23ME-00610 Is a First-in-Class Monoclonal Antibody That Targets the CD200R1 Immune Checkpoint to Enhance T-Cell–Mediated Antitumor Activity

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BACKGROUND: CD200R1

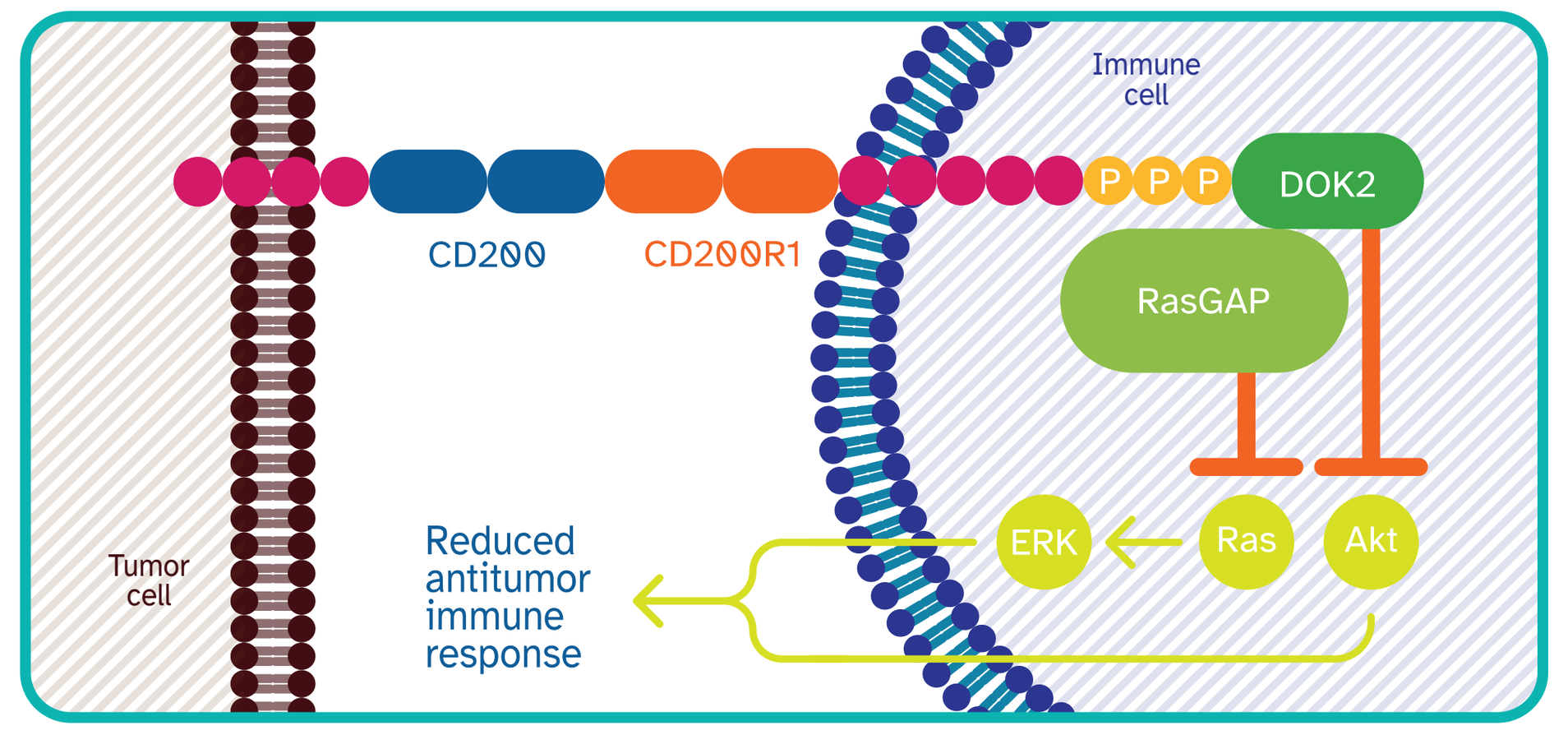
Genetic signature

- Using the 23andMe genetic database, a novel immuno-oncology genetic signature associated with CD200R1, CD200, and its signaling adaptor protein, DOK2, was identified, suggesting that it plays a role in antitumor immunity (Poster #603)

CD200R1 inhibitory pathway

- CD200R1 is an inhibitory receptor predominantly expressed on immune-cell subsets, such as T cells and myeloid cells[1]
- CD200, the only known ligand of CD200R1 in humans, is expressed on both normal and cancer cells[1-3]
- Binding of CD200 to CD200R1 initiates an intracellular signaling cascade mediated by the recruitment of adapter protein DOK2. This cascade has been shown to downregulate the production of proinflammatory cytokines by activated T cells and/or myeloid cells, potentially contributing to an immunosuppressive tumor microenvironment (TME; **Figure 1**)[4-8]

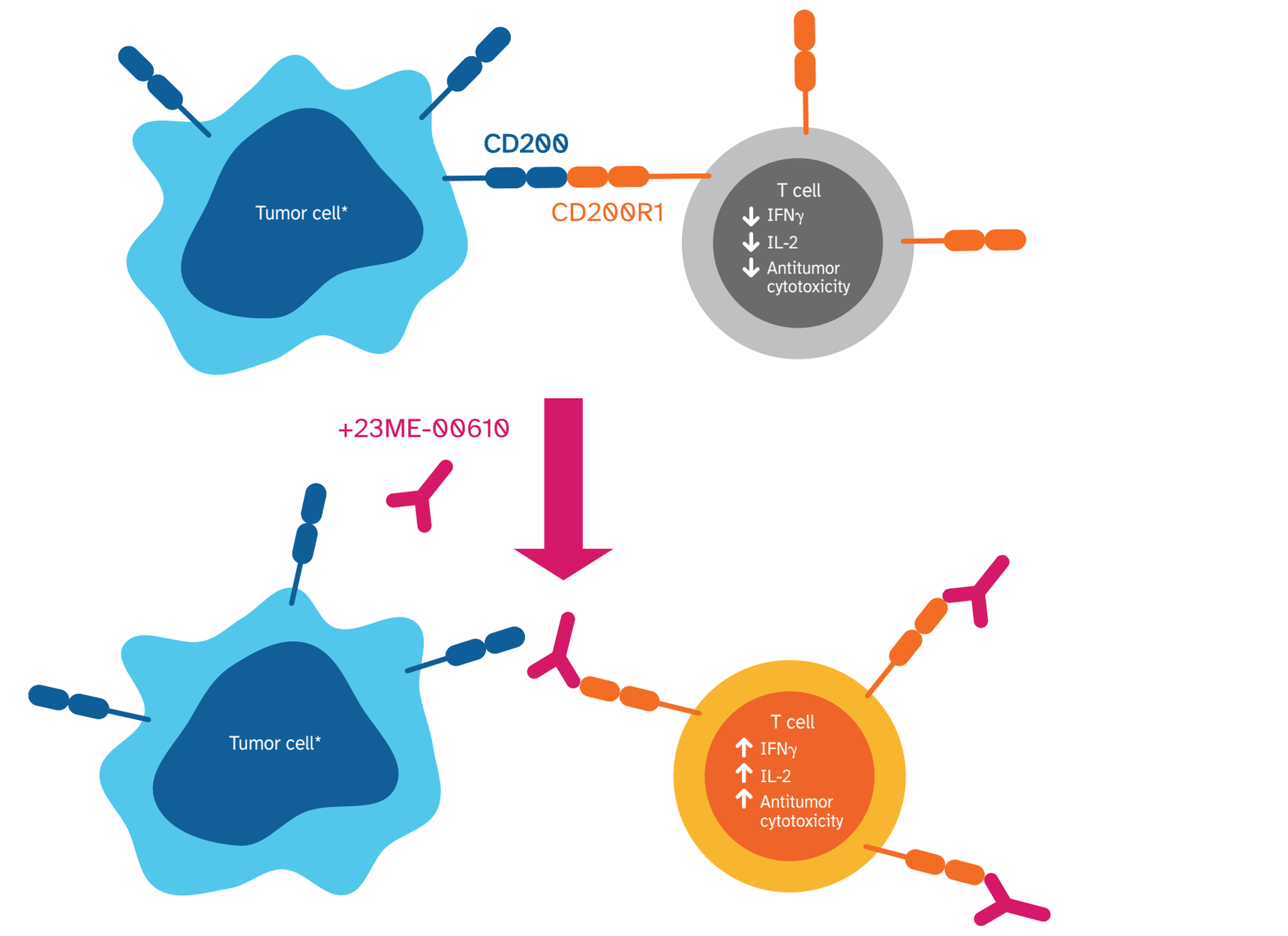
Figure 1. CD200-CD200R1 signaling cascade.



Hypothesis

- 23ME-00610 is a fully humanized, effectorless IgG1 antibody that binds to CD200R1, blocks CD200 binding, and potentiates immune-cell function in preclinical studies
- We hypothesize that blocking the CD200R1 checkpoint can restore T-cell cytotoxic activity to prevent or reverse immune-cell tolerance in the TME (**Figure 2**)

Figure 2. Proposed mechanism of action of 23ME-00610.



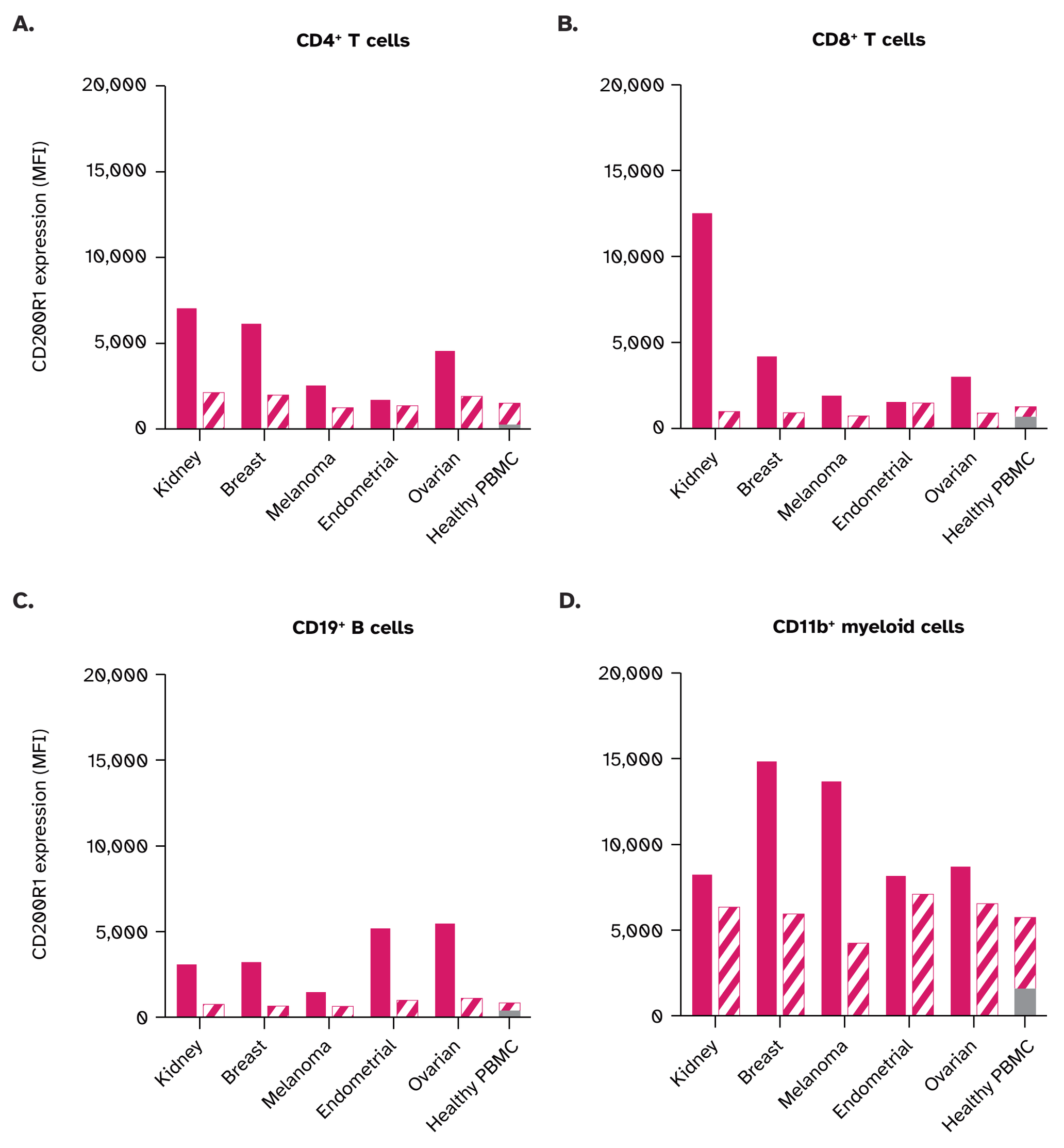
*CD200-expressing cell types include tumor, stroma, and endothelial.

IFN, interferon; IL, interleukin.

RESULTS

Increased CD200R1 expression on TILs may contribute to an immunosuppressive microenvironment

Figure 3. CD200R1 expression on PBMC and TIL subsets compared to peripheral immune cells from matched donors.

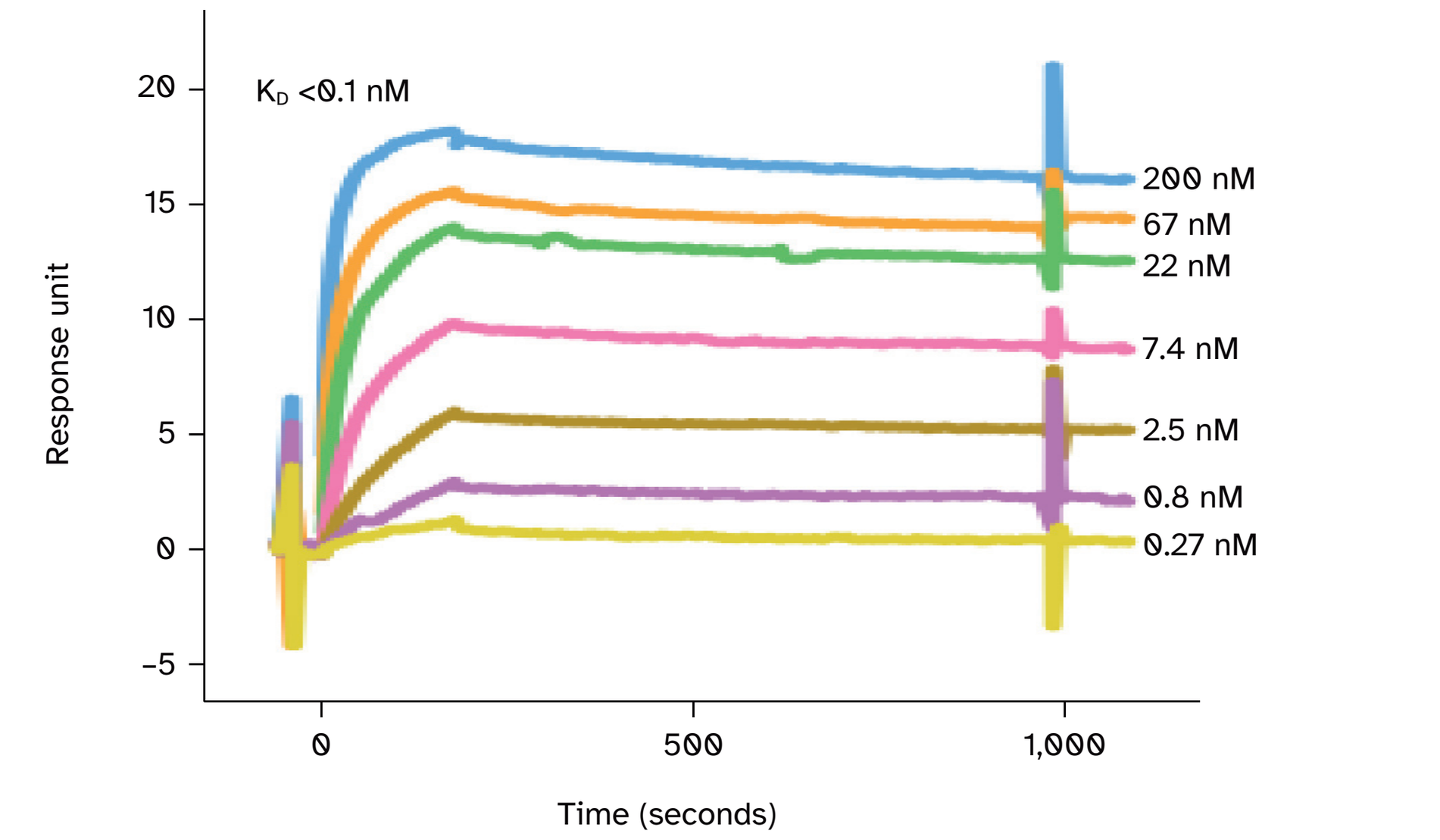


CD200R1 expression among different tumor types was compared on TIL subsets (pink bars) with PBMCs from the same donor (patterned bar) and was controlled for isotopic variance (gray bar).

MFI, mean fluorescence intensity; PBMC, peripheral blood mononuclear cell; TIL, tumor-infiltrating lymphocyte.

23ME-00610 binds with high affinity to CD200R1

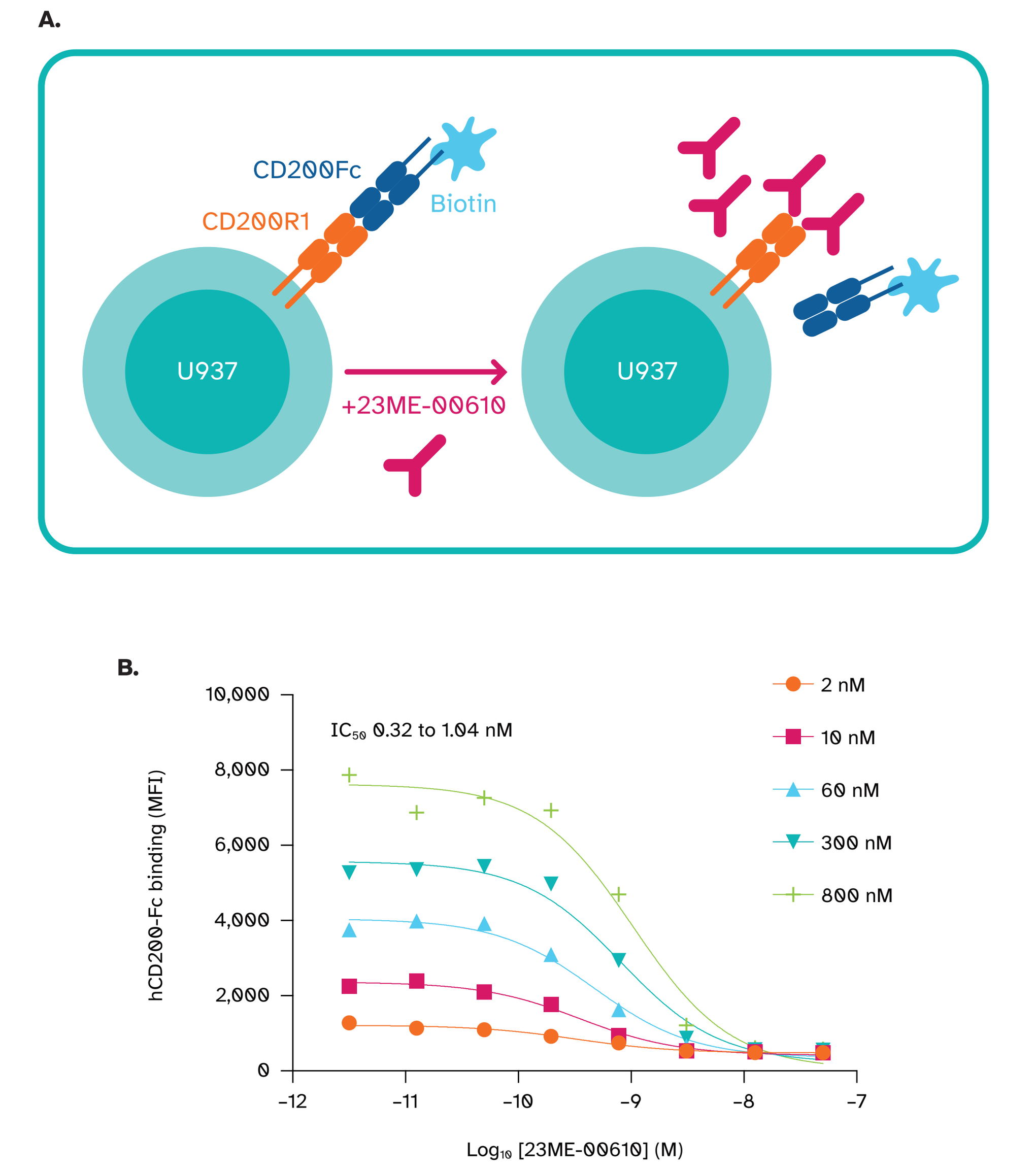
Figure 4. 23ME-00610 binds to CD200R1.



Binding of 23ME-00610 to immobilized human CD200R1 on a CAP chip was evaluated by surface plasmon resonance. A representative sensorgram is shown from 1 of 3 independent experiments using the most prevalent isoform of human CD200R1 (CD200R1-iso4-Alt).[9,10]

23ME-00610 blocks binding of CD200 to CD200R1

Figure 5. 23ME-00610 displaces pre-bound CD200.

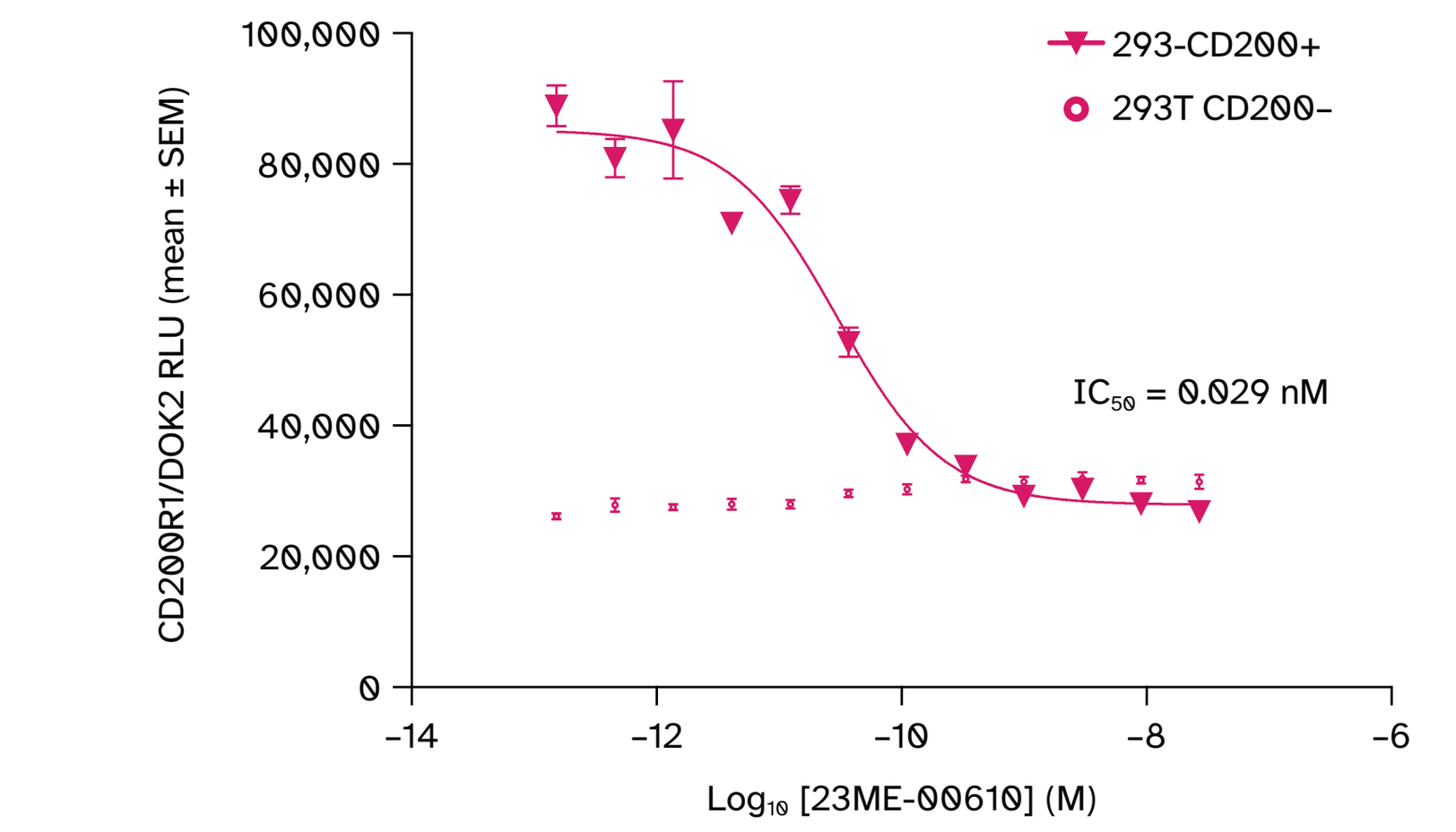


U937 cells engineered with CD200R1 were pre-incubated with biotinylated CD200 Fc (2-800 nM) followed by incubation with 23ME-00610 at 4°C for 30 minutes at a range of concentrations. MFI values of streptavidin detection are reported.

MFI, mean fluorescence intensity.

23ME-00610 inhibits downstream signaling through DOK2

Figure 6. 23ME-00610 blocks DOK2 recruitment to CD200R1 in a reporter T-cell line.

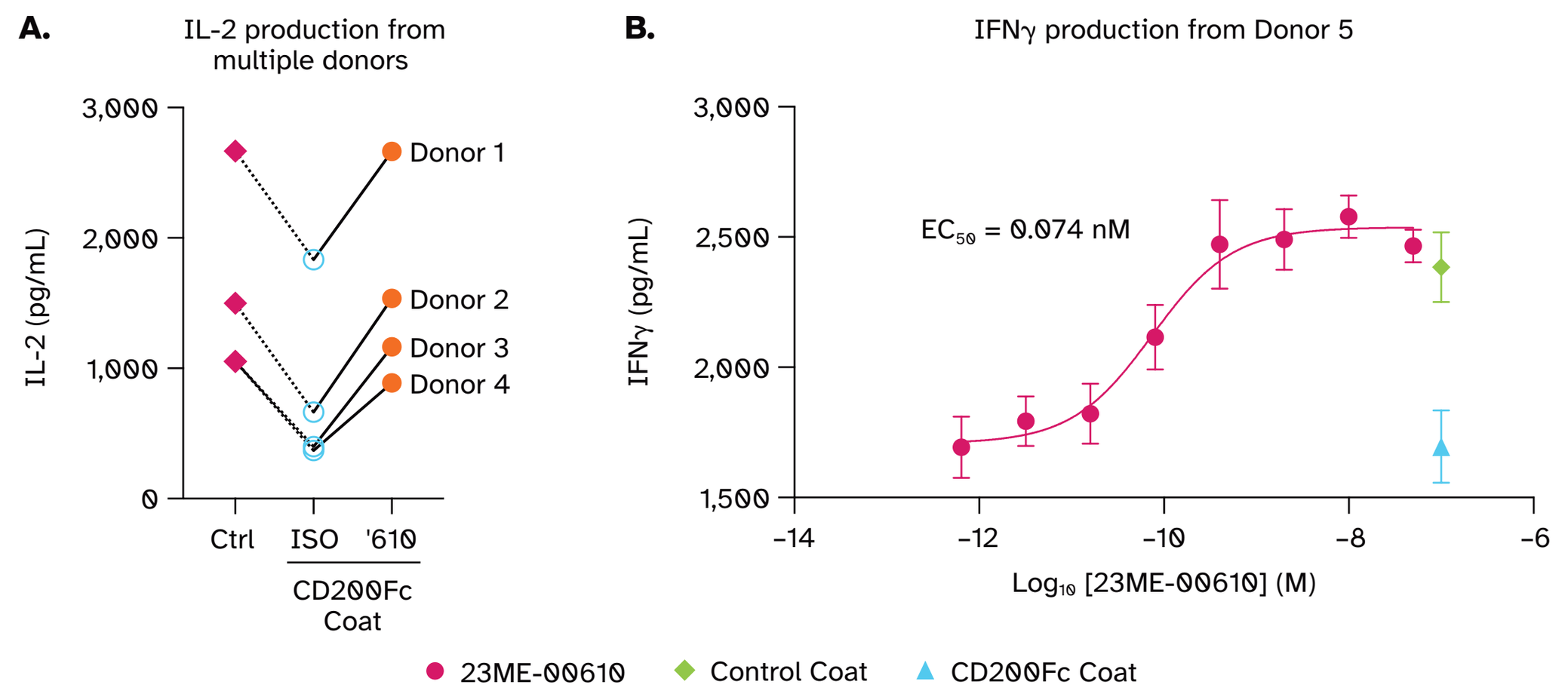


Jurkat cells were engineered to express CD200R1 and DOK2, each modified at the C terminus with a separate ß-galactosidase enzyme fragment (Eurofins Discovery PathHunter). This T-cell line, when co-cultured with HEK293 cells expressing CD200, induces a chemiluminescent signal reflective of CD200R1 signaling. Serial dilutions of 23ME-00610 were added to the co-culture to evaluate blocking of CD200-induced recruitment of DOK2 to CD200R1. HEK293T cells with no CD200 endogenous expression were used to determine the signal baseline.

SEM, standard error of the mean.

23ME-00610 rescues cytokine secretion from chronically stimulated T cells

Figure 7. Exogenous CD200 inhibitory effect on T cells is reversed with 23ME-00610 treatment in multiple donors.

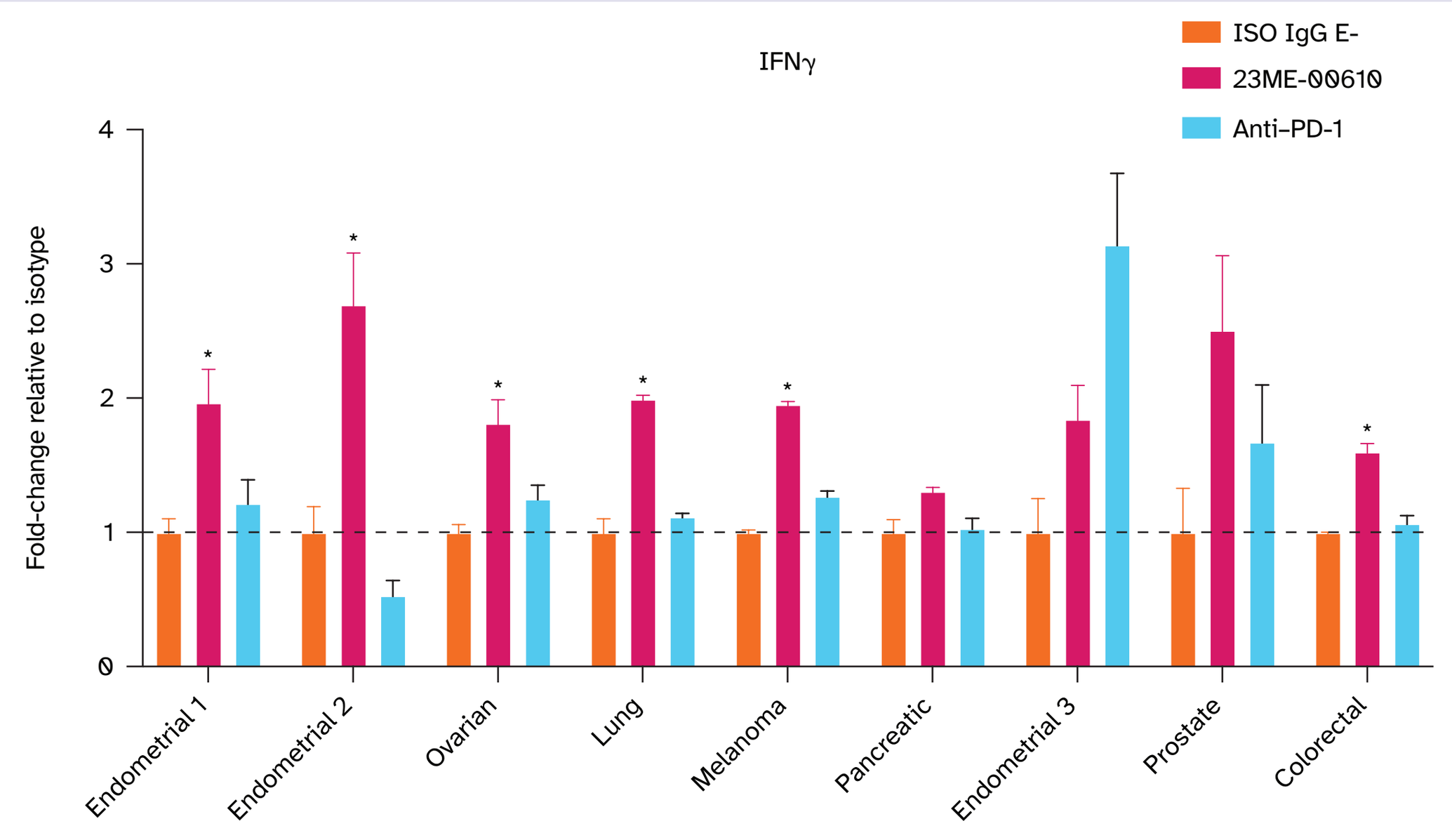


Human pan T cells isolated from healthy donors were chronically stimulated with phytohemagglutinin and IL-2 for 7 days. Cells were harvested, rested of stimulants, and then primed with human IL-4 for 24 hours. Cells were plated with 50 nM antibodies in plates coated with anti-CD3 and CD200 Fc or isotype-matched Fc control (Control Coat, diamonds) for 24 to 72 hours (**Figure 7A**). Mean IL-2 secretion levels were graphed for each donor. A representative dose-response curve for 23ME-00610 rescue of IFNγ secretion is graphed for the mean of biologic quadruplicates ± SEM from 1 donor (**Figure 7B**).

'610, 23ME-00610; Ctrl, Control Fc Coat; IFN, interferon; IL, interleukin; ISO, isotype; SEM, standard error of the mean.

23ME-00610 increases IFNγ secretion from cancer patient PBMCs

Figure 8. Blocking the CD200R1 pathway enhanced IFNγ production from SEB-stimulated PBMCs compared to isotype control and anti-PD1 in the majority of samples tested.

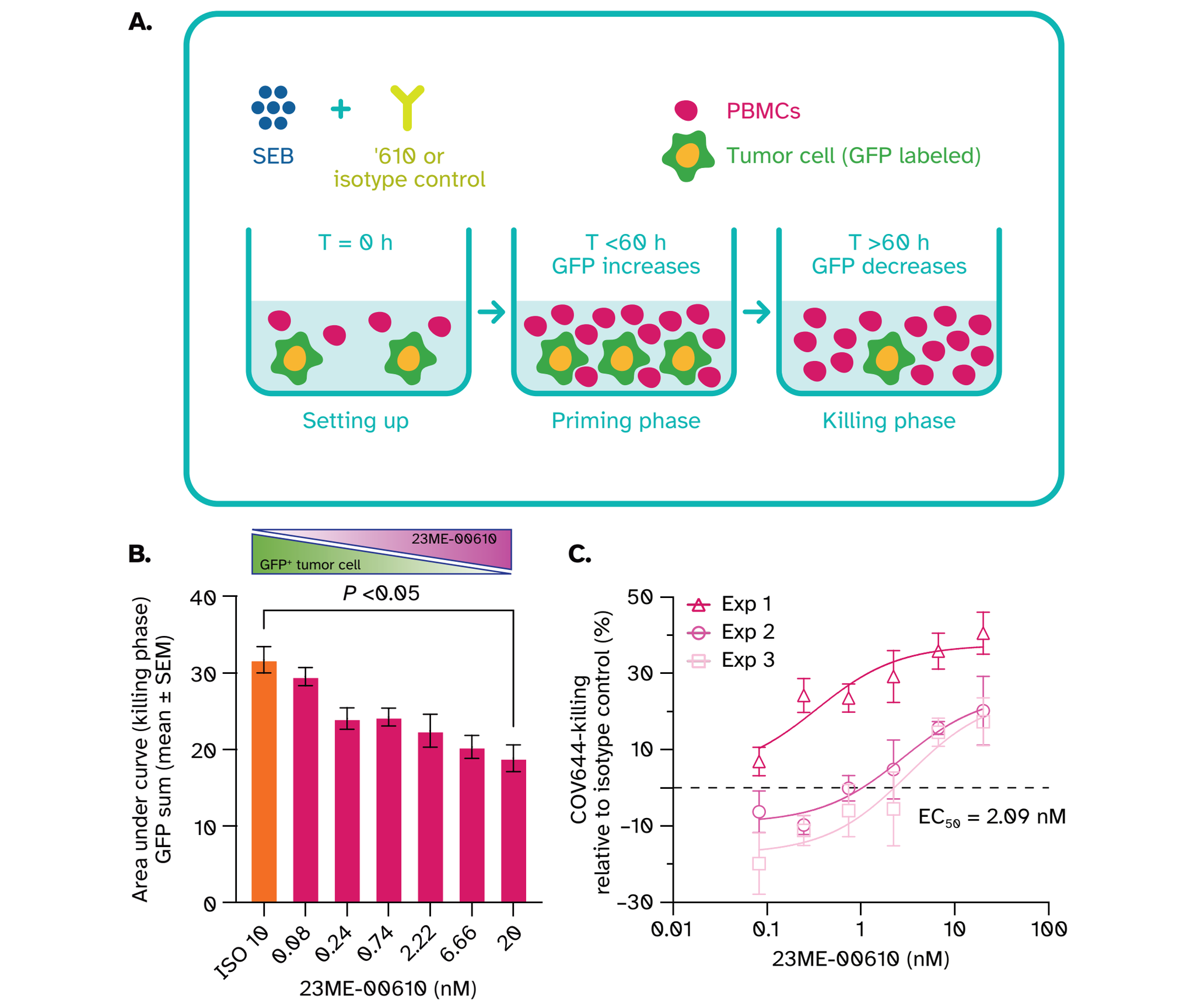


PBMCs from each respective patient were incubated with 100 nM of 23ME-00610, anti–PD-1, or isotype control. Cells were stimulated with SEB. IFNγ levels were determined by enzyme-linked immunosorbent assay. Mean biologic triplicates were normalized to isotype control.
*$P < 0.05$.

IFN, interferon; ISO, isotope; PBMC, peripheral blood mononuclear cell; PD-1, programmed death–1; SEB, staphylococcal enterotoxin B.

23ME-00610 augments killing of CD200-expressing tumor cells

Figure 9. 23ME-00610 enhances PBMC-mediated tumor cell killing in a dose-dependent manner.



Tumor cell (COV-644-GFP) killing by PBMCs without exogenous CD200 or anti-CD3 was evaluated upon treatment with 23ME-00610 or isotype control (**Figure 8A**) CD200R1⁺ PBMCs primed with 5 ng/mL SEB (bar graph; Exp 1, **Figures 8B** and **8C**) or 1 ng/mL SEB (Exp 2 and 3, **Figure 8C**) and treated with a titration of 23ME-00610 are shown. A dose-dependent reduction of tumor-cell GFP signal was measured using GFP area under the curve during the killing phase time frame.

'610, 23ME-00610; GFP, green fluorescent protein; ISO, isotope; PBMC, peripheral blood mononuclear cell; SEB, staphylococcal enterotoxin B; SEM, standard error of the mean; T, time.

CONCLUSIONS

- **CD200R1 expression was elevated on immune cells in tumors compared to the periphery, and the inhibitory function of this pathway on T cells was confirmed, suggesting the CD200R1/CD200 axis contributes to the immunosuppressive TME**
- **23ME-00610 is a high-affinity, first-in-class, anti-CD200R1 antibody with immune-activating properties, including:**
  - **Prevention of CD200-mediated suppression of chronically stimulated T cells**
  - **Enhancement of cytokine secretion from PBMCs isolated from cancer patients**
  - **PBMC-mediated tumor cell killing**
- **These data demonstrate that 23ME-00610 has the potential to reverse CD200-mediated immune suppression in the TME and restore T-cell killing of cancer cells**
- **The influence of CD200R1-expressing myeloid cells on antitumor immunity warrants further investigation**
- **The safety, pharmacokinetics, and anti-cancer activity of 23ME-00610 are currently being evaluated in patients with advanced solid tumors in a phase 1 clinical trial (ClinicalTrials.gov Identifier: NCT05199272)**

REFERENCES

1. Wright GJ, et al. *Immunity*. 2000;13(2):233-242.
2. Wright GJ, et al. *Immunology*. 2001;102(2):173-179.
3. Wright GJ, et al. *J Immunol*. 2003;171(6):3034-3046.
4. Mihrshahi R, et al. *J Immunol*. 2009;183(8):4879-4886.
5. Timmerman LM, et al. *PLoS One*. 2021;16(3):e0244770.
6. Misstear K, et al. *J Virol*. 2012;86(11):6246-6257.
7. Salek-Ardakani S, et al. *Eur J Immunol*. 2019;49(9):1380-1390.
8. Choueiry F, et al. *J Immunother Cancer*. 2020;8:e000189.
9. National Center for Biotechnology Information. Cell surface glycoprotein CD200 receptor 1 isoform a precursor [homo sapiens]. NCBI Reference Sequence: NP_620161.1. https://www.ncbi.nlm.nih.gov/protein/NP_620161. Accessed September 2018.
10. Ensembl. Gene: CD200R1. https://useast.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000163606;r=3:112921205-112975103. Accessed September 2018.

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SUPPORT

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