

Affibody-Mediated PNA-Based Pretargeted Cotreatment Improves Survival of Trastuzumab-Treated Mice Bearing HER2-Expressing Xenografts

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Treatment of patients with human epidermal growth factor receptor 2 (HER2)-expressing tumors using the monoclonal antibody trastuzumab increases survival. The Affibody-based peptide nucleic acid (PNA)-mediated pretargeted radionuclide therapy has demonstrated efficacy against HER2-expressing xenografts in mice. Structural studies suggest that Affibody molecules and trastuzumab bind to different epitopes on HER2. The aim of this study was to test the hypothesis that a combination of PNA-mediated pretargeted radionuclide therapy and trastuzumab treatment of HER2-expressing xenografts can extend survival compared with monotherapies. **Methods:** Mutual interference of the primary pretargeting probe Z_{HER2:342}-SR-HP1 and trastuzumab in binding to HER2-expressing cell lines was investigated in vitro. Experimental therapy evaluated the survival of mice bearing HER2-expressing SKOV-3 xenografts after treatment with vehicle, trastuzumab only, pretargeting using Affibody-PNA chimera Z_{HER2:342}-SR-HP1 and complementary probe ¹⁷⁷Lu-HP2, and combination of trastuzumab and pretargeting. The ethical permit limited the study to 90 d. The animals' weights were monitored during the study. After study termination, samples of liver and kidneys were evaluated by a veterinary pathologist for toxicity signs. **Results:** The presence of a large molar excess of trastuzumab had no influence on the affinity of Z_{HER2:342}-SR-HP1 binding to HER2-expressing cells in vitro. The affinity of trastuzumab was not affected by a large excess of Z_{HER2:342}-SR-HP1. The median survival of mice treated with trastuzumab (75.5 d) was significantly longer than the survival of mice treated with a vehicle (59.5 d). Median survival of mice treated with pretargeting was not reached by day 90. Six mice of 10 in this group survived, and 2 had complete remission. All mice in the combination treatment group survived, and tumors in 7 mice had disappeared at study termination. There was no significant difference between animal weights in the different treatment groups. No significant pathologic alterations were detected in livers and kidneys of treated animals. **Conclusion:** Treatment of mice bearing HER2-expressing xenografts with the combination of trastuzumab and Affibody-mediated PNA-based radionuclide pretargeting significantly increased survival compared with monotherapies. Cotreatment was not toxic for normal tissues.

Key Words: trastuzumab; PNA pretargeting; Affibody molecules; radionuclide therapy; HER2

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Human epidermal growth factor receptor 2 (HER2) is a transmembrane tyrosine kinase receptor overexpressed in about 20%–30% of breast cancer (1), 18% of gastric and gastroesophageal cancer (2), and 9%–32% of ovarian carcinoma cases (3). The humanized monoclonal antibody trastuzumab binds to domain IV in the extracellular part of HER2. HER2-targeted therapy using trastuzumab together with nontargeted chemotherapy is a standard combination for treatment of patients with HER2-positive breast, gastric, and gastroesophageal cancers (4–6). However, resistance to trastuzumab treatment is often developed despite preserved HER2 expression (7).

In vitro studies have demonstrated that treatment with trastuzumab increases the sensitivity of breast cancer cell lines to radiation (8,9). Clinical data suggest that adding trastuzumab to local adjuvant radiotherapy significantly reduces the risk of breast cancer locoregional recurrence (10,11). It would also be attractive to combine radiation therapy and trastuzumab medication in a systemic treatment of disseminated HER2-expressing cancers. HER2-targeted radionuclide therapy might be a solution for such a combination treatment. However, the straightforward use of monoclonal antibodies labeled with cytotoxic radionuclides is problematic for the treatment of solid tumors (12,13). The slow clearance of bulky antibodies from the circulation results in high exposure of the radiosensitive bone marrow, which prevents sufficient delivery of radionuclides to tumors. The use of smaller targeting agents such as Affibody (Affibody AB) molecules (7 kDa) might be an alternative. Safety, tolerability, and excellent targeting of HER2-expressing breast cancer metastases using Affibody molecules have been confirmed in clinics (14). However, direct application of radiolabeled Affibody molecules for radionuclide therapy is excluded because of their high reabsorption in the kidneys (15). To avoid renal reabsorption, an Affibody-based pretargeting approach was investigated, and peptide nucleic acid (PNA)-mediated pretargeting appeared to be the most efficient of the tested strategies (16). PNAs are synthetic DNA analogs with charge-neutral and flexible peptide-like backbones (Supplemental Fig. 1A; supplemental materials are available at <http://jnm.snmjournals.org>) and are highly stable in human serum and cellular extracts (17). The pretargeting system is based on 2 complementary

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15-meric PNA probes: the primary *HP1* probe and the secondary *HP2* probe (18). The secondary *HP2* probe contains both a DOTA chelator and tyrosine, which enable labeling with a variety of radiometals and radioiodine (16,19). The primary *HP1* probe is site-specifically conjugated to the HER2-targeting Affibody molecule $Z_{\text{HER2:342}}$, forming the $Z_{\text{HER2:342}}\text{-SR-HP1}$ Affibody-PNA chimera. A DOTA chelator is also incorporated into $Z_{\text{HER2:342}}\text{-SR-HP1}$, which enables labeling of this agent for preclinical development and for imaging within therapeutic applications (20). The sequences of $Z_{\text{HER2:342}}\text{-SR-HP1}$ and *HP2* are shown in Supplemental Figure 1B. This Affibody molecule-based PNA-mediated approach reduced renal uptake 70-fold compared with direct targeting using ^{177}Lu -labeled parental Affibody molecules (16). A schematic illustration of the PNA-mediated pretargeted therapy approach is shown in Supplemental Figure 1C. Experimental therapy using $Z_{\text{HER2:342}}\text{-SR-HP1}/^{177}\text{Lu-HP2}$ pretargeting significantly increased the median survival of mice bearing HER2-expressing xenografts without bone marrow and renal toxicity (21).

Importantly, structural studies (22) have shown that the epitope of the anti-HER2 Affibody binding to HER2 is distant from the epitope of trastuzumab (Fig. 1). This distance creates a precondition for independent binding of trastuzumab and the primary probe without mutual interference and should enable cotreatment using trastuzumab and Affibody-mediated pretargeting.

The goal of this study was to test the hypothesis that Affibody-mediated PNA-based pretargeting cotreatment can improve the survival of mice with HER2-expressing xenografts treated with trastuzumab. We evaluated interference between the binding of trastuzumab and $Z_{\text{HER2:342}}\text{-SR-HP1}$ to HER2-expressing cancer cell lines in vitro. Further, we compared the effect on tumor growth and survival of mice treated by cotargeting with the effects of treatment with trastuzumab and pretargeting alone.

MATERIALS AND METHODS

Carrier-free $^{177}\text{LuCl}_3$ was purchased from Curium. HER2-expressing ovarian cancer SKOV-3 and breast cancer BT474 cells were purchased from the American Type Culture Collection. Cells were cultured at 37°C in 5% CO_2 in RPMI medium (Flow Laboratories) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 IU/mL penicillin, and 100 $\mu\text{g/mL}$ streptomycin.

Radiolabeling

The PNA probes (*HP1* and *HP2*) and the HER2-binding Affibody were produced, purified, and characterized (Supplemental Figs. 2–5) as described earlier (18). The primary probe $Z_{\text{HER2:342}}\text{-SR-HP1}$ was prepared from the *HP1* probe and the Affibody molecule using an optimized conjugation protocol (23). The secondary probe *HP2* was radiolabeled with ^{177}Lu using a previously described method (23). For in vitro experiments, the primary probe $Z_{\text{HER2:342}}\text{-SR-HP1}$ was labeled with ^{177}Lu and trastuzumab was labeled with ^{125}I as previously described (24,25).

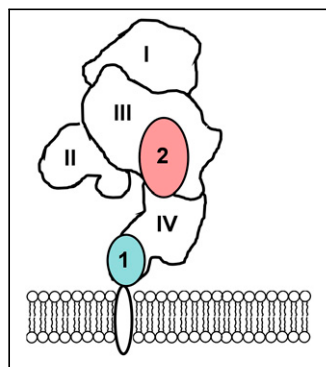


FIGURE 1. Epitopes for binding of trastuzumab (1) and Z_{HER2} Affibody molecule (2) to extracellular domain of HER2 (22).

In Vitro Studies

To check whether trastuzumab and $Z_{\text{HER2:342}}\text{-SR-HP1}$ interfere with each other's

binding to HER2-expressing cell lines in vitro, the following assay was performed. Cells were seeded into cell culture dishes with a density of 10^6 cells per dish. Four sets of 3 dishes were used for each conjugate. For blocking, nonlabeled $Z_{\text{HER2:342}}\text{-SR-HP1}$, trastuzumab, or the control anti-VEGF antibody bevacizumab (both from F. Hoffmann-La Roche) were added to sets of 3 dishes each to obtain concentrations of 200 nM. An equal volume of medium was added to the fourth set of dishes. After incubation at room temperature for 15 min, $^{177}\text{Lu-}Z_{\text{HER2:342}}\text{-SR-HP1}$ or ^{125}I -trastuzumab was added to all dishes to obtain a concentration of 1 nM. The cells were incubated for 1 h at 37°C. Thereafter, the cells were washed, the cells were detached by trypsin, and the cell-associated radioactivity was measured.

To evaluate the mutual interference of the targeting agents further, the affinity of their interaction with SKOV-3 cells in vitro was measured using a LigandTracer Yellow instrument (Ridgeview Instruments AB) as described earlier (19). Two concentrations of $^{177}\text{Lu-}Z_{\text{HER2:342}}\text{-SR-HP1}$ (1 and 3 nM) were used to estimate an association rate. The measurements were performed either in the presence of trastuzumab (70 nM) or in its absence. The association rate of ^{125}I -trastuzumab was measured with concentrations of 1 and 3 nM, in the presence of $Z_{\text{HER2:342}}\text{-SR-HP1}$ (140 nM) or in its absence. The calculations of affinities and their visualization were performed using the InteractionMap software (Ridgeview Diagnostics AB). Experiments were performed in duplicate.

Experimental In Vivo Therapy

Animal experiments were performed in accordance with the national legislation for work with laboratory animals. Approval was granted by the Ethical Committee for Animal Research in Uppsala. According to the ethical permit, the therapy should not continue longer than 90 d.

Female BALB/c nu/nu mice (Scanbur) were subcutaneously (abdomen area) implanted with 10^7 SKOV-3 cells. The subcutaneous xenograft model was selected because it permits more exact tumor volume measurement than a disseminated tumor model. The mice were randomly divided into 4 groups of 10 animals each. Treatment started 1 wk after tumor implantation. The injected activity and number of injections were calculated using mouse dosimetry data (21) to provide the absorbed doses: approximately 100 Gy to tumor, 20 Gy to kidney, and 1 G to bone marrow. Group A (control) was subcutaneously injected with 100 μL of 0.5% bovine serum albumin in phosphate-buffered saline. Group B (trastuzumab treatment) was subcutaneously (neck area) injected with 6 injections of trastuzumab (4 mg/kg for 2 wk followed by 2 mg/kg weekly, that is, the same dosing as in clinics (26)). Group C (radionuclide pretargeting treatment) was intravenously injected with 100 μg (7.6 nmol) of $Z_{\text{HER2:342}}\text{-SR-HP1}$ and 16 h later with 3.5 μg (0.68 nmol/16 MBq) of $^{177}\text{Lu-HP2}$ in a 100- μL solution containing 0.5% bovine serum albumin and 4 mg of Gelofusine (Hausmann Laboratories Ltd.) once a week. In total, 6 injections were performed. Group D (cotreatment) was treated with pretargeting (same as group C) in combination with trastuzumab (the same as group B). The tumor volumes at the start of treatment were 91 ± 25 , 99 ± 42 , 91 ± 34 , and $97 \pm 30 \text{ mm}^3$, for mice treated with 0.5% bovine serum albumin/phosphate-buffered saline, trastuzumab, pretargeting, and combined trastuzumab and pretargeting, respectively (no significant difference in ANOVA, Supplemental Fig. 6A).

Throughout the experiment, tumor volumes and body weights were monitored twice per week. The tumor volumes were determined by caliper measurement of the largest longitudinal (length) and transverse (width) diameter and calculated by the following formula: tumor volume = $\frac{1}{2} [\text{length (mm)}] \times [\text{width (mm)}]^2$.

The animals were euthanized when tumors reached a size of 1,000 mm^3 or became ulcerated, or if an animal's weight dropped by more than 10% during 1 wk or by more than 15% since the study began. Ninety days after treatment started, all animals were euthanized using xylazine/ketamine anesthesia. After euthanasia, tumor, kidneys, and liver were excised for subsequent histologic and immunohistochemistry evaluations.

The evaluations were performed at the Swedish National Veterinary Institute. The samples were formalin-fixed and paraffin-embedded. Tumor sections (4 μ m) were stained with hematoxylin and eosin for histologic evaluation and using the HercepTest Kit (Agilent) to determine HER2 expression.

Imaging During Experimental Therapy

To confirm efficient tumor pretargeting, SPECT/CT imaging of 2 mice from group C and 2 mice from group D was performed 24 h after every injection of ^{177}Lu -HP2. Imaging was performed using a nano-Scan SPECT/CT device (Mediso Medical Imaging Systems) under sevoflurane anesthesia as described earlier (21).

Statistical Analysis

Data were analyzed by an unpaired, 2-tailed *t* test or 1-way ANOVA with Bonferroni adjustment for multiple comparisons. Survival data were analyzed by a log-rank test. Tumor ulceration was considered to be identical to the tumor size endpoint in the test. Prism (version 7.03 for Microsoft Windows; GraphPad Software) was used to determine significant statistical differences ($P < 0.05$).

RESULTS

Radiolabeling

The correct molecular weights of the primary and secondary probes were confirmed using mass spectrometry (Supplemental Figs. 2 and 3). The purity of the probes was over 95% (Supplemental Figs. 4 and 5). Radiolabeling of HP2 with ^{177}Lu was achieved in high yields (>95%) at a maximum molar activity of 23.5 MBq/nmol.

In Vitro Studies

The results of the mutual blocking assay of trastuzumab and $Z_{\text{HER2:342}}\text{-SR-HP1}$ to HER2-expressing cell lines are presented in Figure 2. The cell-associated radioactivity of ^{125}I -trastuzumab for both HER2-expressing cell lines was significantly ($P < 0.005$) decreased when HER2 receptors were saturated with nonlabeled trastuzumab. No significant difference in binding was observed when cells were saturated with the primary agent $Z_{\text{HER2:342}}\text{-SR-HP1}$ or bevacizumab (Fig. 2A). The cell-associated radioactivity of ^{177}Lu - $Z_{\text{HER2:342}}\text{-SR-HP1}$ to both HER2-expressing cell lines was significantly ($P < 0.0001$) decreased when HER2 receptors were saturated with $Z_{\text{HER2:342}}\text{-SR-HP1}$. The binding of ^{177}Lu - $Z_{\text{HER2:342}}\text{-SR-HP1}$ was also significantly decreased by pretreatment with trastuzumab ($P < 0.05$). However, the decrease was much smaller in this case and was at the same level after treatment with the control anti-VEGF antibody bevacizumab (Fig. 2B).

The data concerning the effect of trastuzumab presence on the binding affinity of ^{177}Lu - $Z_{\text{HER2:342}}\text{-SR-HP1}$ to HER2 receptors are presented in Table 1 and Supplemental Figure 7. The data concerning the influence of $Z_{\text{HER2:342}}\text{-SR-HP1}$ presence on the binding affinity of ^{125}I -trastuzumab to HER2 receptors are presented in Table 2 and Supplemental Figure 8. No interference of trastuzumab presence on the binding affinity of ^{177}Lu - $Z_{\text{HER2:342}}\text{-SR-HP1}$, or of $Z_{\text{HER2:342}}\text{-SR-HP1}$ presence on the binding affinity of ^{125}I -trastuzumab, to HER2 receptors was observed.

Experimental In Vivo Therapy

SPECT/CT imaging (Fig. 3) demonstrated efficient delivery of ^{177}Lu to tumors. The tumor uptake in mice treated with both pretargeting and trastuzumab was on the same level as in mice treated with pretargeting alone (Supplemental Fig. 9). Six pretargeted treatment cycles did not decrease the maximum uptake of ^{177}Lu -HP2 in tumors (Supplemental Fig. 9).

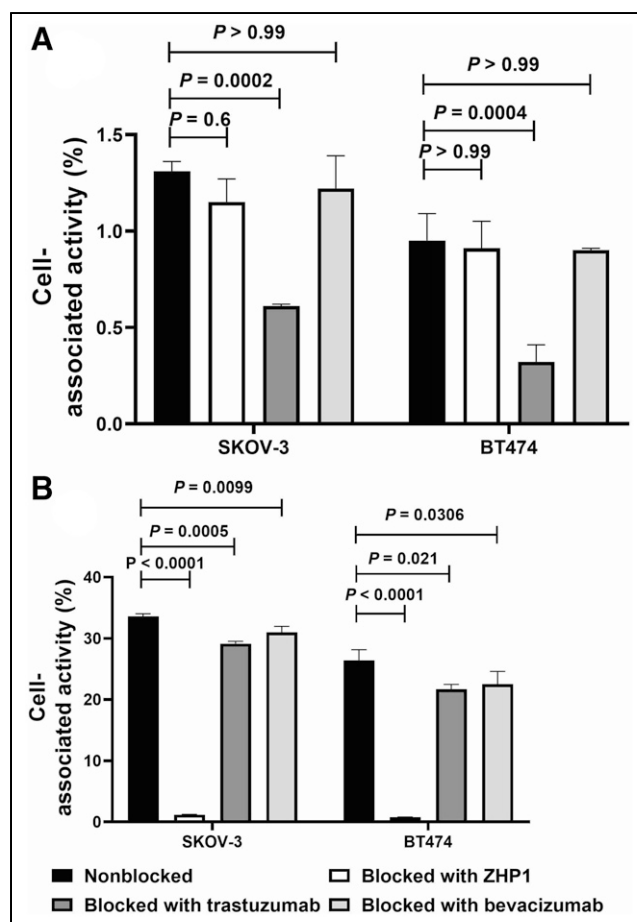


FIGURE 2. In vitro binding specificity of ^{125}I -trastuzumab (A) and ^{177}Lu - $Z_{\text{HER2:342}}\text{-SR-HP1}$ (B) on HER2-expressing SKOV-3 and BT-474 cells. For both experiments, 1 nM of labeled conjugate and 200 nM of blocking agents were used. Data are presented as average value from 3 samples \pm SD. ZHP1 = $Z_{\text{HER2:342}}\text{-SR-HP1}$.

Information concerning tumor growth, body weight, and survival in different groups of animals is presented in Figures 4 and 5. All treatment modalities slowed the tumor growth rate. Seven days after the first injection, the average tumor volume in all treatment groups was significantly smaller than the average volume in the control group (group A) (Supplemental Fig. 6B). The median survival was the shortest in the control group (59.5 d), and all animals in this group were euthanized by day 74 (Fig. 5A). The treatment with trastuzumab alone (group B) significantly increased ($P < 0.05$) the median survival to 75.5 d (Fig. 5A). Two of 4 surviving mice had very small, less than 10 mm^3 , tumors. Pretargeted monotherapy (group C) was somewhat more efficient than monotherapy with trastuzumab. The median survival in group C was not reached within the permitted experiment time, but there was no significant difference between survival in groups C and D (Figs. 4 and 5). Tumors in 2 of 6 surviving mice disappeared completely at the time of euthanasia, and 1 mouse had a tumor smaller than 10 mm^3 . The combination of pretargeting and trastuzumab treatment (group D) was the most efficient, according to the results of the log-rank test (Fig. 5A). All mice survived until study termination. Seven mice had complete remission at that moment, and 2 mice had a tumor smaller than 10 mm^3 . Still, the small tumors contained viable tumor cells undergoing mitosis (Supplemental Fig. 10).

TABLE 1

Association Rate (k_a), Dissociation Rate (k_d), and Equilibrium Dissociation (K_D) Constants for Interaction Between ^{177}Lu -Z_{HER2:342}-SR-HP1 and HER2-Expressing SKOV-3 Cells in Presence and Absence of Trastuzumab Determined Using Interaction Map Analysis of LigandTracer Sensorgrams

Parameter	k_a [(1/M \times s) $\times 10^4$]	k_d [(1/s) $\times 10^{-6}$]	K_D (pM)
^{177}Lu -Z _{HER2:342} -SR-HP1 only	3.5 ± 0.4	2.6 ± 0.6	78 ± 28
^{177}Lu -Z _{HER2:342} -SR-HP1 with trastuzumab	3.5 ± 0.3	2.90 ± 0.04	85 ± 7

TABLE 2

Association Rate (k_a), Dissociation Rate (k_d), and Apparent Equilibrium Dissociation (K_D) Constants for Interaction Between ^{125}I -Trastuzumab and HER2-Expressing SKOV-3 Cells in Presence and Absence of Z_{HER2:342}-SR-HP1 Determined Using Interaction Map Analysis of LigandTracer Sensorgrams

Parameter	k_a [(1/M \times s) $\times 10^5$]	k_d [(1/s) $\times 10^{-6}$]	K_D (pM)
^{125}I -trastuzumab only	5.6 ± 0.0	2 ± 0.7	3.7 ± 1.2
^{125}I -trastuzumab with ZHER2:342-SR-HP1	4.5 ± 0.2	1.9 ± 0.1	4.4 ± 0.7

Although tumors from groups A, B, and C retained HER2 expression on a 3+ level, the remaining tumors from group D had heterogeneous HER2 expression (Supplemental Fig. 11).

When the outcomes were categorized as no response (animals were euthanized before the study endpoint), response (animals with

visible tumors survived until the study endpoint), and remission (no visible tumors at the study endpoint), a Pearson χ^2 test revealed significant differences among all groups (Supplemental Fig. 12).

The therapy was well tolerated. The average weight of the animals in the treatment groups did not differ significantly (ANOVA, $P < 0.05$) from the average weight in the control group at any time point (Fig. 5B). According to the histopathology evaluation, there was no evidence of renal toxicity in the treatment groups. No structures of the renal parenchyma in the treated animals differed from these structures in the kidneys of mice in the control group (Supplemental Fig. 13). In some livers of mice from the treatment groups, scattered mitoses were found among the hepatocytes, a finding that could indicate an ongoing regenerative activity (Supplemental Fig. 14). In the livers of 2 mice from the combined-treatment group, infiltrates of mononuclear leukocytes were observed in a few portal areas. According to the pathologist, these changes were very subtle.

DISCUSSION

Targeted therapies offer the advantage of a specific antitumor effect and minimize systemic toxicity. Therefore, there is an apparent trend to increase the targeted component in the treatment, such as by combining trastuzumab and pertuzumab treatment already in the first-line therapy of advanced breast cancer (27). In addition, the combination of 2 different therapeutics with different mechanisms of action has a potential to suppress resistance development (28). The use of radionuclide targeting is an attractive addition to trastuzumab because this antibody sensitizes the tumor to irradiation. Such a combination requires an absence of mutual interference of targeting agents in the binding to HER2. Trastuzumab does not interfere with binding of the small monomeric form of the anti-HER2 Affibody in vitro or in vivo (29). However, coupling of HP1 makes the Z_{HER2:342}-SR-HP1 chimera bulkier, and interference could not be excluded. The results of the binding test (Fig. 2A) showed that there was no significant decrease in cell-associated radioactivity of ^{125}I -trastuzumab when HER2-expressing cancer cells were pretreated with a large molar excess of Z_{HER2:342}-SR-HP1. There was a small but significant reduction of cell-bound radioactivity of ^{177}Lu -Z_{HER2:342}-SR-HP1 when cells were pretreated with an excess of trastuzumab.

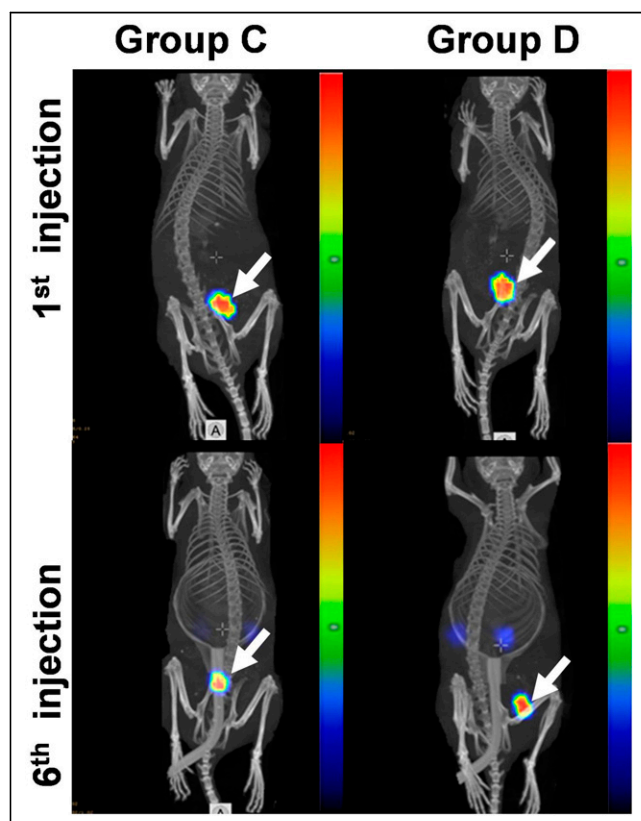


FIGURE 3. Representative SPECT/CT images (maximum-intensity projections) of mouse from treatment group C and mouse from treatment group D. Imaging was performed 24 h after first injection and sixth injection of ^{177}Lu -HP2. Arrows point at tumors. Linear relative scale (arbitrary units normalized to a maximum count rate) is applied.

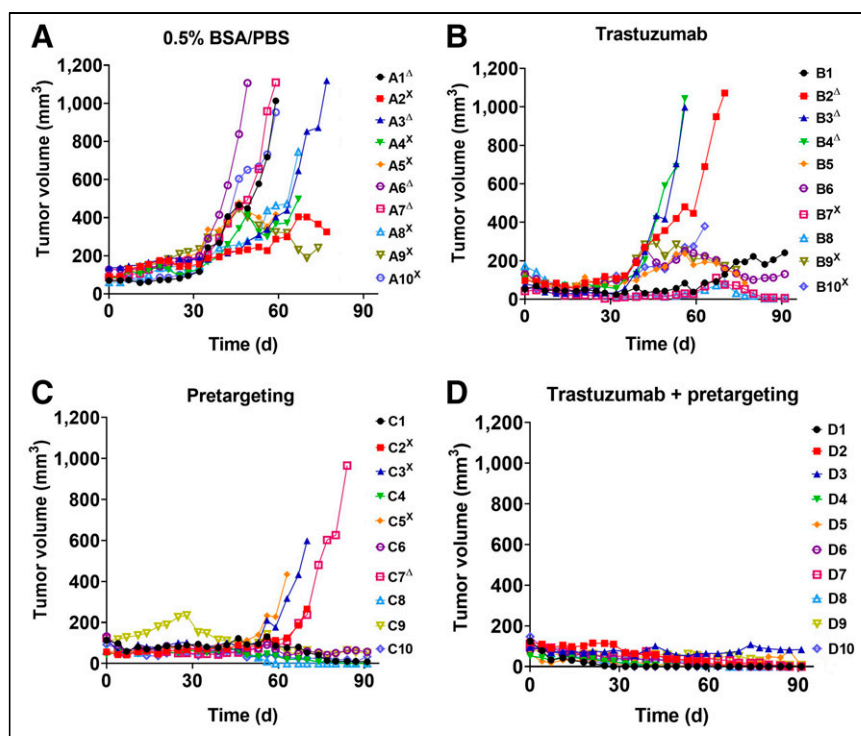


FIGURE 4. Experimental therapy with tumor volume growth curves for individual mice in each group. SKOV-3 cells were subcutaneously implanted into belly of nude BALB/c nu/nu-mice. Mice were treated with 0.5% bovine serum albumin/phosphate-buffered saline (BSA/PBS) (control) (A), trastuzumab only (B), pretargeting only (C), and combination of trastuzumab and pretargeting (D). ^ΔMice were euthanized when volume of subcutaneous xenografts exceeded 1,000 mm³. [×]Mice were euthanized when bleeding ulcers on xenografts were observed.

However, treatment with the control antibody bevacizumab (which does not bind to HER2) had a similar effect. Thus, this phenomenon should not be associated with the blocking of ¹⁷⁷Lu-Z_{HER2:342}-SR-HP1 binding to the target. The measurement by LigandTracer did not show any impact of trastuzumab presence on the affinity of ¹⁷⁷Lu-Z_{HER2:342}-SR-HP1 binding to HER2-expressing cells (Table 2; Supplemental Fig. 7) or of Z_{HER2:342}-SR-HP1 presence on the affinity of ¹²⁵I-trastuzumab binding (Table 2; Supplemental Fig. 8). No influence of trastuzumab treatment on pretargeted delivery of ¹⁷⁷Lu to tumors was observed by SPECT imaging performed during therapy (Fig. 3; Supplemental Fig. 9). The level of uptake was approximately the

appreciably more radioresistant than 2 other cell lines with high HER2 expression, SK-BR-3 and BT-474 (30), and can be considered the worst-case model for radionuclide therapy.

A precondition for a successful combination treatment is an additive effect of the drugs on tumors but different toxicity profiles. This requirement was fulfilled in the proposed therapy. No toxicity of anti-HER2 Affibody molecules was detected in rodents (31). No Affibody-caused toxicity has been found in clinical studies (14), nor has PNA toxicity has been found in clinics (32). The only toxicity in the pretargeted radionuclide therapy is expected from the radionuclide, and the kidneys are expected to be the dose-limiting organ (21). The most severe side effect of trastuzumab is cardiotoxicity (33). Hence, the toxicity profiles of the proposed therapeutics are different. Pathology investigation did not reveal any serious side effects in this study. HER2-targeted therapy remains an area of intensive development (34). As far as we know, our approach is the only one that does not rely on radioresistance of mice but keeps the dose limits to both kidneys and bone marrow within limits accepted in clinics.

CONCLUSION

We have shown that combined injection of the monoclonal antibody trastuzumab with Affibody-mediated PNA-based pretargeting

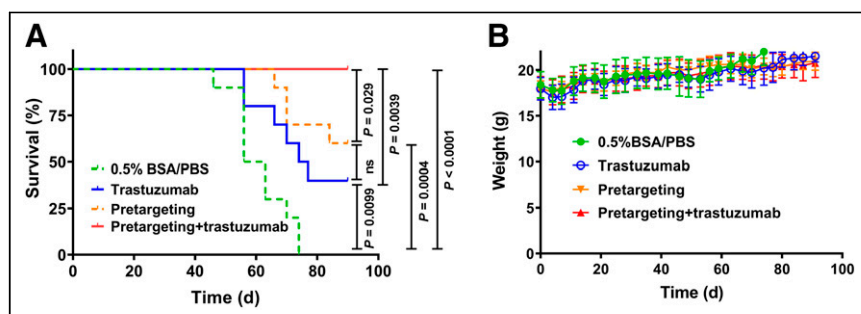


FIGURE 5. (A) Survival of BALB/C nu/nu-mice with SKOV-3 xenografts treated with pretargeting plus trastuzumab, pretargeting alone, trastuzumab alone, or 0.5% bovine serum albumin in phosphate-buffered saline (0.5% BSA/PBS). (B) Average animal weight during therapy. Data are presented as average value \pm SD. BSA = bovine serum albumin; PBS = phosphate-buffered saline.

significantly increased the median survival in mice bearing HER2-expressing tumors compared with trastuzumab only. This makes the combination of trastuzumab and pretargeting a promising candidate for clinical translation.

DISCLOSURE

This work has been supported by grants from the Swedish Cancer Society, Swedish Research Council, and Swedish Agency for Innovation VINNOVA. Vladimir Tolmachev and Anna Orlova are members of the Technical Advisory Board of Affibody AB (Solna, Sweden) and have shares in this company. No other potential conflict of interest relevant to this article was reported.

KEY POINTS

QUESTION: Does the addition of PNA-mediated Affibody-based pretargeted therapy increase the efficacy of trastuzumab treatment of HER2-expressing xenografts?

PERTINENT FINDINGS: The combination of PNA-mediated Affibody-based radionuclide pretargeting and trastuzumab therapy has a stronger antitumor effect than either modality alone. The combination treatment is not associated with any additional toxicity.

IMPLICATIONS FOR PATIENT CARE: Data from this study support further development of combination targeting therapy, which might improve the survival of patients with HER2-expressing cancer.

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