CRB-601, an $\alpha_v\beta_8$ blocking antibody, prevents activation of TGFβ and exhibits anti-tumor activity associated with immune cell remodeling of the tumor microenvironment

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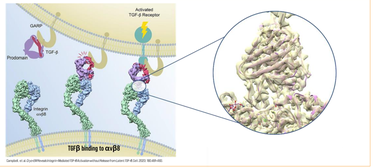
Background

- Transforming growth factor-beta (TGFβ) is a promising immunotherapeutic target in cancer given the association of increased TGFβ signaling in the tumor microenvironment (TME) with immune cell exclusion, and poor clinical outcomes.
- TGFβ is expressed as a latent form (L-TGFβ) and presented on cell surfaces by L-TGFβ binding proteins (e.g., GARP and LRRC33) as part of the large latent complex, whereupon it is activated by binding to integrins, including integrin $\alpha_v\beta_8$ (Figure 1).
- Corbus Pharmaceuticals is developing a humanized monoclonal antibody, CRB-601, that binds with high specificity and affinity to $\alpha_v\beta_8$ and blocks the critical interaction with L-TGFβ that promotes an immune excluded phenotype.
- In the present study, we explored the relationship between CRB-601 antitumor activity and serum concentration. Furthermore, we investigated the antitumor mechanism of CRB-601, including tumor $\alpha_v\beta_8$ receptor occupancy, the impact on TGFβ pathway signaling, and tumor immune cell population.

Figure 1. Model of $\alpha_v\beta_8$ -mediated activation of latent TGFβ and inhibition by CRB-601



- $\alpha_v\beta_8$ surveys the TME for cells displaying L-TGFβ on their cell surface by co-expression of L-TGFβ binding proteins such as GARP.
- Binding of $\alpha_v\beta_8$ to an L-TGFβ-expressing cell exposes the active domain on one fingertip of the mature TGFβ homodimer, allowing binding to TGFβ-R2 and initiation of the TGFβ signaling cascade.
- CRB-601 binds at the same site otherwise occupied by L-TGFβ, thereby preventing its activation.
- A cryo-electron microscopy structure of the antibody C6D4,[1] a murine variant of CRB-601 with the same binding specificity, shows strong overlap with the L-TGFβ binding pocket at the $\alpha_v\beta_8$ interface.[2,3]

Methods

- Tumor growth was evaluated in mice bearing orthotopically implanted murine breast cancer EMT6 or colon cancer MC38 and treated with CRB-601 or the combination.
- $\alpha_v\beta_8$ receptor occupancy (RO) was measured *ex vivo* in dissociated tumors by flow cytometry (FC).
- Tumor-infiltrating immune cell populations were characterized by FC, and pSMAD2/3 was measured by Western blot.

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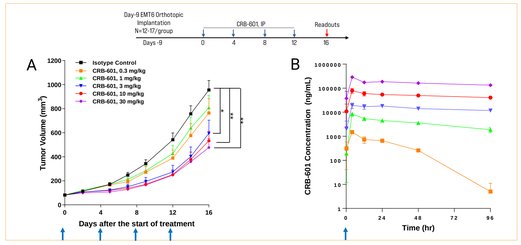
Results

CRB-601 Treatment Shows Dose- and Exposure-Dependent Antitumor Activity

Figure 2. Dose-dependent antitumor activity of integrin $\alpha_v\beta_8$ monoclonal antibody CRB-601 in the mouse EMT6 breast cancer model



*p<0.05, **p<0.01 by 1-way ANOVA multiple comparison.

CRB-601 Plasma Concentration Increased Proportionately with Dose

Table 1. PK parameters of CRB-601 in BALB/c mice bearing EMT6 tumors following the first CRB-601 treatment

Dose (mg/kg)	Plasma $C_{average}$ (ng/mL)	Plasma $C_{average}$ (N-fold EC_{50})
0.1	94	0.051
0.3	395	0.22
1	3,744	2.0
3	14,625	8.0
10	50,237	28
30	165,691	91

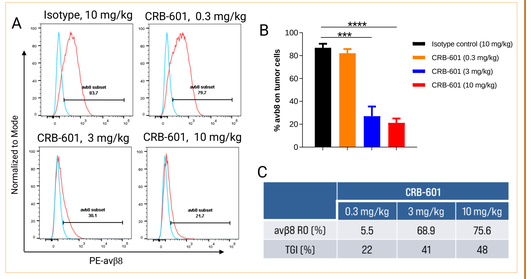
In the maximum effect range (3–30 mg/kg), plasma $C_{average}$ levels reached significant multiples of the CRB-601 binding affinity.

A. BALB/c female mice (n=12–17/group) bearing established orthotopically implanted EMT6 murine breast tumor (81 ± 4 mm^3) were treated by intraperitoneal injection with 10 mg/kg isotype control or 0.3, 1, 3, 10 and 30 mg/kg CRB-601 on Days 0, 4, 8 and 12.

B. Serum concentration-time profiles of CRB-601. Blood was collected at 0.17, 4, 12, 24, 48 and 96 hours after the first treatment (n=4 per time point), and CRB-601 serum concentrations were determined by a sandwich ELISA.

Dose-Dependent Antitumor Activity Correlates with Tumor Cell Surface $\alpha_v\beta_8$ Occupancy by CRB-601

Figure 3. Correlation between dose-dependent antitumor activity and tumor cell surface $\alpha_v\beta_8$ occupancy by CRB-601 in the mouse EMT6 breast cancer model



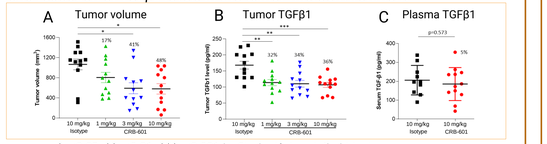
	CRB-601		
	0.3 mg/kg	3 mg/kg	10 mg/kg
avβ8 RO (%)	5.5	68.9	75.6
TGI (%)	22	41	48

***p<0.001, ****p<0.0001 by Student's two-tailed t test.

A, B and C. Receptor occupancy (RO) was measured using a CRB-601-competitive humanized anti-$\alpha_v\beta_8$ mAb (competitor) to detect unoccupied sites. Briefly, $CD45^{Neg}$ single cell suspensions from treated EMT-6 tumor nodules (n=3/group) were incubated with 1 µg/mL of the competitor mAb. This was then detected with PE goat anti-human IgG by FC. Since both CRB-601 and the competitor mAb bind to $\alpha_v\beta_8$ on EMT6 tumor cells, occupancy of $\alpha_v\beta_8$ sites during in-life treatment of these tumors with CRB-601 reduces competitor binding, as shown by the reduction in MFI with increasing CRB-601 dose. As a reference, the percentage of competitor mAb detected when isotype control treated tumors were stained was established as a 100% receptor availability. In the 0.3, 3 and 10 mg/kg treated tumor cohorts we observed 6%, 69% and 76% receptor occupancy at the increasing concentrations which correlated to TGI values of 22%, 41% and 48% in these same dose groups.

Dose-Dependent Antitumor Activity of CRB-601 Correlates with Lower Levels of TGFβ1 in the Tumor Microenvironment

Figure 4. Correlation between dose-dependent antitumor activity of CRB-601 and reduction of TGFβ1 levels in the tumor microenvironment

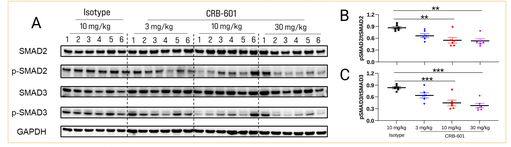


*p<0.05, **p<0.01, ***p<0.001 by Student's two-tailed t test.

Whole-tumor lysates and serum samples (n=12/group) collected on Day 16 after CRB-601 treatment on Days 0, 4, 8 and 12 were analyzed for TGFβ1 levels using enzyme-linked immunosorbent assay. (**A**) Dose-dependent antitumor activity of CRB-601 is correlated with (**B**) dose-dependent reduction of tumor TGFβ1 levels, while (**C**) systemic TGFβ1 is not impacted.

CRB-601 Treatment Inhibits TGFβR-SMAD2/3 Signaling in Tumors

Figure 5. Inhibition of TGFβ-SMAD2/3 signaling by CRB-601



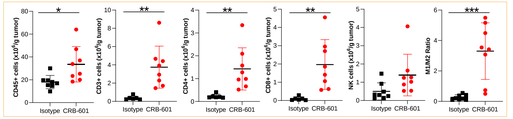
**p<0.001, ***p<0.0001 by 1-way ANOVA multiplex comparison.

Whole-tumor lysates from EMT6 tumor (n=6/group) collected on Day 16 after CRB-601 treatment on Days 0, 4, 8 and 12 were analyzed by immunoblotting for total (t) and phosphorylated (p) SMAD2 and SMAD3. GAPDH served as loading control.

The expression of pSMAD2 (A, B) and pSMAD3 (A, C) was markedly decreased in a dose-dependent manner.

CRB-601 Treatment Modifies Tumor Microenvironment

Figure 6. Remodeling of the tumor microenvironment by CRB-601 treatment

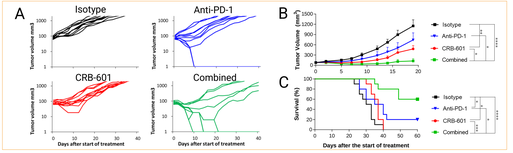


* p<0.05, ** p<0.01, ***p<0.001 by Student's two-tailed t test

BALB/c mice (n=8/group) bearing established orthotopically implanted EMT6 were treated with 10 mg/kg isotype control mCRB-601 by intraperitoneal injection on days 0, 3 and 6. Tumor infiltrating lymphocytes dissociated from tumors collected on days 10 were analyzed by flow cytometry for T cells, NK cells, M1 and M2 polarized macrophages

CRB-601 Treatment Enhances the Efficacy of Anti-PD-1 Therapy in the EMT6 Tumor Model

Figure 7. CRB-601 potentiates anti-PD-1 immunotherapy in the EMT6 tumor model

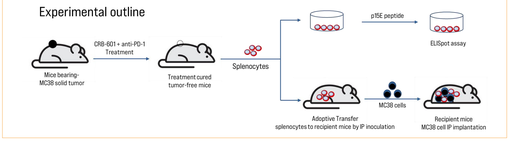


*p<0.05, **p<0.001, ***p<0.001, ****p<0.0001 by Student's two-tailed test or by log-rank test.

BALB/c (n=10/group) bearing orthotopically implanted EMT6 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-PD-1 and CRB-601 twice weekly for a total of 3 weeks. The measurement of tumor growth was conducted three times every week. The growth curves of tumors for (A) individual and (B) average are displayed, along with (C) Kaplan-Meier survival curves.

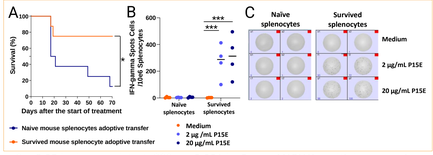
CRB-601 in Combination with Anti-PD-1 Induced Long-Lasting Tumor-Specific Cytotoxic T Cells

Figure 8. Evaluation of long-term antitumor immunity in cured mice after CRB-601 and anti-PD-1 combination treatment



C57BL/6 mice bearing fully established subcutaneous MC38 tumor (197 ± 13 mm^3) were treated with CRB-601 in combination with anti-PD-1 twice weekly for 3 weeks. Five million fresh splenocytes collected from mice (n=4) cured by combination treatment 270 days after treatment or 5 x 10^6 splenocytes collected from naïve C57BL/6 mice (n=4) were adoptively transferred to naïve C57BL/6 mice (n=8) by I.P injection on day -1. The receiving mice were then inoculated with 0.5 x 10^6 MC38 cells on day 0.

Figure 9. Evaluation of long-term antitumor immunity in cured mice after CRB-601 and anti-PD-1 combination treatment



*p<0.05 by long-rank test, *** p < 0.001 by 2-way ANOVA multiple comparison.

A. Adoptive transfer of the fresh splenocytes from MC38-bearing mice cured after combination treatment partially protected the receiving mice from MC38 inoculation. Mouse survival was monitored for up to 75 days after MC38 inoculation.

B and C. ELISpot assay demonstrated a significant increase of IFN-γ producing MC38 associated antigen specific CD8+ T cells in MC38 bearing mice cured after combination treatment. IFN-γ ELISPOT was used to quantify the tumor reactivity in the splenocytes. A total of 2x10^5 splenocytes from the treatment survived mice or naïve mice were analyzed for their ability to produce IFN-γ in the absence or presence of 2 µg/mL and 20 µg/mL MC38 associated T-cell epitope p15E.

Conclusions

CRB-601 is a potent and selective integrin $\alpha_v\beta_8$ blocking monoclonal antibody that enhances the activity of immune checkpoint inhibitors *in vivo* and holds promise as a potential combination partner for immunotherapy.

The main findings of CRB-601 are as follows:

- Exhibited dose-dependent antitumor activity which correlated with systemic exposure and increased occupancy of tumor $\alpha_v\beta_8$.
- Decreased TGFβ-1 and inhibited SMAD2/3 signaling in tumors.
- Modified the tumor microenvironment, leading to a significant increase in tumor-infiltrating T cells, NK cells and M1 polarized macrophages.
- Enhanced the efficacy of anti-PD-1 immunotherapy.
- In combination with anti-PD-1 induced long-lasting tumor-specific cytotoxic T cells.

References

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2. Takasaka, N. et al. (2018) J. Clin. Invest. Insight 3(20) e122591.
3. Seed et al. (2021) Sci. Immunol. 6, eabf0558.

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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.