Therapeutic Activity of Anti-AXL Antibody against Triple-Negative Breast Cancer Patient-Derived Xenografts and Metastasis

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Abstract

Purpose: AXL receptor tyrosine kinase has been described as a relevant molecular marker and a key player in invasiveness, especially in triple-negative breast cancer (TNBC).

Experimental Design: We evaluate the antitumor efficacy of the anti-AXL monoclonal antibody 20G7-D9 in several TNBC cell xenografts or patient-derived xenograft (PDX) models and decipher the underlying mechanisms. In a dataset of 254 basal-like breast cancer samples, genes correlated with AXL expression are enriched in EMT, migration, and invasion signaling pathways.

Results: Treatment with 20G7-D9 inhibited tumor growth and bone metastasis formation in AXL-positive TNBC cell xenografts or PDX, but not in AXL-negative PDX, highlighting AXL role in cancer growth and invasion. In vitro stimulation of AXL-positive cancer cells by its ligand GAS6 induced the expression of several EMT-associated genes (SNAIL, SLUG, and VIM) through an intracellular signaling implicating the transcription factor FRA-1, important in cell invasion and plasticity, and increased their migration/invasion capacity. 20G7-D9 induced AXL degradation and inhibited all AXL/GAS6–dependent cell signaling implicated in EMT and in cell migration/invasion.


Introduction

Breast cancer is the most common female malignancy in western countries. Triple-negative breast cancers (TNBC) account for 15% to 20% of all breast cancer cases and are defined by the absence of estrogen receptors (ER), progesterone receptors (PR), and HER2 expression. TNBC prognosis is poor and chemotherapy remains the sole therapeutic option (1). No targeted therapy is available for TNBC due to the lack of identified molecular targets in most of these cancers. Moreover, TNBC is associated with a significant higher probability and faster occurrence of relapse compared with other breast cancer subgroups. Epithelial-to-mesenchymal transition (EMT) is believed to be a main contributor to the invasion process (2, 3). During EMT, cells lose some of their epithelial characteristics, typically E-cadherin downregulation, and acquire mesenchymal phenotypic marks, characterized by upregulation of vimentin, N-cadherin, and fibronectin. Transcriptional regulators activated in the EMT program include TWIST1, ZEB1, and SNAIL transcription factors (SNAI1/SNAI2). These regulators are expressed in many tumors and have an important role in breast cancer metastasis formation (4).

Several studies reported that AXL, a member of the TAM (TYRO3, AXL, MER) receptor tyrosine kinase family, has a major role in the control of tumor cell migration and invasion (5–8). AXL was first identified as a transforming gene in cells from patients with chronic myelogenous leukemia (9). Following binding to its ligand, growth arrest–specific 6 (GAS6) leads to TAM receptors dimerization and, consequently, to the activation of various intracellular pathways that regulate many biological processes, such as cell invasion and proliferation, angiogenesis, and drug resistance (10).

High AXL expression has been reported in many cancers and is associated with tumor progression and shorter overall survival (10, 11). In breast cancer, Gjerdrum and colleagues found that AXL expression is increased in metastatic lesions compared with matched primary tumors suggesting a crucial role in breast cancer cell invasiveness and metastatic potential (12). However, it is unclear whether AXL directly induces EMT by regulating EMT...
effectors or whether it is a target of these EMT regulators. Indeed, AXL downregulation in breast cancer using siRNAs inhibits AKT phosphorylation and decreases cell invasion, motility, and metastasis. On the other hand, ectopic expression of AXL in human mammary epithelial cells leads to a downregulation of E-cadherin expression and increased expression of mesenchymal markers (13). Along similar lines, transfection of SLUG and SNAIL genes in MCF10A breast cancer cells induces the acquisition of mesenchymal markers and loss of epithelial characteristics together with increased AXL expression (12).

In this study, we examined the potential therapeutic effect of a recently developed anti-AXL mAb (20G7-D9) in TNBC cell xenografts and in basal-like patient-derived xenografts (PDX). We observed a dual role of our antibody consisting of inhibiting tumor growth and AXL/GAS6-dependent migration/invasion of cancer cells. In fact, we demonstrated, in vitro, that stimulation of several TNBC cell lines with recombinant human GAS6 (rhGAS6) induced the expression of EMT transcription factors, mesenchymal markers, and phosphorylation of FRA-1, a key transcription factor in cancer cell invasion, migration, and plasticity (15). Treatment with 20G7-D9 resulted in the degradation of AXL receptor, avoiding any AXL/GAS6-dependent signaling to occur and, in vivo, reducing spreading of MDA-MB-231-Luc to bones after intracardiac injection. In conclusion, in AXL-positive TNBC tumors, AXL and GAS6, have a critical role in the maintenance of the EMT phenotype. Therefore, AXL should be considered as a therapeutic target of choice for tumor growth and metastasis inhibition.

Materials and Methods

Cell lines and reagents

The MDA-MB-231, MDA-MB-435, MDA-MB-436, MDA-MB-453, MDA-MB-468, BT549, BT20, HCC38, HS578T, HBL-100 breast cancer cell lines were obtained from ATCC. Cells were cultured for at most 3 months and then cultures were renewed by thawing a new vial from our master cell bank. Authentication by the MycoAlert Mycoplasma Detection Kit (Lonza) were routinely performed. All cell lines were cultured following the ATCC recommendations. Control (sh-Luc) and anti-AXL shRNAs were previously described (14). Luciferase-transfected MDA-MB-231 cells (MDA-MB-231-Luc) were previously described (16). RhGAS6 and the goat anti-human GAS6 antibody were purchased from R&D Systems. The anti-GAPDH antibody was from Millipore. All other antibodies used in Western blotting were from Cell Signaling Technology. The anti-FRA1 siRNA corresponds to the 124–144 coding region relative to the first nucleotide of the start codon (15). The specific anti-human AXL murine IgG1 antibody 20G7-D9 that induces AXL internalization and degradation was previously described (14).

Western blotting

Western blotting was performed using protein lysates from cell lines or PDXs, as previously described (17). Western blot analysis was performed using the G:BOX iChem imaging system (Syngene). Protein expression was quantitated with the ImageJ software and the Student t test was used to evaluate differences.

Boydien chamber migration and invasion assays

Cancer cells were serum-starved in the presence or absence of 50 μg/mL 20G7-D9 overnight. This incubation time was chosen as we previously showed a massive degradation of AXL at this time point (14). After trypsin digestion, cells were counted and migration assays were performed by seeding 3 × 104 cells resuspended in serum-free DMEM in 8-μm pore size BD Falcon 24 Fluoroblock Transwell inserts. For GAS6-dependent migration, 200 ng/mL rhGAS6 was added to the bottom chamber containing the migration medium (DMEM/5% serum). Invasion assays were carried out using Matrigel Matrix Growth Factor Reduced (BD Biosciences) and 5 × 104 cells. After 4 hours of migration and 24 hours of invasion, cells in the bottom chamber were stained with 4 μg/mL calcein and then counted under an inverted fluorescence microscope.

In vivo studies

All in vivo experiments were performed with compliance with the French regulations and ethical guidelines for experimental animal studies in an accredited establishment (agreement no. C34-172-27). MDA-MB-231 or MDA-MB-436 cells (5 × 106) were injected subcutaneously in 6-week-old female athymic mice (Harlan). When tumors reached a minimum volume of 100 mm3, tumor-bearing mice were randomized in different treatment groups (n = 6) and treated with 200 μg of 20G7-D9 or saline by intraperitoneal injection twice a week for four consecutive weeks. Tumors were measured using a caliper and volume was calculated using the formula V = (tumor length × tumor width × tumor depth)/2, until the tumor volume reached 2,000 mm3. For metastasis studies, 5 × 105 MDA-MB-231-Luc cells were injected intracardially in anesthetized BALB/c nude mice. Intraperitoneal treatments with 200 μg 20G7-D9/injection or saline were done twice a week for four consecutive weeks. Bone metastases were quantified by in vivo bioluminescence imaging at day 25, as described previously (16). For PDX models, approximately 60 mm3 of B3804 and B3467 tumors were transplanted in the interscapular fat pads of Swiss nude female mice (Charles River Laboratories) and treated with 200 μg 20G7-D9 twice weekly or saline, when

Translational Relevance

The receptor tyrosine kinase AXL is overexpressed in several cancers and particularly in breast cancer, the most common female malignancy in western countries. Triple-negative breast cancer (TNBC), which accounts for 15% to 20% of all breast cancer cases, has a poor prognosis and chemotherapy is the only available therapeutic option due to the lack of identified molecular targets. In this preclinical study, we show that in vivo AXL immunotargeting with the specific therapeutic antibody 20G7-D9 significantly decreases the growth of TNBC cell xenografts and AXL-positive patient-derived breast tumor xenografts (PDX). Moreover, 20G7-D9 inhibits breast cancer cell migration in vitro and bone metastasis development in vivo. Mechanistically, the 20G7-D9 antibody acts by downregulating AXL expression at cell membranes and consequently inhibiting cell migration, invasion, and epithelial-to-mesenchymal transition (EMT) induced by the AXL ligand GAS6. AXL might therefore be a target for immunotherapy with the antibody 20G7-D9 in TNBC.
tumor volume reached a minimum volume of 100 mm$^3$. Tumor volumes were measured as described above.

PDX and basal-like breast cancer transcriptome analysis

Expression profiling of PDXs was performed using Affymetrix U133plus2. Raw data were normalized using robust multi-array average (RMA). Data were aggregated around HUGO gene symbols with max argument (18). The CIT classification developed by our group was applied using the R script referred in the publication (19). The dataset of 1,809 breast cancer samples was downloaded from http://kmplot.com/analysis/index.php?p=download. The CIT classification R script was run on the 1,809 breast cancer samples. The AXL expression level was displayed for each molecular subtypes (Supplementary Fig. S2). The 254 samples identified as Basal-like were extracted. The 100 genes most correlated with AXL expression in the 254 basal-like tumors were obtained using the gene neighbor command of the GenePattern suite (Broad Institute, Boston) with standard settings (including Pearson correlation). Annotations were made with the GSEA software (Broad Institute, Boston), first with hallmark gene sets (Fig. 3) and then with the MSigDB collections.

Statistical analyses

A linear mixed regression model was used to determine the relationship between tumor growth and number of days after implantation. The fixed part of the model included variables...
corresponding to the number of days after implantation and the different treatment groups. Interaction terms were built into the model. Random intercepts and random slopes were included to take into account the time effect. The model coefficients were estimated by maximum likelihood and considered significant at the 0.05 level.

Statistical analysis was carried out using the STATA 10.0 software.

**Results**

The anti-AXL 20G7-D9 mAb inhibits growth of AXL-positive TNBC cell xenografts

Before testing the effect of the 20G7-D9 anti-AXL mAbs in TNBC xenografts, we assessed AXL surface expression by flow cytometry (Fig. 1A) and TAM receptors expression by Western blotting (Supplementary Fig. S1) in 10 TNBC cell lines and found that AXL was significantly expressed in 7. These results are consistent with previous studies reporting that AXL is often upregulated in basal B and occasionally in basal A TNBC (20, 21). Moreover, the cell lines in which AXL was most overexpressed (MDA-MB-231, BT549, HBL-100, BT20, and HS578T) are considered as highly invasive (22). Interestingly, we analyzed AXL mRNA expression in a wide range of breast cancer tumors and we did not observe a significant upregulation of this receptor in basal-like breast tumors in comparison with the other breast cancer subtypes (Supplementary Fig. S2).

**Figure 2.** Effect of the anti-AXL 20G7-D9 monoclonal antibody in mice with basal-like TNBC PDX. A, AXL mRNA expression (arbitrary units) in 10 basal-like TNBC PDXs based on Affymetrix microarray data. B, Western blot analysis of AXL, GAS6, and several EMT factors [VIM (Vimentin), MMP9, MMP1, FRA-1, TWIST, SNAIL, SLUG]. TUBULIN served as loading control. C, Average passage duration for the first three passages in PDXs with high and low AXL protein level. D, Mice engrafted with the AXL-positive PDX B3804 were treated or not with 200 μg of 20G7-D9 twice/week. Results are presented as the mean tumor volume (±SEM) in each group (n = 6). E, Mice harboring the AXL-negative PDX B3467 were treated or not with 200 μg of 20G7-D9 twice/week. Results are presented as the mean tumor volume (±SEM) in each group (n = 10). F, Bone metastasis development inhibition by the anti-AXL mAb 20G7-D9. The day after intracardiac injection of 5 × 10^6 MDA-MB-231-luc cells, mice (n = 7/group) were treated or not with 200 μg of 20G7-D9 or saline twice per week for 4 weeks. The bioluminescence intensity was measured weekly after intraperitoneal injection of 100 mg/kg luciferin. Results are presented as the bioluminescence signal (p/s) of treated and untreated mice at day 25. G, Representative bioluminescence images of treated (D9) and untreated mice. This experiment has been reproduced twice. **, P < 0.01; ***, P < 0.001.
Treatment of mice harboring established MDA-MB-436 (basal B, moderate AXL expression) and MDA-MB-231 (basal B, high AXL expression) xenografts with 20G7-D9 for 4 weeks led to a statistically significant growth inhibition in both models compared with saline-treated animals \( (P = 0.015 \text{ and } 0.047, \text{ respectively}) \). Specifically, the mean tumor volume in 20G7-D9–treated mice was reduced by 62.5% at day 53 in the MDA-MB-436 model, and by 57% at day 68 in the MDA-MB-231 model (Fig. 1B).

The anti-AXL 20G7-D9 mAb inhibits metastatic spread of MDA-MB-231 cells to bone

As AXL expression has been linked to EMT and metastasis formation (13), we next assessed whether 20G7-D9 could inhibit in vivo migration and invasion of cancer cells. To this aim, one day after intracardiac injection of MDA-MB-231-Luc cells, mice were treated with 20G7-D9 or saline (control) for 4 weeks (biweekly injection) and at day 25, the development of MDA-MB-231-Luc metastases in bone was quantified using a luciferin/luciferase assay. The bioluminescence intensity was significantly lower in the group of mice treated with 20G7-D9 than in untreated controls (Fig. 2F; \( P = 0.0038 \)). This result was confirmed by bioluminescence imaging performed at the end of the 4 weeks of treatment (Fig. 2G).

AXL expression in basal-like PDxs and basal-like breast tumors is correlated with invasive and mesenchymal gene signatures

The finding that treatment with 20G7-D9 reduces cancer cell invasion to bone in mice with TNBC cell xenografts was in accordance with the AXL gene being a major actor in EMT induction. Accordingly, Western blot analysis of the expression of EMT markers involved in breast cancer metastasis formation in TNBC PDxs showed that all AXL-positive PDxs expressed FRA1,
SNAI1, and TWIST1, whereas SLUG was detected only in three of them (Fig. 2B). Generally, all AXL-positive PDXs expressed molecules involved in EMT, with the exception of GAS6, MMP1, and VIMENTIN that were not correlated with AXL expression. Moreover, microarray analysis of the 100 genes most correlated with AXL expression in 254 basal-like breast cancers (Supplementary Fig. S3) using the MSigDB hallmark gene signatures indicated a strong enrichment in genes that define the EMT and TGFβ1 pathways (Fig. 3A). Particularly, 8 of the 12 genes most correlated with AXL expression, were annotated as EMT markers and included TGFβ1 and VEGFC (Fig. 3B). Further annotation analysis with the MSigDB collections indicated a strong enrichment in signal transduction and cell proliferation. Membrane components, including the integrin pathway and focal adhesion processes, were also overrepresented. Significant overlaps were also found with gene signatures present in adult stem cells, early embryonic stem cells, or epithelial cell lines submitted to EMT-inducing factor TGFβ1 and mesenchymal breast cancer cell lines. These enrichments reveal the influence of AXL in human tumors through integrin-mediated signaling impacting cell proliferation and EMT in accordance with our in vitro experiments (Supplementary Table S1). These findings in basal-like breast cancer samples and PDXs suggest that high AXL expression is associated with acquisition of mesenchymal features. The extent of this effect might depend on the cell context and tumor environment.

The anti-AXL 20G7-D9 mAb inhibits in vitro migration and invasion of TNBC cells

To further understand how 20G7-D9 inhibits metastasis formation, we then performed Boyden chamber assays using the AXL-positive MDA-MB-231 and HBL-100 cell lines (basal B TNBC). Preincubation with 20G7-D9 inhibited migration and invasion by more than 50% in both lines compared with untreated cells (Fig. 4A and B). Conversely, addition of rhGAS6 in the bottom chamber increased significantly the migration and invasion of both cell lines compared with untreated cells (Fig. 4A and B). This effect was abolished by preincubation with the anti-AXL antibody. We then assessed whether 20G7-D9 could inhibit degradation of the extracellular matrix by breast cancer cells using in situ zymography. Preincubation of MDA-MB-231 cells with 20G7-D9 significantly reduced the number of invadopodia and the areas of focal gelatin degradation compared with untreated cells (Supplementary Fig. S4A and S4B). Collectively, these data suggest that GAS6 and AXL are involved in TNBC cell migration and invasion.

GAS6 stimulation triggers expression of EMT factors

To further investigate the role of GAS6/AXL signaling in EMT and metastasis formation, we incubated MDA-MB-231, HBL-100 (two cell lines with high AXL expression), and MDA-MB-436 (moderate AXL expression) cells with rhGAS6 and then assessed AXL and FRA-1 phosphorylation and the expression of EMT effectors by Western blotting. GAS6 induced phosphorylation of AXL and FRA-1 (at S265) and increased AXL expression in all three cell lines (Fig. 5A). Moreover, expression of SNAI1, SLUG, TWIST1, and ZEB1/2 was also increased in the three TNBC cell lines upon addition of GAS6 (Fig. 5A and B, quantification in Supplementary Fig. S5). These effects were abolished when AXL was knocked down by shRNA in MDA-MB-231 cells. Similarly, FRA1 silencing in MDA-MB-231 cells inhibited ZEB1 and SNAI1 upregulation following stimulation with GAS6 (Fig. 5C). This is in agreement with the finding that FRA-1 can induce EMT-related genes, such as SLUG and ZEB1/2 (23, 24), and suggests that some of the effects of activated AXL could result from increased expression of phosphorylated FRA-1.

Finally, incubation with GAS6 also induced expression of the mesenchymal markers vimentin, MMP1 and MMP9 in HBL100 and MDA-MB-436 cells (Fig. 5B). In MDA-MB-231 cells, induction was less clear-cut due to the high basal expression of these markers. Nevertheless, AXL silencing reduced the expression level of the three EMT markers (Fig. 5B). Moreover, the level of induction of these EMT markers varied according to the TNBC cell line and could be explained by the involvement of other, still unidentified factors in AXL/GAS6 signaling. Globally, these results confirm an important role of GAS6 and AXL in the expression of EMT markers involved in breast cancer invasion.

**Figure 4.**

20G7-D9 inhibits TNBC cell migration (A) and invasion (B) in vitro. MDA-MB-231 and HBL-100 cells were preincubated with 20G7-D9 overnight, trypsinized, and plated in the top compartment of a Boyden chamber in serum-free medium with or without 200 ng/mL rhGAS6. At the end of the experiment, cells were stained with calcein to evaluate cell migration/invasion. ***, P < 0.01; ****, P < 0.001.
The anti-AXL mAb dramatically reduces the mesenchymal phenotype of breast cancer cells

We then asked whether 20G7-D9 could influence EMT marker expression and migration of MDA-MB-231 cells. Incubation with 20G7-D9 induced downregulation of AXL (14) and consequently its expression could not be induced by GAS6 (Fig. 6A and D). Similarly, GAS6-induced AXL phosphorylation was reduced compared with untreated cells.

Figure 5.
GAS6 regulates expression of EMT markers in TNBC. The effect of incubation with rhGAS6 on the expression of EMT transcription factors (A) and mesenchymal factors (B) was analyzed by Western blotting. MDA-MB-231-shCONTROL, -shAXL, HBL-100, and MDA-MB-436 cells were incubated with 200 ng/mL of rhGAS6 for the indicated times. GAPDH served as loading control. C, FRA-1 silencing in MDA-MB-231 cells inhibits the GAS6-induced expression of EMT transcription factors. Cells were stimulated with 200 ng/mL of rhGAS6 for 30 minutes. Control cells express a scrambled siRNA. TUBULIN and GAPDH served as loading control. Quantification of each protein band was normalized to GAPDH or TUBULIN and then represented as fold change relative to nontreated siCTRL MDA-MB-231 cells.
Figure 6.
Effect of the anti-AXL mAb 20G7-D9 on AXL activation (A) and on the expression of EMT effectors (B) and their targets (C). MDA-MD-231 cells were incubated with 20G7-D9 or an isotype control antibody (Px) overnight. AXL phosphorylation (Tyr702) was analyzed by Western blotting after 30-minute incubation with 200 ng/mL rhGAS6. Expression of FRA-1 (and its phosphorylation on Ser265), ZEB-1, ZEB-2, TWIST, SLUG, SNAIL, and of the mesenchymal markers Vimentin, MMP1, and MMP9 was analyzed after 120-minute incubation with 200 ng/mL rhGAS6. GAPDH served as loading control. All Western blots experiments were repeated at least three times. D, Quantification of each protein band was normalized to GAPDH and then represented as fold change relative to the nontreated cell band (NT = 1). Statistical analyses were determined between the effect of GAS6 (200 ng/mL) and 20G7-D9 (24 hours) + GAS6 (200 ng/mL) on MDA-MB-231 cells (*, P < 0.05; **, P < 0.01).
A significant inhibition of the GAS6-induced upregulation of the EMT inducers SNAIL, SLUG, TWIST, and ZEB1/2 as well as of FRA-1 was observed in MDA-MB-231 cells preincubated with 20G7-D9 (Fig. 6B and D) compared with untreated cells. GAS6-induced FRA-1 phosphorylation was also strongly inhibited upon AXL downregulation by 20G7-D9. Finally, the anti-AXL antibody treatment also reduced GAS6 effect on the expression of the mesenchymal markers vimentin, MMP1, and MMP9 (Fig. 6C and D). In BT20 cell line, GAS6 treatment induced a strong downregulation of E-cadherin that is abrogated by pre-treatment with 20G7-D9 anti-AXL antibody (Supplementary Fig. S6).

Discussion

Among the large number of drug approved for breast cancer, the use of targeted therapies [hormonal, tyrosine kinase inhibitors (TKI), or antibodies] highlighted the existence of an aggressive breast cancer subtype, the TNBC, characterized by frequent and rapid relapse. Although this subtype is sensitive to chemotherapy, the TNBC, characterized by frequent and rapid relapse. Although this subtype is sensitive to chemotherapy, AXL seems a valuable therapeutic target because it drives both tumor growth and cancer cell migration/invasion.

In breast cancer, AXL plays a dual role in TNBC as inducer and target of EMT signaling during metastasis formation. This GAS6-induced signaling, as well as the basic expression of EMT markers, vary in different TNBC cell lines studied and could imply other intracellular factors that remain to be identified.

Furthermore, we observed the involvement of the transcription factor FRA-1, an AP-1 family member, in GAS6/AXL signaling. Several studies suggest that FRA-1 can regulate cancer cell motility and invasion. Direct or indirect transcriptional FRA-1 targets include proteins that promote extracellular matrix degradation (MMP1, MMP9; ref. 15), EMT transcription factors (SLUG and ZEB1/2; refs. 24, 39), and proteins involved in cell adhesion or migratory signaling (CD44, VEGF, c-MET; ref. 40). In bladder cancer, FRA-1 promotes AXL expression which mediates FRA-1 effect on cancer cell motility (41). Our findings that in TNBC cell lines activation by GAS6 increases FRA-1 expression and FRA-1 knockdown abolishes GAS6-induced upregulation by GAS6/AXL signaling bring new insights into the cross-talk between these proteins in EMT signaling.

Although TNBC cell lines are frequently AXL-positives, we observed that AXL expression in patients was not specific to this breast cancer subtype, confirming previous studies (22). It would be thus interesting to extend the characterization of AXL, as well as the therapeutic use of our antibody, in other breast cancer subtypes, like luminal models, to know whether the receptor has the same behavior and implication in EMT signaling.

Several of the basal-like PDxs we established express elevated levels of AXL and interesting those with the highest AXL expression level grow faster in vivo. Moreover, analysis of the genes correlated with AXL upregulation in basal-like breast tumors indicated that most of these genes are involved in cancer cell invasion and EMT, TGFβ signaling, mesenchymal markers, stem cells, cytoskeleton, and ECM remodeling. Of note, the transcription factor FRA-1 induces EMT through TGFβ modulation (42). AXL is also overexpressed in breast cancer stem cells (13) and EMT features are often associated with stemness (43).
In conclusion, our results highlight the expression and role of AXL and its ligand GAS6 in TNBC cell invasion. Our results suggest that AXL is a modulator, and not only a target, of EMT signaling. The use of anti-AXL antibodies, which degrade the receptor, is an interesting approach to inhibit metastatic process in such breast cancers.

Disclosure of Potential Conflicts of Interest

W. Leconet, C. Labouret, A. Pélegrin, and B. Robert are listed as coinventors on a patent on the development of a murine antibody (20G7-D9) against the human receptor tyrosine kinase AXL for cancer therapy, which is owned by the French Research Agency of Health (INSERM). University of Montpellier, and Regional Clinical Cancer Center (ICM), and licensed to Oribase-Pharma. No potential conflicts of interest were disclosed by the other authors.

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