Reply to Soto-Pantoja et al. and Zhao et al.: Targeting CD47 on human solid tumors

We recently demonstrated that anti-CD47 monoclonal antibodies (mAbs) promote the phagocytosis of solid tumor cells in vitro and inhibit the growth and metastasis of human solid tumors in xenotransplantation models (1). We also observed a significant inhibition of tumor growth in a syngeneic mouse tumor model treated with MIAP410, an anti-mouse CD47 (mCD47) mAb. In contrast, a second blocking anti-mCD47 mAb (MIAP301) produced a moderate, yet insignificant, antitumor effect. The reasons for the lack of MIAP301 activity in this model are unclear, but could simply be due to the statistical power of the study.

MIAP410 was previously reported to not block mCD47–mSIRP α interactions (2). In contrast, we have determined that MIAP410 does block mCD47–mSIRP α interactions (Fig. 1). This discrepancy may be attributable to the N-terminal GST-CD47 fusion protein used in the prior blocking assays (2). Polypeptide additions at the N terminus of CD47 prevent the N-terminal glutamine residue of CD47 from forming pyroglutamic acid. As seen in the SIRP α –CD47 complex crystal structure (3), this residue is critical to the CD47–SIRP α binding interface. As MIAP410 binds endogenous mCD47 and blocks mSIRP α , it is likely that MIAP410 binds the free CD47 N terminus. MIAP410 has been inconsistent in its ability to inhibit tumor growth in some mouse colon cancer (CT26) and melanoma (B16) models. We have also observed splenomegaly in mice treated with MIAP410, likely a consequence of phagocytosis of red blood cells (RBCs) by splenic macrophages following blockade of CD47 signaling. We are currently investigating the factors that influence MIAP410 efficacy.

Soto-Pantoja et al. point out that we demonstrated that anti-CD47 mAbs enable the phagocytosis of tumor cells by macrophages expressing a SIRP α allele that does not interact with human CD47 (4, 5). This observation is seemingly inconsistent with our hypothesis that CD47–SIRP α interactions protect tumor cells from phagocytosis and warrants further investigation.

Both Soto-Pantoja et al. and Zhao et al. reference the results of Zhao et al. (5–7). As we previously demonstrated (8), Zhao et al. observe an enhanced antitumor response when a potent ADCC-inducing antibody is used in the absence of CD47–SIRP α signaling. However, caution must be applied when interpreting results in SIRP α mutant mice. Unlike WT mice, SIRP α mutant mice do not phagocytose CD47^{−/−} RBCs in vitro or eliminate transfused CD47^{−/−} RBCs in vivo (9). Thus, it is not surprising that transplanted melanoma cells form tumors with equivalent efficiency to that in WT mice. In our studies (1), we did not evaluate the potential of anti-CD47 mAbs to enable ADCC, nor did Zhao et al. evaluate phagocytosis in their models. Although we used mouse IgG1 isotype anti-CD47 mAbs in most of our studies to minimize the potential induction of ADCC, our results

Fig. 1. Anti-CD47 clone MIAP410 blocks the mouse CD47–SIRP α interaction. Yeast cells expressing the extracellular domain of mouse CD47 were incubated with 50 nM mouse SIRP α IgV-streptavidin tetramers conjugated to AlexaFluor 647 in the presence of titrating concentrations of MIAP410. Blocking of CD47 was detected by a decrease in SIRP α binding as assessed by mean fluorescence intensity (MFI). A sigmoidal dose–response relationship was observed for blocking with an IC_{50} of 50.22 nM. Error bars show SD of three independent samples.

do not preclude the possibility that both phagocytosis and ADCC contribute to the efficacy of anti-CD47 mAbs. We are aware that although adding ADCC effector mechanisms to our anti-CD47 mAbs might improve tumor killing, it might also cause pathology for the many CD47-expressing normal cells in the body.

We strongly disagree with Zhao et al. (7) that targeting the CD47–SIRP α interaction with anti-CD47 mAbs does not produce a therapeutic effect in the absence of a second, cancer-specific antibody. We have demonstrated that anti-CD47 mAbs alone enabled the phagocytosis of ≥ 24 patient tumor samples in vitro and inhibited tumor growth or metastasis of ≥ 10 solid tumors in vivo. We believe this result is beyond sufficient to demonstrate that a second antibody is not required to produce a therapeutic response. A second, cancer-specific antibody may further enhance the efficacy of anti-CD47 mAbs, but it is not required (8). It is possible that blockade of CD47–SIRP α interactions, in the absence of an intact Fc region, may not be as effective at producing a significant antitumor response. However, the members of the van den Berg laboratory themselves have shown that F(ab')₂ fragments prepared from anti-CD47 mAbs can induce the phagocytosis of human cells (10). In no way do these issues detract from the conclusion of our papers (1), that anti-CD47 mAbs can inhibit the growth and metastasis of solid tumors, especially of human origin.

We urge the readers to consult our previous response to other issues raised by Zhao et al. (11).

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Conflict of interest statement: I.L.W. filed US Patent Application no. 12/321,215 entitled “Methods for Manipulating Phagocytosis Mediated by CD47.” I.L.W. owns Amgen stock and is a Director of Stem Cells.

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