511 ACTIVITY OF ANTI-CD47 ANTIBODIES IS ENHANCED THROUGH FC OPTIMIZATION

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Background The CD47/SIRPa axis plays a crucial role in cancer immunosurveillance.[1 2] While anti-CD47 antibodies have shown promise in several preclinical models,[1 3–8] results from early phase clinical trials have shown limited clinical benefit,[9–11] suggesting that the sole blockade of CD47 by the antibody Fab domain might not be sufficient for effective tumor control. A critical question that remains to be answered is whether interactions between the antibody Fc and Fc gamma receptors (FcgRs) also contribute to their antitumor activity.[12 13] Our study aims to investigate the role of the Fc domain in the in vivo antitumor activity of anti-CD47 antibodies using immunocompetent species-matched models, overcoming limitations of previous studies conducted in immunocompromised models or with interspecies differences between mouse (m) and human (h) CD47, SIRPa and FcγRs.

Methods We modified the Fc domain of the anti-mCD47 antibody MIAP301 to generate antibodies with varying affinity to mFcgRs: 1) MIAP301-mIgG2a Fc, binding to preferentially to activating mFcgRs, 2) MIAP301-mIgG1 Fc, binding to the inhibitory mFcgRIIB, and 3) MIAP301-mIgG1-D265A Fc, which lacks binding to any mFcgRs. We evaluated the antitumor activity of these antibodies in MC38 and B16 tumor models in immunocompetent C57BL/6J mice and mice lacking activating FcgRs.[15] Additionally, we generated a mouse humanized for the expression of hCD47, hSIRPa and hFcgRs by CRISPR/Cas9-mediated gene-targeting strategy, and by backcrossing to our hFcgR mice.[16] We used this model to compare the antitumor activities of the anti-hCD47 antibody Magrolimab (5F9-hIgG4).[14] and an Fc-optimized variant that enhances binding for all the activating hFcgRs (5F9-GAALIE) generated in the lab.

Results The MIAP301-mIgG2a Fc variant led to the most significant reduction in tumor burden when compared to the control or other Fc variants in WT mice in both MC38 and B16 models. This therapeutic effect was abrogated in mice lacking activating FcgRs (figure 1). The CD47/SIRPa/FcgR humanized mice recapitulate the expression profile of CD47 and SIRPa found in human cells (figure 2). Furthermore, increasing dosing concentrations of both 5F9-hIgG4 and 5F9-GAALIE antibodies led to on-target anemia and thrombocytopenia in hCD47/hSIRPa/hFcgR mice, recapitulating results from clinical trials (figure 3). Intratumoral administration of the Fc optimized 5F9-GAALIE results in enhanced long-term antitumor immunity, abscopal antitumor effect, and minimal on-target toxicity when compared to 5F9-hIgG4 or control alone or in combination with PD-1 blockade (figure 4).

Conclusions The antitumor activities of anti-CD47 antibodies require interactions with activating FcgRs, highlighting the importance of Fc optimization in the development of effective anti-CD47 therapies.

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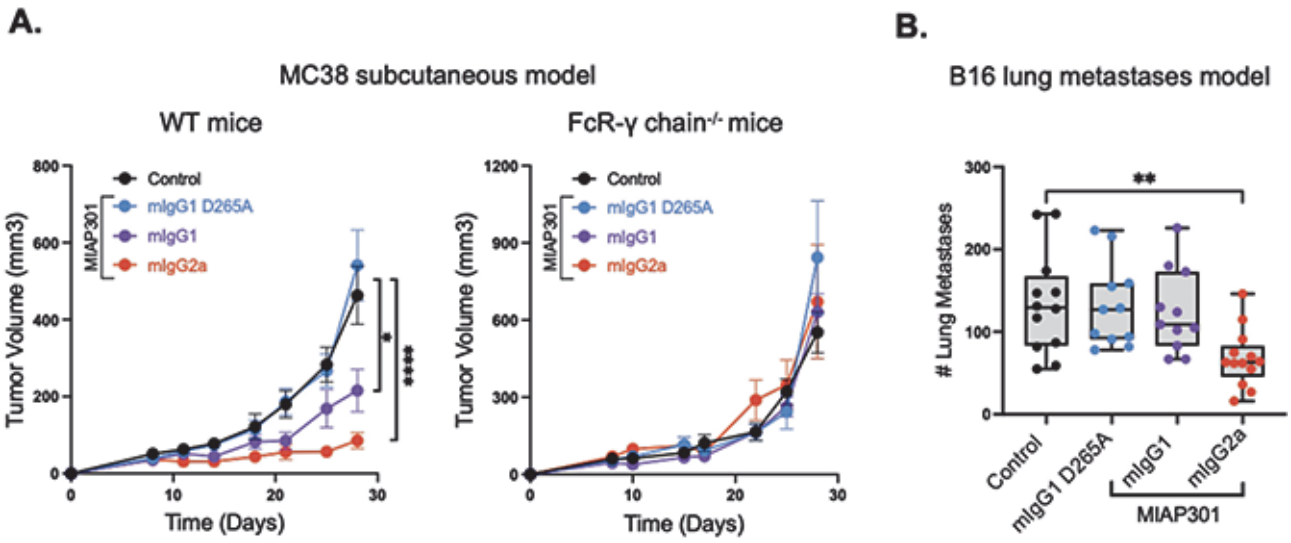
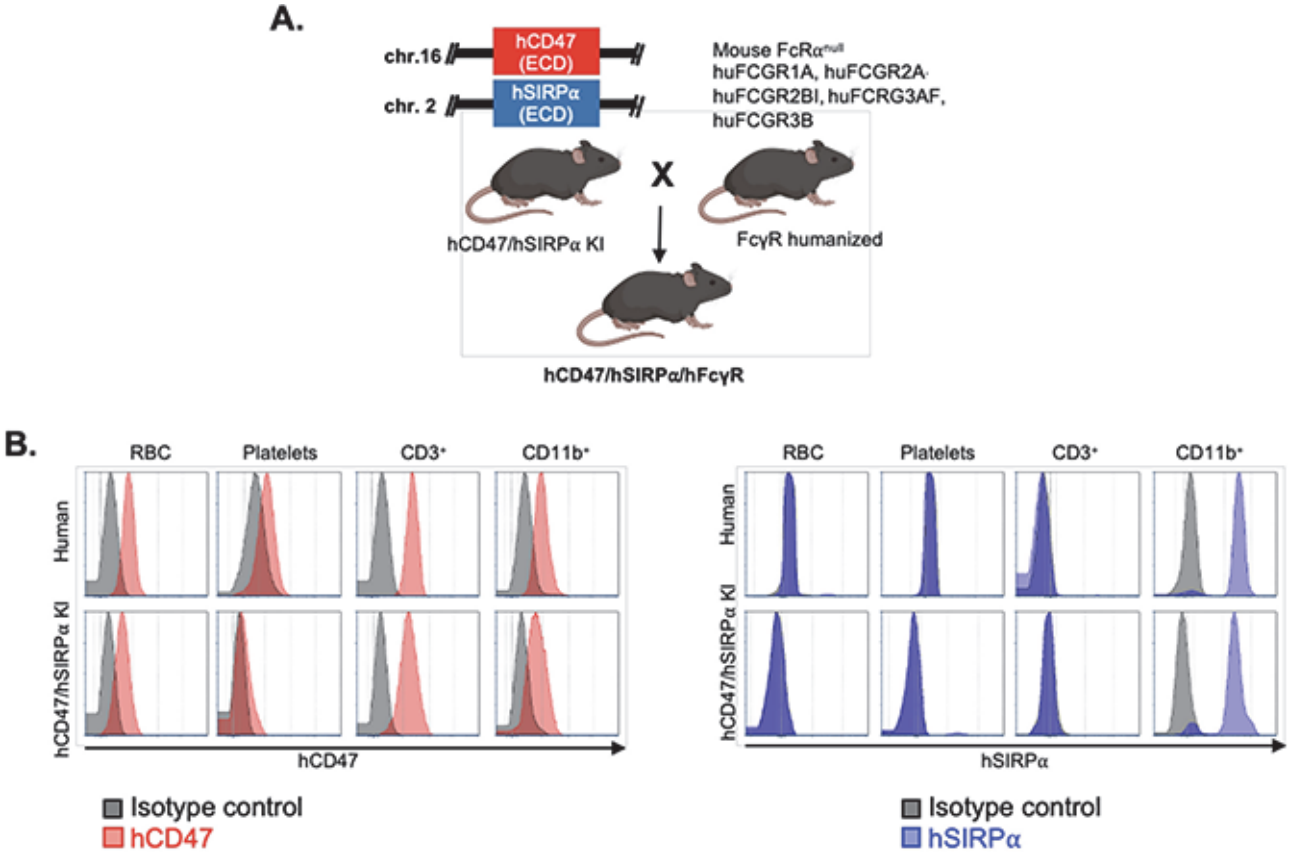
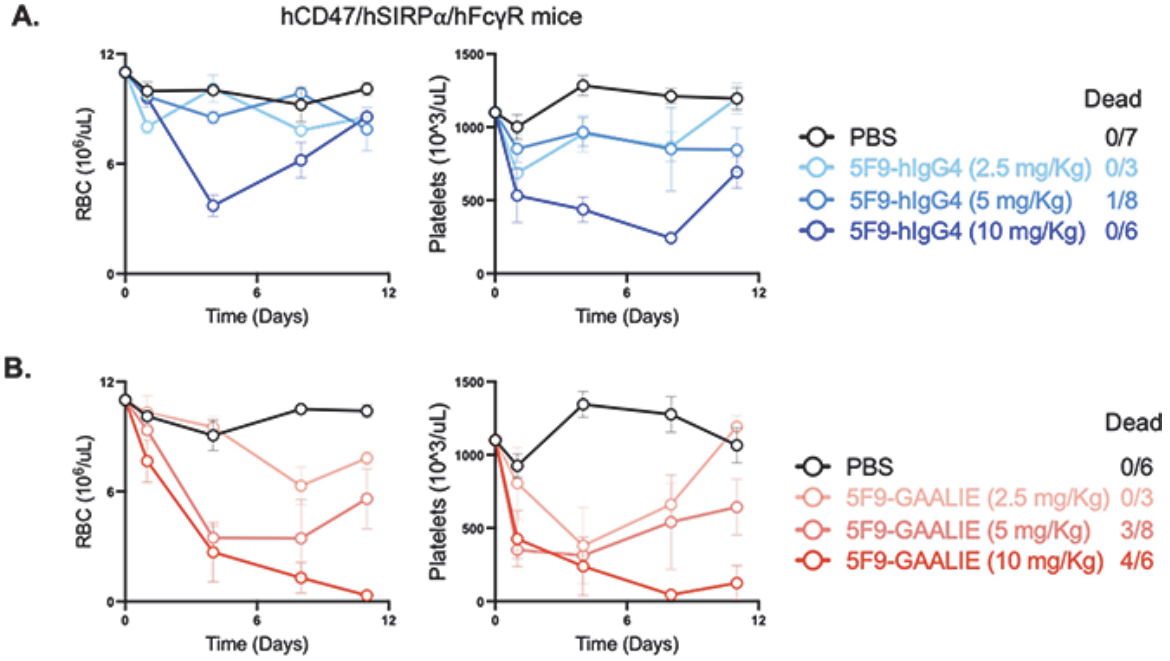


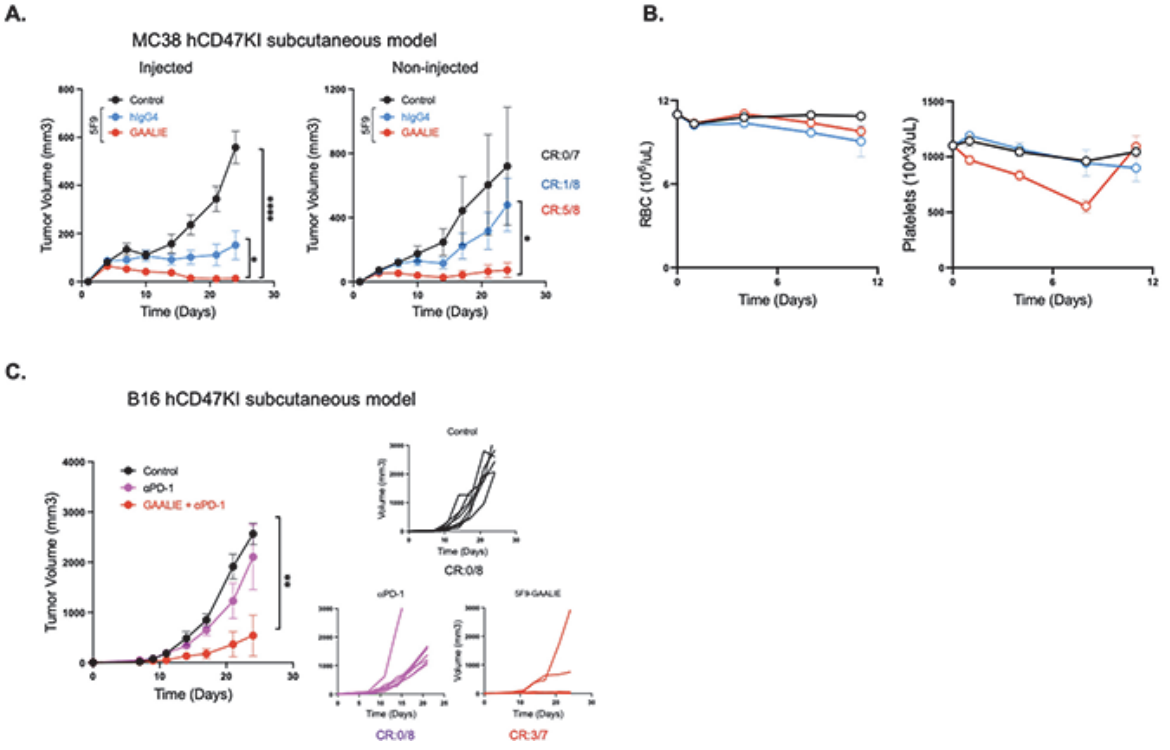
Abstract 511 Figure 1 Engagement of activating FcgRs enhances in vivo antitumor activity of antibodies blocking mouse CD47. (A) Average growth ± SEM of sq. MC38 tumors in WT (right) or FcR-y chain mice (left), treated with Fc variants of anti-mCD47 ab (MIAP301) or control (50ug IT, d. 8,10,14 and 18). (B) Average of lung metastases (Min to Max) of WT mice inoculated IV with B16 tumor cells, treated with anti-mCD47 ab Fc variants (MIAP301) or control (20 mg/Kg IP, d. 1,4,7 and 11).



Abstract 511 Figure 2 Generation of humanized mouse model for CD47, SIRPα, and FcyRs. (A) Schematic drawing showing the generation of the hCD47/hSIRPa/hFcyR mice. (B) Flow cytometry analysis of hCD47 and hSIRPα in red blood cells (RBC), platelets, CD3+ and CD11b+ leucocytes isolated from peripheral blood of human (top) and hCD47/hSIRPα KI mice (bottom).



Abstract 511 Figure 3 Humanized mouse recapitulates toxicity profile of fully humanized anti-CD47 antibodies. (A) Peripheral RBC and platelet count from hCD47/hSIRPa/hFcyR mice after treatment with increasing doses of 5F9-hIgG4 or (B) 5F9- GAALIE ab (2.5, 5, and 10 mg/Kg d. 0, 4, 8 and 11)



Abstract 511 Figure 4 Fc-optimized humanized anti-CD47 Ab promotes effective in vivo antitumor activity (A) Average growth ± SEM of injected (left) and non-injected contralateral (right) sq. MC38 hCD47 KI tumors in hCD47/hSIRPa/hFcyR, treated with 5F9-hIgG4, 5F9-GAALIE ab or control (50ug IT, d. 7, 9, and 12). (B) Serial measurements of peripheral RBC and platelets from hCD47/hSIRPa/hFcyR mice after treatment with 5F9-hIgG4 or 5F9-GAALIE (d. 0 indicates the day when treatment was started). (C) Average growth + SEM of sq. B16 hCD47 KI tumors in hCD47/hSIRPa/hFcyR, treated with 5F9-hIgG4, 5F9-GAALIE ab alone (50ug IT, d. 8,10,14 and 18), or in combination with anti-PD1 ab (RMP1–14, 200 ug IP d. 8, 10, 14 and 18), or control.

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