A PI3K p110α-selective inhibitor enhances the efficacy of anti-HER2/neu antibody therapy against breast cancer in mice

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ABSTRACT

Combination therapies with phosphoinositide 3-kinase (PI3K) inhibitors and trastuzumab (anti-human epidermal growth factor receptor [HER2/neu antibody) are effective against HER2+ breast cancer. Isoform-selective PI3K inhibitors elicit anti-tumor immune responses that are distinct from those induced by inhibitors of class I PI3K isoforms (pan-PI3K inhibitors). The present study investigated the therapeutic effect and potential for stimulating anti-tumor immunity of combined therapy with an anti-HER2/neu antibody and pan-PI3K inhibitor (GDC-0941) or a PI3K p110α isoform-selective inhibitor (A66) in mouse models of breast cancer. The anti-neu antibody inhibited tumor growth and enhanced anti-tumor immunity in HER2/neu+ breast cancer TUBO models, whereas GDC-0941 or A66 alone did not. Anti-neu antibody and PI3K inhibitor synergistically promoted anti-tumor immunity by increasing functional T cell production. In the presence of the anti-neu antibody, A66 was more effective than GDC-0941 at increasing the fraction of CD4+, CD8+, and IFN-γ+CD8+ T cells in the tumor-infiltrating lymphocyte population. Detection of IFN-γ levels by enzyme-linked immunospot assay showed that the numbers of tumor-specific T cells against neu and non-neu tumor antigens were increased by combined PI3K inhibitor plus anti-neu antibody treatment, with A66 exhibiting more potent effects than GDC-0941. In a TUBO (neu+) and TUBO-P2J (neu−) mixed tumor model representing immunohistochemistry 2+ tumors, A66 suppressed tumor growth and prolonged survival to a greater extent than GDC-0941 when combined with anti-neu antibody. These results demonstrate that a PI3K p110α-isofor-selective inhibitor is an effective adjunct to trastuzumab in the treatment of HER2-positive breast cancer.

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1. Introduction

Breast cancer is the most common cancer and a major cause of cancer-related death in women. The main treatment is chemotherapy against molecular targets expressed on the surface of breast cancer cells, including human epidermal growth factor receptor (HER2) (also known as neu and ERBB2). HER2 gene amplification or protein overexpression is detected in 15%–20% of breast cancer patients and is associated with aggressive disease, high recurrence rate, and reduced survival.1,2 Several drugs targeting HER2 have been developed such as trastuzumab (Herceptin), a humanized recombinant monocalonal antibody against HER2 that has demonstrated efficacy in mouse models and in patients with HER2-amplified breast cancer.3-4 However, non-responders to trastuzumab are increasingly observed due to the emergence of resistance during the course of therapy. Combination therapy with trastuzumab is a possible strategy for overcoming this resistance and thereby improving HER2+ breast cancer outcome.

The phosphoinositide 3-kinase (PI3K) signaling pathway plays an important role in cell proliferation and survival in response to oncogenic changes and growth factors such as HER2. This pathway is aberrantly activated in most human cancers including breast cancer, and is therefore a promising therapeutic target.5 Given that PI3K signaling functions downstream of HER26 and is upregulated in trastuzumab-treated breast cancer, its hyperactivation has been proposed as a mechanism underlying trastuzumab resistance.7 Several clinical studies are currently investigating the potential of PI3K inhibitors to overcome resistance to HER2-targeted chemotherapy.8 PI3Ks are divided into three classes according to structures and substrate preference.9 Only class I PI3Ks—which utilize phosphatidylinositol 4,5-bisphosphate as a substrate to generate phosphatidylinositol 3,4,5-triphosphate (PIP3)—have been
linked to cancer. Several class I PI3K inhibitors have been developed as anti-cancer drugs; these include compounds that target specific class I PI3K catalytic isoforms (p110α, p110β, p110γ, or p110δ) and pan-PI3K inhibitors that have similar potency against all class I PI3K catalytic isoforms. Since activating mutations in the PIK3CA gene encoding p110α are common in solid tumors, p110α-selective inhibitors have received the most attention. Pre-clinical data indicate that these compounds are as effective as pan-PI3K inhibitors at suppressing the growth of PIK3CA-mutant as well as HER2-amplified tumor cells. However, these studies did not consider the PIK3CA as the most attention. Pre-clinical data indicate that these compounds may have clinical benefits.

To investigate this possibility, in this study we examined the therapeutic effects and capacity for anti-tumor immunity of combination therapy with an anti-HER2/neu antibody and PI3K p110α-selective inhibitor in mouse models of HER2/neu-positive breast cancer. Moreover, the therapeutic efficacy of the combination treatment was evaluated in mixed tumor models that were Immunohistochemistry (IHC)2+ for HER2 and mimicked clinical tumor heterogeneity.

2. Materials and methods

2.1. Reagents

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise noted. The mouse monoclonal anti-neu antibody (clone 7.16.4) was produced in our laboratory. GDC-0941 (pan-PI3K inhibitor, M1715) and A66 (p110α-selective inhibitor, M1819) were obtained from Abmole Bioscience (Houston, TX, USA). GDC-0941 was reconstituted in 5% dimethylsulfoxide and 0.5% hydroxypropyl methylcellulose, and A66 was dissolved in an aqueous solution of 10% 2-hydroxypropyl-β-cyclodextrin. Anti-CD8 depleting antibody (YTS 169.4) and anti-CD4 depleting antibody (GK1.5) were purchased from BioXcell (West Lebanon, NH, USA).

2.2. Mice

Female BALB/c mice (5–6 weeks of age) were purchased from Orient Bio (Daejeon, Korea) and were used for experiments when they reached a body weight of 17–20 g (6–8 weeks of age). The mice were allowed to acclimate before experiments under specific pathogen-free conditions at the animal care facility of the College of Medicine (Inje University). Experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Inje University (protocol no. 2015-002).

2.3. Cell lines

The TUBO mammary carcinoma cell line was cloned from a spontaneous mammary tumor in a BALB Neu transgenic mouse provided by Joseph Lustgarten (Mayo Clinic, Phoenix, AZ, USA). The TUBO-P2J cell line was established from a metastatic lung nodule of an anti-neu antibody-treated TUBO-bearing mouse. The TUBO and TUBO-P2J cell lines were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum (FBS; HyClone, Logan, UT, USA), 10% NCTC-109 medium, 2 mM l-glutamine, 0.1 mM minimal essential medium non-essential amino acids, 100 U/ml penicillin, and 100 mg/ml streptomycin. The cells were maintained in a humidified incubator at 37°C and 5% CO2 and were tested monthly for mycoplasma using the PCR Mycoplasma Detection kit (Takara Bio, Otsu, Japan). Cell lines passaged for fewer than 2 months after thawing were used for experiments.

2.4. Cell viability assay

Viable cells were quantitated with the In Vitro Toxicology Assay kit (TOX6; Sigma-Aldrich) according to the manufacturer’s instructions. Briefly, 1 × 10⁴ cells/well were seeded in 96-well culture plates and allowed to attach. Indicated doses of anti-neu antibody and/or PI3K inhibitor were administered for 72 h. Cells were then fixed with 10% trichloroacetic acid for 1 h at 4°C, stained with sulforhodamine B for 15 min, and washed three times with 1% acetic acid. The incorporated dye was solubilized with 10 mM Tris base (pH 8.8). Absorbance was measured at 565 nm with an EL800 microplate reader (Bio-Tek Instruments, Winooski, VT, USA).

2.5. T cell proliferation assay

Antigen-specific or non-specific T cell proliferation was evaluated with the carboxyfluorescein diacetate (CFSE) proliferation assay. Splenocytes were isolated from CL4-hemagglutinin (HA) T cell receptor (TCR) transgenic or naive BALB/C mice and labeled with CFSE for 4 min at 37°C, then cultured in the presence of anti-neu antibody and/or PI3K inhibitor for indicated times. The cells were activated with 0.1 μg/mL HA512-520 peptide (IYSTVASSL) or 0.5 μg/mL CD3/CD28 antibody, then harvested and analyzed by flow cytometry using a FACS Canto II instrument (BD Biosciences, Franklin Lakes, NJ, USA) and FlowJo software (Tree Star, Ashland, OR, USA).

2.6. In vivo treatment

TUBO cells (5 × 10⁵ cells/mouse) were subcutaneously (s.c.) injected into the backs of 6- to 8-week-old mice anesthetized with a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). For tumor re-challenge experiments, mice that were tumor-free for at least 1 month after complete rejection of the treatment or surgical tumor removal were re-challenged by separate s.c. injections of 5 × 10⁵ TUBO cells (left upper back) and 5 × 10⁴ TUBO-P2J cells (right upper back) at sites differing from that of the primary tumor. For the heterogeneous mixed-tumor model, TUBO (5 × 10⁵) and TUBO-P2J (1.25 × 10⁵) cells mixed at a
ratio of 4000:1 were injected into mice. Tumor volume was calculated as \((a \times b \times c)/2\), where \(a-c\) are the measurements along three orthogonal axes. When the tumor volume reached approximately 150 mm\(^3\), mice were treated with a vehicle control antibody (mIgG), anti-neu antibody, PI3K inhibitor, or anti-neu antibody/PI3K inhibitor combination. An anti-neu antibody (clone 7.16.4) was intraperitoneally (i.p.) administered at doses of 200 and 100 \(\mu\)g on indicated days. PI3K inhibitor—either GDC-0941 (125 mg/kg) or A66 (100 mg/kg)—was administered daily by oral gavage starting at the first day of the anti-neu antibody injection for indicated periods. For T cell depletion experiments, 200 \(\mu\)g of anti-CD8 or -CD4 antibody were i.p. injected on days -1 and 3 of anti-neu antibody treatment (day 17 and 21 in the TUBO tumor model and days 14 and 18 in the mixed tumor model after tumor cell inoculation). Blood samples were collected at three time points (days 18, 28, and 38 in the TUBO tumor model and days 15, 25, and 35 in the mixed tumor model) and the percentage of CD4\(^+\) or CD8\(^+\) T cells was determined by flow cytometry.

### 2.7. Flow cytometry

Tumor-infiltrating lymphocyte (TIL) populations were evaluated by flow cytometry.\(^4\) Briefly, single-cell suspensions were obtained by collagenase digestion and incubated with fluorochrome-conjugated monoclonal antibodies against surface markers after incubation with the Fc blocker 2.4G2. Antibodies against mouse CD45 (clone 30-F11), CD3 (clone 14-2C11), CD8\(\alpha\) (clone 53-6.7), and CD4 (clone GK1.5) conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), and PE-Cy7, respectively, were purchased from eBioscience (San Diego, CA, USA). Intracellular labeling was performed using the BD Cytofix/Cytoperm\(^\text{TM}\) kit (BD Biosciences) according to the manufacturer’s instructions. Briefly, 1.5 \(\times\) 10\(^6\) cells/ml were stimulated with phorbol myristate acetate (50 ng/ml) plus ionomycin (750 ng/ml) in Golgi Plug\(^\text{TM}\)-containing medium for 5 h at 37°C, then labeled with anti-mouse CD45-APC, CD3-FITC, CD8-PeCy7, and CD4-FITC antibodies. After washing, the cells were fixed with Cytofix/ Cytoperm solution and Perm/Wash\(^\text{TM}\) buffer and then labeled with an anti-mouse IFN-\(\gamma\)-PE (clone XMG1.2), FoxP3-PE (clone FJK-16) antibody, or phosphorylated (p)AKT (S473)-PE (clone SDRNR) antibodies (all from eBioscience), and analyzed by flow cytometry.

### 2.8. Measurement of IFN-\(\gamma\) secretion by T cells by enzyme-linked immunospot (ELISPOT) assay

The ELISPOT assay was performed using the Mouse IFN-\(\gamma\) ELISPOT kit (BD Biosciences) according to the manufacturer’s instructions. Briefly, ELISPOT 96-well plates were coated overnight at 4°C with capture antibody and then blocked with 10% FBS in RPMI 1640 medium. Splenocytes (4 \(\times\) 10\(^5\)) were co-cultured at a 10:1 ratio with tumor antigen-pulsed bone marrow-derived dendritic cells (BMDCs) that had been pulsed overnight with triplicates of freeze-thawed lysed TUBO or TUBO-P2J cells. Concanaavalin A (5 \(\mu\)g/ml) was used as a positive control to confirm the gamma interferon responsiveness of cells cultured with tumor antigen-pulsed BMDCs. After a 24-h incubation, cells were removed from the wells by washing, and a biotinylated anti-IFN-\(\gamma\) antibody (clone XMG1.2) was added to the wells. Spots were developed using streptavidin-conjugated horseradish peroxidase and 3-amino-9-ethyl-carbazole substrate. IFN-\(\gamma\) spots were counted with an ELISPOT plate reader (AID Autoimmun Diagnostika, Strassberg, Germany).

### 2.9. Statistical analysis

Results are presented as mean \(\pm\) SEM, and were analyzed with the unpaired Student’s t and log-rank tests using Prism v. 5 software for Windows (GraphPad Inc., San Diego, CA, USA). Animal survival is presented as a Kaplan-Meier survival curve and was evaluated with the log rank test.

### 3. Results

#### 3.1. Combined PI3K inhibitor and anti-neu antibody treatment enhances T cell infiltration into tumors and induction of tumor-specific T cells

To evaluate the anti-tumor activity of PI3K inhibitor combined with anti-neu antibody, we performed an in vitro cell viability assay in TUBO tumor cells treated with anti-neu antibody and PI3K inhibitors. The antibody alone slightly reduced cell viability while a pan-PI3K inhibitor (GDC-0941) alone strongly induced tumor cell death (Fig. 1A). The combination of anti-neu antibody and GDC-0941 had an additive effect on anti-tumor activity. In contrast to the pan-PI3K inhibitor, a PI3K p110\(\alpha\) isofrom-selective inhibitor (A66) alone had less effect on cell viability; however, anti-neu antibody combined with A66 acted synergistically to inhibit tumor cell survival (Fig. 1A).

Mice bearing TUBO tumors were treated with anti-neu antibody or PI3K inhibitor (GDC-0941 or A66) alone, or with anti-neu antibody plus PI3K inhibitor. Tumor growth was potently inhibited by anti-neu antibody, whereas a slight but non-significant decrease was observed upon treatment with GDC-0941 or A66. Combining either inhibitor with the antibody strongly suppressed tumor growth. Immediately after A66 plus anti-neu antibody treatment, tumors regressed more rapidly as compared to other treatment groups, although no difference was observed between treatment with the inhibitor/antibody combination and antibody alone (Fig. 1B). At > 30 days after complete rejection of the treatment or surgical tumor removal, tumor-free mice were re-challenged with TUBO cells. There were no tumors established in the re-challenged mice upon treatment with anti-neu antibody alone or in combination with PI3K inhibitor (data not shown).

TILs in tumors from TUBO tumor-bearing mice were analyzed by flow cytometry on day 11 after the first anti-neu antibody treatment. The CD4\(^+\) cell population in tumors was unaltered by antibody and/or inhibitor treatment (Fig. 1C and Suppl. Fig. 1A). However, the CD3\(^+\)CD4\(^+\) and CD3\(^+\)CD8\(^+\) fractions of the CD45\(^+\) cell population were increased in the presence of anti-neu antibody with or without PI3K inhibitor (Fig. 1C and Suppl. Fig. 1B). When A66 was used in combination with anti-neu antibody, the sizes of the CD8\(^+\) and CD4\(^+\) T cell populations were increased relative to those in control mice treated with vehicle or antibody alone. Similar albeit less potent...
effects were observed using GDC-0941 in combination with the antibody. The percentage of IFN-γ+CD8+ T cells was increased by anti-neu antibody treatment alone (Fig. 1C). GDC-0941 or A66 in combination with the antibody also increased the IFN-γ+CD8+ T cell fraction, while no obvious effect was observed with either inhibitor alone (Fig. 1C and Suppl. Fig. 1B). In addition, anti-neu antibody combined with either PI3K inhibitor increased the number of IFN-γ+CD8+ T cells as compared to antibody treatment alone. The percentages of CD45+ immune cells, CD8+ and CD4+ T cells, and IFN-γ+CD8+ T cells in draining lymph nodes (DLNs) were unchanged by anti-neu antibody and/or PI3K inhibitor treatment (Suppl. Fig. 1C).

We next evaluated whether either PI3K inhibitor in combination with anti-neu antibody could induce tumor-specific T cells with the IFN-γ ELISPOT assay using splenocytes isolated from TUBO tumor-bearing mice, with or without treatment with each agent. Consistent with the above findings, the number of TUBO-specific IFN-γ+ T cells was increased by antibody treatment alone, and this was potentiated by PI3K inhibitor (Fig. 1D). Interestingly, A66 had a more potent effect than GDC-0941 on the numbers of IFN-γ+ T cells specific to TUBO tumor cells when administered in conjunction with the antibody.

3.2. CD8+ T cells are essential for combination therapy with a PI3K inhibitor and the anti-neu antibody in a TUBO tumor model

To determine whether T cells are essential for combination therapy-induced tumor regression, we evaluated the effects of this treatment under conditions where T cells were depleted using anti-CD8 or anti-CD4 depleting antibody. These antibodies depleted the corresponding T cell population (Suppl. Fig. 2A). CD8+ T cells were more critical than CD4+ T cells for the control of tumor mass by antibody and/or inhibitor treatment, although both T cell populations played important roles (Fig. 2 and Suppl. Fig. 2B). Interestingly, the anti-tumor activity
of the combined anti-neu antibody/GDC-0941 treatment was maintained even in the absence of each T cell subset (Fig. 2A).

3.3. PI3K p110α-selective inhibitor A66 is effective in HER2/neu IHC2+ tumor models when combined with anti-neu antibody

HER2 status in breast cancer is evaluated by IHC and in situ hybridization (ISH). IHC3+ cancers (strong complete membrane staining in > 10% of tumor cells) are susceptible to herceptin (anti-HER2 antibody) treatment; however, IHC2+ cancer (weak to moderate complete staining in > 10% of tumor cells) are less susceptible unless the HER2 gene is amplified (ISH+). In a previous study we developed a preclinical tumor model mimicking intra-tumoral heterogeneity using TUBO mice and TUBO-P2J cells; this tumor model was HER2/neu IHC2+ and was partially responsive to anti-neu antibody treatment. In addition, these tumor-bearing mice died from spontaneous lung metastasis since TUBO-P2J cells have high metastatic potential and unlike TUBO cells, are resistant to PI3K inhibitors. Treatment with 1 μM GDC-0941 or A66 did not induce death in TUBO-P2J cells (Fig. 3A). On the other hand, viability was reduced at 10 μM GDC-0941, although this is higher than the effective dose of GDC-0941 for PI3K inhibition. No additional nor synergic effects on TUBO-P2J cell viability were observed upon anti-neu antibody and PI3K inhibitor treatment (Fig. 3A).

Using these mixed-tumor mouse models, we evaluated the effect of combined anti-neu antibody and PI3K inhibitor therapy on tumor growth and survival. Treatment with antibody alone suppressed primary tumor growth, which was not the case with inhibitor alone (data not shown). A66 in combination with the antibody reduced primary tumor growth as compared to antibody alone or vehicle. However, GDC-0941 decreased the antitumor activity of anti-neu antibody when the two agents were administered in combination (Fig. 3B).

Furthermore, A66 in combination with the antibody prolonged survival (Fig. 3C). Similar to the observed effects on primary tumor growth, GDC-0941 in combination with anti-neu antibody did not increase survival relative to anti-neu antibody alone. We analyzed TIL populations using tumors from mixed tumor-bearing mice on day 11 after the first anti-neu antibody treatment. Similar to results from the TUBO-bearing mouse model, the CD3+CD4+ and CD3+CD8+ fractions of the CD45+ cell population as well as the percentage of IFN-γCD8+ T cells were increased by anti-neu antibody treatment in the absence or presence of A66 in the mixed tumor mouse model (Fig. 3D and Suppl. Fig. 3A). Interestingly, GDC-0941 in combination with the antibody did not cause a significant change in the CD3+CD8+ T cell population as compared to the vehicle control, although it increased the number of CD3+CD4+ and IFN-γ+CD8+ T cells (Fig. 3D).

The results of the IFN-γ ELISPOT assay with splenocytes isolated from mixed tumor-bearing mice showed that the numbers of TUBO and TUBO-P2J-specific IFN-γ+ T cells were increased by anti-neu antibody alone or in combination with PI3K inhibitor (Fig. 3E). However, the increase in the number of tumor-specific IFN-γ+ T cells was not due to a change of splenic T cell numbers (Suppl. Fig. 3B). The combination of A66 and antibody resulted in a synergistic increase in the number of tumor-specific T cells against neu and non-neu tumor antigens.

3.4. CD8+ T cells are indispensable for combination therapy with PI3K inhibitor and anti-neu antibody in HER2/neu IHC2+ tumor models

To determine the impact of T cell subsets in the mixed tumor model, we evaluated tumor regression following combination therapy with anti-neu antibody and PI3K inhibitor under conditions of T cell depletion. Anti-CD8 or CD4 depleting antibody depleted the corresponding T cell population (Suppl.
Fig. 4A). Under CD8<sup>+</sup> T cell depletion, the anti-tumor efficacy of anti-neu antibody alone or in combination with GDC-0941 was slightly reduced, whereas that of antibody plus A66 was completely abolished (Fig. 4 and Suppl. Fig. 4B). Depletion of CD4<sup>+</sup> T cells partly suppressed the anti-tumor activity of the antibody/A66 combination but had no effect on that of antibody alone or combined with GDC-0941.

3.5. PI3K p110α inhibitor A66 preserves AKT activation in CD8<sup>+</sup> TILs

To investigate why A66 in combination with anti-neu antibody elicited a better clinical outcome and more tumor-specific T cells as compared to the GDC-0941/anti-neu antibody combination, we examined the phosphorylation status of AKT in
CD8$^+$ TILs. In both TUBO and mixed tumor models, anti-neu antibody treatment increased the pAKT$^+$ fraction of CD8$^+$ T cells, which was markedly decreased in the presence of GDC-0941 (Fig. 5). However, no difference was observed between groups treated with antibody alone or in combination with A66 in terms of the percentage of pAKT$^+$CD8$^+$ T cells.

We also evaluated the effects of PI3K inhibition on T cell proliferation induced by antigen-specific or non-specific stimuli. The proliferation of T cells stimulated with either anti-CD3/CD28 antibody or HA peptide was unaltered by A66 but was strongly inhibited by GDC-0941 treatment (Fig. 6).

4. Discussion

Trastuzumab is a standard cancer drug for the treatment of HER2-amplified breast cancer; results from clinical trials have confirmed that it can improve overall survival in metastatic breast cancer. However, trastuzumab resistance can occur within 1 year of trastuzumab treatment in these patients. Combination therapy with trastuzumab is one strategy to overcome this resistance. Here we tested the anti-tumor efficacy of selective (A66) and pan-PI3K (GDC-0941) inhibitors in combination with an anti-neu antibody (trastuzumab in humans) in mouse breast cancer models, and examined the effects of these treatments on anti-tumor immunity. The PI3K inhibitors synergized with anti-neu antibody to inhibit tumor growth and enhance anti-tumor immunity, with A66 showing a more potent effect in combination with the antibody than GDC-0941. Thus, the p110$\alpha$ isoform-selective inhibitor has the following advantages over the pan-PI3K inhibitor when administered in combination with anti-neu antibody: 1) increased size of the TIL population; 2) tumor-specific T cell induction; and 3) therapeutic effects in a heterogeneous mixed-tumor model.

Several mechanisms have been proposed to explain trastuzumab resistance, including 1) HER2 proteolysis resulting in constitutive kinase activity or the masking of trastuzumab-binding sites on HER2; 2) alternative activation of other receptor-tyrosine kinases; and 3) changes in signaling downstream of HER2. Trastuzumab resistance can in theory be overcome by targeting these mechanisms; indeed, combinations of pertuzumab, lapatinib, vascular endothelial growth factor inhibitors, and HER2 downstream signaling inhibitors have been investi-
and p110α/gated.25-29 Targeting the PI3K/AKT/mTOR pathway may also be an effective strategy for overcoming resistance to HER2-based therapy in breast cancer.30 Inhibiting PI3K signaling can also block pathway hyperactivation caused by gain-of-function mutations in the PI3K and AKT1 genes, AKT2 amplification, and PTEN loss in breast cancer.31 Given that a hallmark of cancer cells is an elevation in PI3P levels, targeting class I PI3Ks is critical in cancer drug discovery. To this end, several compounds such as pictilisib (GDC-0941), buparlisib (BKM120), XL147 (SAR245408; Exelixis/Sano) or GX15-070 are currently being evaluated in clinical trials in patients with HER2+ breast cancer who previously received trastuzumab therapy.32-35

Although pan-PI3K inhibitors can be effective anti-cancer compounds, the problems of side effects and toxicity have yet to be resolved. Targeting a specific isoform of PI3K is one solution. The p110δ and p110γ isoforms of PI3K are predominantly expressed in immune cells, whereas p110α and p110β isoforms are ubiquitously expressed. Thus, targeting p110δ and p110γ with pan-PI3K inhibitors may inhibit immune functions; indeed, GDC-0941 and ZSTK474 have been shown to suppress lymphocyte proliferation, ADCC, and cytokine production.17,18 On the other hand, some studies have reported that p110δ and p110γ inhibitors enhance antitumor responses.36 Based on these emerging evidences for immunomodulatory actions of p110δ and p110γ inhibitors, further study is necessary to discriminate the role of these isoforms from pan-PI3K actions in tumor cells and their microenvironments. It was recently shown that activating mutations in PIK3CA encoding p110α are common in solid tumors, and that inhibitors of p110α are less immunosuppressive than those targeting other isoforms and pan-PI3K inhibitors, but are as effective as the latter at suppressing the growth of PIK3CA-mutant tumor cells and HER2-amplified tumor cells.10,11 Genetic ablation of p110α blocked tumor formation in both polyoma middle T antigen and HER2/neu-transgenic models of breast cancer, with p110β inhibition having a negligible effect.37 The effects of PI3K inhibitors on other systems should be considered when administering PI3K inhibitors in combination with trastuzumab since PI3K signaling functions in most cell types, including immune cells (T cells) and because anti-tumor immunity is inducible by and mediates the effects of anti-HER2/neu antibody therapy.12-14 Thus, administration of p110α-selective inhibitors is a reasonable anti-tumor strategy that can also preserve or increase anti-tumor immunity induced by anti-neu antibody.

The p110δ and p110γ isoforms of PI3K play critical roles in T cell development; p110δ is critical for the differentiation of mature T cells,39 while a pan-PI3K inhibitor suppressed T cell expansion and cytokine production following stimulation by phytohemagglutinin or antigens, which were preserved by p110α-selective inhibition.15 Thus, pan-PI3K inhibitor treatment may prevent the induction of anti-tumor immunity by anti-HER2/neu antibody, thereby limiting its therapeutic effects. Interestingly, our data showed that PI3K inhibition did not block the therapeutic effects of anti-neu antibody in the TUBO tumor model. In fact, combination therapy with PI3K inhibitors and anti-neu antibody increased the size of functional T cell populations in tumors and the numbers of tumor-specific T cells relative to anti-neu antibody monotherapy. However, in the HER2/neu IHC2+ heterogeneous mixed tumor model that only partially responds to anti-neu antibody,32 pan-PI3K inhibitor diminished whereas p110α-selective PI3K inhibitor enhanced the anti-tumor activity of anti-neu antibody.

PI3K signaling plays important roles in effector and memory CD8+ T cell differentiation,40 and is essential for T cell expansion. This can explain the observed enhancement of the anti-tumor activity of anti-neu antibody when administered in combination with A66. Although further study is required to clarify the role of each PI3K isoform in CD8+ T cell differentiation, we speculate that p110α is not critical for CD8+ T cell functions for following reasons: 1) A66 was less effective than GDC-0941 in decreasing AKT phosphorylation in CD8+ T cells; 2) TILs and tumor-specific T cells were most abundant in mice treated with A66 and anti-neu antibody; and 3) CD4+ and CD8+ T cells were required for the anti-tumor effects of A66 but not GDC-0941 combination treatment. In addition, GDC-0941 strongly inhibited proliferation in cells stimulated via the TCR by HA peptide or anti-CD3/CD28 antibodies, while TCR-stimulated T cells treated with A66 showed no reduction in proliferation.

Regulatory T (Treg) cells, and myeloid-derived suppressor cells (MDSCs) are important components of the immunosuppressive tumor microenvironment. We observed no differences between anti-neu antibody monotherapy and combination therapy with either PI3K inhibitor in terms of MDSC numbers (data not shown). In the case of Foxp3+ Treg cells, GDC-0941/ anti-neu antibody combination therapy increased the size of the Treg population in tumors but not in DLNs, whereas no
effect was observed upon treatment with a single agent or the A66/anti-neu antibody combination in the TUBO model (Suppl. Fig. 5). PI3K signaling is thought to play an important role in regulating the balance between Treg and effector T cell differentiation. However, it was also demonstrated that low PI3K activity is detrimental to Treg function. Additional studies are needed to determine whether the GDC-0941-dependent increase in Treg population size in the presence of anti-neu antibody was responsible for the lower therapeutic effect relative to that observed with the A66/anti-neu antibody combination. Since there were no differences in the percentage of Treg cells among treatment groups in the mixed tumor model (Suppl. Fig. 5C), it remains to be determined whether this phenomenon is due to the expression level of neu antigen or to the animal models that were used.

The results presented here suggest that PI3K signaling can be targeted to increase the efficacy of anti-HER2/neo antibody therapy. Importantly, PI3K inhibition synergized with anti-neu antibody in terms of anti-tumor immunity and activity, with the p110alpha isoform-selective inhibitor A66 being more effective in combination therapy than the pan-PI3K inhibitor. Our findings indicate that adjunctive use of the PI3K p110alpha isoform-selective inhibitor is an effective strategy for the treatment of HER2+ breast cancer that is either susceptible or resistant to trastuzumab.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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