CRB-601: A Highly Potent and Selective Integrin αvβ8 Blocking Antibody with Anti-Tumoral Properties

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AACR Poster 5611

Abstract

TGFβ is a secreted protein produced by multiple lineages of leukocytes and tumors that promotes cancer progression primarily via the suppression of both the innate and adaptive immune systems. This makes TGFβ a promising immuno-therapeutic target in cancer. It is ubiquitously expressed in a latent (L-TGFβ) form and L-TGFβ has been shown to promote an immune suppressive phenotype within the tumor micro-environment (**1**). Integrin αvβ8 specifically binds to L-TGFβ. This interaction is essential for the activation of L-TGFβ-mediated signals in a variety of immune cell types. Interestingly, it has been recently shown that integrin αvβ8-mediated TGFβ activation can act directly through L-TGFβ and does not require the release of active TGFβ (**2**). Inhibition of integrin αvβ8-mediated TGFβ activation has been shown to block immunosuppressive regulatory T cell differentiation and enhance the recruitment of cytotoxic T cells into the tumor microenvironment (**3, 4**).

Here, we demonstrate by Surface Plasmon Resonance (SPR) that our clinical candidate CRB-601, a monoclonal antibody selective inhibitor of integrin αvβ8 has a high affinity and specificity for the integrin αvβ8 complex. Additionally, we evaluated the anti-tumoral properties of CRB-601, and its murine compatible version, mCRB-601, as a monotherapy, as well as in combination with anti-PD-1 therapy in an MC38 syngeneic mouse tumor model. Findings from this study highlight the importance of integrin αvβ8 blockade to modulate the immune landscape within the tumor and to enhance response to immune checkpoint therapy.

Background

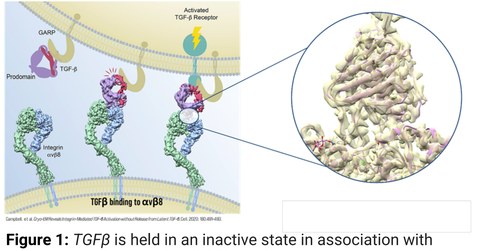

Figure 1: *TGFβ* is held in an inactive state in association with latency associate peptide (LAP) and is presented on cell surfaces by latent transforming growth factor β binding proteins (e.g. LTBP1, GARP). Upon binding of the LAP-*TGFβ* complex to the αvβ8 integrin, *TGFβ* is now capable of activating the *TGFβ* receptor and the associated SMAD signaling pathway, leading to expression of *TGFβ* target genes. CRB-601 was specifically designed to bind at the *TGFβ* activation site on *αvβ8* (cryoEM, inset), thereby blocking *αvβ8*-dependent activation (**1**).

In Vitro Binding

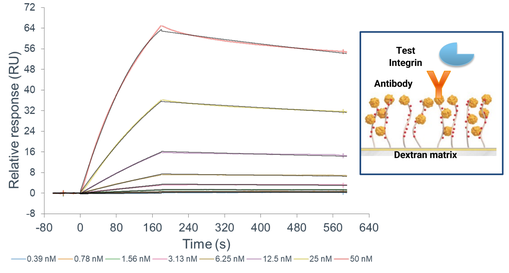

Figure 2. SPR Binding Assay. Integrin binding of CRB-601, and its murine compatible version, mCRB-601, was assessed at 37°C. mAbs were immobilized on an anti-Fc CM5 sensor chip, and integrins (0.39-50 nM for $\alpha_v\beta_8$ and 200 nM for others) were injected at a flow rate of 30 μL/min for a contact time of 180 s and dissociation time of 400 s. Data were fit (grey lines) to a 1:1 antibody:ligand binding model to determine k_a, k_d and Kd.

Table 1. Surface Plasmon Resonance Binding Affinities (Kd, nM) to Human $\alpha_v\beta_x$ and Murine $\alpha_v\beta_8$ Integrins

Antibody	$\alpha_v\beta_1$	$\alpha_v\beta_3$	$\alpha_v\beta_5$	$\alpha_v\beta_6$	$\alpha_v\beta_8$	$m\alpha_v\beta_8$
CRB-601	>200	>200	>200	>200	1.4	1.4
mCRB-601	ND	ND	ND	ND	10.2	10.8

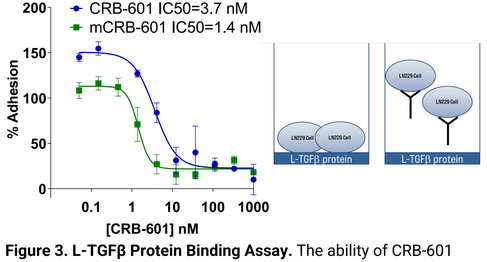

Figure 3. L-TGFβ Protein Binding Assay. The ability of CRB-601 and mCRB-601 to block binding of L-TGFβ to $\alpha_v\beta_8$ was measured in a cell-based competition assay. L-TGFβ was immobilized on a polystyrene plate and incubated with LN229 cells expressing $\alpha_v\beta_8$ and increasing concentrations of CRB-601 for 30 mins at 37°C. Bound LN229 cells were quantified by Crystal Violet. Data were fit to a 4-parameter displacement curve to determine IC_{50}.

Anti-Tumor Activity in MC-38 Syngeneic Model

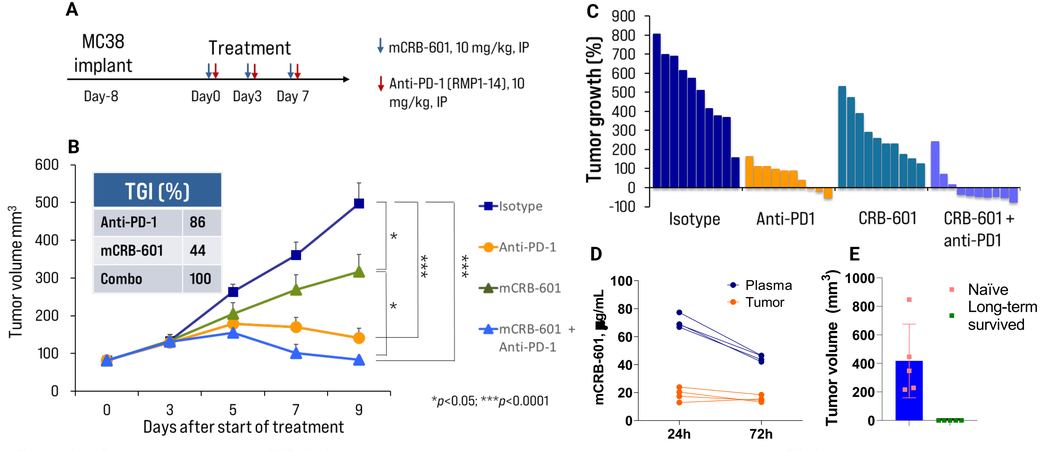

Figure 4: $\alpha_v\beta_8$-blocking antibody mCRB-601 inhibits MC38 tumor growth and augments the efficacy of anti-PD-1 immunotherapy. **A.** Experimental schema: C57BL/6 mice were inoculated subcutaneously (sc) with $0.3x10^6$ MC38 murine colon carcinoma cells. When tumors reached 82 ± 6 mm^3 (mean ± sem), mice were randomized and treated by intraperitoneal injection (n=10 per treatment) with 10 mg/kg isotype control, anti-mouse PD-1 mAb (RMP1-14), mCRB-601 or combination of mCRB-601and anti-mouse PD-1 mAb on days 0, 3 and 7. **B**. Tumor growth curves and tumor growth inhibition (TGI,%). **C**. Change in tumor volume compared to baseline, individual mice. **D**. Biodistribution of mCRB-601 in plasma and tumor at 24 and 72 hr post ip injection of 10 mg/kg CRB-601. **E**. Tumor re-challenge in MC38 tumor-free mice 52 days after initiation of mCRB-601 and anti-PD-1 combination treatment, and in naïve C57BL/6 control mice. Mice (n=5/gp) were inoculated sc with 0.3 x 10^6 MC38 cells, and followed for (30) days. Tumors did not grow in mice previously treated with the mCRB-601 and anti-PD-1 mAb combination. All *p* values are calculated by one-way ANOVA followed by Tukey's multiple-comparison test. *p <0.05, ***p<0.0001.

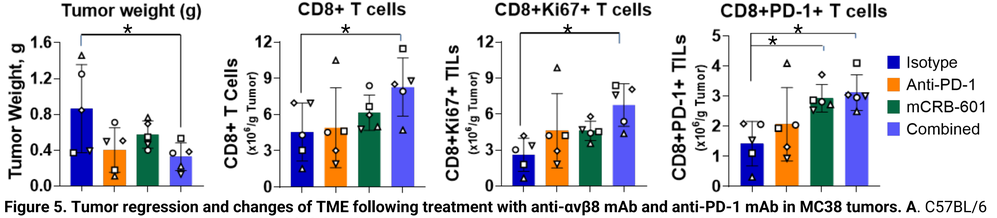

Figure 5. Tumor regression and changes of TME following treatment with anti-αvβ8 mAb and anti-PD-1 mAb in MC38 tumors. A. C57BL/6 mice were inoculated subcutaneously with $0.3x10^6$ MC38 cells on day -14. When tumors reached 283 ± 41 mm3 (mean ± sem), mice (n=5/gp) were randomized and treated by intraperitoneal injection with 10 mg/kg Isotype control, anti-mouse PD-1 mAb (RMP1-14), mCRB-601 or combination of anti-mouse PD-1 mAb and mCRB-601 on days 0 and 3. Mice were sacrificed on day 7, tumor nodules were collected and weighed. Flow cytometry analysis of dissociated tumors was analyzed on (**B**) CD8+ tumor-infiltrating lymphocytes, (**C**) Ki67+ expanding CD8+ T cells and (**D**) PD-1 expression CD8+ T cells. The *p* value was calculated with the two-tailed Student's *t* test. *p <0.05.

Conclusions

- CRB-601 exhibits low nM affinity to human and murine $\alpha_v\beta_8$ and high selectivity with no appreciable binding to other RGD-binding integrin proteins.
- mCRB-601 significantly inhibits MC38 tumor growth as a single agent and enhances the efficacy of anti-PD-1 immunotherapy
- mCRB-601 treatment effects on tumor growth, alone and in combination with anti-PD-1, correlated to increased infiltration into the tumor microenvironment of CD8+ T-cells exhibiting activation markers, suggesting an augmented immune contribution to tumor clearance.
- The combination of mCRB-601 and anti-PD-1 therapy protected mice from tumor rechallenge
- CRB-601 is a potent and selective integrin αvβ8 blocking monoclonal antibody that enhances the activity of immune checkpoint inhibitors *in vivo* and holds promise as a potential combination partner for immunotherapy. Investigational New Drug (IND) enabling studies are currently underway.

References

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Disclosures and Acknowledgements

- This study was sponsored by Corbus Pharmaceuticals, Inc. Authors DW, EH and AK are employees and/or shareholders of Corbus Pharmaceuticals
- We thank Dr. Steven Nishimura and 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.