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AXL – a new player in resistance to HER2 blockade

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ABSTRACT

HER2 is a driver in solid tumors, mainly breast, oesophageal and gastric cancer, through activation of oncogenic signaling pathways such as PI3K or MAPK. HER2 overexpression associates with aggressive disease and poor prognosis. Despite targeted anti-HER2 therapy has improved outcomes and is the current standard of care, resistance emerge in some patients, requiring additional therapeutic strategies. Several mechanisms, including the upregulation of receptors tyrosine kinases such as AXL, are involved in resistance. AXL signaling leads to cancer cell proliferation, survival, migration, invasion and angiogenesis and correlates with poor prognosis. In addition, AXL overexpression accompanied by a mesenchymal phenotype result in resistance to chemotherapy and targeted therapies. Preclinical studies show that AXL drives anti-HER2 resistance and metastasis through dimerization with HER2 and activation of downstream pathways in breast cancer. Moreover, AXL inhibition restores response to HER2 blockade *in vitro* and *in vivo*. Limited data in gastric and oesophageal cancer also support these evidences. Furthermore, AXL shows a strong value as a prognostic and predictive biomarker in HER2+ breast cancer patients, adding a remarkable translational relevance. Therefore, current studies enforce the potential of co-targeting AXL and HER2 to overcome resistance and supports the use of AXL inhibitors in the clinic.

Background

HER2 (gene name, *ERBB2*) transmembrane glycoprotein receptor is a member of the human epidermal growth factor receptor (HER) family together with receptors HER1, HER3, and HER4. Homo- or heterodimerization of these receptors activate key downstream pathways, being HER2-HER3 the most prevalent and potent heterodimer in signaling [1].

HER2 has been widely recognized to be a relevant oncogenic driver across several solid tumors [2]. Its gene amplification, mutations or overexpression cause a potent oncogenic signal through several downstream pathways, including phosphatidil-inositol-3-kinase (PI3K) and mitogen-activated phosphokinases (MAPK), promoting cancer cell proliferation and preventing apoptosis, causing cell growth, survival and differentiation [3]. However, despite the broad presence of HER2 alterations in solid tumors, anti-HER2 treatment has been well established in breast, gastric and gastroesophageal junction carcinomas. HER2-positive (HER2+) breast cancer (BC) represents 15–20 % of breast malignancies. This subtype is characterized by an aggressive behaviour and high risk of recurrence [3–5]. Besides, in gastric cancer, overexpression of HER2 represents 12–23 % of the cases and it varies with the histologic subtype (more frequent in intestinal than in diffuse) and tumor grade

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(greater in moderately differentiated than in poorly differentiated) [6].

In this context, several therapeutic agents such as monoclonal antibodies, tyrosine kinase inhibitors or antibody drug conjugates (ADCs) have been developed and are currently used in the clinical practice for HER2+ BC [5]. Trastuzumab, a monoclonal antibody against HER2, is the leading-class compound that changed the scenario of *HER2* amplified tumors since it was approved by FDA in 1998 [7–8]. More recently, other strategies such as the combination of pertuzumab plus trastuzumab as a first line treatment, the ADCs trastuzumab-DM1 (T-DM1) or trastuzumab-deruxtecan have entered the scene as principal therapeutics options [9–11].

Nowadays, anti-HER2 treatment represents the golden standard for both early and metastatic HER2+ BC [5]. Furthermore, the combination of trastuzumab to platinum-based chemotherapy is the optimal treatment to first-line for HER2 amplified advanced gastric/gastroesophageal cancer [12], and recently trastuzumab-deruxtecan has been approved to second or later line for this setting of patients while no benefit in improving clinical outcome was observed with other anti-HER2 agents already approved for BC [13–14].

Apart from BC and gastric cancer, HER2 has been recently identified as a driver in other types of cancer. For example, trastuzumab has emerged as an effective therapy approach for patients with advanced stage and recurrent HER2+ endometrial carcinoma, increasing progression-free (PFS) and overall survival (OS) when combined with the standard chemotherapy regimen [15]. Moreover, the available evidence supports the use of anti-HER2 therapy in biliary tract cancer with HER2 amplification that lacks other therapeutic options [16–17]. The identification of HER2 amplification is also recommended in *RAS* wild-type colorectal cancer to detect those patients who may benefit from HER2 blockade after first-line progression [18]. Finally, while HER2 exon 20 insertion mutations are rare in lung cancer, HER2 targeted ADCs have demonstrated activity. In fact, trastuzumab-deruxtecan could be recommended for patients following prior first-line therapy [19–20].

Interestingly, novel drugs are currently in development such as bispecific antibodies (BsAbs). These antibodies have two distinct antigen-binding sites that can bind to different antigens or different epitopes on the same antigen. For example, an antibody against two distinct HER2 epitopes has shown promising results and distinct mechanisms of action [21]. Among several BsAbs, T cell engaging bsAb is a new class of therapeutic agents designed to simultaneously bind to the T cells receptor and to a tumor-associated or specific antigens [22]. Subsequently, with the progress in antibody engineering and biology, the diverse concept and constructs of BsAbs are evolving.

Despite most of the patients experience a clear benefit consisting on increased disease-free survival (DFS), PFS and OS, a subset of them develop resistance and ultimately experience disease progression, requiring additional therapeutic strategies [23–24]. Hence, the identification of those potential mechanisms responsible for primary or acquired anti-HER2 resistance is a fundamental unmet need to improve and personalize the therapeutic strategy for HER2 dependent disease.

The inhibition of a specific target often activates horizontal or longitudinal pathways in an attempt to escape cancer cell death. In the case of HER2+ BC, activation of several receptors tyrosine kinase (RTK) has been described as a mechanism of resistance to anti-HER2 agents. Among them, the upregulation of other members of HER family is a frequently event. In particular, HER1 upregulation and the subsequent dimerization with HER2 attenuate the anti-cancer effect of trastuzumab *in vitro* [25–28], as well as the upregulation of HER3 associates to shorter DFS and OS in HER2+ BC patients [29–31].

Several preclinical and clinical studies demonstrate that molecular alterations in other RTKs beyond HER family members can cause resistance to anti-HER2 drugs in BC, such as MET amplification [32–35], Insulin-like growth factor 1 receptor (IGF1R) [36–40], Fibroblast Growth Factor Receptor (FGFR) [41–43],Erythropoietin receptor (EPOR) [44–45], Ephrin type-A receptor 2 (EPHA2) [46–47], Vascular Endothelial Growth Factor Receptor (VEGFR) [48–50], and AXL [51–52].

Similarly, the molecular alterations previously described in BC have been also identified to be responsible for worse prognosis in HER2+ gastric cancer. However, in this type of tumor heterogeneity limit the use of a tailored agent [53–57].

Given this complexity of cancer molecular biology, the perspective of combining an anti-HER2 drug with specific novel RTKs inhibitors represent a promising strategy to overcome primary or acquired resistance. In this scenario, AXL, an important RTK associated with cancer progression and chemoresistance, has been recently described as one of the main players also in resistance to HER2 blockade and its potential mechanism took the attention of the scientific community.

AXL receptor tyrosine kinase signaling pathway

The *AXL* gene, which is localized at chromosome 19q13.2, was first identified in chronic myeloid leukaemia and encodes for 98 kDa AXL protein, that after post-translational regulation presents a final weight of 100–140 kDa. AXL RTK, also named as UFO due to its initially unknown function, contains an extracellular domain with two immunoglobulin-like motifs at N-terminal, two fibronectin type III-like motifs involved in ligand binding, a transmembrane domain and an intracellular domain essential for tyrosine kinase activity [58–59].

AXL activation has been described through both ligand-dependent with GAS6 and ligand-independent through crosstalk with other transmembrane RTKs such as FGFR, HER1 and MET in solid tumors [58–63]. Upon dimerization and phosphorylation, AXL activates downstream signaling pathways such as PI3K/AKT/mTOR, JAK/STAT, NF-κB, and RAS/RAF/MEK/ERK that play major roles in tumor cell survival, migration, invasion, anoikis and angiogenesis [64].

AXL upregulation is not mediated by genomic amplifications or activating mutations, suggesting that its upregulation may be caused by other mechanisms. Among them, AXL transcriptional activation by Fos/cJun/AP1 [65–66], Sp1/Sp3 [67], YAP1 [68–69], Fra-1 [70] and MZF1 [71] has been widely described to increased *AXL* expression. Furthermore, some microRNAs, such as miR-34a [72], miR-199a [72], miR-202-5p [73] and miR-432 [74] also regulate *AXL* expression.

Role of AXL in cancer

Several studies focused on the role of TAM family members in different cancers and context. TAM RTKs and, particularly AXL, have been directly linked to epithelial to mesenchymal transition (EMT), and strongly associated to cancer progression, metastasis and drug resistance [64,75–77]. AXL upregulation together with a mesenchymal phenotype has been recognized to be responsible of acquired resistance to several cytotoxic agents, immunotherapies and targeted therapies across several tumor types [78]. In addition, association between AXL and targeted therapy resistance has been reported in different types of cancer such as oesophageal, head and neck [79], myeloid leukemia [80], neuroblastoma [81], melanoma [82–84] and lung cancer [85–88]. In the case of triple negative BC, preclinical studies showed that AXL could be responsible for chemo- and radio-resistance and HER1-targeted therapy resistance [60–61,89–91].

Hyperactivation or aberrant expression of AXL and its ligand GAS6 have been observed and correlated with worse prognosis and metastasis in different types of cancer such as leukaemia, breast, lung, melanoma, pancreatic, renal, prostate, ovarian and oesophageal cancer. However, despite interesting preclinical and translational evidences, the role of AXL as an oncogenic driver has not been yet completely elucidated [60,92–93].

Apart from the important role of AXL in cancer progression, it has been recently acknowledged another role as a mediator of resistance to HER2 blockade in te context of HER2+ tumors.

AXL as a driver of resistance to anti-HER2 therapies in preclinical models

Breast cancer

AXL was first identified as a potential mechanism of resistance to anti-HER2 agents in 2009 [52]. In this study, *in vitro* acquired lapatinib-resistant BT474 HER2+/ER + BC cells showed upregulation of AXL in comparison to sensitive cells, and genetic knockdown or treatment with foretinib (AXL, MET and VEGFR inhibitor) restore lapatinib response. In this case, AXL activate the PI3K pathway through p85 that induces cell proliferation despite HER2 blockade [52]. In another study, foretinib also showed potent inhibition of the growth in the *in vitro* lapatinib-resistant cells with PI3K/AKT activation. In this work, resistant cells acquired mesenchymal traits, which, that support the association of AXL and an aggressive metastatic phenotype [94]. However, forenitinb is a multikinase inhibitor that targets Met, RON, AXL, and VEGFR2, thus it cannot be completely demonstrated that its effect on lapatinib-resistant cells is exclusively through AXL inhibition [95].

In line with these studies showing AXL as a mechanism of resistance to lapatinib in HER2+ BC, AXL overexpression has been described an important mechanism of resistance to trastuzumab. In particular, AXL was increased in acquired trastuzumab-resistant HER2+ BC cell lines both at mRNA and protein level, compared with their sensitive counterparts [96]. Moreover, both genetic AXL knockdown and pharmacological inhibition with TP-0903 (a highly selective inhibitor) were able to increase trastuzumab response *in vitro*, and AXL gain of function was sufficient to decrease trastuzumab response [51].

In these acquired trastuzumab-resistant cell lines, AXL upregulation occurred in the context of an EMT associated transcriptional program. Indeed, trastuzumab-resistant cell lines show increased migration and invasion capability and an EMT-like phenotype, which are associated to metastatic behaviour. In addition, AXL gain and loss of function was sufficient to modulate the mesenchymal-like phenotype and migration and invasion capacity in three independent models [51]. These preclinical results agree with those found by Goyette *et al.* where inhibition of AXL with small molecule inhibitor (R428) was sufficient to reduce cell invasion *in vitro*. The combination of R428 with HER2 blockade decreased the number of circulating tumor cells and lung metastatic burden in MMTV-NIC mouse model of HER2+ BC [97]. Therefore, these studies demonstrate the importance of AXL in the metastatic cascade in HER2+ BC and suggest that simultaneous inhibition of AXL and HER2 could be a potential approach to abrogate metastatic spread [51,97].

A relevant biological question is how AXL is activated. Several studies reported that AXL could be activated both in a dependent or independent manner of its ligand GAS6. Exploration of the mechanism underlying trastuzumab resistance through AXL revealed that it happens in a ligand independent manner [51], which supports previous results where AXL role on metastatic potential was also independent of GAS6 [97]. In this case, AXL activation arises through AXL-HER2 heterodimerization [51,97]. This heterodimer leads to an increase of PI3K/AKT and MAPK/ERK cascades which had been previously reported to give resistance to anti-HER2 therapies [98–100]. This is consistent with previous studies showing that AXL can drive oncogenic signaling of each of these pathways in other cancer types [101]. Furthermore, AXL inhibition in combination with HER2 blockade achieved a significantly great inhibition of these pathways and restored response to anti-HER2 agents [51]. These results suggest that activation of HER2 downstream pathways by AXL can be one main mechanism leading to trastuzumab resistance in these models. Moreover, trastuzumab interferes with ligand-independent HER2 dimerization and has preferential activity against HER2 homodimers. Therefore, the increased number of AXL/HER2 heterodimers in cells would lead to a decrease in treatment response [102].

These observations were also validated in acquired trastuzumab-resistant patient-derived tumor xenograft (PDX) HER2+ BC models *in vitro* and *in vivo*. Importantly, the combination of AXL inhibitor TP-0903 and trastuzumab achieved complete regression in trastuzumab resistant tumors *in vivo*, whereas any single agent had no significant effect. Moreover, even after a long period of follow up, tumor did not relapse in the combination group. Altogether, these results suggests that the combination of HER2 and AXL inhibition might avoid treatment escape by tumor cells with acquired trastuzumab resistance, leading to a potential long-term benefit for HER2+ BC patients [51].

Gastric and oesophageal cancer

Despite molecular classification, only HER2 amplification and microsatellite instability-high (MSI-H) can be considered as drivers for advanced gastroesophageal adenocarcinoma. Nevertheless, several clinical trials testing various anti-HER2 agents have been performed in this type of cancer being trastuzumab and trastuzumab-deruxtecan the only ones that showed clinical evidence [12–13]. The high heterogeneity, loss of HER2 and the complex molecular scenario seems to be responsible for these failures.

The role of AXL as responsible for resistance also emerged from preclinical investigations in gastric cancer. Afatinib (Tyrosine kinase inhibitor against EGFR and HER2)-resistant HER2+ gastric cancer cell lines showed AXL amplification. Interestingly, Combination treatment of afatinib plus cabozantinib, a multikinase inhibitor against MET and AXL, increased response of acquired afatinib-resistant cells *in vitro* and *in vivo* models [103].

In oesophageal squamous cell carcinoma (ESCC), the rate of HER2 overexpression is less than 10 % and the efficacy of HER2 blockade has not been fully demonstrated [104–105]. As it was observed in other cancers, acquired lapatinib-resistant ESCC cells presented increased levels of AXL and combination treatment of lapatinib or afatinib plus foretinib showed synergetic effect *in vitro* [106]. Therefore, the role of AXL in this setting of patients should be further investigated and clarified.

Translational relevance of AXL in HER2+ cancers

In the clinical setting, AXL independently predicts worse survival and is associated with metastasis in BC [92,107–109]. AXL is currently recognized as a prognostic biomarker of triple negative BC and other solid tumors [92,110]. Currently, there are 29 clinical trials evaluating the toxicities and efficacy of AXL inhibitors as single agents or in combination with different therapies in solid tumors (Table 1). However, there is still a lack of clinical information on the role of AXL as a biomarker in HER2+ cancers.

In this scenario, a positive correlation was observed for the first time between *AXL* expression and increased risk of metastasis, EMT signature expression and a reduced survival in HER2+ BC patient samples [97]. These results are in line with those in which *AXL* expression correlated with the mesenchymal marker *VIM* in patient's samples of triple negative BC and other cancers [90,93,109].

Furthermore, a strong positive correlation was also found between *AXL* and *VIM* expression in HER2+ BC patients. In particular, *AXL* is highly expressed in primary tumor samples at time of diagnosis in those patients who experienced a relapse versus those free of disease. Moreover, *AXL* expression significantly correlates with shorter DFS and OS in this subtype of BC and presents a strong potential as a biomarker to discriminate patients who do or do not respond to HER2 blockade [51].

Interestingly, none of the previous studies found correlation between AXL and GAS6 expression nor between *GAS6* and prognosis in HER2+ BC patient's samples, supporting the hypothesis of a ligand independent mechanism of HER2 blockade resistance through AXL [51,97].

Lastly, the association of AXL with acquisition of trastuzumab resistance in HER2+ BC has been also evaluated in a cohort of patients enrolled in the PAMELA trial, a phase II multicentric clinical trial, providing an additional translational value to the previous preclinical

Table 1
Current clinical trials evaluating AXL inhibitors in solid tumors.

AXL inhibitor	Targets	Treatment	Conditions	Phase/ Reference
TP0903 (Dubermatinib)	AXL	Single agent	Solid tumors	Phase 1 NCT02729298
SLC-391	AXL	Single agent	Solid tumors	Phase 1 NCT03990454
INCB081776	AXL, MER	+INCMGA00012	Solid tumors	Phase 1 NCT0352142
PF-07265807	AXL, MER	+ Sasanlimab + Axitinib	Solid tumors	Phase 1 NCT04458259
RXDX-106	AXL, MER, TYRO3	Single agent	Solid tumors	Phase 1 NCT03454243
BPI-9016 M	MET, AXL	Single agent	Solid tumors	Phase 1 NCT02478866
MGCD516 (Sitravatinib)	MET, AXL, MER, VEGFR, PDGFR, DDR2, TRK, Eph	Single agent	Solid tumors	Phase 1 NCT02219711
Q702	AXL Mer	Single agent	Solid tumors	Phase 1 NCT04648254
	CSF1R	+Pembrolizumab	Solid tumors	Phase 1/2 NCT05438420
R428 (Bemcentinib)	AXL	+Pembrolizumab	Advanced Lung cancer	Phase 2 NCT03184571
		+Pembrolizumab	Mesothelioma	Phase 2 NCT03654833
			Triple negative BC	Phase 2 NCT03184558
			Glioblastoma	Phase 1 NCT03965494
		+ Nab-paclitaxel + Gemcitabine + Cisplatin	Pancreatic cancer	Phase 1/2 NCT03649321
		+ Erlotinib	Non-Small Cell Lung Cancer	Phase 1/2 NCT02424617
BMS907351 (Cabozantinib)	AXL MET RET VEGFR2	+ Lanreotide	Neuroendocrine tumors	Phase 2 NCT04427787
		+ Niraparib	Urothelial Cancer	Phase 1–2 NCT03425201
		Single agent	Hepatocellular Carcinoma	Phase 2 NCT04316182
		+ Nivolumab	Colorectal Cancer	Phase 2 NCT04963283
		+ Ipilimumab + Nivolumab	Melanoma	Phase 2 NCT05200143
		+ Lanreotide	Neuroendocrine tumors	Phase 2 NCT04427787
ADCT-601 (Mipasetamab uzoptirine)	AXL	+ Gemcitabine	Solid tumors	Phase 1 NCT05389462
HuMax-AXL-ADC (Enapotamb vedotin)	AXL	Single agent	Ovarian Cancer Cervical Cancer Endometrial Cancer NSCLC Tyroid Cancer Melanoma Sarcoma	Phase 1/2 NCT02988817
CAB-AXL-ADC (BA3011)	AXL	+PD1-1 inhibitor	Solid tumors	Phase 1/2 NCT03425279
			Non-Small Cell Lung Cancer	Phase 2 NCT04681131
TILs/ CAR-TILS	HER2, Mesothelin, PSCA, MUC1, Lewis-Y, GPC3, AXL, EGFR, Claudin18.2/6, ROR1, GD1 or B7-H3	Cell therapy	Solid tumors	Phase 1 NCT04842812
CAR-T cells	PSCA, MUC1, TGFβ, HER2, Mesothelin, Lewis-Y, GPC3, AXL, EGFR, Claudin18.2 or B7H3	Cell therapy	Solid tumors	Phase 1 NCT03198052
CCT301-38 (CAR-T)	AXL	Single agent	Sarcomas	Phase 1 NCT05128786
			Renal Cell Carcinoma	Phase 1/2 NCT03393936

results [51,111]. In this trial, patients with stage I-IIIA HER2+ BC, received lapatinib and trastuzumab for 18 weeks; and in hormone receptor-positive patients were additionally given hormonotherapy. A remarkable observation from this study is that *AXL* expression exhibits an increase in residual disease during dual anti-HER2 therapy. This biological acquisition is relatively rapid in the first two weeks of treatment, suggesting the importance of AXL upregulation in anti-HER2 resistance acquisition and the consequent high risk of metastases. This underlines that the selective pressure of the therapy may shape cancer evolution, leading to selection of tumor subclones enriched with aberrations causing drug resistance.

However, little clinical information is currently available from

HER2+ disease beyond BC. Only a correlation between AXL expression and poor prognosis in ESCC patients was observed and, interestingly, co-expression of AXL and HER2 increased the hazard of recurrence and death [106], which is in line with previously suggested crosstalk between AXL and HER2 [51–52,97].

Collectively, clinical data point out AXL as a predictive biomarker of anti-HER2 therapy response. Thus, targeting it with specific agents could help in overcoming resistance.

Future prospective of AXL inhibition

Several novel drugs targeting AXL including small molecule inhibitors, antibodies, aptamers and others are currently under development and under evaluation in different trials in solid tumors [112–113]. Among them, foretinib have been tested in a phase II trial for triple negative BC patients and showed a clinical benefit suggesting that may have clinical activity as a single, non-cytotoxic agent in this group of patients [114]. Bosutinib, an inhibitor of the BCR-ABL1 tyrosine kinase with activity against AXL, has been evaluated as single agent in a phase II trial and showed promising efficacy in prolonging time to progression. Bemcentinib and TP0903, which are in phase I/II trials for solid tumors, are possibly the most specific AXL inhibitors [114–116]. Recently, antibody-drug conjugates or chimeric antigen receptor (CAR) T cells against AXL are being under development in phase I clinical trials for solid tumors.

Nevertheless, because of the recent identification of AXL upregulation as a mechanism of anti-HER2 resistance, the simultaneous inhibition of HER2 and AXL should be further investigated in clinical trials. Currently, the only available result come from a phase I trial with metastatic HER2+ BC patient treated with foretinib and lapatinib. Although the objective of the trial was toxicity, a limited efficacy was observed probably due to the small cohort and the lack of molecular selection [117]. Future prospective studies will help to determine the potential value of AXL as a biomarker of treatment response and as a therapeutic target in HER2+ BC, but also as a promising anti-cancer approach in different types of cancer.

Despite these results, translational findings provide a strong rationale for developing and testing AXL inhibitors for clinical use in AXL-upregulated HER2+ BC patients to either prevent or overcome resistance to trastuzumab. In particular, these studies have shed the light on how HER2+ cells could ***acquire resistance to trastuzumab through AXL activation and demonstrated that simultaneous inhibition of AXL and HER2 is a potential therapeutic strategy in acquired trastuzumab-resistant HER2+ BC (Fig. 1) [51]. Therefore, independently of the AXL inhibition strategy, testing this approach together with trastuzumab in selected HER2+ BC patients would be of special interest. Nevertheless, future studies to better understand AXL activation in HER2+ disease will extend the knowledge of its contribution of acquisition to anti-HER2 therapy resistance.

It is important to note that, beyond its role in cancer cells, AXL is expressed by immune cells such as natural killer, dendritic cells and macrophages, playing also a role in the microenvironment by promoting immunosuppression and resolution of inflammation [77,118]. Moreover, AXL has a direct role in modulation of the immune system and promotes reprogramming of the metastatic niche in favour to an initial EMT and a subsequent MET (mesenchymal to epithelial transition) at metastatic sites in BC. Besides, AXL promotes the release of cytokines that further contribute to decreasing the antitumor immune response [119]. In triple negative BC, AXL also cooperates to promote evasion of antitumor immunity in mice models, while TAM-family inhibitors induce anti-tumor immune response and act synergistically with anti-PD1 blockade [120–121].

Regarding HER2+ disease, clinical trials of combined immune checkpoint blockade plus trastuzumab showed modest benefit [122]. However, recent studies evaluated the role of AXL in the tumor microenvironment in HER2+ BC *in vivo* models and demonstrated that targeting AXL during immunotherapy enhanced anti–PD1 therapy response by promoting a proinflammatory tumor microenvironment [123]. Furthermore, simultaneous targeting of macrophages and HER2 represents a potential therapeutic strategy in HER2+ BC that have progressed to anti-HER2 agents. The combination of anti-CD47 macrophage checkpoint immunotherapy and trastuzumab overcomes resistance to

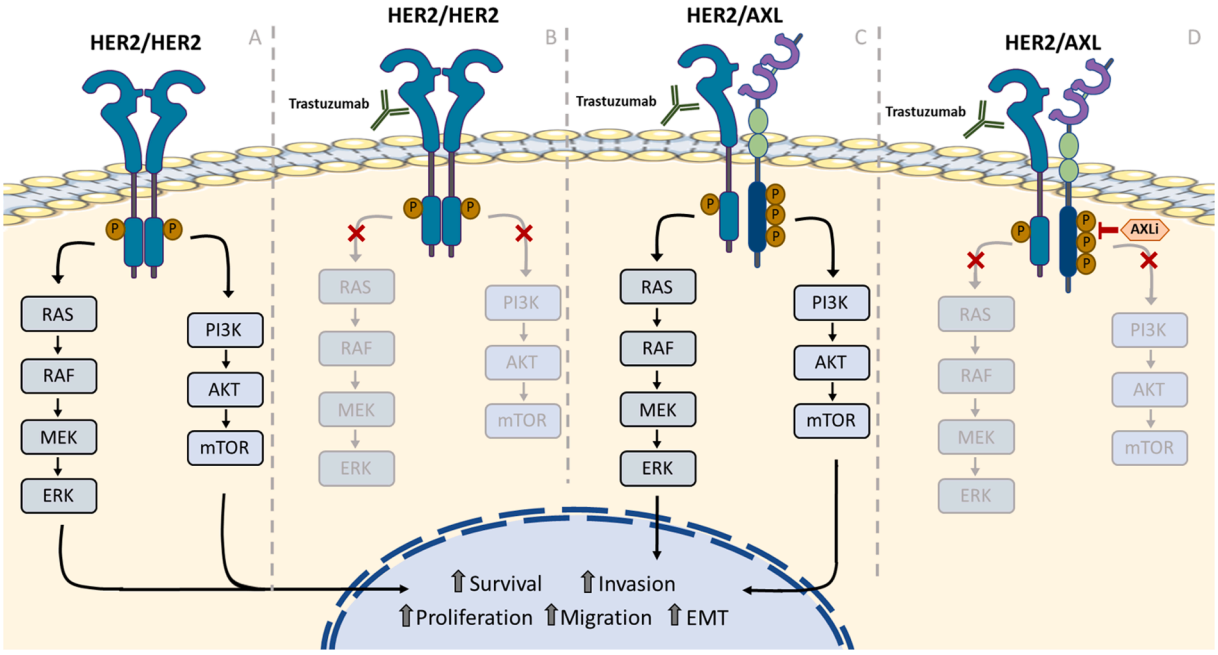

Fig. 1. Proposed mechanism of AXL–driven resistance to HER2 blockade in HER2+ solid tumors. A) HER2 homodimerization activates PI3K/AKT and MAPK/ERK signaling pathways leading to survival, invasion, migration, proliferation and EMT. B) Trastuzumab interferes HER2 signaling through different mechanisms such as inhibition of dimerization, receptor internalization, inhibition of PI3K/AKT pathway and antibody-dependent cellular cytotoxicity (not shown). C) AXL heterodimerization with HER2 activates PI3K/AKT and MAPK/ERK signaling pathways in a GAS6-independent manner bypassing HER2 blockade. D) Simultaneous pharmacological inhibition of AXL and HER2 in tumor cells would prevent PI3K/AKT and MAPK/ERK pathway activation.

anti-HER2 therapies [124]. Therefore, these results support the hypothesis that AXL inhibition can overcome trastuzumab resistance not only in a tumor intrinsic manner but also by the modulation of the tumor microenvironment on different immune cell populations. However, further studies to elucidate this emerging role of AXL in the stroma are required.

As a result, past and current studies open the door to a new area of investigation that need to clarify the role of AXL in anti-HER2 resistance, the combinatorial use of AXL inhibition plus HER2 blockade as a therapeutic strategy, and the emerging role of AXL as a a potential strategy to improve response not only to HER2 targeted therapies but also to immunotherapy in HER2+ disease.

CRediT authorship contribution statement

Anna Adam-Artigues: Conceptualization. **Enrique J. Arenas:** . **Joaquín Arribas:** . **Aleix Prat:** . **Juan Miguel Cejalvo:** Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Juan M. Cejalvo reports speakers' fees from Novartis and Pfizer Inc. and travel expenses from Roche, Lilly, Novartis and Pfizer Inc. outside the submitted work.

Dr. Prat has a patent (HER2DX) licensed to Reveal Genomics, has a patent (WO 2018/103834) licensed to Reveal Genomics, and is an equity stockholder in Reveal Genomics. He has declared personal honoraria from Pfizer, Novartis, Roche, MSD Oncology, Lilly and Daiichi Sankyo, travel, accommodations, and expenses paid by Daiichi Sankyo, research funding from Nanostring Technologies, Roche and Novartis, consulting/advisory role for Nanostring Technologies, Roche, Novartis, Pfizer, Oncolytics Biotech, Amgen, Lilly, MSD, PUMA and Daiichi Sankyo, Inc. outside the submitted work.

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