# New Insights into Targeting the CD200R1 Pathway in T and NK Cells Using 23ME-00610 as a Single Agent or in Combination

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## BACKGROUND

### Genetic Signature

- Using the 23andMe database, pleiotropic causal variants with opposing effect on risks for cancer and immune diseases, referred to as an immuno-oncology signature, were observed in 3 components of the CD200R1 pathway, including CD200R1, its ligand CD200 and the signaling adaptor protein DOK2.[1]

### CD200R1

- CD200R1 is expressed on immune cells and inhibits the adaptive and innate immune system.[1-4]
- CD200 is expressed on both normal and cancer cells.[5]
- Disruption of the CD200-CD200R1 axis in preclinical models increases the severity of autoimmune disease and inhibits tumor growth, consistent with our human genetic data.[1,6]

### 23ME-00610

- 23ME-00610 ('610) is a high-affinity, humanized and effectorless monoclonal antibody that binds to CD200R1 and blocks its interaction with CD200.[1]
- 23ME-00610 is currently being evaluated as a monotherapy in tumor-specific expansion cohorts, including ovarian carcinoma and clear cell renal cell carcinoma (ccRCC), in the Phase 2a portion of a Phase 1/2a clinical study (NCT05199272).

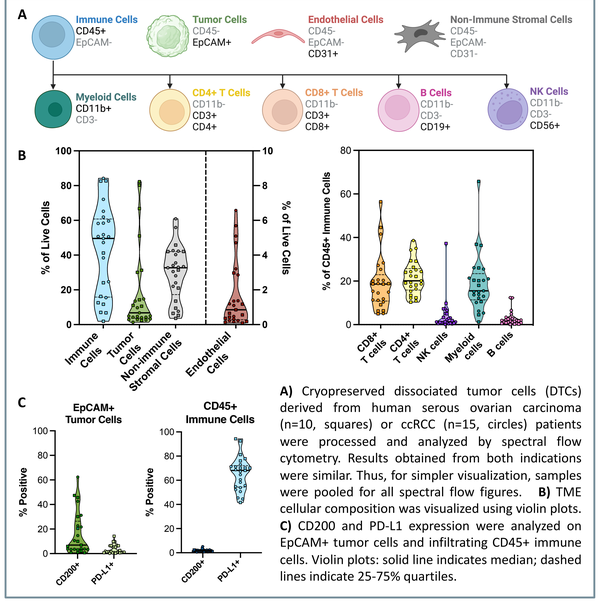
## OBJECTIVES

- Characterize the expression of CD200R1 and PD-1 pathway components on different cell subsets within serous ovarian carcinoma and ccRCC tumors from advanced-stage patients.
- Interrogate 23ME-00610 activity on immune cells and evaluate preclinical combinations of 23ME-00610 with anti-PD-1 and anti-VEGF.

## RESULTS

### CD200 has a Differentiated Expression Pattern from PD-L1

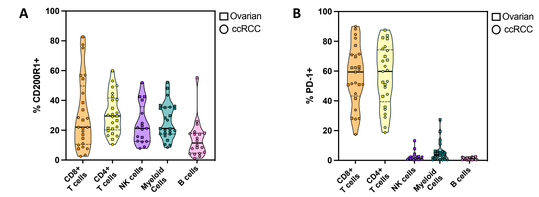
**Figure 1. Immunophenotyping of Dissociated Tumor Cells Reveals Differentiated Expression Pattern between CD200 and PD-L1**




**A)** Cryopreserved dissociated tumor cells (DTCs) derived from human serous ovarian carcinoma (n=10, squares) or ccRCC (n=15, circles) patients were processed and analyzed by spectral flow cytometry. Results obtained from both indications were similar. Thus, for simpler visualization, samples were pooled for all spectral flow figures. **B)** TME cellular composition was visualized using violin plots. **C)** CD200 and PD-L1 expression were analyzed on EpCAM+ tumor cells and infiltrating CD45+ immune cells. Violin plots: solid line indicates median; dashed lines indicate 25-75% quartiles.

### CD200R1 is Broadly Expressed on Tumor-Infiltrating Immune Cells

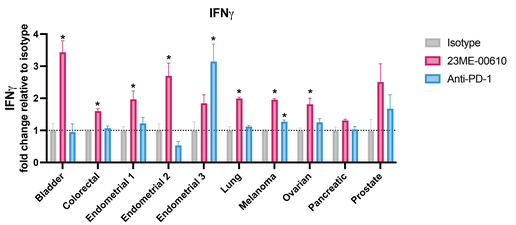
**Figure 2. CD200R1 and PD-1 have Distinct Expression Profiles Across Tumor-Infiltrating Immune Cell Subsets**




Expression of **A)** CD200R1 and **B)** PD-1 on immune cells infiltrating serous ovarian (n=10, squares) and ccRCC (n=15, circles) tumor patient samples was determined by spectral flow cytometry. Only samples with immune cell subsets containing over 450 events were used for accurate receptor expression analysis. Solid line: median; dashed lines: 25-75% quartiles.

### 23ME-00610 Enhances IFNγ from Cancer Patient PBMCs

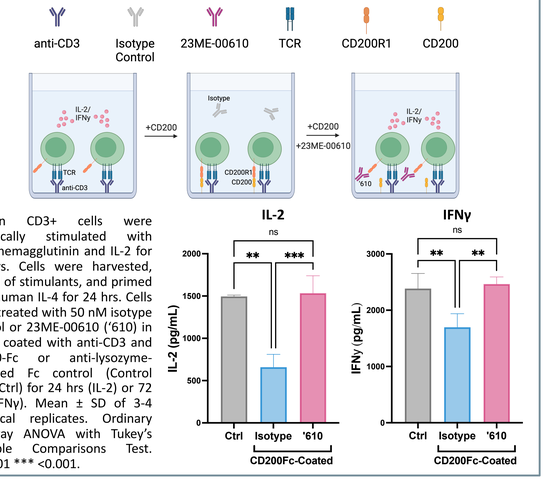
**Figure 3. 23ME-00610 Differentially Activates Immune Cells Compared to Anti-PD-1**




PBMCs obtained from cancer patients (8 of 10 prior to treatment) were purchased from Discovery Life Sciences and incubated with 100 nM of 23ME-00610, anti-PD-1 or isotype control and stimulated with staphylococcal enterotoxin B (SEB). IFNγ levels were determined by enzyme-linked immunosorbent assay. Mean technical triplicates were normalized to isotype control. Plotted is mean ± SEM. Ordinary one-way ANOVA with Tukey's Multiple Comparisons Test. *P-value ≤ 0.05 compared to control.

### 23ME-00610 Enhances T Cell Anti-Tumor Activity

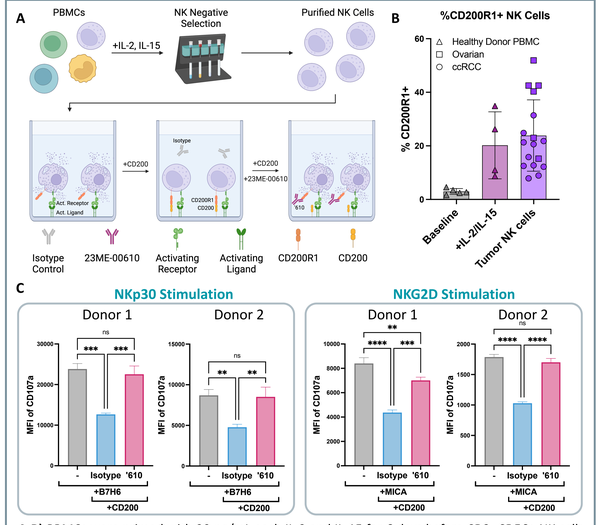
**Figure 4. 23ME-00610 Rescues the Immunosuppressive Activity of CD200 on T Cells**




Human CD3+ cells were chronically stimulated with phytohemagglutinin and IL-2 for 7 days. Cells were harvested, rested of stimulants, and primed with human IL-4 for 24 hrs. Cells were treated with 50 nM isotype control or 23ME-00610 ('610) in plates coated with anti-CD3 and CD200-Fc or anti-lysozyme-matched Fc control (Control Coat, Ctrl) for 24 hrs (IL-2) or 72 hrs (IFNγ). Mean ± SD of 3-4 technical replicates. Ordinary one-way ANOVA with Tukey's Multiple Comparisons Test. **<0.01 *** <0.001.

### 23ME-00610 Enhances NK Cell Anti-Tumor Activity

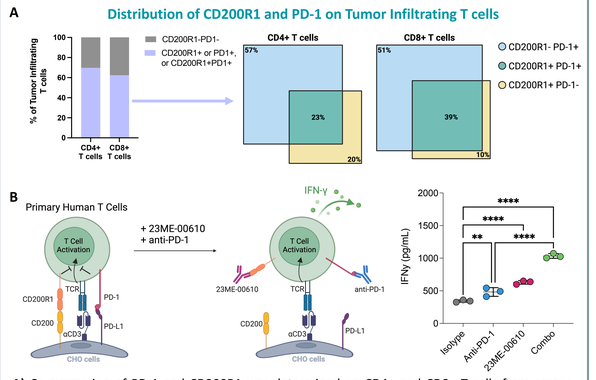
**Figure 5. 23ME-00610 Rescues the Immunosuppressive Activity of CD200 on NK Cells**




**A-B)** PBMCs were primed with 20 ng/mL each IL-2 and IL-15 for 6 days before CD3- CD56+ NK cells were isolated. CD200R1 expression on NK cells was measured pre- and post-priming and compared to expression on NK cells from dissociated tumors. **A,C)** Isolated NK cells with high CD200R1 expression were treated with 3 µg/mL isotype control or 23ME-00610 ('610) in plates coated with immobilized activating ligand (B7H6-Fc or MICA-Fc) and CD200-Fc in the presence of monensin and PE-αCD107a. NK cell degranulation (CD107a) was measure by flow cytometry after 5 hours. Mean ± SD of three technical triplicates. Ordinary one-way ANOVA with Tukey's Multiple Comparisons Test, **p<0.01, ***p<0.001, ****p<0.0001.

### Potential for 23ME-00610 to Combine with Anti-PD-1

**Figure 6. 23ME-00610 Synergizes with anti-PD-1 to Enhance IFNγ Secretion from Primary Human T cells**




**A)** Co-expression of PD-1 and CD200R1 was determined on CD4+ and CD8+ T cells from serous ovarian (n=10) and ccRCC (n=15) tumor patient samples. Mean values of the data are plotted. **B)** Human Pan T cells were stimulated with anti-CD3/anti-CD28 Dynabeads for 5 days in the presence of IL-2 (20 ng/mL) and IL-4 (100 ng/mL). They were then rested in IL-4 (100 ng/mL) for 1 day prior to treatment with 2 µg/mL antibody and co-cultured with CHO cells engineered to express CD200 and PD-L1. One day following co-culture, IFNγ levels were measured by MSD. Mean ± SD of three technical triplicates for one of four representative donors shown. Ordinary one-way ANOVA with Tukey's Multiple Comparisons Test, **p<0.01, ***P<0.001, ****p<0.0001.

### Potential for 23ME-00610 to Combine with Anti-Angiogenics

**Figure 7. Blockade of the CD200R1 Signaling Pathway Combines with Anti-VEGF to Inhibit Tumor Growth**

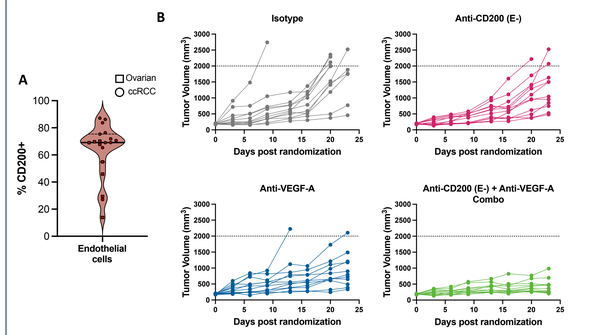



**Table 1. Ratio of adjusted Area Under the Curve (aAUC)**

| Test (X:Y) | Observed Ratio (Y/X) | Upper 95% CI | FDR Adjusted P-Value |
|---|---|---|---|
| Isotype : Anti-CD200 (E-) | 0.78 | 1.01 | 0.086 |
| Isotype : Anti-VEGF-A | 0.79 | 1.04 | 0.097 |
| Isotype : Combo | 0.45 | 0.59 | <0.0001 |
| **Anti-CD200 (E-) : Combo** | 0.58 | 0.72 | **<0.001** |
| **Anti-VEGF : Combo** | 0.57 | 0.75 | **<0.001** |

**A)** CD200 expression was determined on CD45-EpCAM-CD31+ endothelial cells from human serous ovarian (n=8, squares) and ccRCC (n=11, circles) patient tumor samples. Solid line: median; dashed lines: 25-75% quartiles. **B)** DBA/2 mice were subcutaneously injected with S91 cancer cells and randomized to treatment groups (N=12/group) when the mean tumor size reached approximately 200mm$^3$. Mice were treated twice weekly with isotype, effectorless (E-) CD200 blocking antibody, anti-VEGF-A, or a combination ("Combo"). Individual mice were sacrificed when individual tumor volume exceeded 2000mm$^3$. An adjusted Area Under the Curve (aAUC)[7] was calculated for each mouse to quantitatively assess tumor volume growth over time. Statistical analysis was performed using a one-sided test to determine if the ratio of mean adjusted Area Under the Curve (aAUC) between groups was significantly less than 1, indicating lower tumor growth, with significance assessed at an FDR-adjusted alpha level of 0.05.

## CONCLUSIONS

- CD200R1 is a dominant immune checkpoint and differentiated from PD-1, based on both the pattern of expression on tumor infiltrating immune cells from patient tumors and the pattern of activation on patient PBMCs.
  - CD200R1 was broadly expressed on tumor-infiltrating immune cells, including T cells and NK cells, whereas expression of PD-1 was predominantly restricted to T cells.
  - 23ME-00610 differentially enhanced IFNγ secretion from cancer patient PBMCs relative to anti-PD-1, and 23ME-00610 enhanced both T and NK cell anti-tumor activity.
- Our preclinical results support the potential for 23ME-00610 to combine with anti-PD-1 and anti-angiogenics.
  - CD200R1 is an independent immunosuppressive pathway from PD-1, with potential for synergism in patients with cancer based on preclinical combination data with primary human T cells.
  - CD200 is expressed on both tumor cells and endothelial cells, and blockade of the CD200R1 pathway synergized with anti-VEGF to inhibit tumor growth in a preclinical mouse model.

## ACKNOWLEDGEMENTS

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