AGEN1721, A FIRST-IN-CLASS FC-ENHANCED BIFUNCTIONAL ANTIBODY TARGETING FAP AND TGFB, REMODELS THE TUMOR MICROENVIRONMENT TO OVERCOME CANCER-ASSOCIATED FIBROBLAST-MEDIATED IMMUNE SUPPRESSION

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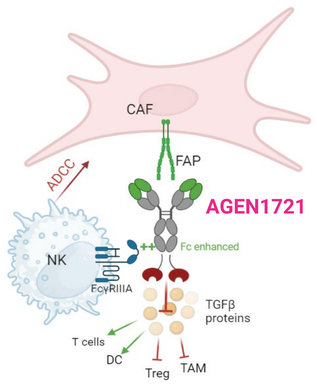
AGEN1721 Remodels the Tumor Stroma by Simultaneously Targeting Cancer-Associated Fibroblasts and TGFβ Pathways

AGEN1721: A Bifunctional Fc-enhanced Anti-FAP Antibody with an Optimized TGFβRII Trap



1. **Depletes immunosuppressive FAP+ CAFs**

   Fc-enhanced to potently deplete FAP+ cancer-associated fibroblasts (CAFs) in the TME that inhibit T cell infiltration and tumor killing[1]

2. **Neutralizes TGFβ in the TME**

   Preferentially inhibits the immunosuppressive effects of TGFβ in the TME[2] to enhance innate and adaptive anti-tumor immunity.

3. **Promotes T-cell Infiltration and Activation**

   Remodels the tumor stroma, reducing desmoplasia and barriers associated with T-cell infiltration in immune excluded tumors[3]

AGEN1721 converts immune-excluded tumors into a 'hot' immune-infiltrated TMEs

FAP is expressed across multiple solid tumors and its co-expression with TGFβ1 correlates with poor prognosis

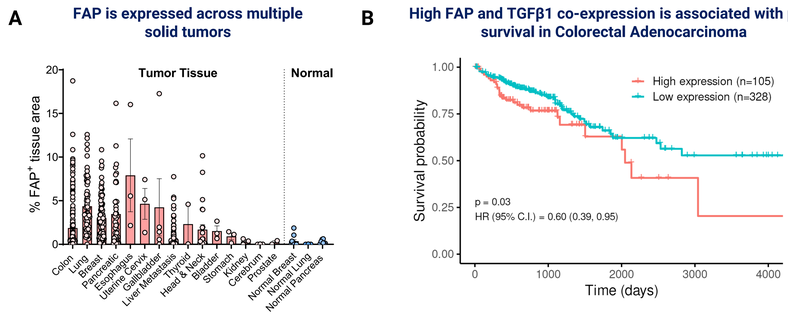


Figure 1. FAP expression in solid tumors correlates with poor prognosis when co-expressed with TGFβ1. (A) FAP overexpression detected by IHC microarray across multiple solid tumors, with minimal expression in normal tissues. **(B)** High FAP and TGFβ1 co-expression correlates with poor prognosis in colorectal adenocarcinoma patients. RNA sequencing and survival data from 597 colorectal cancer samples were obtained from TCGA, curated by the Human Protein Atlas[4]. Patient groups were stratified using maximally selected rank statistics (maxstat R package).

FAP expression correlates with CD8+ T cell exclusion from the tumor microenvironment in patients with colorectal cancer (CRC)

High FAP expression is associated with a stromal barrier that limits CD8+ T-cell infiltration in the TME

Low FAP expression correlates with greater CD8+ T-cell infiltration in the TME

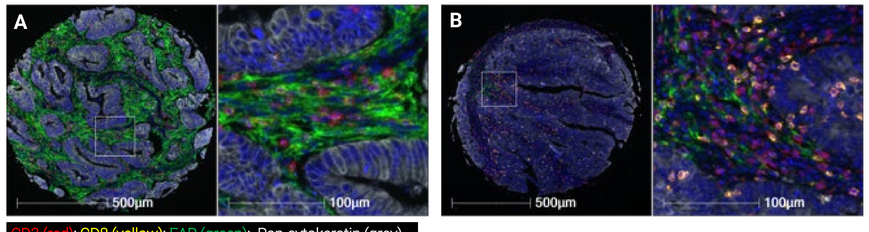


Figure 2. FAP expression inversely correlates with T cell infiltration in colorectal cancer. Multiplex immunofluorescence of human CRC tissue showing CD3 (red), CD8 (yellow), FAP (green), and pan-cytokeratin (grey). **(A)** High stromal FAP expression correlates with limited CD8+ T cell infiltration and reduced tumor cell proximity (left: full view; right: magnified region). **(B)** Low FAP expression correlates with enhanced CD8+ T cell infiltration and tumor penetration (left: full view; right: magnified region). Scale bars shown.

References: 1. Jaeghere et al. Trends in Cancer 2019; 2. Mariathasan et al . Nature 2018; 3. YT Liu et al. Theranostics, 2021; 4. Uhlen M et al. Science 2017; 5. Hothorn and Zeileis, Biometrics 2008

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AGEN1721 binds with high affinity to FAP and TGFβ

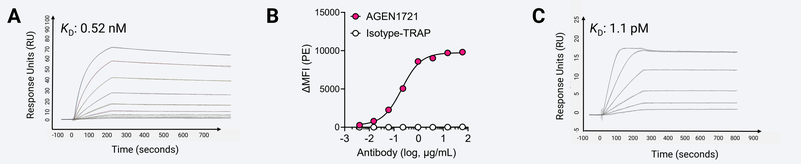


Figure 3. AGEN1721 demonstrates high-affinity binding to both human FAP and TGFβ. (A) Surface plasmon resonance (SPR) analysis of AGEN1721 binding to human FAP protein. Binding affinity (K_D) was determined using a dose range of human FAP with AGEN1721 captured on a CM5 chip. **(B)** Binding of AGEN1721 (pink circle) or Isotype-TRAP (clear circle) to CHO cells expressing human FAP by flow cytometry. Binding was detected using a PE-conjugated anti-human IgG1 secondary antibody. **(C)** SPR analysis showing AGEN1721 binding to human TGFβ1.

AGEN1721 is Fc-enhanced to improve binding to high and low affinity activating Fcγ receptors

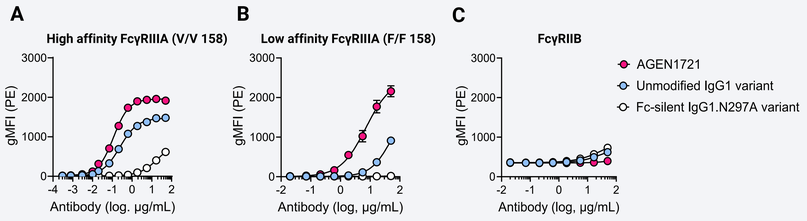


Figure 4. Enhanced Fc domain of AGEN1721 demonstrates improved binding to activating Fcγ receptors. Binding of AGEN1721 (pink circle), anti-FAP-hIgG1-TRAP (unmodified Fc variant, blue circle) and anti-FAP-hIgG1.N297A-TRAP (reduced Fc variant, white circle) to CHO cells expressing human (**A**) FcγRIIIA (variant V158), (**B**) FcγRIIIA (variant F158) or (**C**) FcγRIIB. Cells were incubated with a dose range of the indicated antibodies. Binding was detected using a FITC-labelled goat anti-human IgG antibody and analyzed by flow cytometry.

AGEN1721 promotes superior ADCC-mediated depletion of FAP+ cells compared to conventional IgG1 anti-FAP antibodies

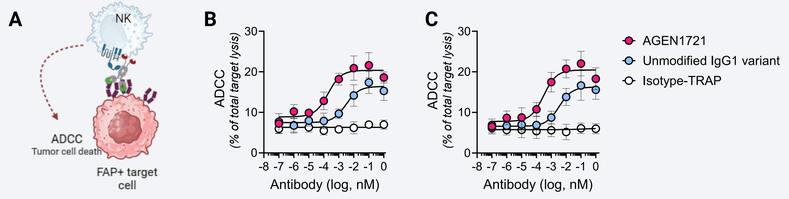


Figure 5. AGEN1721 enhances NK cell-mediated cytotoxicity. (A) Schematic of primary human PBMC-derived NK cells activated with IL-2/IL-15 overnight and co-cultured with **(B)** U138MG or **(C)** WI-38 cells. Cells were treated with a dose range of AGEN1721 (pink circle), an unmodified IgG1 Fc version of AGEN1721 (blue circle) or isotype control-TRAP (clear circle). Cell death assessed by calcein release after 3 hours.

AGEN1721 neutralizes TGFβ-mediated tumorigenic functions

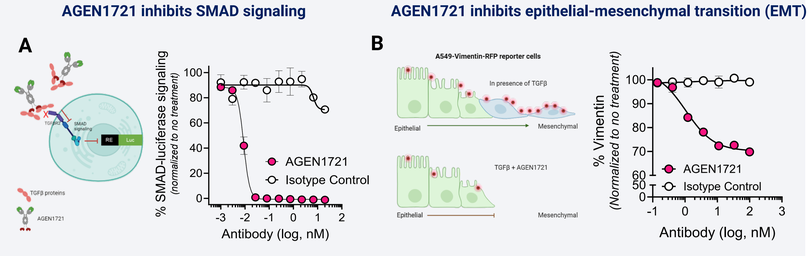


Figure 6. AGEN1721 inhibits TGF-β-induced SMAD signaling and epithelial-mesenchymal transition (EMT). (A) TGFβ-induced SMAD signaling in HEK 293 luciferase reporter cells treated with increasing concentrations of AGEN1721 (pink circle) or isotype control (clear circle). **(B)** A549-VIM-RFP reporter cells were incubated with a dose range of AGEN1721 (pink circle), or isotype control (clear circle) and TGFβ1 for 24 hours. Vimentin expression was assessed by flow cytometry and normalized to non-treated A549 cells. Similar results were obtained with other forms of TGFβ (data not shown).

AGEN1721 mouse surrogate combines with PD-1 blockade to enhance anti-tumor immunity in treatment-resistant CRC tumor-bearing mice

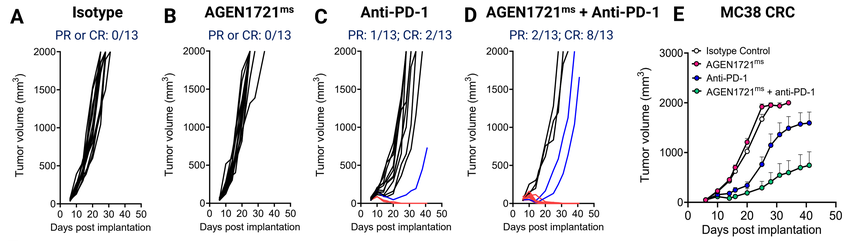
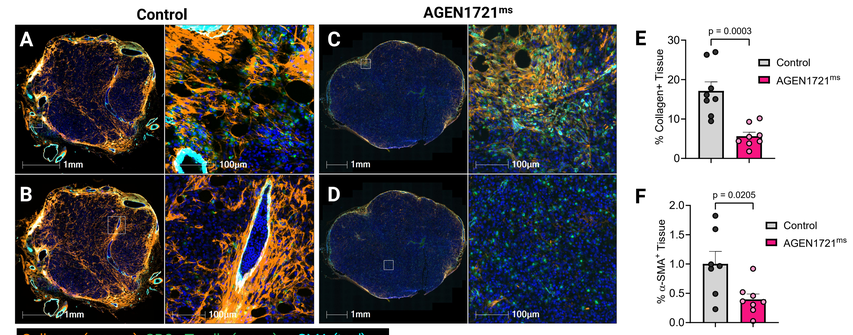


Figure 7. A mouse surrogate of AGEN1721 (AGEN1721ms) enhances anti-PD-1 efficacy in MC38-tumor-bearing mice. C57/BL/6 female mice bearing MC38 tumors (~60 mm^3; n=13 mice/group) implanted in the mammary fat pad were treated with **(A)** 200 µg of isotype control **(B)** 240 µg of AGEN1721ms **(C)** 200 µg of anti-PD-1 or **(D)** AGEN1721ms + anti-PD-1 antibodies twice a week for two weeks. Individual tumor growth curves for each treatment group are shown. Partial response (PR; blue); Complete response (CR; red). **(E)** Mean tumor growth curves and **(F)** survival for all treatment groups are shown.

AGEN1721ms remodels the tumor microenvironment by reducing stromal mass and enhancing T cell infiltration and activation



AGEN1721ms selectively increases CD4+ and CD8+ T cell infiltration in the tumor and tumor-draining lymph nodes

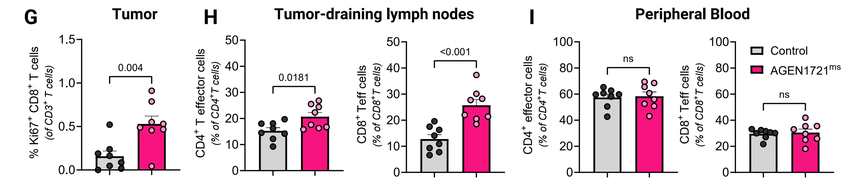


Figure 8. AGEN1721ms reduces stromal markers and enhances T cell infiltration in the tumor microenvironment of MC38-tumor bearing mice. Multiplex immunofluorescence images showing the expression of collagen (orange), CD3+ T cells (green), and α-SMA (teal) in MC38 colorectal tumors from (**A,B**) isotype control and (**C,D**) AGEN1721ms treated-mice. In the control group, CD3+ T-cells are predominantly at the tumor periphery with minimal central infiltration. AGEN1721ms enhances CD3+ T-cell infiltration throughout the tumor. Immunofluorescence data were quantified and normalized to tissue area to determine percent **(E)** collagen and **(F)** α-SMA area. **(G)** Percent Ki67+ CD8 T cells of total CD3 T cells were quantified from immunofluorescent-stained images. Percent CD4+ and CD8+ CD62L- effector cells in the **(H)** tumor draining lymph nodes and **(I)** peripheral blood were assessed by flow cytometry. Data represent mean ± SEM (n = 8 mice per group). Statistical significance was calculated using a Mann-Whitney test.

Summary

- Co-expression of FAP and TGFβ promotes immune exclusion, suppression, and resistance to therapy
- AGEN1721 depletes FAP+ CAFs and neutralizes TGFβ to remodel the TME from 'cold' immune-excluded to 'hot' immune-infiltrated tumors
- AGEN1721ms combines with anti-PD-1 to enhance anti-tumor immunity in MC38 colorectal tumor-bearing mice
- Developmental packages complete to support clinical evaluation of AGEN1721