Monoclonal Antibody against Cell Surface GRP78 as a Novel Agent in Suppressing PI3K/AKT Signaling, Tumor Growth, and Metastasis

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Abstract

Purpose: The ER chaperone GRP78 translocates to the surface of tumor cells and promotes survival, metastasis, and resistance to therapy. An oncogenic function of cell surface GRP78 has been attributed to the activation of the phosphoinositide 3-kinase (PI3K) pathway. We intend to use a novel anti-GRP78 monoclonal antibody (MAb159) to attenuate PI3K signaling and inhibit tumor growth and metastasis.

Experimental Design: MAb159 was characterized biochemically. Antitumor activity was tested in cancer cell culture, tumor xenograft models, tumor metastasis models, and spontaneous tumor models. Cancer cells and tumor tissues were analyzed for PI3K activity. MAb159 was humanized and validated for diagnostic and therapeutic application.

Results: MAb159 specifically recognized surface GRP78, triggered GRP78 endocytosis, and localized to tumors but not to normal organs in vivo. MAb159 inhibited tumor cell proliferation and enhanced tumor cell death both in vitro and in vivo. In MAb159-treated tumors, PI3K signaling was inhibited without compensatory MAPK pathway activation. Furthermore, MAb159 halted or reversed tumor progression in the spontaneous PTEN–loss-driven prostate and leukemia tumor models, and inhibited tumor growth and metastasis in xenograft models. Humanized MAb159, which retains high affinity, tumor specific localization, and the antitumor activity, was nontoxic in mice, and had desirable pharmacokinetics.

Conclusions: GRP78-specific antibody MAb159 modulates the PI3K pathway and inhibits tumor growth and metastasis. Humanized MAb159 will enter human trials shortly.

Introduction

Cancer cells are characterized by metabolic alterations and the tumor microenvironment is often marked with impaired blood flow and hypoxia (1, 2), all of which can elicit endoplasmic reticulum (ER) stress. Tumor cells adapt to these adverse conditions by activating the unfolded protein response (UPR), with induction of GRP78 as a major prosurvival arm of the UPR signaling pathways (1, 2). GRP78, also referred to as BiP/HSPA5, is a 78-kD glucose–regulated protein with potent antiapoptotic properties that plays critical roles in cancer cell survival, tumor progression, metastasis, and resistance to therapy (3–5). Knockdown of GRP78 by siRNA in cancer cells as well as tumor-associated endothelial cells reduced their proliferative rate and sensitized them to chemotherapeutic treatment (6, 7). Genetically altered GRP78 mouse models further demonstrated the critical role of GRP78 in cancer in vivo. For instance, GRP78 haploinsufficiency delayed tumor development, growth, and inhibited metastasis (8, 9). In mice harboring biallelic conditional knockout of both GRP78 and tumor suppressor PTEN in the prostate epithelium, prostate tumorigenesis was potently arrested (10). In addition, inducible heterozygous knockout of GRP78 in the hematopoietic system suppresses PTEN-null leukemogenesis with no harmful effect on hematopoiesis (11). Strikingly, in both the prostate and leukemia models, phosphoinositide 3-kinase (PI3K)/AKT signaling resulting from the...
GRP78 Antibody Inhibits PI3K and Tumor Growth

**Translational Relevance**

A major obstacle in cancer therapy is the damage to normal organs by conventional chemotherapy and radiotherapy. This highlights the need for therapy that specifically targets and kills cancer cells while sparing normal cells. One emerging target is GRP78, which is preferentially expressed on cancer cell surface and promotes tumor cell survival and metastasis. We have developed a monoclonal antibody MAb159 against GRP78 to target surface GRP78 and block its oncogenic functions. MAb159 effectively images tumors in vivo, suppresses PI3K/AKT signaling, induces apoptosis, and induces tumor regression in xenografts and spontaneous tumor models. The humanized MAb159 retains its GRP78-binding affinity and efficacy and is nontoxic to normal organs. We plan to initiate human clinical trial shortly.

loss of PTEN was greatly impaired with only partial loss of GRP78 (10, 11). Collectively, these studies establish GRP78 as a novel regulator of the PI3K/AKT oncogenic signaling and a target for anticancer therapy.

Although traditionally GRP78 has been regarded as an ER lumenal protein, evidence has accumulated that a fraction of GRP78 can exist on the plasma membrane of specific cell types (12-14) and that ER stress actively promotes cell surface localization of GRP78 (15). Whereas the physiologic function of GRP78 on the cell surface is still emerging, recent studies show that cell surface GRP78 forms complexes with specific protein partners, regulating both proliferation and viability (14, 16), suggesting that cell surface GRP78 presents an opportunity for therapeutic targeting (17-19).

Here we report the generation of a monoclonal antibody (mAb; MAb159) against human GRP78 that shows antitumor activity with no toxicity and also inhibits PI3K signaling. This antibody opens up a unique opportunity to study both the biology of cell surface GRP78 and its therapeutic potential.

**Materials and Methods**

**Antibodies, reagents, and cell lines**

A549, HT29, Colo205, MCF7, 4T1, and 293T cell lines were obtained from the American Type Culture Collection. C4-2B cell was kindly provided by Michael Stallcup (University of South California), and H249 was kindly provided by Dr. Ravi Salgia of the University of Chicago. The generation of the B16-Fluc-A1 melanoma cell line has been described previously (9). All these cells were propagated in RPMI-1640 supplemented with 10% FBS, 100 units/mL of penicillin, and 100 μg/mL streptomycin from Cellgro. The CE1 cell line was kindly provided by Dr. Pradip Roy-Burman and cultured as previously described (20). These cell lines have been validated by HLA typing and molecular phenotyping relative to the respective primary tumors. Detailed information about antibodies and reagents used in this study can be found in Supplementary Materials and Methods.

**Generation of mAbs and affinity analysis**

The procedure for generation of mAbs is described in Supplementary Materials and Methods. The affinity of mAbs to antigen was determined by Scatchard assay as described previously (21).

**In vivo and ex vivo near-infrared fluorescence imaging**

H249 tumor-bearing mice were injected with Cy5.5-labeled humanized MAb159 or control antibody. In vivo fluorescence imaging was performed using the Xenogen Lumina XR Imaging System and analyzed using the IVIS Living Imaging 3.0 software. Twenty-eight hours after injection, the tumors and organs were harvested for ex vivo fluorescence imaging. Please see Supplementary Materials and Methods for detailed procedure.

**PTEN-null models**

The prostate-specific PTEN knockout model has been described previously (22). Mice were treated with MAb159, and prostate tumors were monitored by luminescence imaging (xenogen). The Pten (floxed/floxed); Mx-1 cre leu-kemic model and the protocols for flow cytometry for analysis of leukemic blasts and peripheral blood counts have been described previously (11). Please see Supplementary Materials and Methods for detailed procedure.

**Murine tumor xenograft models**

The procedures for murine tumor xenograft studies and immunohistochemical analysis were as described previously (21) and are further described in Supplementary Materials and Methods.

**Statistical Analysis**

The statistical significance of differences in different samples or groups was determined using an unpaired two-tailed Student t test. Results were considered significantly different if the P value was less than 0.05.

**Results**

**Generation of mAbs specific to surface GRP78**

We immunized mice with a hexahistidine-tagged and secreted form of human GRP78 (Supplementary Fig. S1A), and screened a panel of mAbs capable of binding the native form of GRP78. The criteria for the desirable antibody were high-affinity binding to native cell surface GRP78 and the ability to endocytose. MAb159 fulfilled these requirements. MAb159 is highly specific for GRP78, as the binding of MAb159 to GRP78 was completely abolished by purified soluble GRP78 protein (Supplementary Fig. S1B). In addition, we performed an immunoprecipitation study with MAb159 and confirmed that GRP78 can be pulled down from cell lysate using mass spectrometric analysis (data not shown). MAb159 has no cross-reactivity to GRP78’s closest paralog HSP70.
surface GRP78. These data implicate that MAb159 led to internalization of inhibitor specific for clathrin-mediated endocytosis (23). GRP78 was markedly reduced after MAb159 treatment (Fig. 1A, left). Under glucose-starvation conditions, which mimicked nutrient deprivation in the tumor microenvironment, greater amount of MAb159 was recruited to cancer cell surface. This is consistent with the previous findings that surface GRP78 significantly increases when the cell is under stress (14, 15). In addition, we determined whether MAb159 preferably localizes to tumor and not normal organs by tracking biotin-labeled MAb159 in HT29 xenograft tumor–bearing mice in vivo. Biotin-MAb159 was only seen in the tumor, not in the normal organs, including heart, liver, and kidney (Fig. 1A, right).

When incubated with cells at 37°C, MAb159 underwent endocytosis and was localized to the intracellular clathrin-coated endosomes compared with a fine ring-like appearance at the cell surface when incubated at 4°C (Fig. 1B, top; Supplementary Fig. S2A). We next examined the surface GRP78 level with a second antibody and found that surface GRP78 was markedly reduced after MAb159 treatment (Fig. 1B, bottom). Moreover, this reduction of GRP78 can be reverted with chlorpromazine (Supplementary Fig. S2B), an inhibitor specific for clathrin-mediated endocytosis (23). These data implicate that MAb159 led to internalization of surface GRP78.

MAb159 induces tumor cell apoptosis and inhibits PI3K signaling

The effect of MAb159 on tumor cells was first tested in vitro. Before incubation with MAb159, cells from the breast carcinoma cell line MCF7 and colon cancer cell line HT29 were cultured in glucose-free medium to enhance surface GRP78 level and thus the efficacy of the antibody. MAb159 significantly reduced the cell viability in both MTT (Fig. 1C, top) and clonogenic assay (Supplementary Fig. S3A), correlating with increased apoptosis as determined by terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL; Fig. 1C, bottom) and M30 apoptosis assay (Supplementary Fig. S3B). In addition, we have measured activated caspase levels and showed that MAb159 treatment activates caspases 8 and 9 (Supplementary Fig. S3C), which represent the activation of extrinsic and intrinsic apoptotic pathways, respectively. This suggests that, at least in part, the loss of cell viability was initiated at the cell membrane.

The PI3K signaling pathway regulates many biologic events in the cells, including cell survival, and GRP78 has previously been shown to modulate its activity. Therefore, we examined whether MAb159-treated cell–surface-GRP78-expressing tumor cells have alterations in PI3K activity as measured by the changes in phosphorylated AKT and S6 levels. We found, indeed, that both phosphorylated AKT and S6 levels were reduced in antibody-treated cells compared with the controls (Fig. 1D). Because MAb159-induced GRP78 endocytosis led to the loss of cell surface GRP78 and inhibition of pAKT signaling, we anticipate that blocking GRP78 endocytosis would antagonize the activity of MAb159. We, however, were unable to conduct this...
increased (Fig. 2B), and vessel density (CD31 staining) had reduced, apoptosis (TUNEL assay) was significantly reduced. In the MAb159-treated group, tumor volume (Fig. 2A) decreased by 85%. The combination of MAb159 and irinotecan inhibited tumor growth by 54% compared with the control group. Irinotecan mono-administered alone, MAb159 inhibited tumor growth by 50%, 58%, and 78% tumor growth inhibition in these models, respectively (Fig. 2A).

To further test if the combination of GRP78-targeted therapy and conventional chemotherapy leads to greater efficacy, we combined MAb159 and irinotecan in a colon cancer xenograft model. The colon cancer model was chosen because GRP78 overexpression is reported to be associated with colorectal carcinogenesis (25). When administered alone, MAb159 inhibited tumor growth by 54% compared with the control group. Irinotecan monotherapy inhibited tumor growth by 85%. The combination therapy caused tumor regression to 47% of the starting tumor volume (Fig. 2A).

**MAb159 inhibits tumor growth and causes tumor regression in various xenograft tumor models**

The efficacy of the GRP78 antibody MAb159 in vivo was examined in various xenograft tumor models including HT29 (colon cancer), H249 (small cell lung carcinoma), and A549 (lung adenocarcinoma). These cells have relatively high (4.6%–9.4%; Supplementary Fig. S5) surface GRP78 expression compared with normal cells (15). MAb159 treatment led to 50%, 58%, and 78% tumor growth inhibition in these models, respectively (Fig. 2A).

To further test if the combination of GRP78-targeted therapy and conventional chemotherapy leads to greater efficacy, we combined MAb159 and irinotecan in a colon cancer xenograft model. The colon cancer model was chosen because GRP78 overexpression is reported to be associated with colorectal carcinogenesis (25). When administered alone, MAb159 inhibited tumor growth by 54% compared with the control group. Irinotecan monotherapy inhibited tumor growth by 85%. The combination therapy caused tumor regression to 47% of the starting tumor volume (Fig. 2A).

**MAb159 reduces proliferation, induces apoptosis, impairs tumor vasculature, and inhibits PI3K signaling in tumor xenografts**

At the end of the xenograft experiments, tumors were harvested for analysis. In the MAb159-treated group, the proliferation index (Ki67 staining) was markedly reduced. Apoptosis (TUNEL assay) was significantly increased (Fig. 2B), and vessel density (CD31 staining) had a modest decrease (Supplementary Fig. S6A). In addition, MAb159 treatment led to a marked reduction in phosphorylated AKT, mTOR, and S6 (Fig. 2B–D; Supplementary Fig. S6B), indicating inhibition of PI3K signaling. Meanwhile, there was no increase in phosphorylated ERK1/2 and Src (Fig. 2C and D), indicating that these compensatory pathways, often induced with PI3K inhibitors and resistance to therapy, were not activated. In addition, it is notable that the systemic administration of the antibody was well tolerated, as measured by the animal food intake, body weight (data not shown), and microscopic examination of vital organs (Supplementary Fig. S7).

**MAb159 inhibits tumor metastasis**

GRP78 promotes growth of blood vessels in the tumor accompanied by tumor growth and metastasis (9). Targeting surface GRP78 with a peptide conjugated to proapoptotic molecules inhibits metastases (19). Therefore, we tested MAb159 in an orthotopic tumor model using mouse breast adenocarcinoma cell 4T1 (26, 27). Here, 4T1 cells were implanted into the #4 fat pads of isogenic BALB/c mice, which were treated biweekly with MAb159 at a dose of 10 mg/kg or with saline. Within 7 days, MAb159 inhibited primary tumor growth and secondary metastasis to #9 fat pads (n = 2; Supplementary Fig. S8A). After 13 days, metastatic tumors were present on the liver surface of control animals (n = 3) but not in MAb159-treated mice (n = 3; Fig. 3A, left). Further focal liver necrosis was seen in control but not in MAb159-treated animals (data not shown; Fig. 3A). By day 14 in all control mice, both primary tumor and #9 fat pad secondary tumors had become large and invaded through the body wall into the underlying peritoneal cavity. In comparison, MAb159-treated mice exhibited complete or near-complete primary tumor regres- sion (data not shown; Supplementary Fig. S8B). There was no visible contralateral metastasis in any of the MAb159-treated animals. Histologic evaluation of lung metastasis showed that the majority of the lung volume of control animals was occupied by metastatic breast cancer that resulted in internal hemorrhaging in 50% of control lungs (Fig. 3A, right). Lung metastasis was significantly inhibited by MAb159 treatment (Fig. 3B), and there was no evidence of internal hemorrhaging in any of the M159-treated animals (Fig. 3A, right). Analysis of breast tumor tissues shows that MAb159 significantly reduced the pS6 level, indicative of inhibition of PI3K signaling (Supplementary Fig. S8C).

We also examined the effect of MAb159 on the metastatic growth of a syngeneic melanoma cancer cell line (9). B16-Fluc-A1 melanoma cells stably expressing luciferase were injected intravenously. Tumor metastasis and progression in the lungs was monitored live with a whole-animal luminescence imaging system. MAb159 treatment significantly reduced the formation of lung tumors (Fig. 3C, left). At the end of the experiment, lungs were harvested and pigmented tumors were observed on the lung surface. Compared with the control group, the lungs from MAb159-treated mice had significantly fewer tumors (Fig. 3C, right).
MAb159 suppresses PTEN deletion–induced prostate cancer progression and leukemogenesis

We tested MAb159 in the setting of constitutively active PI3K in the PTEN knockout spontaneous tumor models, including inducible PTEN knockout in the prostate and hematopoietic system. In the PTEN knockout spontaneous prostate cancer model, PTEN deletion is achieved with the induction of Cre under probasin promoter. In addition, luciferase expression is induced by the same Cre; therefore, PTEN-deficient prostate cells can be imaged with a luminescence imaging system (22). Tumor develops in the prostate in 2 to 3 months. Control immunoglobulin G (IgG) or MAb159 was given to 2-month-old PTEN null mice 2 times a week at a dose of 10 mg/kg. Tumor progression was monitored with live-animal luminescence imaging. In the MAb159-treated group, there was marked tumor suppression compared to the control group.
In contrast, mice in the control IgG-treated group uniformly progressed. Histologic analysis of dorsolateral prostate indicates that the control IgG-treated prostate had extensive adenocarcinoma (Fig. 4B). In contrast, the MAb159-treated prostate only had mild prostate intraepithelial neoplasia (Fig. 4B). Further immunohistochemical analysis shows that MAb159 significantly reduced pAKT and pS6 levels, suggesting inhibition of PI3K signaling (Fig. 4B). There was an insignificant decrease in phosphorylated ERK in the MAb159-treated group (Fig. 4B). In addition, we studied the efficacy of MAb159 in the xenograft model of a hormone refractory-mouse prostate cancer cell line CE1 that is established from a postcastration-recurrent tumor of PTEN-deficient mouse (20). More than 50% CE1 tumor growth inhibition was achieved with MAb159 treatment (Supplementary Fig. S9).

As reported previously, inducible knockout of PTEN in the hematopoietic system leads to the development of myeloproliferative disorders and eventual leukemia, which is suppressed by GRP78 haploinsufficiency (11, 28). These PTEN-deficient mice when treated with control IgG had morbid hunched posture, whereas those treated with MAb159 appeared normal (Fig. 5A). PTEN deficiency led to significant increase in leukemic blast cells in the bone marrow, as well as increase in spleen weight. PTEN-deficient mice treated with MAb159 showed a prominent reduction in leukemic blast cells and spleen weight compared to the control IgG group.
in spleen size and percentage of blast cells in the bone marrow, compared with those injected with control IgG (Fig. 5B), and restoration of white blood cells, lymphocytes, monocytes, and granulocytes similar to the level of wild-type mice (Fig. 5C). The effect on PI3K signaling was measured by the level of phosphorylated AKT with Western blot analysis (Fig. 5D). Consistent with suppression of AKT activation following inducible heterozygous ablation of GRP78 in the same PTEN null model (11), MAb159 decreased phosphorylated AKT to normal levels in PTEN-deficient mice.

Affinity, activity, and specificity of humanized MAb159

We selected the antibody with the desired features suitable for therapeutic development. Next, we prepared humanized MAb159 to avoid potential immunogenicity in humans. This antibody has affinity to GRP78 (Fig. 6A) and efficacy in tumor growth inhibition (Fig. 6B) comparable with the parental murine antibody.

To determine the tumor-specific localization of humanized MAb159, we imaged tumor-bearing live mice injected with Cy5.5-labeled humanized MAb159. We found that 28 hours after injection, humanized MAb159 preferentially localized to H249 tumors but not to mouse organs (Fig. 6C, left). At the end of the study, the normal organs together with tumors were harvested for an ex vivo imaging. There was a dramatic difference in the signal intensity in the tumor between control IgG and humanized MAb159 (Fig. 6C, right). The tissues were further subjected to immunohistochemical analysis using a human Fc-specific antibody, confirming the specific localization of humanized MAb159, but not control IgG, to the tumor (Fig. 6D).

Pharmacokinetics and toxicology studies of humanized MAb159

We performed pharmacokinetics and toxicology studies of humanized MAb159 in the mouse. A single 10 mg/kg dose of humanized Mab159 was administered intravenously, and serum levels of the antibody were measured at designated time points. A mean maximum serum concentration ($C_{max}$) of 61.5 µg/mL was achieved. The mean serum half-life was more than 3 days (Supplementary Table S1). At 10 mg/kg of humanized MAb159, an area under curve (AUC) of 4045 ± 1026 µg.h/mL was achieved. Toxicology study was conducted in C57BL/7 mice treated 2 times a week for 5 weeks with 10 mg/kg humanized MAb159. Overall, there was no significant toxicity found in either the blood or vital organs of humanized MAb159-treated mice (Supplementary Table...
No histologic abnormalities were seen except for mild inflammation in the pancreas of a control and humanized MAb159-treated mouse. These results provide good safety and pharmacokinetics data to proceed with clinical trials.

**Discussion**

Cell surface GRP78 is a multifunctional receptor and a potential target for cancer therapy. For example, proapoptotic moieties or cytotoxic agents were conjugated onto peptides with high affinity for GRP78 to successfully target and kill cancer cells (12, 19). Recently, an unconjugated peptidic GRP78 ligand also demonstrated toxicity to prostate cancer cell (29). A human monoclonal IgM antibody against cell surface GRP78 isolated from a cancer patient is capable of inducing lipid accumulation and apoptosis in cancer cells (30). Here, we screened and identified a novel mAb that recognizes both human and mouse GRP78 with high specificity and potently inhibits tumor growth and causes tumor regression in xenograft tumor models and spontaneous tumor models. In addition, MAb159 suppresses tumor metastasis in multiple models. Furthermore, the humanized form of this antibody is efficacious and without toxicity.

Cell surface GRP78 is an upstream regulator of PI3K/AKT signaling. GRP78 interacts with Cripto and α2-macroglobulin on the cell surface and is required for these factors to activate downstream PI3K/AKT signaling (16, 31, 32). Recently, a murine mAb against GRP78 was reported to suppress AKT activation in a melanoma model (33). Here, we show that surface GRP78 is in the same complex as the p85 subunit of PI3K, and treatment of cells with the GRP78-specific antibody MAb159 led to marked reduction in PI3K signaling in both cultured cells and multiple tumor models. The spontaneous tumor models include PTEN deletion–induced spontaneous prostate cancer and leukemia models. Furthermore, in tumor xenografts subjected to long-term treatment with MAb159 for up to 30 days, inhibition of the PI3K pathway was sustained, and there was no evidence for induction of escape mechanisms of resistance to PI3K inhibition such as MAPK (34). Altogether, these highlight the importance of surface GRP78 in PI3K/AKT signaling and support the clinical investigation of MAb159 for PI3K-driven tumors. However, cell surface GRP78 may also regulate other critical oncogenic pathways. For example, MAb159 is active to cell lines with Kras mutation (A549) and Braf mutation (HT29 and Colo205). In the light of proapoptotic activity of MAb159, we are also investigating the role of surface GRP78 in the extrinsic apoptotic pathway.

Recent studies reported that surface GRP78 is highly elevated in tumor-associated vasculature and is required for endothelial cell proliferation and survival (7, 9). These findings indicate that cell surface GRP78-targeting agents will have dual function: targeting tumor cell and tumor vasculature. Consistently, we have observed that MAb159 suppresses PTEN loss-induced spontaneous leukemia. A, hunched posture of PTENf/f,Mx1-Cre mice treated with IgG compared with normal posture of those treated with MAb159. B, quantification of the spleen weight and leukemic blast cell percentages of wild-type (WT; n = 4), cPf/f treated with IgG (n = 5), and cPf/f treated with MAb159 (n = 8). All data are presented as mean ± SE C, complete blood count with tail peripheral blood from WT (n = 4), cPf/f treated with IgG (n = 5), and cPf/f treated with MAb159 (n = 8). All data are presented as mean ± SE *, P < 0.05; **, P < 0.01, as determined by an unpaired two-tailed Student t test. D, results of representative Western blot analysis using bone marrow cell lysates for detection of the indicated protein levels. For each group, samples from two mice were used for analysis.
reduced the density of tumor endothelial cell and angiogenesis-dependent tumor metastasis, supporting dual targeting.

Antibody tracking and in vivo imaging studies showed that MAb159 is strictly localized to the tumor and not normal organs, indicating that antibody targeting is highly specific. In agreement with this, formal toxicology study in mice showed that the humanized antibody was well tolerated and did not induce any noticeable organ toxicity or changes in blood counts or blood chemistry.

Another potential application of MAb159 lies in its ability to be used for in vivo imaging. MAb159 specifically recognizes surface GRP78 and, thus, can be used to image the tumor for personalized medicine and determine whether the amount of surface GRP78 in the tumor predicts disease progression and response to therapy. Clinical trials will incorporate patient imaging as a screening process for inclusion of study subjects.

In conclusion, we have developed a novel antibody MAb159 targeting surface GRP78 expressed on tumor cells and tumor endothelial cells. This antibody disrupts the PI3K signaling pathway and induces apoptosis in tumor cells, while sparing normal cells. It has the potential to be both a therapeutic and diagnostic agent in cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Liu, X. Li, W. Gao, Y. Zhou, S. Wey, S.K. Mitra, D. Dong, S. Louie, P.S. Conti, Z. Li, A.S. Lee, P. Gill
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