

AR170, a novel PD-1xVEGFxIL-2v tri-specific immunocytokine to redefine next generation cancer immunotherapy



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Introduction

Bispecific antibodies (BsAbs) that simultaneously target PD-1 and VEGF have shown promise in extending the efficacy of PD-1 inhibition alone, yet despite improved treatment response and prolonged progression-free survival, overall survival advantage over anti-PD-1 monotherapy is so far limited. Interleukin-2 (IL-2) combination can markedly potentiate the efficacy of PD-1xVEGF BsAbs, but challenges remain due to systemic toxicity. To overcome this limitation, AR170 was designed as a novel tri-specific immunocytokine, designated as “Multi-AbKine”, that simultaneously engages PD-1, VEGF, and the IL-2 receptor by incorporating an optimized cis-acting IL-2 variant (IL-2v) and complete Fc-silencing.

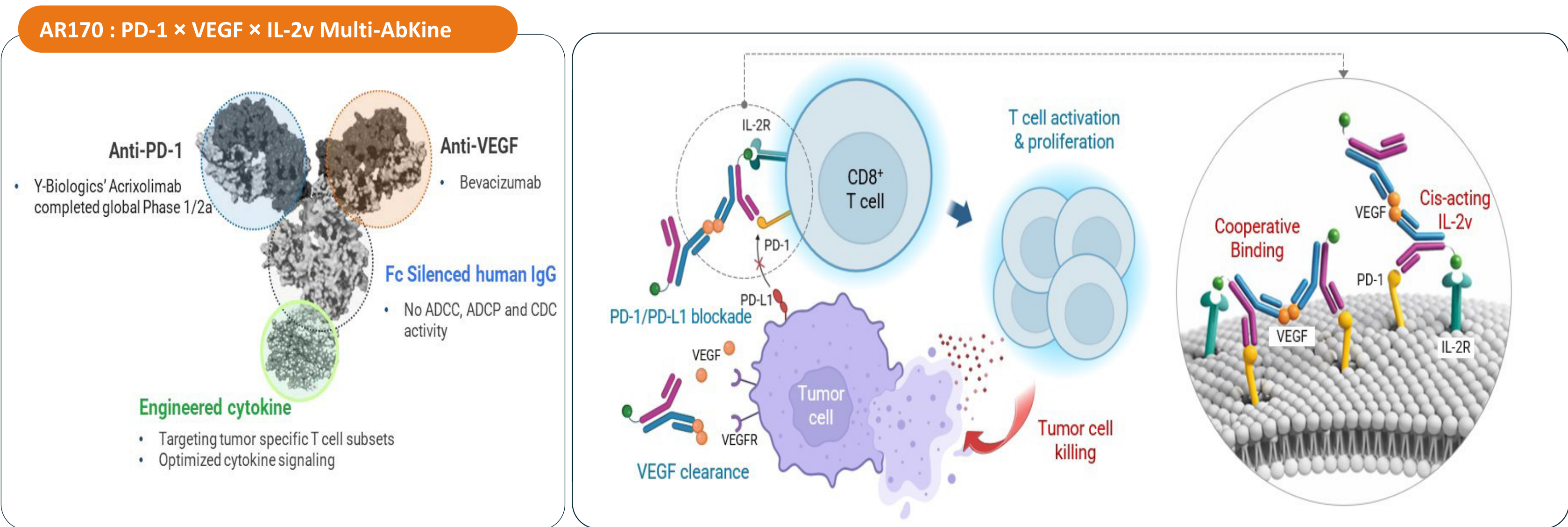


Figure 1. Built on an anti-PD-1 backbone, AR170 integrates both a bispecific axis and a cytokine axis within a single molecule, enabling improved response rates while addressing anti-PD-1 resistance. AR170 blocks PD-1/PD-L1 interactions and neutralizes VEGF to enhance T cell-mediated antitumor immunity, while the fused IL-2v domain functions in a cis-acting manner to selectively expand tumor-infiltrating T cells.

AR170 exhibits comparable binding affinities to a PD-1xVEGF competitor, and can simultaneously bind to both hPD-1 and hVEGF with complete Fc-silencing

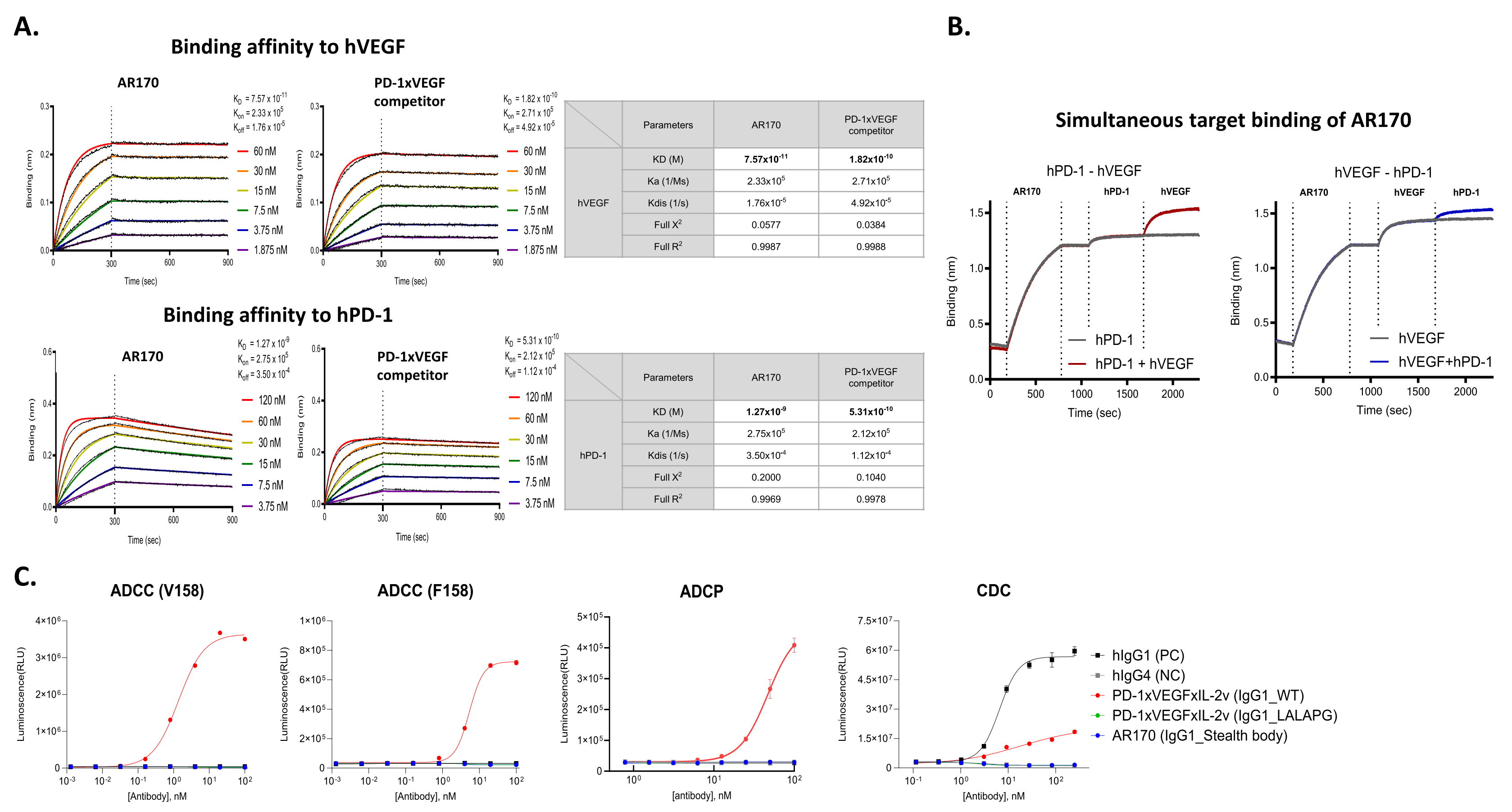


Figure 2. AR170 dual-target binding properties and silenced Fc-mediated effector functions (A) Binding kinetics of AR170 and a competitor PD-1xVEGF antibody to human VEGF and human PD-1 as measured by BLI. (B) AR170 was sequentially exposed to hPD-1 and hVEGF (left) or to hVEGF and hPD-1 (right) as analyzed via BLI. (C) ADCC (V158/F158 variants), ADCP, and CDC activities were determined by measuring luminescence (RLU) as a readout of effector function following incubation of target cells with serial dilutions of each antibody.

AR170 retains VEGF/VEGFR blockade comparable to a competitor PD-1xVEGF BsAb and, in the presence of VEGF, shows enhanced PD-1 binding and markedly increased PD-1/PD-L1 inhibitory activity

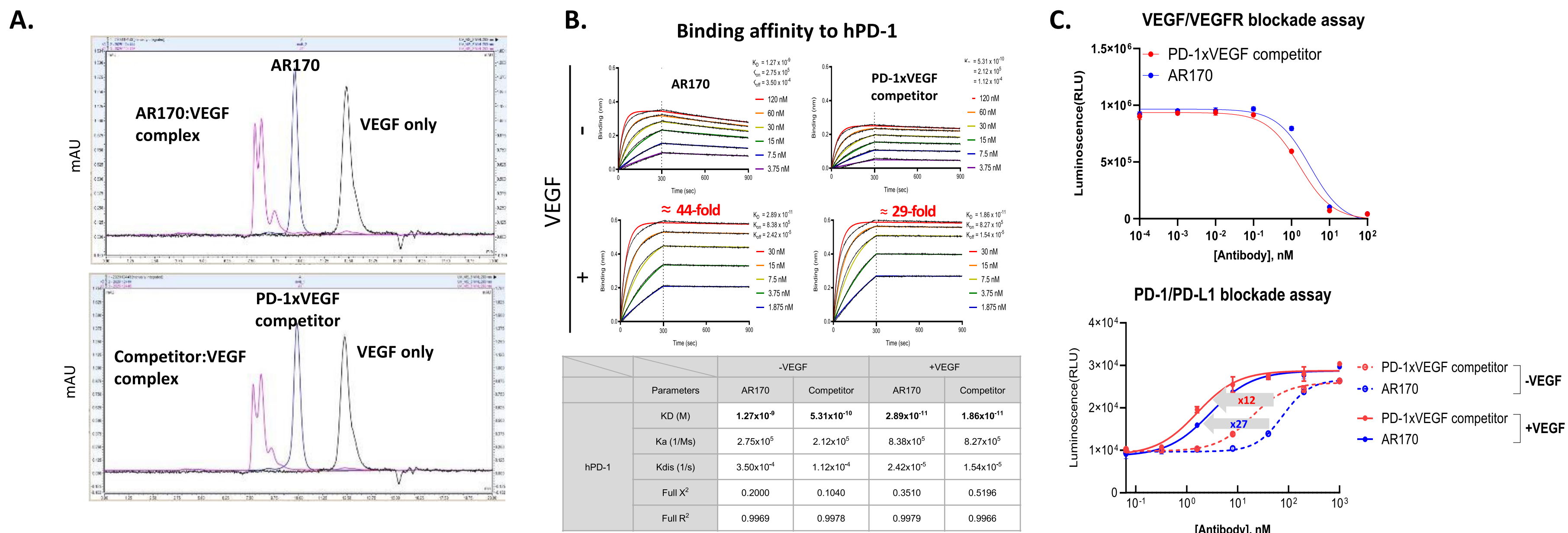


Figure 3. AR170 undergoes VEGF-induced complex formation, PD-1 binding in the presence of VEGF, and PD-1/PD-L1 and VEGF/VEGFR blockade (A) Size-exclusion chromatography profiles of AR170 and a PD-1xVEGF competitor in the presence of hVEGF. (B) Binding affinities of AR170 and competitor BsAb for hPD-1 in the presence or absence of hVEGF, measured by BLI. (C) Inhibition of PD-1/PD-L1 and VEGF/VEGFR signaling pathways by AR170 and competitor BsAb, as determined by reporter gene assays.

AR170 restores and enhances antitumor T cell functions in primed and exhausted human PBMCs

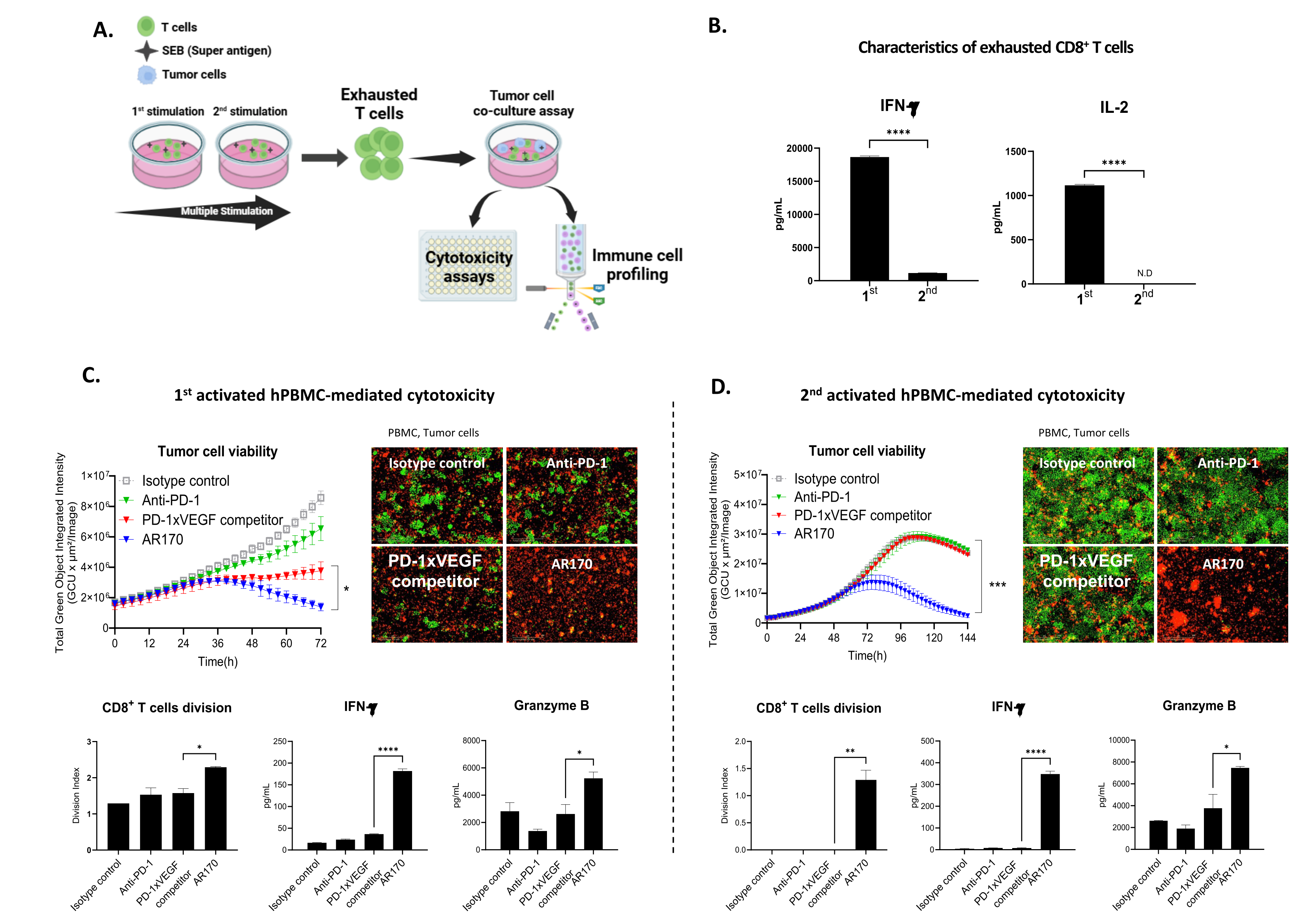


Figure 5. Effect of AR170 on primary and secondary SEB-induced activation of PBMCs and tumor cell killing in a tumor–PBMC co-culture system (A) Schematic of the hPBMC-mediated cytotoxicity assay. (B) Cytokine production by human PBMCs after primary and secondary SEB stimulation. Human PBMCs were subjected to a single (1st) or repeated (2nd) challenge with SEB, and the resulting IFN-γ and IL-2 production was measured in the supernatants via ELISA and compared to competitor (unpaired two-tailed Student's t-test, *p < 0.05). (C) and (D) hPBMCs were primed once (1st) or restimulated (2nd; D) with SEB and co-cultured with tumor cells in the presence of isotype control, anti-PD-1, competitor, or AR170. Tumor cell viability was quantified by live-cell imaging, CD8⁺ T cell division index was determined by flow cytometry, and levels of IFN-γ and granzyme B were measured using ELISA (one-way ANOVA followed by Dunnett's multiple comparisons test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

AR170 preferentially expands PD-1⁺ T cells by inducing IL-2R/pSTAT5 signaling through its cis-acting IL-2v, and this activity is further augmented in the presence of VEGF

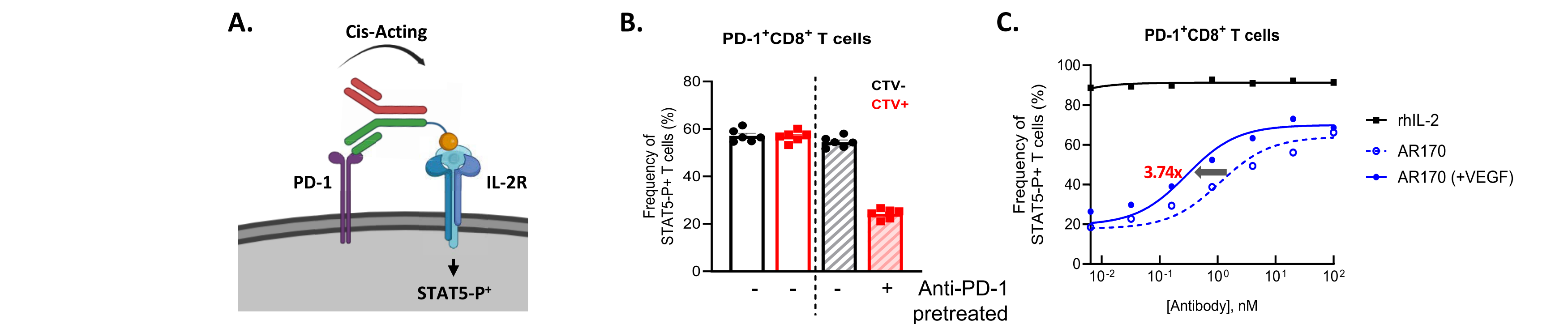
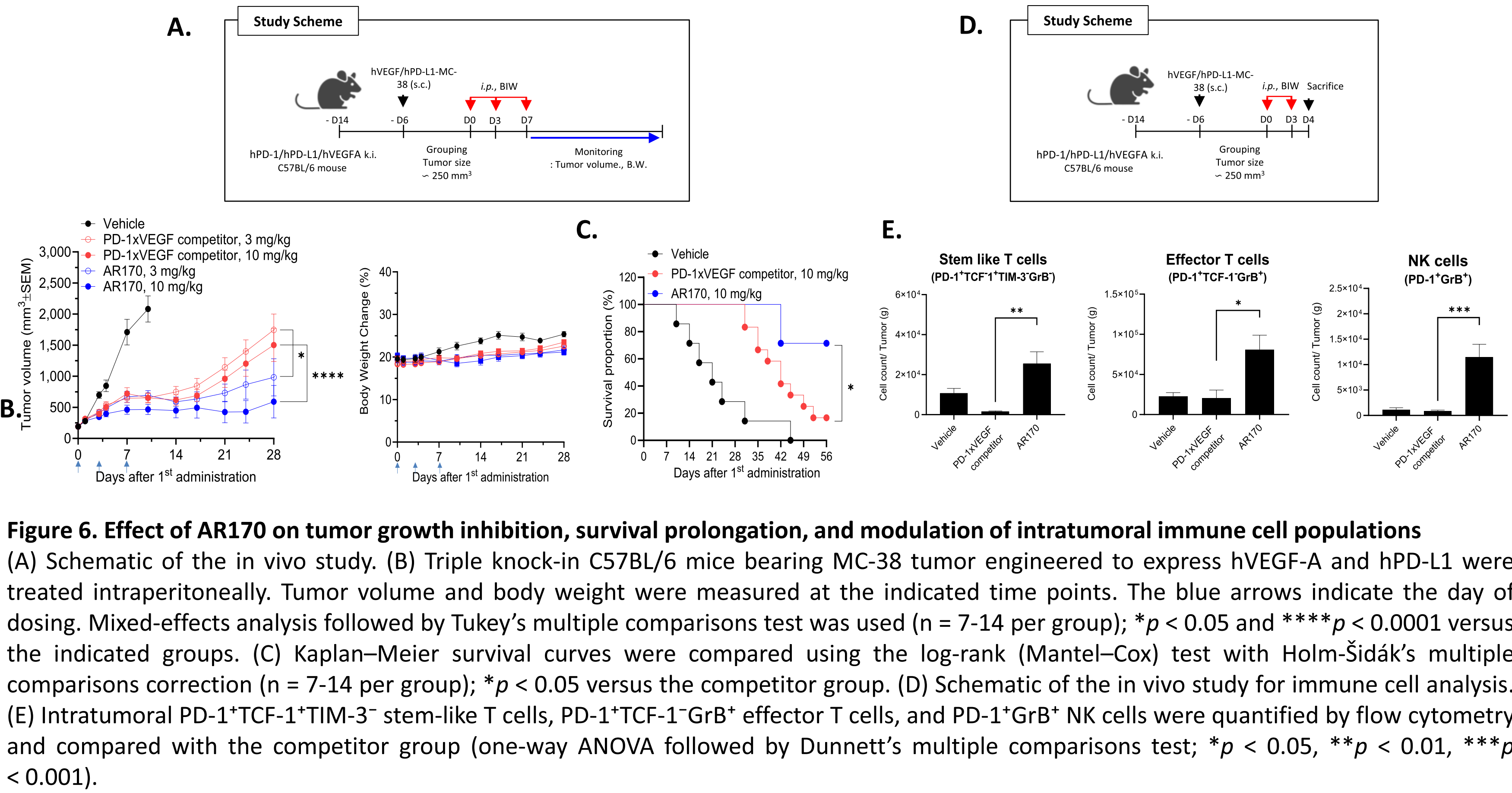


Figure 4. PD-1-dependent targeted cis-acting IL-2 delivery by AR170 and STAT5 signaling in the presence of VEGF (A) Schematic of cis-acting PD-1 targeting and activation of tumor reactive PD-1⁺ CD8⁺ T cells by AR170. (B) The frequency of pSTAT5⁺ CD8⁺ T cells was analyzed by flow cytometry after AR170 exposure, following co-culture with either unlabeled PD-1⁺ T cells or CTV-labeled PD-1-preblocked T cells. (C) Activated PD-1⁺ CD8⁺ T cells treated with AR170 in the absence or presence of VEGF. PD-1⁺ CD8⁺ T cells were stimulated with increasing concentrations of AR170 or rIL-2, and the frequency of pSTAT5⁺ cells was quantified by flow cytometry.

AR170 suppresses tumor growth, improves survival, and enhances the activity of stem-like and effector T cells, as well as NK cells, in a syngeneic mouse tumor model



AR170 shows a favorable safety profile in cynomolgus monkeys

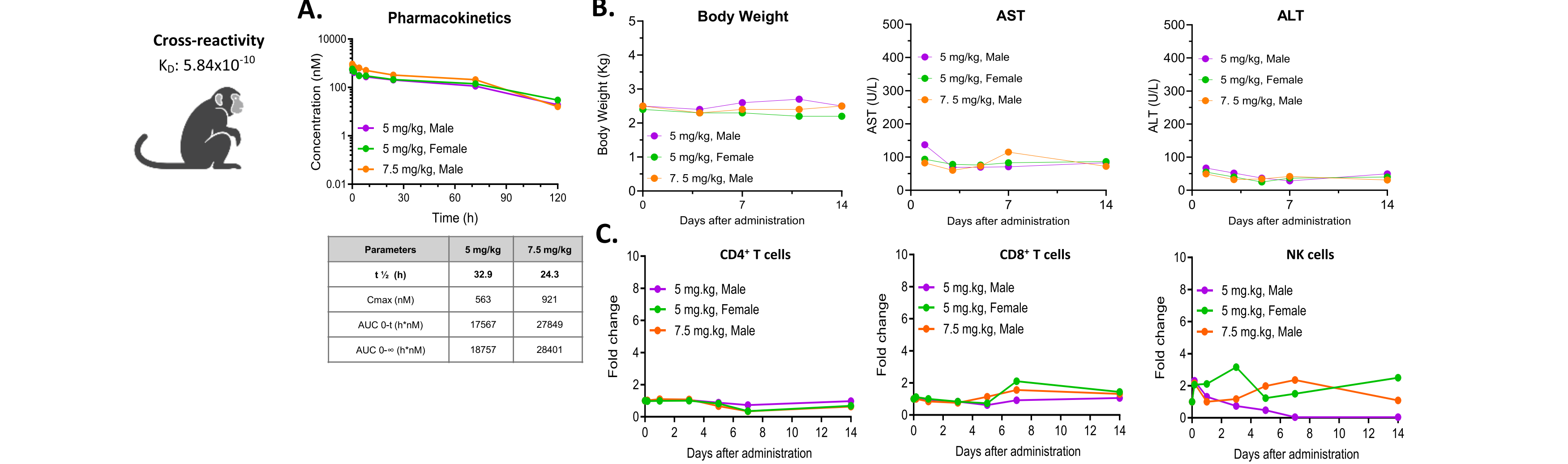


Figure 7. AR170 pharmacokinetics, systemic safety, and effect on peripheral immune cell populations (A) AR170 was administered as a single intravenous dose of 5 or 7.5 mg/kg. Serum concentrations were measured to generate concentration–time profiles, and pharmacokinetic parameters were derived by noncompartmental analysis. (B) Body weight and serum AST and ALT levels were measured after a single dose of AR170. (C) Fold changes in circulating CD4⁺ T cells, CD8⁺ T cells, and NK cells were measured. This study is ongoing.

Conclusion

AR170 is a next-generation cancer immunotherapy that uses a novel IL-2v fusion approach to surpass the efficacy limitations of PD-1xVEGF bispecific antibodies.