Dissecting Breast Cancer Complexity: Specific Biological Features and Vulnerabilities of Triple Positive Breast Cancer Tumors

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Editorial

Tumor heterogeneity and complexity are recognized as some of the major barriers of precision medicine. The paradigm of target therapies is to hit specific "Achilles' heels" of tumors in order to achieve the maximum of therapeutic efficacy with the minimum of toxicity. This might not be realized until we will be able to recognize and specifically treat each relevant tumor subtype harboring its own biological features and vulnerabilities.

In this regard, Breast cancer (BC) is very heterogeneous. In clinical practice, the ability to identify three main groups of tumours, including hormonal receptor positive, HER2 overexpressed, and triple negative tumours, each requiring different therapeutic approaches, is determined mainly by Immunohistochemical staining, and occasionally by FISH testing for the c-erbB2 coding gene [1].

Several early milestone studies, mainly based on mRNA expression profiling, provided a more complex portrait of breast cancer heterogeneity, identified six molecular subtypes: Luminal A, Luminal B, HER2 enriched, Basal like, Normal like and Claudin-low breast cancers [2].

Subsequently, with the advent of a number of different high-throughput technologies, able to provide comprehensive details also on DNA mutational status as well as on epigenetic and proteomic tumour's landscape, it has become clear that each molecular subtype exhibits a considerable range of intrinsic molecular/genomic variability [3].

This intragroup heterogeneity is even broader within clinically immunohistochemical defined tumour subgroups.

In particular, the clinically defined HER2 positive (HER2+) breast cancer is a composite disease, as in nearly 50% of HER2-positive disease, there is the coexistence of both expression of estrogen receptor/progesterone receptor (HR) and hyperexpression/amplification of HER2 [4].

In vitro and *in vivo* models suggest the existence of a cross-talk between these two pathways, which could in turn affect the natural history, response to therapy and outcome of patients affected by this specific subset of BC.

A study from the Cancer Genome Atlas Network, clearly confirmed, on a molecular basis, the existence of at least two subtypes of clinically defined HER2 positive breast cancer. These include an HER2 enriched-mRNA-subtype, which shows a HER2-driven phenotype, and a Luminal-mRNA-subtype, which shows a more luminal-like phenotype with higher expression of the typical Luminal genes, comprising GATA3, BCL2 and estrogen receptor gene ESR1 and harboring *GATA3* mutations too [3].

It is also known that the coexistence of both HR and HER2 over-activated pathways influences the natural history of disease and patients' outcome.

A retrospective analysis from the HERA trial demonstrated, for the HER2+/HR- cohort of tumors, a very high risk of early recurrence, in contrast with HER2+/HR+ disease, characterized by a relatively consistent risk of relapse over time [4,5].

Numerous studies have provided evidences that HER2 pathways may directly or indirectly contribute to the development of resistance to Hormone Therapies (HT).

On the other hand, the HR expression in HER2-positive tumors may be involved in the development of de novo and/or acquired resistance to anti-HER2 therapies [6].

Preclinical studies showed that in HER2+/HR+ cell lines the antiproliferative and pro-apoptotic effect of the sustained inhibition of HER2 with lapatinib or the combination of lapatinib and trastuzumab was suddenly overcome by HR pathway activation, which became the primary controller of cancer survival and proliferation [7,8].

Consistently with these preclinical data, studies in the neoadjuvant setting show a correlation between anti-HER2 therapies activity and HR status. The so-called CTneoBC pooled analysis, including 12 international neoadjuvant trials, clearly demonstrated an association between HR status and the percentage of pCR obtained with treatments containing trastuzumab (50.3% of pCR in HER2+/HR- vs. 30.9% of pCR in HER2+/HR+ p <.003) [9].

More recent neoadjuvant clinical trials, exploring the activity of dual blocking strategy in HER2+ disease (NeoALTTO and NeoSphere), also confirmed a different response rates to anti-HER2 drugs in HR+ vs. HR- tumors. In NeoALTTO trial, in-breast pCRs were consistently higher in HR- than in HR+ disease treated with trastuzumab (36.5% vs. 22.7%), lapatinib (33.7% vs. 16.1%) or with their combination (61.3% vs. 41.6%) [10].

Similarly, in the NeoSphere trial pCRs were higher in HR- disease (63.2%) rather than HR+ (26%) in the docetaxel-trastuzumab-pertuzumab arm but also in the chemo-free dual HER2 blockade arm (27.3% vs. 5.9%) [11].

Also in the adjuvant setting, several studies demonstrate less benefits for patients with HER2+/HR+ disease compared with patients with HER2+/HR-negative tumors who received anti-HER2 therapy.

In a retrospective subgroup analysis of the HERA trial, a slightly lower magnitude of effect for adjuvant trastuzumab was observed in the experimental arm compared with the observational arm in patients with HER2+/HR+ tumors (3-year DFS percentage: 84% vs. 78%; HR 0.68; 95% CI:0.51–0.89) compared to results in the patients with HER2+/HR- tumors (3-year DFS percentage: 76% vs. 70%; HR 0.62; 95% CI:0.5–0.77) [5].

A more recent retrospective analysis from HERA trial showed that the degree of HER2 amplification and estrogen receptor levels strongly influenced the responsiveness of HER2+/HR+ tumors to trastuzumab [12]. Significant reduction of therapeutic benefit with trastuzumab was observed in HR+ patients with low fluorescence in situ hybridization (FISH) ratios (>or =2 to <5). Hazard Ratio for DFS was 0.89 (CI 0.65-1.21) in low FISH ratio/HR+ group versus 0.62 (0.47-0.82) in high FISH ratio/HR+ tumors. Interestingly FISH ratio levels did not influence the outcome of anti HER2 therapy in patients with HER+/HR- disease [12].

Furthermore, when the outcome of HER2+/HR+ breast cancers was analysed according to the ESR1 mRNA expression levels, it was found that a higher ESR1 mRNA levels define a group of patients with low benefit to anti-HER-2 therapy, regardless HER2 levels. The Hazard ratio for DFS for tumours in the lower tertile of ESR1 expression level was 0.36 (0.21-0.60) vs. 1.07 (CI 0.59-1.93) for tumours in the higher tertile (interaction p value= 0.01) [12].

Consistently with these findings, another similar analysis from NSABP B31 trial, using gene expression data, has identified a group of HER2+/HR+ tumors characterized by high ESR1 and "intermediate" HER2 transcript levels that had no significant benefit from adjuvant trastuzumab therapy [13].

Taken together, these data seem to indicate the fact that HER2+/HR+ BCs is, in turn, a heterogeneous disease characterized by different responsiveness to anti-HER2 therapies as a function of different degree of addiction to HER2 or HR pathways. Stratification of HER2+/HR+ BCs according to degree of HER2 amplification and estrogen receptor expression levels, as recently performed in retrospective analysis of HERA trial, seems to help to identify two different subgroups with different treatment outcome, but probably need to be further refined.

The different outcome to anti-HER2 therapies showed by HER2+/HR+ tumours compared with HER2+/HR- BCs discussed above is a confirmation, from a clinical perspective, of the distinctive features of triple positive tumours clearly identified in preclinical and molecular studies.

Similarly, there are data showing significant differences between HER2+/HR+ and luminal like/HER2- tumours in terms of sensitivity to endocrine therapies (HTs).

In general subgroup analysis from phase III trials evaluating efficacy of HTs, reported an absolute poorer outcome for HER2+/HR+ tumours compared with HR+/HER2- ones, showing quite consistently shorter DFS in adjuvant trials as well as lower response rate and shorter PFS in trials for advanced disease [14-16].

Taken together these data showed that, for concurrently HER2 and ER driven cancers, it is becoming apparent that new treatment approaches will be necessary to obtain durable disease control or even cures.

As preclinical and clinical data so far available consistently suggest that HR+/HER2+ disease could derive the highest therapeutic benefit from the concurrent dual HER2 blockade (i.e trastuzumab plus lapatinib or pertuzumab) together with ER inhibition, a number of clinical trials, in both neoadjuvant and metastatic setting, have been designed to further test this hypothesis.

Recently, at 2016 San Antonio Breast Cancer Symposium, Arpino MG. and colleagues presented the results of PERTAIN, an open-label randomized phase II study comparing Pertuzumab versus placebo given in combination with Trastuzumab and an Aromatase Inhibitor, as first line treatment in 258 post-menopausal patients with HER2+/HR+ locally advanced or metastatic breast cancers. Treatment was given with or without an induction chemotherapy with taxanes at investigator's discretion for 18 to 24 months before starting AI therapy [17].

The results showed that a complete blockade of both pathways yields a better outcome than the partial blockade, with a median PFS that was 18.8 months for the pertuzumab containing combination compared with 15.8 months for trastuzumab and AI arm (HR, 0.65; 95% CI, 0.48-0.89; P = .007). Moreover, only patients treated with the three drugs combination reached complete responses, with a CR rate of 7,3% vs. 0,9% in the Trastuzumab + AI arm alone [16].

Ultimately, we expect that multi-drugs combination therapies will be needed, in order to obtain highest efficacy and overcame the mechanisms of potential resistance.

However, the large number of potential resistance mechanisms will likely necessitate the use of more drugs simultaneously but this approach could be hampered by unacceptable toxicity profile.

Several ongoing phase II-III trials, in both neoadjuvant and

metastatic setting, are testing the strategy to reduce or avoid chemotherapy and relative toxicity, in order to implement multi-drugs combinations able to inhibit HER2 and ER pathways more efficiently. In the majority of cases, chemotherapy is confined to a short induction phase or is completely replaced by a three or four drugs combination target therapies that concomitantly inhibit HER2 and ER as well as specific pathways known to act as resistance mechanisms.

In particular, concomitant inhibition of HER2, ER and the cell cycle regulators CDK4/6 or Pi3K/AKT/mTOR pathways represents a great hope as both seems play a major role in either anti-HER2 as well as endocrine resistance mechanisms [18,19].

Two ongoing trials are testing the efficacy and tolerability of a four-drugs combination that allows to completely block HER2 signalling through trastuzumab and pertuzumab, ER activity with AI or fulvestrant and CDK4-6 with palbociclib.

In the first one, the phase III PATINA trial (NCT02947685), HER2+/HR+ untreated metastatic breast cancer patients, after the completion of a maximum of 8 cycles of induction chemotherapy plus anti HER2 therapy, will be randomized to receive palbociclib or placebo in combination with Trastuzumab, Pertuzumab and an endocrine therapy with AI or Fulvestrant.

The second one, the NA-PHER2 (NCT02530424) single arm phase II trial, is testing the activity of the four drugs combination without chemotherapy in the neoadjuvant setting. Results of these trials are awaited in order to understand the success of such type of new strategies that exploit all the available knowledge of the biological features of tumours in order to focus therapeutic interventions against the specific tumour vulnerabilities, limiting or even avoiding the use of non specific cytotoxic drugs.

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