICIA phase II clinical trial supports these preclinical findings [67]. In this study, patients with advanced or metastatic HR+/HER2+ disease previously treated with anti-HER2-based chemotherapy were treated palbociclib and trastuzumab with or without letrozole [67]. Tumor samples from 26 patients were PAM50 profiled. As expected, 46.2% of HR+/HER2+ tumors were found to be HER2-E. More importantly, a statistically significant difference in PFS was observed between luminal and non-luminal disease (median PFS 10.5 months vs 3.5 months; hazard ratio = 0.54)[68]. Although this was not a randomized trial, and the difference in survival could be due to inherent prognosis, the data support a rather small absolute benefit from CDK4/6 inhibition in non-luminal disease. Ongoing trials in HR+/HER2+ should consider a pre-planned analysis with this biomarker.

Immune infiltration in non-luminal subtypes

The presence of tumor-infiltrating lymphocytes (TILs) in HER2+ disease has been associated with better survival outcome in early [69–71] and metastatic setting [72]. Moreover, TILs have been associated with higher pCR rates following anti-HER2-based chemotherapy independently of other clinical-pathological variables. Futhermore, TILs in HER2+ disease might predict anti-PD1 monotherapy benefit, as suggested by the results from the PANACEA phase Ib/II trial presented at the 2017 SABCS [73]. In this study, the combination of pembrolizumab and trastuzumab reached an objective response rate (ORR) of 15.2% in unselected patients with trastuzumab-resistant HER2+ BC. Among patients with TILs levels ≥5%, the objective overall response rate was 39% compared to 5% in patients with TILs levels < 5%.

In HER2+ disease, non-luminal subtypes have the highest levels of TILs compared to luminal disease (CHERLOB [57] and SOLTI-PAMELA [74]), implying that these subtypes are more immunogenic. In both studies, continuous TILs at baseline were significantly associated with pCR following anti-HER2 therapy; however, in multivariable models adjusting for PAM50 subtypes, TILs lost their significant association suggesting that the subtype encompasses the information provided by TILs. Future clinical trials in HER2+ disease should explore immune therapies according to intrinsic subtype.

Conclusions

Here, we reviewed the wealth of clinical data that exist today regarding the value of non-luminal disease, defined by PAM50, in HR+ disease. Overall, the data clearly suggest that both non-luminal subtypes provide additional prognostic and predictive information beyond HR and HER2 status. Thus, we can conclude that the clinical validity of both subtypes in HR+ disease has been established in different settings. Now the challenge is to run retrospective and prospective studies in order to obtain Level 1 evidence of the clinical utility of both biomarkers. One important aspect is that the cutoffs to define one subtype versus another are based on biological classification and not clinical results (e.g. benefit to a drug). Thus, for particular indications, the cutoffs may require optimization. This should not be a major issue since

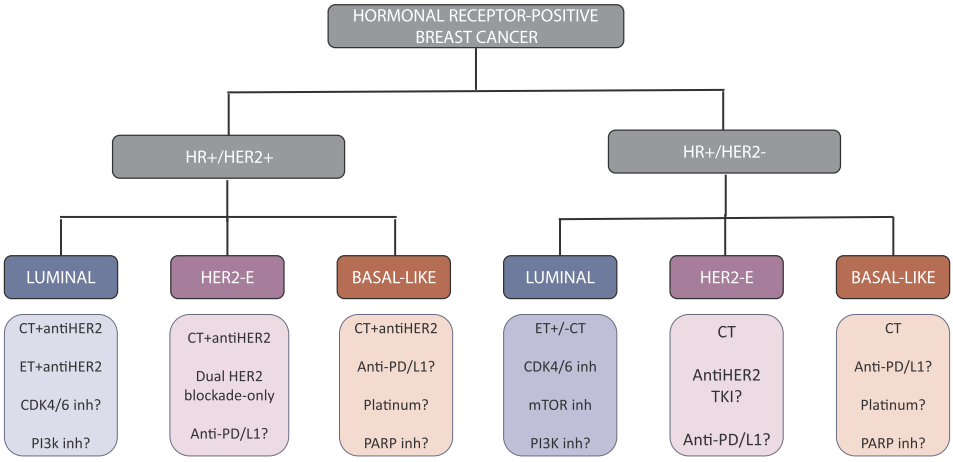

Fig. 4. Proposed Therapeutic Options for HR+ breast cancer. CT, chemotherapy; ET, endocrine therapy; inh, inhibitor; TKI, tirosine-kinase inhibitor.

the "intrinsic" biology is actually evaluated as a continuous variable by the PAM50 assay.

To conclude, multigene assays provide prognostic and predictive information beyond pathological parameters and may support more-informed treatment decisions (Fig. 4). The intrinsic molecular subtypes of BC significantly extend our knowledge about behavior of HR+ disease and may have clinical utility.

Conflict of interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ctrv.2018.04.015.

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