CRB-601, a selective integrin αvβ8-blocking antibody, prevents TGFβ activation, promotes immune cell remodeling, and exhibits potent antitumor activity

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Background

- Increased TGFβ signaling is a key driver of tumor growth and immune cell exclusion. Thus, blocking TGFβ signaling has emerged as a challenging but essential node to regulate to prevent uncontrolled tumor proliferation.
- TGFβ is thought to promote tumor progression via various mechanisms, including epithelial-mesenchymal transition associated with metastasis, neo-angiogenesis that facilitates tumor vascularization, stromal cell and cancer-associated fibroblast proliferation, and immunosuppressive signaling. Collectively, TGFb signaling alters the tumor microenvironment, contributes to immune exclusion, and renders tumors insensitive to many systemic treatments including checkpoint therapies.
- An essential aspect of TGFβ activation is the structural changes that are induced by the binding the integrin αvβ8 to a homodimer of latent-TGFβ that is cell bound as a large latent complex (LLC). This binding exposes the active domain of the mature TGFβ protein enabling the binding and activation of the TGFβ-R2 and triggering TGFβ signaling.
- Recognizing the significant impact of this protein: protein interaction in driving tumorigenesis, Corbus is developing CRB-601 a humanized IgG4 blocking mAb designed to prevent this interaction and reduce the tumor promoting impact of TGFβ signaling and overcomes immune cell exclusion in the TME.
- In the current investigation we explored the impact of CRB-601 on tumor growth and the modulation of the immune milieu in murine syngeneic tumor models. We also explored the profile of biomarkers associated with an anti tumor response across immune cell types, chemokines and cytokines.

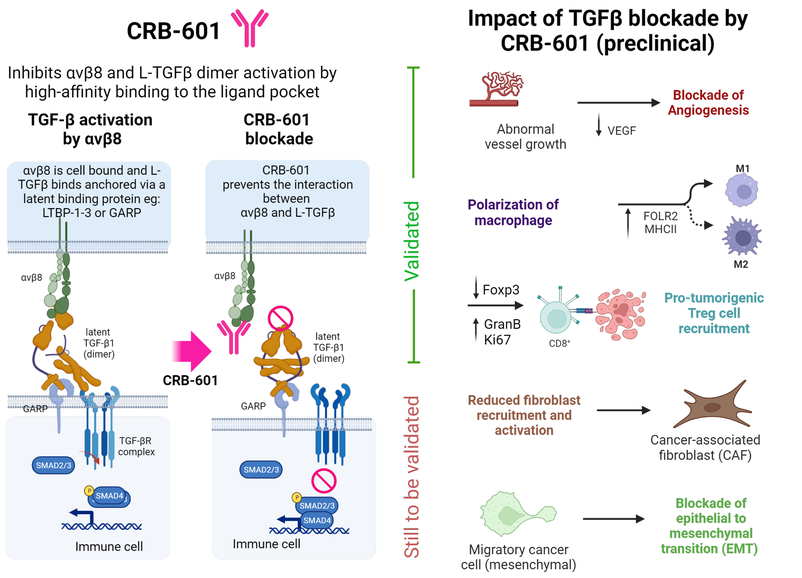


Figure 1. Schematic of the protein interactions between the integrin αvβ8 and the large latent-TGFβ complex in the tumor microenvironment and the role of CRB-601 in blocking this protein interaction. Initial validations suggest that the MOA of CRB-601 prevents TGFβ related angiogenesis, T-reg recruitment and CD8 T-cell reactivation/recruitment.

Prepared in Bio Render

Methods

- The tumor growth inhibition of CRB-601+/- anti PD-1 was assessed in 3 tumor models, MC38, EMT6, and 4T1. Post treatment both tumors and peripheral blood were collected to conduct pharmacodynamic assessments
- Flow Cytometry was used as a primary method to analyze both the periphery and excised tumors for modulation of the immune cell populations. Additionally, tumor specimens underwent Bulk Sequencing and Luminex cytokine assessment, which provided a multifaceted understanding of the tumor's genetic and cytokine profiles.
- A specific immunological assessment, known as the IFN-γ ELISpot, was also performed on the spleens extracted from mice that were rechallenged with the MC38 tumor model to determine the immune system's responsiveness and effectiveness in combating the reintroduced tumor cells.

References

- Cryo-EM reveals integrin-mediated TGF-β activation without release from latent TGF-β. Cell, 180(3), 490-501 (2020).
- Integrin αvβ8–expressing tumor cells evade host immunity by regulating TGF-β activation in immune cells J. Clin. Invest. Insight 3(20) e122591 (2018).
- A tumor-specific mechanism of Treg enrichment mediated by the integrin αvβ8. Sci. Immunol. 6, eabf0558 (2021).

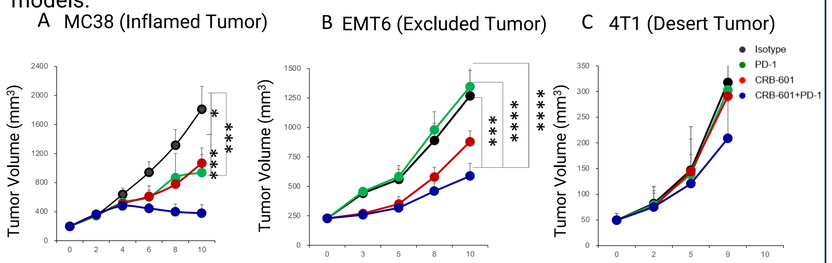
Disclosures and Acknowledgements

- This study was sponsored by Corbus Pharmaceuticals, Inc. Authors MS, DW, VS, RB and AK are employees and/or shareholders of Corbus Pharmaceuticals.
- SR, JB and SN are UCSF employees.
- 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.

Results

CRB-601 Enhances Anti-PD-1 Therapy in Checkpoint Inhibition Sensitive and Resistant Murine Tumor Models

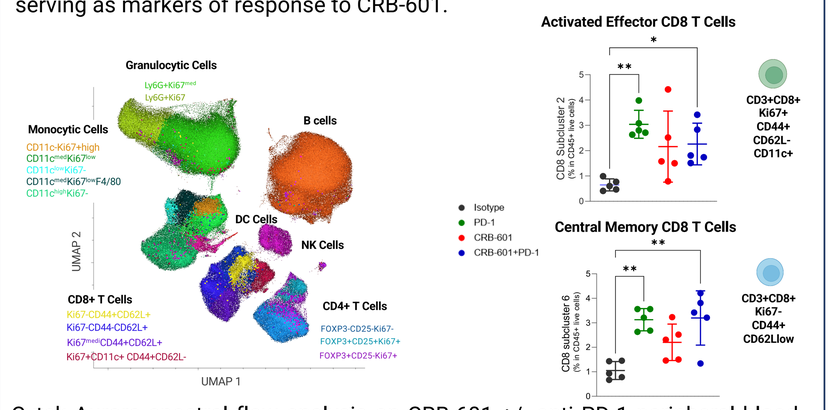
Figure 2. $\alpha_V\beta_8$-blocking antibody CRB-601 enhances anti-PD-1 therapy in both immune checkpoint blockade therapy sensitive and resistant murine tumor models.



(A) Mice (n=10/group) with SubCu implanted MC38 (B) orthotopically implanted EMT6 and (C) SubCu 4T1 tumors were treated with 10 mg/kg isotype control, 10 mg/kg anti-mouse PD-1 (RMP1-14), 10 mg/kg CRB-601. In MC38 and EMT6 models, treatments were started at 200 mm^3 or 50-80 mm^3 for 4T1. All p values are calculated by one-way ANOVA followed by Tukey's multiple-comparison test. *p <0.05, ***p<0.001, ****p<0.0001.

The Increase in Activated CD8+ T Cells in Peripheral Blood Post CRB-601 Treatment Indicates That This Assessment is a Potential PD Biomarker

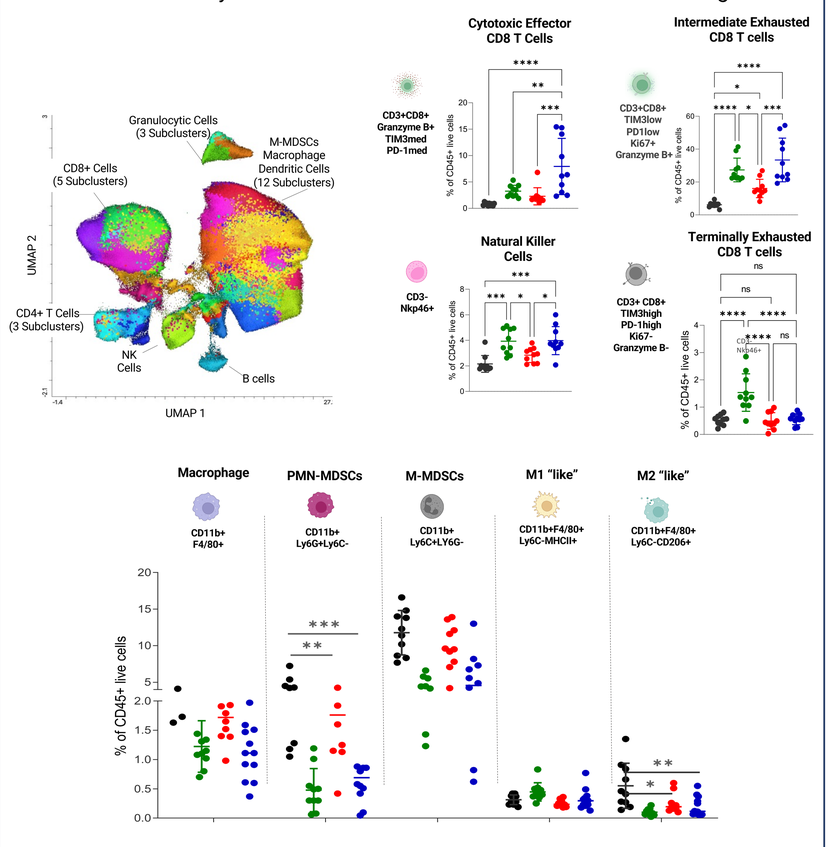
Figure 3. In CRB-601-treated mice, an increase in activated effector CD8 T cells and central memory CD8 T cells in peripheral blood are observed, potentially serving as markers of response to CRB-601.



Cytek Aurora spectral flow analysis on CRB-601 +/- anti PD-1 peripheral blood. 21-color panel, 1.25x10^6 CD45+ cells. 5 mice/gp. Data refinement by (1) Down sampling, (2) UMAP, (3) X-Sift, (4) Euclid, and (5) Cluster Explorer.). p values = one-way ANOVA then Tukey's multiple-comparison *p<0.05, ***p<0.001, ****p<0.0001.

CRB-601 Treatment Elevates Cytotoxic T and Natural Killer Cells While Reducing Immunosuppressive Subsets in Murine Tumor samples

Figure 4. After a 10-Day treatment with CRB-601, immune profiling reveals changes in T-cell and innate immune activities. In combination with antiPD1, CRB-601 enhances cytotoxic effector T-cells and NK cells while reducing PMNs.



Treated disaggregated tumor tissue was assessed by a 22-Ab flow cytometry panel. 1.25x10^6 CD45+ cells, defined 31 immune subclusters through high-dimensional analysis. Data refinement by (1) Down sampling, (2) UMAP, (3) X-Sift, (4) Euclid, and (5) Cluster Explorer techniques. CRB-601 treatment resulted in an increase in Ki67+ Granzyme B-secreting cytotoxic T cells and an intermediate exhausted T-cell population. Interestingly, No escalation of terminal exhaustion markers was observed. Increase in the natural killer population and decrease in PMN-MDSCs and M2 "like" macrophage was observed. p values = one-way ANOVA followed by Tukey's multiple-comparison test. *p <0.05, ***p<0.001, ****p<0.0001.

CRB-601 Enriches the Effector Immune Cell Population in a Murine Tumor

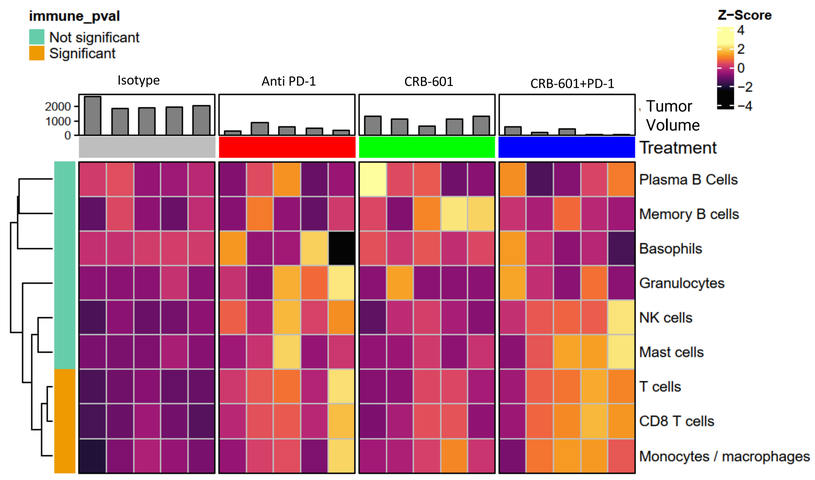
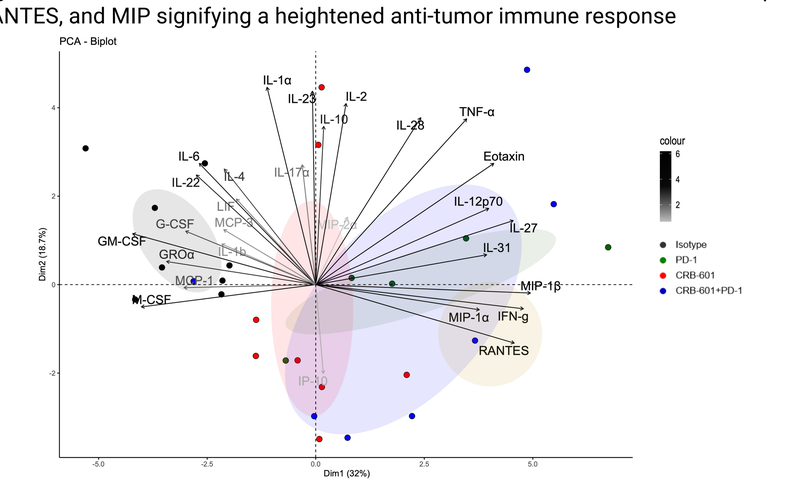


Figure 5. Immune deconvolution of RNA bulk seq tumor samples after treatment with CRB-601 +/- anti-PD-1. Alterations in CD8+ T cells and monocytes/macrophages immune subsets within MC38 tumors.

Immunomodulatory Cytokines Within the Local Tumor Milieu are upregulated with CRB-601 + anti PD-1 treatment

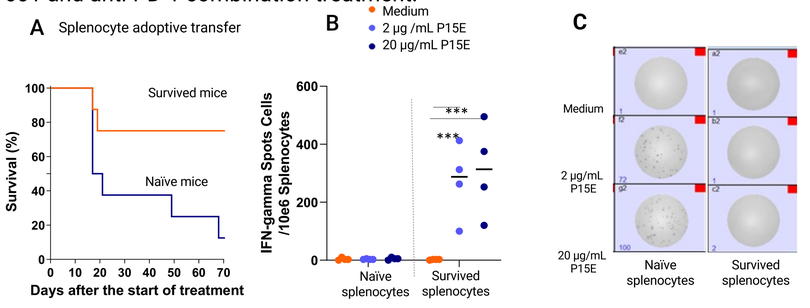
Figure 6: CRB-601 + anti PD-1 treatment resulted in increased levels of IFN-γ, RANTES, and MIP signifying a heightened anti-tumor immune response



Principal component analysis of tumor samples (n=30) based on cytokine levels (n=36). The PCA biplot shows separation based on both the 1st and 2nd principal component between samples treated with either anti-mPD1, CRB-611 or combination therapy (CRB-611 and anti-mPD1) and the control group with the cytokines IL-31, IL-27, MIPs, CCL5/RANTES and IFNγ explaining majority of the variability in the data.

CRB-601 + anti PD-1 treatment Induced A Long-Lasting Tumor-Specific Cytotoxic T Cells Response

Figure 7. Evaluation of long-term antitumor immunity in cured mice after CRB-601 and anti-PD-1 combination treatment.



(A) Naïve animals received 5 x10^6 fresh splenocytes via adoptive transfer from either cured or naive mice to new recipient mice. These transfer mice were then challenged with inoculated of MC38 cells and the ability to protect tumor growth via immune memory. (B) IFN-γ ELISpot quantified tumor production of IFN-γ +/- the MC38-associated T-cell epitope p15E at varying concentrations. (C) A rise in the number of MC38-associated antigen-specific CD8+ T cells secreting IFN-γ was noted in the splenocytes of mice.

Conclusions

- CRB-601 advances immunotherapeutic strategies by antagonizing integrin αvβ8, enhancing the efficacy of immune checkpoint inhibitors in vivo.
- The synergistic administration of CRB-601 with anti-PD-1 agents significantly enhances tumor-specific cytotoxic T-cell responses, suggesting an enriched T-cell precursor population. Concurrently, a noticeable reduction in PMNs count is observed, indicating a transition towards a less immunosuppressive tumor microenvironment. This favorable shift is further strengthened by the elevated IFNγ activity observed, which signals enhanced anti-tumor activity mediated by immune cells, showcasing a promising therapeutic synergy for improved cancer treatment outcomes.
- This notable combination reveals the potential of such synergistic strategies in strengthening anti-tumor immunological responses, thereby emphasizing the promise of this combinatorial approach in advancing the domain of immunotherapy.