CRB-601, a selective integrin avβ8 blocking antibody, exhibits potent anti-tumor activity in anti-PD-1 resistant models

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Abstract

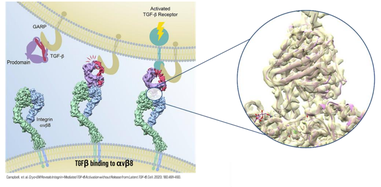
Increased transforming growth factor β (TGFβ) signaling within the tumor microenvironment (TME) is associated with immune exclusion, resistance to checkpoint inhibitor (CPI) therapy, and poor patient outcomes, making TGFβ a promising immunotherapeutic target in cancer (**1**). TGFβ is ubiquitously expressed as a latent form (L-TGFβ) and presented on cell surfaces by L-TGFβ binding proteins (e.g. GARP and LTPB1) as part of the large latent complex (LLC) whereupon it is activated by binding to integrins. Integrin $\alpha_v\beta_8$ specifically binds to L-TGFβ, and this interaction is essential for the activation of the TGFβ-receptor and associated signaling. Corbus Pharmaceuticals is developing a humanized monoclonal antibody, CRB-601, that binds specifically with high affinity to integrin $\alpha_v\beta_8$ and blocks its critical interaction with L-TGFβ.

CRB-601 was selected as a development candidate due to its high binding affinity and specificity to $\alpha_v\beta_8$, and high potency for blocking binding of the $\alpha_v\beta_8$ ligand L-TGFβ. CRB-601 efficacy was evaluated in syngeneic murine tumor models. Mice bearing subcutaneously implanted murine colon carcinoma MC38 and pancreatic ductal adenocarcinoma Pan02, or orthotopically implanted murine breast cancer EMT6 and 4T1 cells were treated with isotype control, anti-mouse PD-1, CRB-601 and the combination of CRB-601 with anti-PD-1. As a single agent, CRB-601 significantly inhibited CPI-sensitive MC38 and resistant EMT6 tumors. In combination with anti-PD-1, CRB-601 not only enhanced efficacy of anti-PD-1 therapy in immune inflamed MC38 tumors, but also reversed the PD-1 therapy resistance in immune excluded EMT6 tumors. Notably, in the Pan02 and 4T1 models, both regarded as "desert tumors" that are non-responsive to current CPIs, combination treatment with CRB-601 and anti-PD-1 mAb significantly enhanced the antitumor efficacy of anti-PD-1 therapy. Flow cytometry analyses of EMT6 tumors showed a reshaped TME, converting immune excluded EMT6 tumors into immune cell-inflamed tumors. Changes included marked increases in infiltration of T cells, NK cells and M1 polarized macrophages in tumors exposed to CRB-601 or the combination.

CRB-601 is a potent and selective integrin $\alpha_v\beta_8$ blocking monoclonal antibody that enhances the activity of CPI treatment *in vivo* and holds promise as a potential combination partner for immunotherapy. Investigational New Drug (IND) enabling studies are currently underway.

Background

TGFβ in its latent form is presented on cell surfaces by L-TGFβ binding proteins (e.g. LTBP1, GARP). Binding of LAP within the LLC by $\alpha_v\beta_8$ integrin activates *TGFβ*. CRB-601 was specifically designed to bind at the *TGFβ* activation site on $\alpha_v\beta_8$ (cryoEM, inset), thereby blocking $\alpha_v\beta_8$-dependent activation (**2,3,4**).



Characterization of CRB-601

Binding specificity

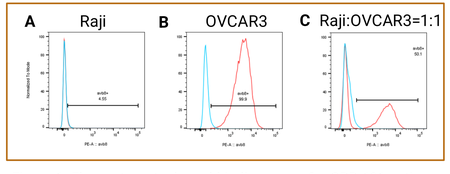


Figure 1. Flow cytometry based binding assay for CRB-601 antigen specificity analysis was performed by incubating 1 µg/ml CRB-601 with $\alpha_v\beta_8$-negative Raji cells (A) and $\alpha_v\beta_8$-positive OVCAR3 cells (B) or 1:1 mixture of the two (C). Raji cells were stained negative, 99.9% OVCAR3 cells express cell-surface $\alpha_v\beta_8$, while 50.1% of Raji and OVCAR3 mixtures (1:1) were stained positive for $\alpha_v\beta_8$.

Binding affinity

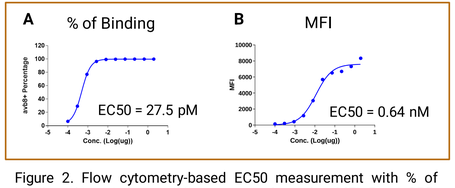
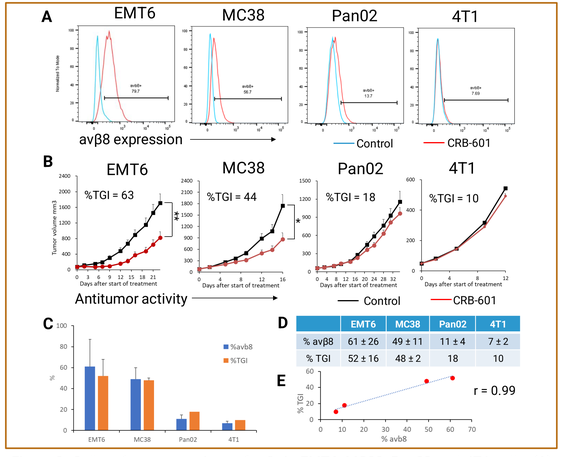


Figure 2. Flow cytometry-based EC50 measurement with % of binding (A) and mean of fluorescence intensity (MFI, B) of CRB-601 in $\alpha_v\beta_8$–positive OVCAR3 cells. Dose-response binding of CRB-601 from 0.001 to 20 µg/ml to OVCAR3 cells was analyzed by flow cytometry assay. The EC50 of 27.5 pM was obtained when % of binding was reported, while the EC50 of 0.64 nM was obtained when MFI was examined (B).

Anti-tumor Activity in CPI Sensitive and Resistant Tumor Models

Relationship between $\alpha_v\beta_8$ expression on tumor cells and antitumor activity of $\alpha_v\beta_8$–blocking antibody CRB-601



	EMT6	MC38	Pan02	4T1
% avβ8	61 ± 26	49 ± 11	11 ± 4	7 ± 2
% TGI	52 ± 16	48 ± 2	18	10

Figure 3. Cell surface expressions of $\alpha_v\beta_8$ in EMT6, MC38, Pan02 and 4T1 cells. (**A**) Representative (from 3 experiments) histogram overlays of CRB-601 stained cells (red) compared with control (light blue). (**B**) Antitumor activity of 10 mg/kg CRB-601 for treatment of implanted $\alpha_v\beta_8$-high EMT6 (4 studies) and MC38 (3 studies), and $\alpha_v\beta_8$-low Pan02 (1 study) and 4T1 (1 study) solid tumors. Representative mean tumor growth curves are shown (n=10 mice/gp., **C, D and E**). Levels of $\alpha_v\beta_8$ expression on tumor cells are closely related to the antitumor activity of CRB-601 in those models. *p* values were calculated by t-test. **p* <0.05, ***p*<0.01.

CRB-601 inhibits tumor growth as a single agent and enhances anti-PD-1 therapy in CPI-sensitive and CPI-resistant murine tumor models

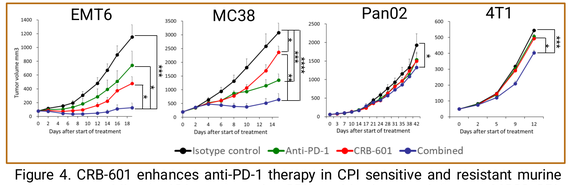


Figure 4. CRB-601 enhances anti-PD-1 therapy in CPI sensitive and resistant murine tumor models. Mice (n=10/group) bearing CPI-sensitive immune-inflamed MC38, CPI-resistant immune-excluded EMT6 and CPI-resistant immune-desert Pan02, 4T1 were treated with 10 mg/kg isotype control, 10 mg/kg anti-mouse PD-1 mAb (RMP1-14), 10 mg/kg CRB-601 or CRB-601 and RMP1-14 in combination twice weekly for 3 weeks. *p* values were calculated by one-way ANOVA or t-test. **p* <0.05, ***p*<0.01, ****p*<0.001, ****p<0.0001.

CRB-601 alone and in combination with anti-PD-1 mAb reverses immune exclusion

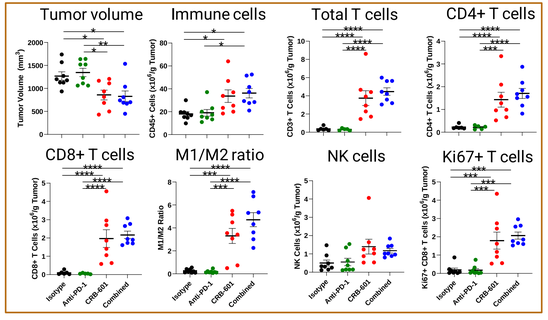


Figure 5. In EMT6 immune excluded tumors, CRB-601 alone or in combination with anti-PD-1 mAb induces infiltration and expansion of T cells, NK cells and M1 polarized macrophages. BALB/c female mice (n=8/group) bearing orthotopically implanted EMT6 murine breast tumors were treated by intraperitoneal injection with 10 mg/kg Isotype control, 10 mg/kg anti-mouse PD-1 mAb (RMP1-14), 10 mg/kg CRB-601 or combination of anti-mouse PD-1 mAb and CRB-601 on days 0, 3 and 6. Tumor infiltrating lymphocytes dissociated from tumors collected on day 10 were analyzed by flow cytometry analysis for T cells, NK cells and M1 or M2 polarized macrophages. The *p* values are calculated by one-way ANOVA followed by Tukey's multiple-comparison test. **p* <0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001.

Treatment with CRB-601 in combination with anti-PD-1 mAb induces long-lasting CD8+ T cell-dependent immunity against cancer

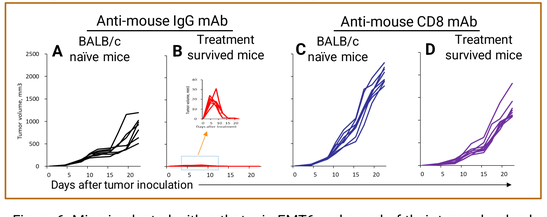


Figure 6. Mice implanted with orthotopic EMT6 and cured of their tumor burden by combination therapy (15 of 30 mice among combination treated groups) were rechallenged by subcutaneous implantation with EMT6 tumor cells two months after original tumor inoculation with (D, n=8) or without (B, n=7) CD8+ T cell depletion. Naïve BALB/c mice subcutaneously inoculated with EMT6 tumor cells with (C, n=8) or without (A, n=8) CD8+ T cell depletion served as tumor growth controls. Mice cured by the combination treatment showed durable antitumor immunity, and rejected tumor rechallenge completely (B). CD8+ T cell depletion abrogated tumor protection in rechallenged animals indicating a T-cell memory response (D).

Conclusions

- CRB-601 is a potent and selective integrin αvβ8 blocking monoclonal antibody
- CRB-601 significantly inhibits tumor growth as a single agent and enhances the efficacy of anti-PD-1 immunotherapy in CPI-sensitive and CPI-resistant tumor models.
- CRB-601 activity was significantly increased in tumors expressing high levels of αvβ8 compared to tumors expressing low levels.
- CRB-601 alone and in combination with anti-PD-1 led to a significant increase in tumor-infiltrating T cells, NK cells and M1 polarized macrophages, suggesting that CRB-601 treatment overcomes immune exclusion in EMT6 tumors.
- CRB-601 holds promise as a potential combination partner for cancer immunotherapy.
- CRB-601 is planned for IND in H1 2023.

References

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- We thank Dr. Steven Nishimura and UCSF colleagues for scientific advice and development of the C6D4F12 antibody.
- CRB-601 is an investigational, pre-clinical stage candidate that has not entered clinical testing and is not approved by the FDA for any indication.