A Bispecific HER2-Targeting FynomAb with Superior Antitumor Activity and Novel Mode of Action

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Abstract

Upregulation of HER2 is a hallmark of 20% to 30% of invasive breast cancers, rendering this receptor an attractive target for cancer therapy. Although HER2-targeting agents have provided substantial clinical benefit as cancer therapeutics, there is a need for the development of new agents aiming at circumventing anti-HER2 resistance. On the basis of the approved antibody pertuzumab, we have created a panel of bispecific FynomAbs, which target two epitopes on HER2. FynomAbs are fusion proteins of an antibody and a Fyn SH3-derived binding protein. One bispecific FynomAb, COVA208, was characterized in detail and showed a remarkable ability to induce rapid HER2 internalization and apoptosis in vitro. Moreover, it elicited a strong inhibition of downstream HER2 signaling by reducing HER2, HER3, and EGFR levels in vitro and in vivo. Importantly, COVA208 demonstrated superior activity in four different xenograft models as compared with the approved antibodies trastuzumab and pertuzumab. The bispecific FynomAb COVA208 has the potential to enhance the clinical efficacy and expand the scope of HER2-directed therapies, and delineates a paradigm for designing a new class of antibody-based therapeutics for other receptor targets.

Introduction

Since the introduction of trastuzumab (Herceptin, Roche) for the treatment of HER2-positive metastatic breast cancer in 1998, HER2-targeted therapies have transformed the clinical practice of HER2-positive tumor treatment. With the recent approval of pertuzumab (Perjeta, Roche; ref. 1), an antibody which binds to domain II of HER2 and blocks its dimerization (2, 3), and the antibody drug conjugate ado-trastuzumab emtansine (Kadcyla, Roche; ref. 4), multiple therapeutic options for patients with HER2-positive breast cancer have become available. Still, the vast majority of patients with metastatic breast cancer relapse after treatment with the currently available HER2-targeted therapies, and there remains a significant medical need for better treatment regimens (5).

The observation that patients relapsing on trastuzumab treatment benefit from the addition of lapatinib, an EGFR/HER2 kinase inhibitor, was a first indication that HER2 blockade is neither complete nor durable with single-agent treatments (6). Furthermore, several reported examples of synergistic activity of anti-HER2 antibody combinations in preclinical animal models support the concept of targeting HER2 with more than one therapeutic agent at the same time (7–10). In particular, the combination of trastuzumab and pertuzumab, the latter of which displayed no significant clinical activity as a single agent (11), showed impressive therapeutic activity in trastuzumab-resistant mouse tumor models (12) and prolonged overall survival in patients with metastatic breast cancer (1). These observations prompted us to explore whether bispecific HER2-targeting FynomAb-antibody fusion proteins (FynomAbs) would allow for an even more effective treatment of HER2-positive tumors.

Fynomers are small 7-kDa globular proteins derived from the SH3 domain of the human Fyn kinase (Fyn SH3) that can be engineered to bind with antibody-like affinity and specificity to virtually any target of choice through random mutation of two loops (RT- and src-loop) on the surface of the Fyn SH3 domain (13–15). The Fyn SH3 domain is a particularly attractive scaffold for the generation of binding proteins because the derived Fynomers (i) achieve very high expression levels, (ii) are monomeric and do not aggregate when stored in solution, (iii) are very stable (Tm ~70 °C), (iv) lack cysteine residues, and (v) are of human origin featuring a framework amino acid sequence completely conserved from mouse to man and are considered to have a low-immunogenic potential (14). Importantly, Fynomers are ideal building blocks to generate multispecific FynomAbs, which are obtained by fusion of Fynomers to any of the four antibody light- or heavy- chain variable domains.
heavy-chain termini. The resulting FynomAbs can be produced with comparable yield as antibodies, and they have favorable biophysical and pharmacokinetic properties (the Fynomer technology is described in Supplementary Methods and refs. 13–15).

Here, we have fused the HER2-specific Fynomer C12, which targets an epitope in domain I of HER2, to all termini of pertuzumab to generate bispecific HER2-targeting FynomAbs (Fig. 1A). These constructs showed superior in vitro and in vivo activity as compared with pertuzumab or trastuzumab, while the simultaneous targeting of both epitopes on HER2 with a mixture of the monospecific agents did not result in superior activity. Interestingly, we found that the activity of the HER2-targeting FynomAbs was depending on the FynomAb architecture, i.e., the relative spatial arrangement of the binding sites of antibody and the Fynomer C12. Further investigations with the optimized FynomAb, COVA208, established that this molecule acts through a novel mode of action, i.e., it is capable of inducing apoptosis and rapid HER2 internalization, and leads to a broad and efficacious blockade of HER2 downstream signaling accompanied by a reduced level of HER3 and EGFR. The therapeutic potential of COVA208 has been demonstrated in four different HER2 mouse models, where COVA208 exhibited promising antitumor activity.

Materials and Methods

Cell lines and reagents
OE19 cell line was purchased from HPA cultures, all other cell lines from ATCC. KPL-4 cell line was a kind gift from professor Kurebayashi (Kawasaki Medical School, Kurashiki, Japan; ref. 16). Cell lines were authenticated by frequent cell morphology analysis and FACS analysis of several surface markers. Chemicals were from Sigma except otherwise indicated, trastuzumab (Herceptin; Roche) was purchased from the pharmacy. Bispecific FynomAbs were transiently expressed in Chinese hamster ovary (CHO) cells and purified on protein A affinity chromatography columns.

In vitro cell assays: viability, apoptosis, antibody-dependent cellular cytoltoxicity
For viability assays, 5,000 to 7,000 cells per well were seeded in 96-well plates, and treated for 5 days. Percent viability relative to PBS control was determined by XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide) assay (Roche).

Caspase assays were performed using a caspase-3/7 kit (Promega). A total of 100 nmol/L anti-HER2 agents were added to 45,000 NCI-N87 cells in 96-well plates, and incubated for 24, 48, or 144 hours. Staurosporine (1 μmol/L; Applichem) served as a positive control.

Antibody-dependent cell-mediated cytoltoxicity (ADCC) was measured by calcein-AM release on BT-474 cells, using human peripheral blood mononuclear cells (PBMC) as effector cells at an E/T ratio of 25:1 as described in Tai and colleagues (17).

Internalization assays
NCI-N87 cells were surface labeled on ice for 1 hour with 100 nmol/L anti-HER2 agent, followed by 5-hour incubation at 37°C. Geldanamycin (1 μmol/L) served as a positive control. Cells were fixed, permeabilized, and antibody distribution analyzed using an anti-human IgG-Alexa488 conjugate (Invitrogen), followed by laser scanning microscopic analysis. Images were processed and merged using Image J (Fiji).

The amount of anti-HER2 agents that localized into distinct dots was quantified in three-dimensional using the software Imaris 7.4.0 (Bitplane). The percentage of anti-HER2 agents present in dots was expressed using the formula

\[
\text{% anti-HER2 agents in dots} = \frac{\text{volume of dots}}{\text{volume of total anti-HER2 staining}} \times 100.
\]

Immunoblot analysis of cell lysates
MCF-7 cells (5 × 10⁵) were starved in medium without serum for 3 hours, then treated with 40 μg/mL anti-HER2...
agents for 1 hour in 6-well plates. After 45 minutes, 2 nmol/L human recombinant heregulin-1b (HRG-1b; R&D Systems) was added for 15 minutes. NCI-N87 (1 x 10^6) were treated with 40 μg/mL anti-HER2 agents for 72 hours in 6-well plates. Total cell lysate was analyzed by immunoblotting. Primary antibodies were purchased from Cell Signaling Technology, except anti-Vinculin, anti-HER3 (both Millipore), and anti-EGFR Y1172 antibodies (Abcam).

**In vivo tumor xenograft and pharmacokinetic studies**

Mouse experiments conducted at Covagen AG were performed in accordance with Swiss guidelines and were approved by the Veterinarian Office of Zürich, Switzerland. For pharmacokinetic analysis, 200 μg anti-HER2 agent was injected i.v. into C57BL/6 mice. Serum concentrations after 10 minutes, 6, 24, 48, 96, 120, 144, and 168 hours were determined by ELISA on immobilized HER2 with the following species: Mouse, Rat, Human, and Goat. For pharmacodynamic analysis, 5 animals from each GXA-3039 treatment group were redosed with 30 mg/kg anti-HER2 agents for 72 hours in 6-well plates. Total cell lysate was analyzed by immunoblotting. Primary antibodies were purchased from Cell Signaling Technology, except anti-Vinculin, anti-HER3 (both Millipore), and anti-EGFR Y1172 antibodies (Abcam).

**Results**

**Bispecific anti-HER2-targeting FynomAbs suppress tumor cell growth in vitro with high potency**

The HER2-specific Fynom C12 is a 63-amino acid polypeptide derived from the SH3 domain of the Fyn kinase. Using recombinant HER2 (extracellular domain, ECD) as target antigen, Fynom C12 was isolated by phage display from a repertoire of mutated Fyn SH3-derived domains (14, 15) in which selected residues located within or in close proximity of either the RT-loop or the src-loop were randomized to one of the 20 natural amino acids (Supplementary Fig. S1A). Fynom C12 was further characterized and showed specific binding to HER2 (recombinant and cell-surface expressed HER2), high stability (T_m = 74°C) and was found to be produced at high levels (>100 mg/L) in E. coli shake flask cultures under nonoptimized conditions (Supplementary Fig. S1B–S1D). Using alanine scanning mutagenesis, the binding site of Fynom C12 on HER2 was mapped to five amino acid residues on the surface of domain I, representing an epitope distinct from pertuzumab within the dimerization domain II (3) or from trastuzumab within domain IV (21; Supplementary Fig. S1E). As expected from the binding site mapping studies, Fynom C12 is neither blocked by pertuzumab nor trastuzumab in competitive cell binding assays, thus confirming that Fynom C12 binds to a different epitope (Supplementary Fig. S1F).

We constructed HER2-targeting bispecific FynomAbs by genetically fusing Fynom C12 to each of the four termini of pertuzumab, thereby generating FynomAbs with different architectures due to the different relative orientation of the two binding specificities (Fig. 1A). All four C12-pertuzumab FynomAbs termed COVA201, COVA202, COVA207, and COVA208 could be purified as described in Materials and Methods at comparable yields as the parental antibody pertuzumab (in the range of 100 mg/L from CHO cell supernatant). The FynomAbs were capable of engaging both the pertuzumab as well as the C12 epitope on HER2-overexpressing cells with similar efficacy (Supplementary Fig. S2A). In addition, all four FynomAbs showed improved binding to HER2-overexpressing cells with EC_{50} values improved by a factor of 4.2 to 7.9 as compared with pertuzumab (Supplementary Fig. S2B). This was in agreement with binding dissociation constants (K_d) determined by BiAcore, which were 7- to 60-fold lower than for pertuzumab (Supplementary Fig. S2C).

The bispecific FynomAbs were tested for cell growth inhibition of HER2-overexpressing NCI-N87 gastric carcinoma cells in vitro (Fig. 1B). Even though targeting the C12 epitope on HER2 with a bivalent Fc-C12 fusion protein did not have any effect and did not synergize with pertuzumab when used in combination, the bispecific FynomAbs potently inhibited tumor cell growth with a maximal reduction of relative cell viability of 39% to 82% as compared with 23% observed for pertuzumab (Fig. 1B). The greatest effect was seen with COVA208 in which the Fynom C12 was fused to the N-terminus of the pertuzumab light chain. To establish whether the superior in vitro activity of COVA208 seen on NCI-N87 cells translates into superior antitumor activity in vivo, we compared the antitumor activity of COVA208 and pertuzumab in vivo in a NCI-N87 tumor xenograft therapy study. COVA208 led to rapid tumor regression, which was not achieved by pertuzumab (P < 0.0001; Fig. 2A). Importantly, COVA208 and pertuzumab had identical pharmacokinetic (PK) profiles and similar serum half-life (244 hours for COVA208 vs. 187 hours for pertuzumab; Fig. 2B). In addition, antibody-dependent cellular cytotoxicity was identical for COVA208 and pertuzumab (Fig. 2C). In addition, COVA208 maintained the favorable biophysical properties of pertuzumab such as long-term stability and monomeric aggregation state (data not shown) and potently suppressed growth of additional cell lines, including OE19 esophageal cancer, Calu-3 lung cancer, MDA-MB-
175 VII breast cancer, and BT-474 breast cancer cells, the latter being highly sensitive to trastuzumab (Supplementary Fig. S3).

COVA208 is unique in its ability to induce apoptosis and rapid internalization of HER2 on the NCI-N87 cell line

We then focused on COVA208 to elucidate its mode of action and its effect on tumor cell biology and HER2 signaling. Apoptosis was investigated in NCI-N87 cells by caspase-3/7 activity assay and by Tunel staining. Upon exposure to COVA208 \textit{in vitro}, NCI-N87 cells markedly upregulated caspase-3/7 activity in a dose-dependent fashion (Fig. 3A and Supplementary Fig. S4A). The activity was clearly above background after 2 days and further increased after 6 days of incubation, which was not observed with trastuzumab or pertuzumab. Around 65% of NCI-N87 cells contained fragmented DNA and were Tunel positive within 3 days after addition of COVA208 (Supplementary Fig. S4B). Addition of soluble HER2 ECD interfered with induction of apoptosis (data not shown), supporting that it is a specific effect of the COVA208–HER2 interaction. Thus, COVA208 potently induces apoptosis in NCI-N87 cells.

To investigate whether COVA208 is able to induce rapid internalization, we surface-labeled NCI-N87 cells with COVA208, pertuzumab, or trastuzumab, and tracked the distribution of the anti-HER2 agents with a fluorescently labeled detection antibody using confocal laser scanning microscopy (Fig. 3B and C). After 5 hours, pertuzumab and trastuzumab remained confined to the cell membrane and showed minimal cell uptake (9.5% or 10%, respectively). In contrast, around 65% of COVA208-relocated to the intracellular area, appearing in a punctate pattern typical of internalized material and resembling the pattern of the positive control pertuzumab plus geldanamycin, a Hsp90 inhibitor which leads to rapid internalization and degradation of HER2 (22). These observations confirm that COVA208 efficiently induces apoptosis and internalizes rapidly into cells, suggesting a novel mode of action.

COVA208 inhibits ligand-induced as well as ligand-independent HER2 downstream signaling

Trastuzumab inhibits ligand-independent activation occurring in HER2-overexpressing tumor cells (23). In contrast, pertuzumab but not trastuzumab is capable of blocking ligand-induced activation (2). We analyzed the effect of COVA208 on HER2 downstream signaling by focusing on key nodes of the HER3–PI3K–AKT and MAPK pathways. MCF-7 cells in the presence of the HER3 ligand HRG-1\textbeta were used as model for ligand-induced activation, and NCI-N87 cells were used as model for ligand-independent activation.

COVA208 retained the activity of the parental antibody pertuzumab and efficiently prevented HRG-1\textbeta-induced phosphorylation of HER3, AKT, and ERK in MCF-7 cells, whereas trastuzumab did not show any inhibitory activity, as expected (Fig. 4A). In NCI-N87 cells, COVA208 reduced the level of basal phosphorylated HER3 (pHER3) and pAKT similar to trastuzumab, whereas pertuzumab did not have any effect (Fig. 4B). Strikingly, COVA208-treated cells displayed a pronounced reduction of total HER3 levels. Thus, in addition to retaining pertuzumab’s ability to inhibit ligand-induced signaling, COVA208 was found to inhibit ligand-independent signaling similar to trastuzumab. However, the mechanism leading to this
effect involves downregulation of total HER3 levels and thus is different from trastuzumab.

**COVA208 elicits a more complete HER2 signaling inhibition as compared with trastuzumab and pertuzumab**

To identify additional HER2 signaling mediators that are specifically modulated by COVA208, lysates of treated NCI-N87 cells were analyzed by reverse phase protein array (RPFA) for differences in 167 phospho-protein or total protein levels (24). COVA208 clustered separately from trastuzumab and pertuzumab on a two-dimensional heat map, indicating that the effect of COVA208 on NCI-N87 cells is distinct from the effect achieved with conventional anti-HER2 antibodies (Supplementary Fig. S5). Of the 167 proteins analyzed, 22 proteins (13.1%) were found to be modulated, (log2 change > 0.5 compared with PBS) by COVA208, trastuzumab, or pertuzumab, of which 15 proteins (68%) were specifically modulated by COVA208 but neither by trastuzumab nor pertuzumab (Supplementary Table S1). RPPA data suggested that COVA208 induced downmodulation of HER2, phosphorylated EGFR, FOXM1, CDC2 (CDK1), cyclin D1, and phosphorylated RB1. In addition, it prevented upregulation of STAT3, JNK (MAPK18), and P38 (MAPK14) as observed with
trastuzumab and pertuzumab. Furthermore, phosphorylated SRC levels were slightly reduced by COVA208. NCI-N87 lysates were analyzed by immunoblot to confirm these findings. First, COVA208 specifically reduced HER2 and pHER2 protein, the latter being at least in part a consequence of reduced HER2 levels (Fig. 4C). Furthermore, COVA208 strongly reduced EGFR and pEGFR levels, which remained unaffected by trastuzumab or pertuzumab. Finally, COVA208 reduced pSRC, FOXM1, and CDC2, and prevented upregulation of pSTAT3, which are all oncogenic proteins or are described to be involved in tumor progression and resistance (25–28). In summary, COVA208 inhibits HER2 downstream signaling in a more complete and more efficacious manner as compared with the anti-HER2 antibodies trastuzumab and pertuzumab.

COVA208 inhibits tumor growth in different HER2-overexpressing xenograft models and downregulates HER2 and HER3 in vivo

In the KPL-4 model, trastuzumab and pertuzumab had only moderate activity on the growth of established xenograft tumors (Fig. 5A), whereas COVA208 strongly inhibited tumor growth ($P$ value $= 0.0009$) and showed significantly better antitumor activity than pertuzumab or trastuzumab ($P$ value $= 0.0279$ and 0.0015, respectively). COVA208 was also highly active in the GXA-3039 model ($P$ value $= 0.0136$) and was clearly superior to pertuzumab ($P$ value $= 0.0279$) and trastuzumab ($P$ value $= 0.0015$; Fig. 5B). Finally, COVA208 showed improved tumor growth inhibition compared with pertuzumab and trastuzumab in the CXF-1991 model, and resulted in a significant tumor growth inhibition ($P$ value $= 0.0045$, Fig. 5C). Thus, we observed significant in vivo activity of COVA208 in three patient-derived tumor models in which trastuzumab or pertuzumab showed no or little activity.

To establish whether HER2, HER3, or EGFR downregulation observed in vitro may contribute to the in vivo activity of COVA208, five GXA-3039 tumors from each treatment group were retreated 48 hours before the treatment groups were intended to be terminated. Tumors were collected and lysates were analyzed by immunoblot (Fig. 6). HER2 and HER3 protein levels were reduced in COVA208-treated tumors, whereas pertuzumab and trastuzumab had no or only a weak effect. This reduction correlated with lower pHER2, pHER3, and pAKT levels. EGFR levels and consequently pEGFR levels were also reduced by COVA208, but the difference was only weak. Hence, COVA208 leads to a reduction in total and phosphorylated HER family receptors, and resulted in decreased pAKT levels in vivo.
Discussion

The widespread use of HER2-targeted therapies has improved the treatment outcome of patients with breast and gastric carcinoma in the clinic. Still, the development of resistance to these therapies limits their clinical efficacy and urges the development of improved HER2-targeted therapies. By fusing the small 7-kDa anti-HER2 Fynomer C12 to the clinically validated antibody pertuzumab, we generated a bispecific HER2-targeting FynomAb with a novel mode of action and superior activity as compared with the parental antibody pertuzumab and also with trastuzumab, the antibody which forms the backbone of today’s clinical management of HER2-positive malignancies. COVA208 was highly active in the KPL-4 xenograft model, a tumor model with an activating PI3KCA mutation, which is associated with trastuzumab resistance in the clinic, as well as in the gastric GXA 3039 model, where trastuzumab showed no antitumor effect.

The in vivo activity of COVA208 may be explained by its unique effect on HER2 trafficking and HER2 downstream signaling. COVA208 induced rapid HER2 internalization in vitro and reduced HER2 levels in vitro and in vivo. In addition, COVA208 treatment was accompanied by a reduction in pHER2 levels in vitro and in vivo, suggesting that COVA208, unlike pertuzumab or trastuzumab, reduces phosphorylated HER2 levels and consequently the activation of oncogenic downstream signaling. This is exemplified by the reduction in pAKT signal in vitro and in vivo, and of pSRC, FOXM1, CDC2, and STAT3 in vitro. This finding is of importance because it is known from other receptors that endocytosis does not necessarily block the capability of receptors to participate in active signaling complexes.

In addition, we have found that COVA208 reduced HER3 and pHER3 surface levels in vitro and in vivo. In light of the importance of HER3 to propagate HER2-mediated signaling and of the mounting evidence that HER3 is involved in resistance to HER2-directed therapies, possibly through the interruption of a negative AKT–HER3 feedback loop, this observation offers the potential that COVA208 may prevent HER3-mediated resistance. Similarly, COVA208 treatment resulted in reduced EGFR levels in vitro, which may prevent resistance through a transition from HER2 to EGFR-dependence of HER2-overexpressing tumors.

Under physiologic conditions, several studies found that HER2 internalizes rather slowly, and monoclonal anti-HER2 agents have little or no effect on internalization (38, 39), even though Lub-de Hooge and colleagues showed that targeting HER2 with trastuzumab (carrying a residualizing111Indium label) may internalize efficiently (40). This contradiction could be explained by the rapid degradation and leakage of catabolites when using non-residualizing labels. Importantly, synergistic activity and accelerated internalization induced by anti-HER2 antibody combinations have previously been established in...
several studies (7–10). This observation was attributed to extensive receptor cross-linking producing large receptor clusters on the cell surface that are targeted for degradation. Because of the observed rapid internalization of COVA208, we speculate that the formation of HER2 clusters is an important underlying mechanism by which COVA208 exerts its activity. However, the Fynomer C12 and the pertuzumab paratope could in principle bind to the same receptor, thereby constraining the receptor in an inactive conformation. We cannot exclude that both binding mechanisms take place and contribute to the activity of COVA208.

More recently, two groups reported on the in vitro and in vivo activity of bispecific HER2-targeting agents (36, 41). To our knowledge, COVA208 is the first bispecific HER2-targeting agent that showed promising activity in patient-derived trastuzumab-resistant tumor models, highlighting the therapeutic potential of COVA208 in metastatic breast cancer. Whereas most of the previous studies showed antibody-mediated downmodulation of HER2 in vitro, we present a comprehensive analysis of the impact of COVA208-induced HER2 downmodulation on downstream signaling and apoptosis and compare it with the clinically validated antibodies trastuzumab and pertuzumab.

On the basis of our findings, we speculate that multiepitope targeting of other disease-associated cell surface receptors with reagents such as designed bispecific FynomAbs is capable of modulating molecular processes such as receptor trafficking, downstream signaling or induction of apoptosis in a novel, unique manner, and may result in superior anticancer therapeutics.

The study presented here also has important implications for the discovery of bi- or multispecific antibodies.

Figure 6. COVA208 reduces HER2 and HER3 levels in vivo.
A, immunoblots assessing the impact of COVA208 on HER family members and on downstream pAKT in human patient-derived GXA-3039 gastric tumor xenografts in vivo. Total tumor lysates were immunoblotted for phosphoprotein and for total protein levels as specified. Vinculin served as loading control. B, quantification of the immunoblot bands shown in A. Every dot represents the signal intensity relative to the vinculin signal for one tumor. Mean (n = 5) and SEM are indicated by whiskers. (*, P = 0.0232 for both comparisons).
Even though the four C12-pertuzumab FynomAbs were essentially indistinguishable in binding to cell surface HER2, we found that the in vitro activity of the bispecific FynomAbs was different and influenced by the FynomAb architecture, thus depending on the position at which the Fynomer was fused to the antibody. This is likely due to the different FynomAb architectures, which lead to different geometries and/or stoichiometries of FynomAb-HER2 complexes. The importance of the molecular architecture has also been shown with monospecific trastuzumab derivatives bearing nonnatural Fab arm arrangement (42) as well as for other EGFR-specific arms (43–45). We also found that the activity of bispecific reagents cannot be predicted from single-agent activities. Together, these observations suggest the investigation of bispecific molecules with different architectures to discover optimal lead candidates.

In summary, we have shown that the fusion of a small Fynomer to a clinically validated antibody can dramatically improve the therapeutic activity of this antibody. Such FynomAbs represent a novel approach to convert existing antibodies into bispecific therapeutic agents with superior efficacy. The anti-HER2 FynomAb COVA208 is a highly active HER2-targeting agent with a novel, differentiated mode of action, which results in a strong antitumor effect in vitro and in vivo in models resistant to conventional anti-HER2 antibodies. COVA208 treatment leads to a qualitatively distinct molecular response from those of trastuzumab or pertuzumab: COVA208 (i) elicits rapid internalization of HER2, (ii) induces apoptosis, and (iii) more effectively inhibits HER2 signaling than pertuzumab or trastuzumab. We thus believe that COVA208 is a promising HER2-targeting agent for further development in metastatic breast cancer.

Disclosure of Potential Conflicts of Interest
S. Brack, I. Attinger-Toller, B. Schade, F. Mourlane, K. Klupsch, R. Woods, J. Bertschinger, and D. Grabulovski have ownership interest (including patents) in Covagen AG. No potential conflicts of interest were disclosed by the other authors.

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