review articles

HER2-Low Breast Cancer: Pathological and Clinical Landscape

Paolo Tarantino, MD[1,2]; Erika Hamilton, MD[3]; Sara M. Tolaney, MD, MPH[4]; Javier Cortes, MD, PhD[5,6]; Stefania Morganti, MD[1,2]; Emanuela Ferraro, MD[1,2]; Antonio Marra, MD[1,2]; Giulia Viale, MD[1,2]; Dario Trapani, MD[1,2]; Fatima Cardoso, MD[7]; Frédérique Penault-Llorca, MD, PhD[8,9]; Giuseppe Viale, MD[1,2]; Fabrice Andrè, MD, PhD[10]; and Giuseppe Curigliano, MD, PhD[1,2]

INTRODUCTION

Breast cancer (BC) is the most commonly diagnosed cancer in women worldwide.[1] It is a heterogeneous disease, comprising distinct biologic entities with different prognosis and oncogenic drivers. Gene expression profiling studies have identified six main BC intrinsic subgroups.[2,3] However, treatment decisions are commonly based on conventional histopathological factors, recognizing four primary clinical subtypes of BC with prognostic and predictive relevance in clinical practice: luminal A-like, luminal B-like (human epidermal growth factor receptor 2 [HER2]-negative), HER2-positive, and triple negative BC (TNBC).[4-8] In particular, in the context of HER2-positive BC (approximately 15% of all BCs[9]), *ERBB2* amplification leads to HER2 overexpression, which confers this subgroup of cancers a more aggressive behavior and worse prognosis, if untreated.[10,11] However, the development of multiple agents targeting HER2 has provided significant clinical benefits in the early- and advanced-stage settings, changing the trajectory of its natural history.[12]

According to the 2018 ASCO and College of American Pathologists HER2 testing guideline update,[13] BC is considered HER2 positive when there is evidence of HER2 overexpression on an immunohistochemistry (IHC) assay (score 3+) or gene amplification on an in situ hybridization (ISH) assay on at least one tumor sample. In the case of a 2+ IHC score, reflex ISH testing is required to define HER2 status, with additional concomitant review of IHC slides in case of particular ISH results (groups 2, 3, and 4) for an integrated assessment. In case of IHC 0 and 1+ results, or IHC 2+ with a negative ISH assay, BC is considered HER2 negative, and no HER2-targeted therapy is recommended, with the exception of dual-probe ISH testing group 3 results (HER2/CEP17 ratio < 2.0 with average HER2 copy number of 4.0 to 5.9 per cell) where tumors that are IHC 2+ are deemed HER2 positive.[13]

Lately, a potential new nomenclature has been proposed for the cases with IHC 1+ or 2+ with negative ISH—namely, HER2-low BC. In clinical practice, these tumors are reported as HER2 negative, either TNBC or luminal-like if hormone receptors (HRs) are expressed, and agents disrupting the HER2 pathway have not been shown to offer clinical benefit.[14,15] More recently, however, on the basis of the benefit observed with novel anti-HER2 compounds, it is suggested that a subset of BCs with low levels of HER2 expression and no detectable *ERBB2* amplification might also derive benefit from targeting HER2. In contrast with HER2-overexpressing tumors, the benefit in this setting might be achieved by alternative pharmacological mechanisms, including the delivery of targeted cytotoxic agents into cancer cells as well as the attraction of immune-competent cells. The clinical development of novel anti-HER2 agents for HER2-low BC has the potential to improve the treatment armamentarium for a subgroup of patients not currently considered candidates for HER2-targeted therapy, thereby potentially expanding these therapies to a much larger number of patients with BC.

In this review, we discuss the evolving landscape of HER2 targeting in HER2-low BC and propose an algorithm to define HER2-low BCs as we begin to rethink the current binary paradigm of HER2 expression from negative or positive to also include intermediate levels of expression, assuming evidence of clinical utility is confirmed. If so, this will require tighter definitions to ensure more accurate characterizations of levels of HER2 expression that would influence clinical decision-making.

CLINICAL DEFINITION OF HER2-LOW BC

Most of the published data and ongoing clinical trials define HER2-low BCs as those with an HER2 IHC score of 1+ or 2+ with negative ISH assay,[16-23] according to the current scoring systems.[13] As a result, more than a half of BCs may qualify as HER2 low (Fig 1).[9] The clinical definition of HER2 low is intrinsically dependent on the testing technique and currently can only be applied with the standard IHC/ISH approach, because clear parameters that would define a tumor as HER2 low using other assays have not been formally established.

In Situ Hybridization for HER2-Low BC

ISH testing to detect HER2 gene amplification using various chromogenic or fluorescence assays[24] and

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on February 4, 2020 and published at ascopubs.org/journal/jco on April 24, 2020: DOI https://doi.org/10.1200/JCO.19.02488

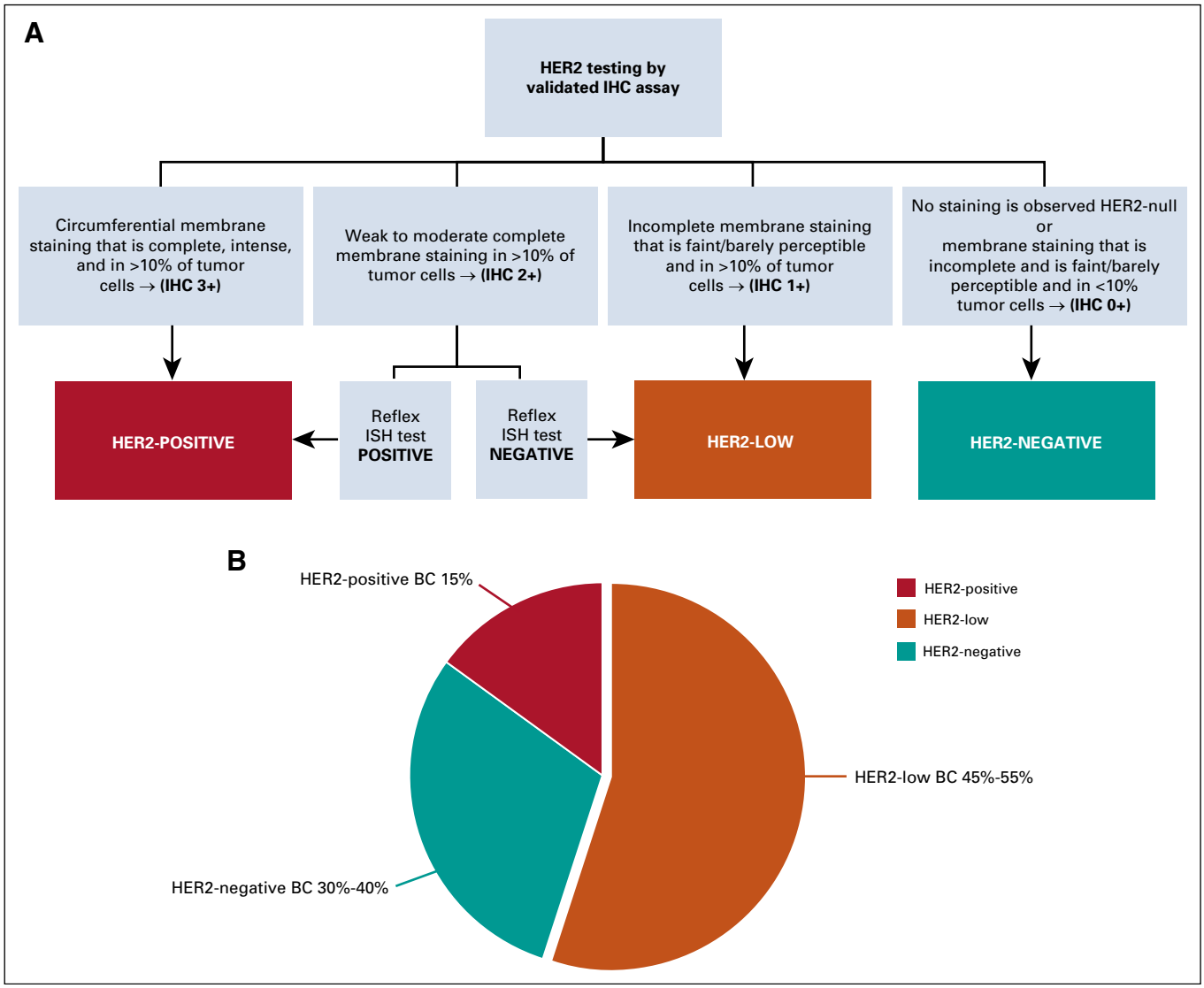

FIG 1. Proposal of an algorithm for defining HER2-low BC. (A) The majority of clinical trials testing anti-HER2 agents in HER2-low BC enroll patients affected by cancers with an HER2 IHC score of 1+ or 2+ with a negative reflex ISH assay, regardless of the molecular subtype (eg, luminal, triple negative). Accordingly, IHC and ISH currently represent the most appropriate techniques to define HER2-low BC. Nevertheless, novel quantitative testing methods (more extensively discussed in the text) might allow a better dissection of HER2 expressions, particularly for HER2-negative samples, dissecting HER2 absence (HER2 null) from faint or barely perceptible staining in < 10% of tumor cells. (B) If activity of these drugs was confirmed, the percentage of patients deriving benefit from anti-HER2 treatments could reach 60% to 70%.[9] BC, breast cancer; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

IHC testing to assess membrane protein expression in a semiquantitative fashion are the established assay platforms used in routine practice to determine HER2 status of the tumor.[13] Abundant evidence from studies using frozen tissue suggests a direct relationship between HER2 gene amplification and protein expression, and essentially no protein overexpression is observed in the absence of gene amplification.[25,26] However, new preclinical evidence suggests that the activity of some anti-HER2 antibody-drug conjugates (ADCs) might be independent from HER2 amplification,[27,28] and antitumoral effects have been observed in BCs with and without gene amplification.[16,29] If so, ISH alone might not be the ideal methodology to predict efficacy to novel anti-HER2 treatments.

IHC to Detect HER2-Low Expression

IHC was used to assess HER2 status in the seminal trials of trastuzumab and remains the most common initial test done in clinical practice.[13] IHC is also the primary technique used to identify patients with HER2-low tumors in clinical trials attempting to enroll patients with HER2-nonamplified cancers for trials of novel anti-HER2 agents (Table 1).[16,29] However, IHC assay is associated with issues

that could ultimately lead to underestimation of the rate of HER2-low tumors.

Factors That May Influence IHC and ISH Test Results

Preanalytical and analytical factors may help explain some of the discordance observed when HER2 testing were done in local and central laboratories in various reported clinical trials.[13] One study assessing the discrepancies in local and centralized assessment of HER2 reported that up to 85% of the patients with tumors originally scored IHC 0 actually were 1+ or 2+, suggesting the value of a quality assessment and report in reference laboratories for HER2-low BC.[30] Therefore, pathologists should adhere to the 2018 ASCO and College of American Pathologists HER2 testing recommendations[13] to ensure accurate and reproducible IHC scoring.

But even then, methodological limitations of the IHC platform as currently deployed could affect results. Formalin fixation may artificially drive down the detection of protein expression, and a semiquantitative assay like IHC might not be sensitive enough to accurately detect low levels of HER2 expression. Hence, an IHC score of 0 may reflect an artifactual limitation of the technique rather than total absence of the HER2 protein on the membrane.[31] To tackle these uncertainties, several novel quantitative assays are under development to enhance the sensitivity of HER2 assessment. For instance, the automated quantitative analysis technology can quantitatively assess HER2 expression by measuring the intensity of antibody-conjugated fluorophores[32,33]; the HERmark technology measures HER2 expression through a proximity-based release of antibody-bound fluorescent tags[34-36]; the quantitative IHC technology converts antibody/antigen complexes into red dots, subsequently counted to quantify HER2 expression[36a]; the time-resolved fluorescence energy transfer technology enables assessment of HER2 expression through the detection of fluorescence emitted by two fluorophores in close proximity.[37] If validated, these assays could improve our ability to identify patients with HER2-low BC.

Quantitative Real-Time Polymerase Chain Reaction for HER2-Low Tumors

Quantitative real-time polymerase chain reaction (RT-qPCR) allows a more quantitative assessment to be made of the expression levels of HER2 mRNA in BC samples and has been evaluated as potential complement or alternative to IHC/ISH, particularly in tumors that do not appear to be clearly positive or negative by standard IHC and FISH. Key advantages of RT-qPCR are the possibility to offer a more standardized, objective, and automated assessment, and the growing ability to perform them in small samples, such as core biopsy specimens. Comparisons with the recommended techniques have yielded conflicting results thus far.[38-41] However, in the context of HER2-low tumors, RT-qPCR could potentially complement the semiquantitative data obtained by IHC. The ultimate clinical utility of such effort will need to be assessed in clinical trials, prospectively and retrospectively.

CLINICAL LANDSCAPE OF HER2-LOW BC

HER2 amplification defines a distinct subtype of BC for which translational, epidemiologic, prognostic, and predictive data have been extensively studied.[10,11] However, much less evidence is available regarding HER2-low BC and on what criteria should be used to define it as a clinically relevant subtype of BC.

Biology of HER2-Low BC: A New Entity?

Quantitative assays have highlighted a continuous distribution of HER2 expressions in nonamplified BC cells, with higher degrees of expression found in luminal tumors and stem cells.[42] Several factors could lead to supraphysiological levels of HER2 expression in BC cells that lack *ERBB2* gene amplification. Crosstalk between HER2 and estrogen receptor pathways,[43,44] along with modifications induced by endocrine treatments,[45,46] may promote HER2 protein overexpression as a potential mechanism of tumor adaptation and treatment resistance. Chemotherapy[47] and radiotherapy[48] also upregulate HER2 in HER2-low BC cells, through the activation of the NF-κB pathway. Interestingly, NF-κB has been reported as a functional player in HER2 upregulation in nonamplified cells when activated by stimuli from the tumor microenvironment.[42] Furthermore, epigenetic changes are implied in the pattern of HER2 upregulation in HER2-low BC cells.[49]

Although preclinical models implicate HER2 as a mechanism of response to several stress stimuli, its oncogenic role in HER2-low BC is still unclear. Thus, evidence to date is insufficient to support defining HER2-low BC as an individual BC subtype with well-characterized features associated with prognosis and responsiveness to therapy.

Clinical Implications of Low HER2 Expression

Multiple retrospective analyses have investigated the prognostic significance of low HER2 expressions, with conflicting results.[50-54] Overall, no solid evidence supports HER2-low status as an independent prognostic factor. As a predictive factor and integral biomarker for trial eligibility, patients with centrally determined HER2-low tumors gained no benefit from adjuvant trastuzumab in the phase III NSABP-B47 trial that tested the addition of trastuzumab to standard adjuvant chemotherapy in patients with early BC.[14] Taken together, the results confirm the limited clinical value of HER2-pathway blockade with trastuzumab in the absence of standard measures of HER2 gene amplification or protein overexpression. Nonetheless, the emergence of new anti-HER2 agents suggests a potential predictive value of HER2-low tumors for novel compounds with unique mechanisms of action (Fig 2). Early promising data suggest that some of these agents are active in

TABLE 1. Ongoing Trials in HER2-Low BC

Drug (Sponsor)	NCT.gov Identifier	Phase; Status	Sample Size	Population	Drug Regimen
Monoclonal antibody					
Margetuximab (MacroGenics, Rockville, MD)	NCT01828021	II; C	25	Pretreated, HER2-low–expressing (IHC 2+ or IHC 1+, FISH-negative, tumors score ≥ 10.5 by HERmark testing) advanced BC; centrally assessed	Margetuximab
Antibody-drug conjugates					
DS8201a (Daiichi Sankyo, Tokyo, Japan)	NCT03523572	Ib; R	99	Pretreated, advanced HER2-low–expressing (IHC 1+ or 2+ ISH-negative) BC	DS8201 + nivolumab
DS8201a (Daiichi Sankyo)	NCT03368196	I; ANR	12	Pretreated advanced BC or adenocarcinoma with any HER2 expression (IHC 1-3+ and or ISH positive)	DS8201
DS8201a (Daiichi Sankyo)	NCT03734029 (DESTINY-Breast04)	III; R	540	Pretreated advanced HER2-low–expressing (IHC 1+ or 2+, ISH-negative) BC; centrally assessed	DS8201 *v* treatment of physician choice (2:1)
DS8201a (Daiichi Sankyo)	NCT04042701	I; R	105	Pretreated, advanced, HER2-low–expressing (IHC 1+ or 2+, ISH-negative) BC; centrally assessed	DS8201 + pembrolizumab
SYD985 (Synthon Biopharmaceuticals, Nijmegen, Netherlands)	NCT02277717	I; C	185	Pretreated, advanced BC HER2-low expressing (IHC 1+ or 2+ ISH-negative); locally assessed	SYD985
ARX788 (Zhejiang Medicine, Shaoxing Zhejiang, China)	NCT02512237	I; UK	80	Advanced HER2-low–expressing (IHC 2+ ISH-negative) BC; locally assessed	ARX788
ARX788 (Zhejiang Medicine)	NCT03255070	I; R	60	Pretreated, advanced, HER2-low–expressing (IHC 2+ ISH negative) BC; locally assessed	ARX788
A166 (Klus Pharma, Cranbury, NJ)	NCT03602079	I/II; R	82	Pretreated, advanced, HER2-low–expressing (IHC 1+ and 2+ without FISH confirmation) BC; locally assessed	A166
PF-06804103 (Pfizer, New York, NY)	NCT03284723	I; R	124	Part 2: Pretreated, advanced, HER2-low–expressing (2+ without FISH confirmation) BC; locally assessed	PF-06804103
FS-1502 (Fosun Pharmaceutical, Shanghai, China)	NCT03944499	I; NYR	92	Dose escalation: Pretreated, advanced, HER2-low–expressing (IHC 1+ and 2+ without FISH confirmation) BC; locally assessed, with central confirmation	FS-1502
XMT-1522 (Mersana Therapeutics, Cambridge, MA)	NCT02952729	I; ANR	120	Pretreated, advanced, HER2-low–expressing (IHC 1+ and 2+ without FISH confirmation) BC; locally assessed	XMT-1522
Vaccines					
HER-2/neu peptide vaccine (National Cancer Institute, Bethesda, MD)	NCT01355393	I/II; ANR	50	Stage II/III HER2-positive BC (IHC 1+ or 2+ or 3+ and/or ISH positive) or stage IV HER2-positive BC treated to NED or stable bone only disease	HER-2/neu peptide vaccine + rintatolimod *v* HER-2/neu peptide vaccine + sargramostim *v* HER-2/neu peptide vaccine + sargramostim + rintatolimod
AdHER2/neu DC vaccine (National Cancer Institute, Bethesda, MD)	NCT01730118	I; ANR	33	Advanced "anti-HER2-naïve" HER2-positive BC (IHC 1+ or 2+ or 3+ and/or FISH positive or equivocal)	AdHER2/neu DC vaccine monotherapy
Bispecific antibodies					

(continued on following page)

TABLE 1. Ongoing Trials in HER2-Low BC (continued)

Drug (Sponsor)	NCT.gov Identifier	Phase; Status	Sample Size	Population	Drug Regimen
MCLA-128 (Merus, Utrecht, the Netherlands)	NCT03321981	II; R	120	Advanced, HR+, HER2-low–expressing BC (IHC 1+ or 2+), progressing during an endocrine treatment	MCLA-128 + endocrine treatment
ZW25 (Zymeworks, Vancouver, British Columbia, Canada)	NCT02892123	I; R	234	Pretreated advanced, HER2-expressing (HER2 1+, 2+, or 3+ by IHC) BC	ZW25
BTRC4017A (Genentech, San Francisco, CA)	NCT03448042	I; R	449	Pretreated HER2-expressing (not further specified) advanced BC, locally assessed	BTRC4017A
IBI315 (Innovent Biologics, Jiangsu, China)	NCT04162327	I; R	191	Pretreated HER2-expressing (not further specified) advanced solid tumors	IBI315

Abbreviations: ANR, active; not recruiting; BC, breast cancer; C, completed; FISH, fluorescent in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH; in situ hybridization; NED, no evidence of disease; NYR, not yet recruiting; R, recruiting; UK, unknown status.

nonamplified tumors, potentially challenging the current paradigm of HER2 targeting in BC. (Table 2)

Potential Implication of HER2 Heterogeneity

Another still-underestimated issue of HER2 expression or overexpression in BC is the clinical implication of intratumoral heterogeneity. Indeed, according to the current recommendations, tumors are considered HER2 positive by IHC if > 10% tumor cells show intense circumferential membrane staining, whereas tumors are considered HER2 positive by ISH testing when they have an aggregate population of amplified cells composing > 10% of the total tumor cell population.

The advent of ADCs has highlighted the possible impact of HER2 intratumoral heterogeneity on response. Available data in the neoadjuvant setting document that patients treated with T-DM1 and pertuzumab are less likely to achieve pathologic complete response and have a higher risk of locoregional progression before surgery as compared with patients receiving chemotherapy and dual HER2 blockade.[55] The data suggest that, in the absence of systemic chemotherapy, the bystander killing effect of T-DM1 may not be sufficient to eradicate tumors with HER2 heterogeneity.[56] As a corollary, it may become imperative to report the actual percentage of overexpressing or amplified tumor cells to inform the choice of anti-HER2 treatments when ADC compounds are a possible option.

HER2-Targeting Therapies in Tumors With HER2 Gene Mutations

Besides HER2 overexpression, additional HER2 aberrations have been proved actionable. In particular, approximately 2% of BCs harbor HER2 mutations, which appear to be enriched in HER2-low tumors.[57] Neratinib was tested in pretreated patients with HER2-mutant, nonamplified mBC and showed signs of efficacy both in monotherapy[58] and in combination with fulvestrant.[59] Following these encouraging results, additional studies were initiated in this setting with other anti-HER2 tyrosine kinase inhibitors, including poziotinib (ClinicalTrials.gov identifier: NCT02544997) and pyrotinib (ClinicalTrials.gov identifier: NCT03412383). Moreover, ADCs showed potential activity in other HER2-mutant tumor histologies,[60] and their investigation in BC is warranted. Overall, some HER2 mutations appear to be an actionable target in BC, and investigation of novel anti-HER2 drugs should be expanded to this subgroup of patients.

THE EXPANDING ARMAMENTARIUM OF ANTI-HER2 AGENTS IN HER2-LOW BC

Monoclonal Antibodies

Anti-HER2 monoclonal antibodies (mAbs) comprise antibodies binding to different domains of HER2. Although no role for trastuzumab in HER2-low BC is suggested,[14] novel anti-HER2 mAbs have had anticancer activity in preclinical models of HER2-low cell lines, prompting further clinical development in this setting.[61-63]

Pertuzumab has limited activity in patients with HER2-low metastatic BC (mBC). In a phase II trial in which the role of pertuzumab monotherapy was assessed in pretreated patients with HER2-low mBC, only two of 78 patients achieved a response.[15] A phase Ib trial tested pertuzumab with paclitaxel and the anti-HER3 mAb lumretuzumab in patients with HER3-expressing HER2-low mBC,[20] but an unacceptable toxicity profile prompted discontinuation of this study.

Margetuximab was tested in a phase I trial of patients with multiple solid tumors overexpressing HER2 by IHC (scores 2+ and 3+), 40% of which were BCs.[64] The trial enrolled 23 patients with HER2-low BC but no signal of activity was seen in this subgroup. A phase II trial testing margetuximab in HER2-low BCs (ClinicalTrials.gov identifier: NCT01828021)

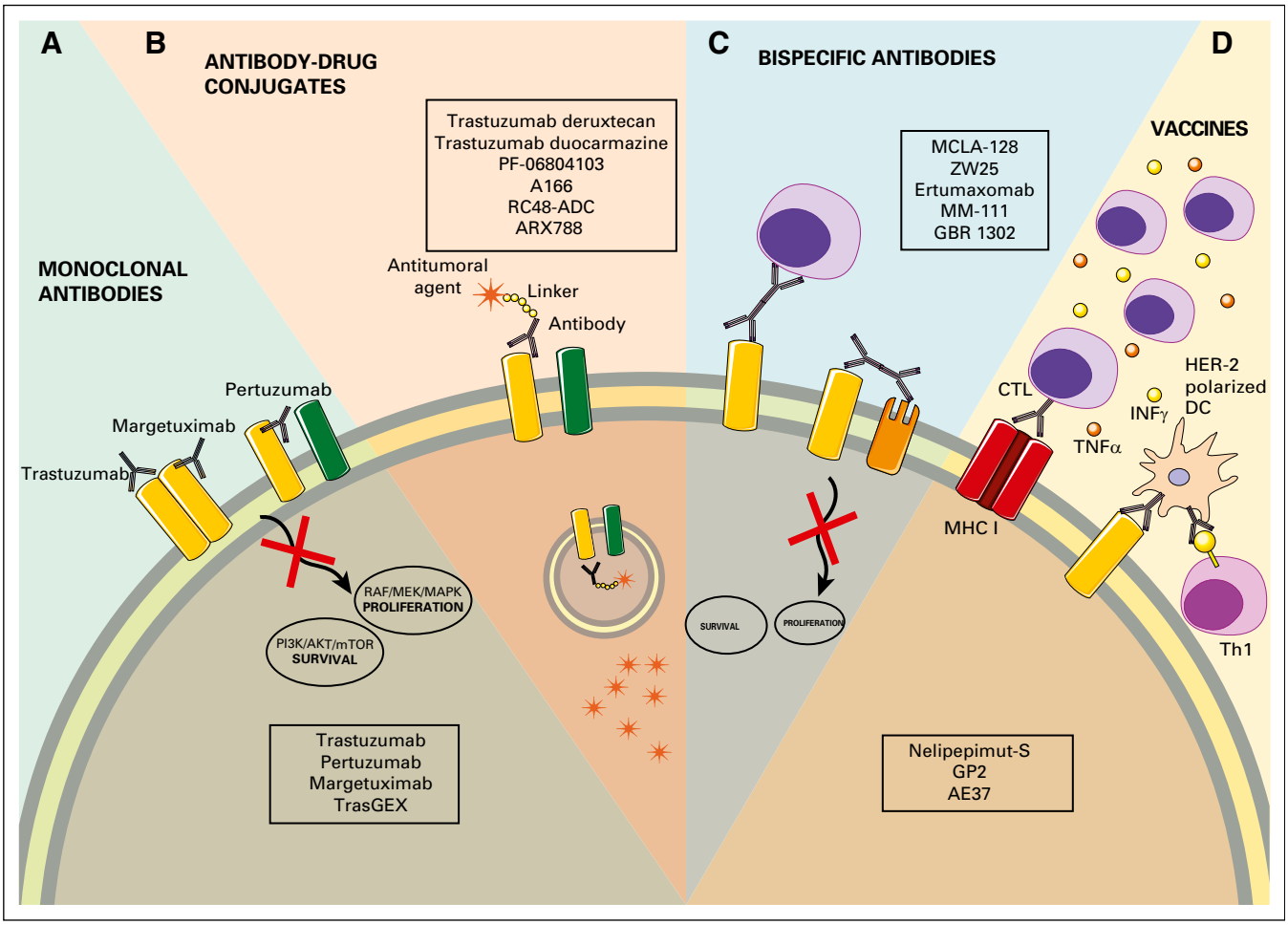

FIG 2. Novel agents and mechanisms enabling the targeting of HER2-low–expressing BC. Novel anti-HER2 drugs enable the targeting of low HER2-expressing cancers through different mechanisms. (A) Monoclonal antibodies engineered to enhance antibody-dependent cellular cytotoxicity, or to more effectively inhibit HER2 heterodimerization, have shown preclinical evidence of activity in HER2-low–expressing BC. (B) Novel antibody-drug conjugates enable exploitation of low HER2 expression to direct cytotoxic molecules to tumor cells, showing promising activity in early clinical trials. (C) Bispecific antibodies allow forced connections between cancer and immune cells and/or suppress multiple signaling pathways, with potential activity in HER2-low–expressing BC cells. (D) Cancer vaccines enhance antitumor immune response against HER2 and are currently being tested in the adjuvant setting to reduce relapses in HER2-low–expressing BC. Abbreviations: CTL, cytotoxic T cell; DC, dendritic cell; HER2, human epidermal growth factor receptor 2; Th1, T-helper lymphocyte.

just completed recruitment and results are pending. Notably, results from the phase III SOPHIA trial, which enrolled pretreated patients with HER2-positive BC, suggest a differential activity of the compound according to the patient's CD16A polymorphisms, highlighting the potential relevance of host factors in HER2 targeting.[65]

Antibody-Drug Conjugates

ADCs are mAbs covalently bound to potent cytotoxic agents and are designed to combine the selectivity of targeted therapy with the cytotoxicity of chemotherapy. Three main elements compose an ADC: a mAb (the vehicle), a cytotoxic agent (the payload), and a synthetic linker. The number of molecules of payload carried by each antibody is defined as the drug-to-antibody ratio (DAR), a critical feature related to both efficacy and safety. Although a low DAR implies a reduction in potency, higher values can affect structure, stability, and antigen-binding capacity.[66]

T-DM1 is composed of trastuzumab and DM1, conjugated through a noncleavable linker, with a DAR of 3.5 molecules per antibody.[67] Some retrospective evidence suggests limited activity of the compound in patients with HER2-low BC. An exploratory analysis was performed in two phase II trials of T-DM1 in HER2-positive BC (4258g and 4374g trials)[68,69] with centralized confirmation of the HER2 status; the revision of HER pathology identified a cohort of patients with HER2-low tumors. The objective response rate (ORR) was significantly higher for HER2-positive BC (33.8% *v* 4.8% in the 4258g trial; 41.3% *v* 20.0% in the 4374g trial) along with improved progression-free survival (PFS; 8.2 *v* 2.6 months in the 4258g trial; 7.3 *v* 2.8 months in the 4374g trial). Subgroup analysis were also performed in

TABLE 2. Available Data of Anti-HER2 Agents Activity in HER2-Low BC

Drug	NCT.gov Identifier	Phase	Number of Patients	Population	Results
Antibody-drug conjugates					
DS8201a (Daiichi Sankyo, Tokyo, Japan)	NCT02564900[29]	I	43	Pretreated, HER2-low–expressing mBC (IHC 1+ or 2+, ISH negative) locally assessed	ORR, 44.2%
					DCR, 85%
					PFS, 12.9 months
SYD985 (Synthon Biopharmaceuticals, Nijmegen, Netherlands)	NCT02277717[16]	I	47 (HR+, 32; TNBC, 17)	Pretreated, HER2-low expressing mBC (IHC 1+ or 2+, ISH negative) centrally assessed using HER2 IHC and dual ISH assays (Ventana; F. Hoffmann-La Roche))	ORR, 28% in HR+
					ORR, 40% in TNBC
					PFS, 4.1 months (HR+)
					PFS, 4.9 months (TNBC)
Monoclonal antibodies					
Pertuzumab (Hoffmann-La Roche, Basel, Switzerland)	NCT02491892[15]	II	78 (HER2 low, 74; HER2 -negative, 4)	Pretreated HER2-low–expressing (IHC 1+ or 2+, FISH negative) and HER2-negative (IHC 0) mBC, centrally assessed	ORR, 2.5% (2 patients)
					DCR, 43%
Trastuzumab(Hoffmann-La Roche, Basel, Switzerland)	NCT01275677[14]	III	3,270	High-risk HER2-low–expressing early BC pretreated with adjuvant chemotherapy (IHC 1+ or 2+, ISH negative); locally assessed	5-year iDFS, 89.6% (T) *v* 89.2% (no T)
Vaccines					
Nelipepimut-S (Sellas Life Sciences Group, New York, NY)	NCT01479244[18] (PRESENT)	III	758 (VG, 376; CG, 382)	Radically resected, HER2-low–expressing (IHC 1+ or 2+ FISH negative) BC, centrally assessed using Bond Oracle HER2 IHC System III (Leica Biosystems) and PathVysion HER-2 DNA Probe Kit (Abbott Laboratories)	3-year DFS, 77.1% (VG) *v* 77.5% (CG)
Nelipepimut-S +trastuzumab (Sellas Life Sciences Group, New York, NY)	NCT01570036[17]	IIb	275 (VG, 136; CG, 139)	HER2-low–expressing, node-positive early BC (IHC 1+ or 2+, FISH negative); locally assessed	24-month DFS, 88% (VG) *v* 82% (CG) in ITT
					24 mo. DFS, 91% (VG) *v* 69.6% (CG) in TNBC
GP2 (NuGenerex Immuno-Oncology, Walton, United Kingdom)	NCT00524277[80]	II	180 (VG, 89; CG, 91)	HER2-expressing (IHC 1-3+ and/or FISH ratio > 1.2), node-positive or high-risk node-negative early BC; locally assessed	Estimated 5-year DFS, 88% (VG) *v* 81% (CG)
AE37 (Antigen Express, Generex Biotechnology, Toronto, Canada)	NCT00524277[90]	II	134 (VG, 68; CG, 66)	HER2-expressing (IHC 1-3+ and/or FISH ratio > 1.2), node-positive or high-risk node-negative early BC; locally assessed	55-month DFS, 89% (VG) *v* 51% (CG) in ITT
					55-month DFS 89% (VG) *v* 0% (CG) in TNBC
Bispecific antibodies					
Ertumaxomab (Fresenius, Germany)	NCT00522457[87]	II	28	Pretreated HR+ HER2-low–expressing (IHC 1+ or 2+, FISH-negative) mBC, locally assessed	ORR, 0%
					DCR, 53.8%
GBR1302(Glenmark Pharmaceuticals, Mumbai, India)	NCT02829372[88]	I	19	Pretreated HER2-positive and HER2-low–expressing (2+, FISH-negative) solid tumors, including BC	ORR, 0%
					DCR, 10%

Abbreviations: CG, control group; DCR, disease-control rate; DFS, disease-free survival; FISH, fluorescent in situ hybridization; HR+, hormone receptor positive; iDFS, invasive disease-free survival; ITT, intention to treat; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; mBC, metastatic breast cancer; ORR, objective response rate; PFS, progression-free survival; PT, patient; T, trastuzumab; TNBC, triple-negative breast cancer; VG, vaccine group.

other TDM1 trials and confirmed a poorer benefit in HER2-low BC.[56,70-72]

In contrast with these disappointing results, novel ADCs have demonstrated encouraging activity in HER2-low BC.

Trastuzumab deruxtecan (DS-8201a) is an HER2-targeting mAb conjugated with the topoisomerase I inhibitor DXd through an enzymatically cleavable linker, with a DAR of eight molecules per antibody. On the basis of the impressive results of the phase II DESTINY-Breast01 trial,[73] the compound was approved in early 2020 by the US Food and Drug Administration for use in pretreated patients with HER2-positive BC. However, the drug was also tested in patients with HER2-low BC in the context of a phase I trial (ClinicalTrials.gov identifier: NCT02564900)[75]. As of February 2019, 54 patients were evaluable for efficacy end points.[29] The confirmed ORR was 37% with a disease control rate of 87%, a median PFS of 11.1 months, and a median duration of response of 10.4 months. Efficacy was observed in all subgroups, including HR-negative and HER2 IHC score 1+ BC. Safety data were reported on the overall BC study population, with the most frequently observed toxicities being gastrointestinal and hematologic, likely related to the chemotherapy backbone. However, there were three fatal cases of pneumonitis. Such adverse effects (AEs) were also described in the DESTINY-Breast01 trial report[73] and further characterized in a pooled analysis of seven studies testing trastuzumab deruxtecan, where pneumonitis incidence was 9.9% (n = 66 of 665) and higher drug doses, along with the Japanese ethnicity of patients, were identified as potential risk factors.[74] A phase III trial testing trastuzumab deruxtecan in HER2-low BC is ongoing,[19] along with two phase Ib trials in combination with nivolumab or pembrolizumab.

Trastuzumab duocarmazine (SYD985) is an HER2-targeting ADC coupling trastuzumab to a potent duocarmycin payload (vc-seco-DUBA) through a cleavable linker, with a DAR of 2.8. The compound was tested in a phase I trial including HR-positive and triple-negative, HER2-low mBC (ClinicalTrials.gov identifier: NCT02277717).[16,76] At the cutoff date of July 2018, 49 patients were evaluable for efficacy analysis. The observed ORRs were 28% and 40% in the HR-positive and TNBC cohorts, respectively, and the median PFS was similar in the two subgroups (4.1 and 4.9 months, respectively). One death from pneumonitis occurred in the dose-escalation phase, with four more cases of any-grade pneumonitis described in the overall population. In the dose-expansion phase, trastuzumab duocarmazine at the 1.2 mg/kg dose was moderately tolerated: grade 3 and 4 AEs were reported in 35% of the patients—mainly neutropenia, fatigue, and conjunctivitis. Any-grade ocular toxicities were common: 71% of the patients experienced conjunctivitis, dry eye, lacrimation, and/or keratitis. Following the promising results observed in this study, a phase III trial (TULIP trial; ClinicalTrials.gov identifier: NCT03262935) was initiated, although it is restricted to HER2-positive BC.

Vaccines

Cancer vaccines aim at preventing or treating tumors by enhancing immune response against tumor-related antigens. In the context of BC, vaccine immunotherapy has been studied mostly to improve outcomes in the early setting. Moving from the consideration that the majority of BCs express HER2 at dramatically higher levels compared with normal tissues,[77] anti-HER2 vaccines were developed and tested in HER2-positive and HER2-low BC.

The nelipepimut-S vaccine consists of the HER2-derived peptide E75 combined with the immunoadjuvant granulocyte-macrophage colony-stimulating factor (GM-CSF). The vaccine binds HLA-A2/A3 molecules on antigen-presenting cells, which then sensitize cytotoxic T lymphocytes against HER2-expressing cells.[78]

The large, phase III PRESENT trial randomly assigned 758 patients with HER2-low BC to GM-CSF with or without nelipepimut-S as an adjuvant treatment. Results did not show a significant difference in disease-free survival (DFS).[18] However, the emergence of preclinical data showing synergy between this vaccine and trastuzumab[79] prompted the initiation of a randomized phase IIb trial testing the combination in high-risk patients with HER2-low early BC. In that trial 275 patients were randomly assigned to adjuvant trastuzumab plus GM-CSF with or without nelipepimut-S. After a median follow-up of 25.7 months, there was a numerical increase in DFS (89.8% *v* 83.8%; HR, 0.62; $P = .18$), and an exploratory analysis showed a potential benefit in the HER2-low TNBC subgroup (n = 97), with a 24-month DFS of 92.6% in the vaccine arm compared with 70.2% in the control arm (HR, 0.26, $P = .013$). Both regimens appeared to be well tolerated, with the majority of AEs consisting of mild skin reactions and fatigue. Additional anti-HER2 vaccines (eg, GP2,[80] AE37[81]) have been tested in the same setting; results are listed in Table 2.

Bispecific Antibodies

Bispecific antibodies (bsAbs) are mAbs targeting two different epitopes, enabling inhibition of multiple oncogenic pathways, to force the connection between cancer and immune cells, and/or to deliver payload into the tumor microenvironment.[82] Several anti-HER2 bsAbs are under development, although only a minority also are being tested in HER2-low disease.[83]

ZW25 is a bsAb that targets HER2 domains 2 and 4, for which in vivo studies demonstrated antitumor activity in HER2-low BC models.[84] A phase I clinical trial (ClinicalTrials.gov identifier: NCT02892123) is evaluating ZW25 administration in HER2-expressing cancers, including a cohort of HER2-low (IHC 2+, ISH-negative) BCs. Preliminary analysis of this trial demonstrated 46% ORR in

patients with HER2-positive BC[85]; efficacy data in HER2-low tumors are pending. Of note, by linking an auristatin payload to ZW25, the new compound ZW49 was obtained, combining the mechanism of action of bsAbs and ADCs. The antibody showed encouraging preclinical activity in HER2-low BC[86] and is currently being tested in a phase I trial, although the trial is restricted to HER2-positive cancers (ClinicalTrials.gov identifier: NCT03821233). Additional anti-HER2 bsAbs (eg, ertumaxomab,[87] GBR1302[88]) have been tested in similar settings; results are listed in Table 2.

BALANCING ACTIVITY AND TOXICITY OF THE NOVEL ANTI-HER2 DRUGS

Despite an articulated landscape of drug development for HER2-low BC, ADCs are the only compounds extensively under development in phase III controlled trials. If the clinical benefit of targeting HER2 in nonamplified cancers is confirmed, a shift in our approach to HER2-targeting therapy can be anticipated for the following reasons:

(1) Independence from oncogene dependency: preclinical evidence shows novel ADCs' activity might not be related to HER2-pathway inhibition but rather to the delivery of cytotoxic payload though a "Trojan horse" pharmacological mechanism.[27] Consequently, they could be active regardless of biologic cell dependency on the HER2 pathway.
(2) Higher DAR: similar to TDM1, most novel ADCs use trastuzumab as the vehicle of cytotoxic drugs; however, engineering evolutions enabled the linking of more cytotoxic molecules to each anti-HER2 antibody, thus enhancing the cytotoxic power of novel ADCs and likely explaining the divergent activity of T-DM1 and novel compounds in HER2-low BC.[27,28]
(3) Cleavable linker: novel ADCs are conjugated via enzymatically cleavable linkers, digested by lysosomal enzymes, which are highly expressed in the tumor microenvironment and tumor cellular endocytic vesicles.[27,28] Cleavable linkers facilitate the killing of bystander HER2-positive cells but also of neighboring non–antigen-expressing cells,[28] potentially overcoming the intratumoral heterogeneity in HER2 expression. T-DM1 was developed with a noncleavable linker, thus possibly explaining the lower activity in this setting.
(4) Payload mechanism of action: in contrast to TDM1, which uses an inhibitor of microtubules assembly as payload, new ADCs are conjugated to direct DNA-targeting agents like alkylators[28] and topoisomerase I inhibitors,[27] exerting more potent cytotoxic activity in BC clones.

Importantly, some of the same reasons explaining new ADCs' activity in HER2-low BC might also justify their emerging toxicities. Indeed, the AE profile observed in the phase I and II trials of trastuzumab deruxtecan and trastuzumab duocarmazine resembles that of their chemotherapy backbone, which could be released in the bloodstream because of the cleavable linker and the high DAR. Common AEs in both trials were myelosuppression, alopecia, stomatitis, and GI toxicities. However, some peculiar AEs were also observed in these trials, including ocular toxicities and potentially severe pneumonitis. Although the underlying mechanism of these toxicities is still unclear, a variety of potential risk factors have been suggested, raising the awareness of the timely recognition of severe events and prompting treatment discontinuation, as appropriate.[16,89]

In conclusion, up to 55% of BCs express low levels of HER2 in the absence of gene amplification; currently, they are clinically classified either as luminal like or TNBCs. Although a variety of anti-HER2 agents are approved for the treatment of HER2-positive BC, none has activity in HER2-low tumors.

The preliminary activity reported in HER2-low tumors with ADCs suggest that some strategies of HER2 targeting might be effective in this population. Nonetheless, several challenges are posed by the emergence of such novel agents and none more than the definition of HER2-low itself, especially when using semiquantitative assays like IHC. In fact, the biologic difference between HER2 IHC 0 and 1+ is unknown and, in some cases, may represent a fixation artifact. However, patients with BCs scored IHC 0 have been excluded from most trials targeting HER2-low cancers; consequently, the activity of novel anti-HER2 compounds in this population is largely unknown. Furthermore, the current HER2 testing methods (ie, IHC and ISH) may prove insufficient to identify tumors with low levels of HER2 expression or pathway activation that could benefit from some of these new therapies. As such, quantitative immune assays and RT-qPCR might enable the detection of these tumors, and it is imperative that all new trials strive to collect sufficient amounts of tumor specimens to allow subsequent testing of these and other methods to assess the HER2 pathway. Finally, a better biologic understanding of the toxicities observed with some of the new drugs is needed.

If controlled trials confirm the promising activity observed in earlier studies with novel anti-HER2 agents in HER2-low BC, a change in the clinical interpretation of HER2 status may need to be pursued, including a re-evaluation of existing HER2 assays and a clear characterization of HER2 expression beyond the current binary HER2-positive and HER2-negative test results that have served us quite well thus far. These new observations may also help expand the targeting of the HER2 pathway to other diseases beyond BC that characteristically express lower levels of HER2. The impact of these new treatment strategies in tumors that have low levels of HER2 expression but have gene-activating mutations will also need to be examined.

AFFILIATIONS

[1]European Institute of Oncology IRCCS, Milan, Italy
[2]University of Milan, Milan, Italy
[3]Sarah Cannon Research Institute/Tennessee Oncology, Nashville, TN
[4]Dana-Farber Cancer Institute, Boston, MA
[5]IOB Institute of Oncology, Quiron Group, Madrid and Barcelona, Spain
[6]Vall d'Hebron Institute of Oncology, Barcelona, Spain
[7]Breast Unit Champalimaud Clinical Center/Champalimaud Foundation, Lisbon, Portugal
[8]Jean Perrin Comprehensive Cancer Center, Department of Pathology and Tumor Biology, Centre Jean Perrin, Clermont-Ferrand, France
[9]UMR INSERM 1240 IMoST, Université Clermont Auvergne, Villejuif, France
[10]Department of Medical Oncology, Gustave Roussy, Villejuif, France

CORRESPONDING AUTHOR

Giuseppe Curigliano, MD, PhD, European Institute of Oncology IRCCS, Via Ripamonti 435, Milan 20141, Italy; Twitter: @curijoey; e-mail: Giuseppe.curigliano@ieo.it.

EQUAL CONTRIBUTION

F.A. and G.C. contributed equally to this work.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

Disclosures provided by the authors and data availability statement (if applicable) are available with this article at DOI https://doi.org/10.1200/JCO.19.02488.

AUTHOR CONTRIBUTIONS

Conception and design: Paolo Tarantino, Erika Hamilton, Javier Cortes, Giulia Viale, Fatima Cardoso, Giuseppe Curigliano
Provision of study material or patients: Javier Cortes
Collection and assembly of data: Paolo Tarantino, Sarah Tolaney, Stefania Morganti, Emanuela Ferraro, Antonio Marra, Giulia Viale, Fatima Cardoso, Frédérique Penault-Llorca, Giuseppe Viale, Giuseppe Curigliano
Data analysis and interpretation: Paolo Tarantino, Erika Hamilton, Giulia Viale, Dario Trapani, Fatima Cardoso, Giuseppe Curigliano
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

HER2-Low Breast Cancer: Pathological and Clinical Landscape

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Erika Hamilton
Consulting or Advisory Role: Pfizer (Inst), Roche (Inst), Eli Lilly (Inst), Puma Biotechnology (Inst), Daiichi Sankyo (Inst), Mersana (Inst), Boehringer Ingelheim (Inst), AstraZeneca (Inst), Novartis (Inst), Silverback Therapeutics (Inst), Black Diamond (Inst)
Research Funding: AstraZeneca (Inst), Hutchison MediPharma (Inst), OncoMed (Inst), MedImmune (Inst), Stem CentRx (Inst), Roche (Inst), Curis (Inst), Verastem (Inst), Zymeworks (Inst), Syndax (Inst), Lycera (Inst), Rgenix (Inst), Novartis (Inst), Mersana (Inst), Millennium (Inst), TapImmune (Inst), Eli Lilly (Inst), BerGenBio (Inst), Medivation (Inst), Pfizer (Inst), Tesaro (Inst), Boehringer Ingelheim (Inst), Eisai (Inst), H3 Biomedicine (Inst), Radius Health (Inst), Acerta Pharma (Inst), Takeda (Inst), Macrogenics (Inst), AbbVie (Inst), Immunomedics (Inst), Fujifilm (Inst), eFFECTOR Therapeutics (Inst), Merus (Inst), Nucana (Inst), Regeneron (Inst), Leap Therapeutics (Inst), Taiho Pharmaceutical (Inst), EMD Serono (Inst), Daiichi Sankyo (Inst), ArQule (Inst), Syros Pharmaceuticals (Inst), Clovis Oncology (Inst), CytomX Therapeutics (Inst), InventisBio (Inst), Deciphera (Inst), Unum Therapeutics (Inst), Sermonix Pharmaceuticals (Inst), Sutro (Inst), Aravive (Inst), Zenith Epigenetics (Inst), Arvinas (Inst), Torque (Inst), Harpoon (Inst), Fochon (Inst), Black Diamond (Inst), Orinove (Inst), Molecular Templates (Inst), Silverback Therapeutics (Inst), Seattle Genetics (Inst), Puma Biotechnology (Inst), Compugen (Inst), G1 Therapeutics (Inst), Karyopharm Therapeutics (Inst), Torque (Inst)
Travel, Accommodations, Expenses: Amgen, AstraZeneca, Bayer, Bristol-Myers Squibb, Clovis Oncology, Eisai, EMD Serono, Eli Lilly, Roche, Genentech, Genzyme, Helsinn Therapeutics, Heron, Lexicon, Medivation, Merck, Novartis, Pfizer, Sysmex, Tesaro, Guardant Health, Foundation Medicine

Sara M. Tolaney
Consulting or Advisory Role: Novartis, Pfizer, Merck, Eli Lilly, Nektar, NanoString Technologies, AstraZeneca, Puma Biotechnology, Genentech, Eisai, Immunomedics, Sanofi, Celldex, Bristol-Myers Squibb, Paxman, Seattle Genetics, Odonate, AbbVie, Silverback Therapeutics, G1 Therapeutics
Research Funding: Roche (Inst), Merck (Inst), Exelixis (Inst), Pfizer (Inst), Eli Lilly (Inst), Novartis (Inst), Bristol-Myers Squibb (Inst), Eisai (Inst), AstraZeneca (Inst), NanoString Technologies (Inst), Cyclacel (Inst), Nektar (Inst), Immunomedics (Inst), Odonate Therapeutics (Inst)
Travel, Accommodations, Expenses: AstraZeneca, Eli Lilly, Merck, Nektar, Novartis, Pfizer, Roche, Immunomedics, Eisai, NanoString Technologies, Puma Biotechnology, Celldex

Javier Cortes
Stock and Other Ownership Interests: MedSIR
Honoraria: Novartis, Eisai, Celgene, Pfizer, Roche, Samsung, Eli Lilly, Merck Sharp & Dohme, Daiichi Sankyo
Consulting or Advisory Role: Celgene, Cellestia Biotech, AstraZeneca, Biothera, Merus, Roche, Seattle Genetics, Daiichi Sankyo, ERYTECH Pharma, Polyphor, Athenex, Eli Lilly, Servier, Merck Sharp & Dohme, GlaxoSmithKline, Leuko, Clovis Oncology, Bioasis
Research Funding: ARIAD (Inst), AstraZeneca (Inst), Baxalta/Servier Affaires (Inst), Bayer (Inst), Eisai Farmaceutica (Inst), Guardant Health (Inst), Merck Sharp & Dohme (Inst), Pfizer (Inst), Puma CO (Inst), Queen Mary University of London (Inst), Roche (Inst), Piqur (Inst)
Travel, Accommodations, Expenses: Roche, Pfizer, Eisai, Novartis, Daiichi Sankyo

Fatima Cardoso
Consulting or Advisory Role: Roche, Novartis, Pfizer, AstraZeneca, Teva, Astellas Pharma, Merus, Celgene, Eisai, Daiichi Sankyo, Genentech, Merck Sharp & Dohme, Sanofi, Pierre Fabre, Macrogenics, Amgen, GE Healthcare, GlaxoSmithKline, Mylan, Mundipharma, Seattle Genetics, Samsung Bioepis, Medscape, Prime Oncology
Travel, Accommodations, Expenses: Pfizer, Roche, AstraZeneca

Frédérique Penault-Llorca
Consulting or Advisory Role: Roche, Puma, Nanostring, Daiichi, AstraZeneca, Pfizer, MSD
Research Funding: Roche (Inst), AstraZeneca (Inst), Nanostring (Inst)

Giuseppe Viale
Honoraria: MSD Oncology, Pfizer
Consulting or Advisory Role: Dako, Roche, Astellas Pharma, Novartis, Bayer, Daiichi Sankyo, MSD Oncology, Menarini, Roche
Research Funding: Roche, Ventana Medical Systems (Inst), Dako/Agilent Technologies (Inst), Cepheid (Inst)
Travel, Accommodations, Expenses: Roche, Celgene

Fabrice Andrè
Research Funding: AstraZeneca (Inst), Novartis (Inst), Pfizer (Inst), Eli Lilly (Inst), Roche (Inst), Daiichi (Inst)
Travel, Accommodations, Expenses: Novartis, Roche, GlaxoSmithKline, AstraZeneca

Giuseppe Curigliano
Honoraria: Ellipses Pharma
Consulting or Advisory Role: Roche, Pfizer, Novartis, Eli Lilly, Foundation Medicine, Bristol-Myers Squibb, Samsung
Speakers' Bureau: Roche, Novartis, Pfizer, Eli Lilly, Foundation Medicine, Samsung, Daiichi Sankyo
Travel, Accommodations, Expenses: Roche, Pfizer

No other potential conflicts of interest were reported.