Discovery of CD200R1 as a Novel Immuno-oncology Target Using Pleiotropic Signals From 23andMe's Genetic and Health Survey Database

Xin Fang*,†, Wei-Jen Chung†, Jill Fenaux, Alice Chen, Nizar Batada, Maike Schmidt, Sophia R. Majeed, Sushil Kumar, Steven Pitts

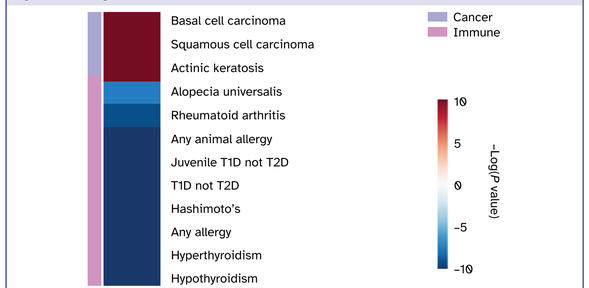
23andMe, Inc., South San Francisco, CA.
*Presenting author.
†These authors contributed equally to this poster: X.F. performed the analysis on gene expression and generated the poster; W-J.C. performed the pleiotropy analysis, gene expression analysis, and identified genetic signatures.

BACKGROUND

Identifying a pleiotropic signature

- Using a genome-wide association study (GWAS) across multiple phenotypes to assess 23andMe's unique genetic database, pleiotropic causal variants with consistent opposing direction of effect in cancer and immune diseases were identified as having an "Immuno-oncology (IO) signature"
- A coding variant of the immune checkpoint cytotoxic T-lymphocyte–associated protein 4 (CTLA4) serves as a proof of concept (**Figure 1**)
- We hypothesized that genes with such a pleiotropic signature play a critical role in the antitumor immune response

Figure 1. IO signature observed for CTLA4 in the 23andMe database.*

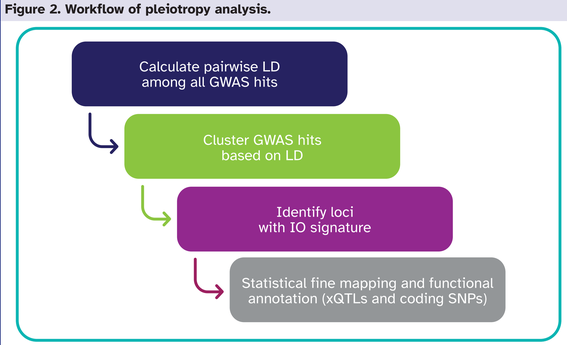


Intensity of the color corresponds to −Log(*P* value) and is capped at 10. The color (blue-red) corresponds with the direction of the effect.
*Consenting customers fill out medical surveys that capture their personal and family history of cancer- and immune-related diseases. We look for genetic variants that associate with these immune-related phenotypes to create a GWAS-PheWAS database.

CTLA4, cytotoxic T-lymphocyte–associated protein 4; GWAS, genome-wide association; IO, immuno-oncology; T1D, type 1 diabetes; T2D, type 2 diabetes.

- To identify novel potential targets for cancer therapies with human genetic support, we conducted further analyses to identify genetic variants with a similar IO signature (**Figure 2**)

Figure 2. Workflow of pleiotropy analysis.



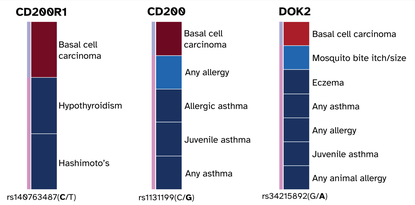
GWAS, genome-wide association study; IO, immuno-oncology; LD, linkage disequilibrium; SNP, single nucleotide polymorphism; xQTL, any quantitative trait loci.

METHODS AND RESULTS

CD200R1 was identified as a potential IO target, as 3 components of the pathway were observed to have an IO signature in the 23andMe database

- An IO signature was observed for 3 genes known to be involved in the same immune pathway: CD200R1 (inhibitory cell surface receptor), CD200 (CD200R1 ligand), and DOK2 (involved in CD200/CD200R1 signaling pathway; **Figure 3A** and **3B**)

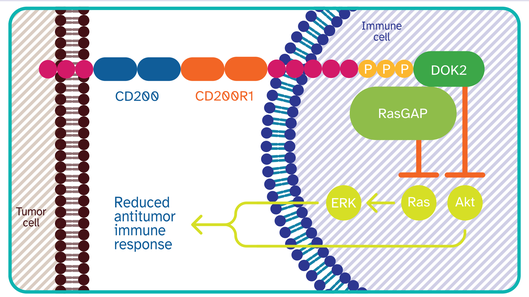
Figure 3A. CD200R1, CD200, and DOK2 IO signature.*



	CD200R1 rs149763487(**C**/T)	CD200 rs1131199(C/**G**)	DOK2 rs34215892(G/**A**)
Variant to gene mapping	eQTL from whole blood	Missense of CD200	Missense of DOK2, eQTL from whole blood

*Test alleles in GWAS are shown in bold text.

Figure 3B. CD200-CD200R1 signaling cascade.



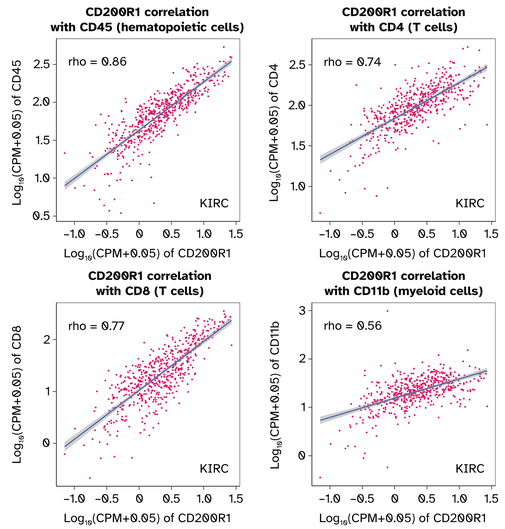
The binding of CD200 to CD200R1 initiates an intracellular signaling cascade mediated by the recruitment of adapter protein DOK2. This cascade has been shown to downregulate the production of proinflammatory cytokines by activated myeloid and/or T cells, potentially contributing to an immunosuppressive tumor microenvironment.[1-5]

eQTL, expression quantitative trait loci; GWAS, genome-wide association study; IO, immuno-oncology.

CD200R1 is co-expressed on tumor-infiltrating lymphocytes (TILs) from The Cancer Genome Atlas (TCGA)

- To confirm the presence of CD200R1 in TILs, the correlation between CD200R1 expression and that of several immune cell marker genes (CD45, CD8, CD4, CD11b) was calculated using RNAseq data from TCGA[6]
- A positive correlation (ranging from 0.51-0.88) between CD45 and CD200R1 expression in 30 out of 32 cancer types examined in TCGA was observed, suggesting that CD200R1 is expressed on TILs in most cancers (clear cell renal carcinoma is shown in **Figure 4** and was chosen because it had high immune infiltration in the TCGA dataset)

Figure 4. CD200R1 expression in immune cells from clear cell renal carcinoma.



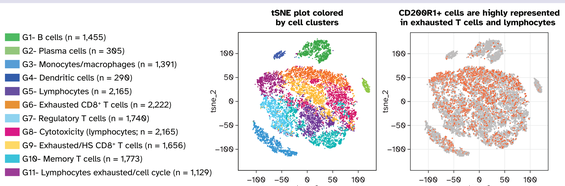
Measurements of CD200R1 and TILs were taken from samples of KIRC.

KIRC, kidney renal clear cell carcinoma; TIL, tumor-infiltrating lymphocyte.

CD200R1 is expressed in immune cell subsets from patients whose cancer did not respond to immune checkpoint inhibitors[7]

- Previously published single-cell RNA-seq data from 48 tumor samples of melanoma patients treated with checkpoint inhibitors anti–programmed death–1 (PD-1) and anti-CTLA4 were reanalyzed to further understand CD200R1 expression on immune cell subsets[7]
- As illustrated in the t-distributed stochastic neighbor embedding plot, CD200R1 is expressed on a variety of immune cells, particularly exhausted T and NK cells, indicating that the CD200R1 pathway contributes to an immunosuppressive tumor microenvironment (**Figure 5**)

Figure 5. Immune cells from melanoma patients treated with anti–PD-1 and anti-CTLA4 express CD200R1.[7]



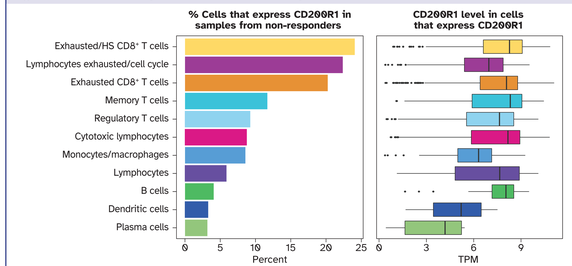
CD200R1 is most highly represented in exhausted CD8+ T cells (G6: 21.8%; G9: 23.4%) and other exhausted lymphocytes (G11: 22.9%).

CTLA4, cytotoxic T-lymphocyte–associated protein 4; PD-1, programmed death–1; tSNE, t-distributed stochastic neighbor embedding.

CD200R1 expression was confirmed on T cells in immune checkpoint inhibitor (ICI) non-responders

- The frequency and level of CD200R1 expression was examined on TIL subsets from 32 ICI non-responders
- Exhausted CD8+ T cells are the most abundant CD200R1-expressing tumor-infiltrating cell type in non-responders (**Figure 6**)[7]
- These observations were similar to a published RNAseq expression analysis,[8] suggesting that CD200R1 is an immune checkpoint that may contribute to ICI resistance[9-11]

Figure 6. CD200R1 expression in ICI non-responders.*,[7]



*Elevated proportion of CD8+ T cells enriched for genes linked to cell exhaustion is observed in non-responders compared to responders.
ICI, immune checkpoint inhibitor; TPM, transcripts per million.

CONCLUSIONS

- **There are multiple instances in the 23andMe database where genetic associations driven by a common causal variant are observed to consistently show opposing direction of effect in cancer and immune diseases—a pleiotropic IO signature**
- **CD200R1 was identified as a critical immune checkpoint**
  - **CD200R1 and its pathway genes CD200 and DOK2 all show pleiotropic IO signatures**
- **CD200R1 is expressed on TILs in the TCGA dataset, suggesting that this pathway contributes to an immunosuppressive tumor microenvironment (also see Poster #602)**
- **CD200R1 is expressed in ICI non-responders, indicating that inhibition of the CD200R1 immune checkpoint has the potential to address resistance to anti–PD-1 and anti–CTLA4 therapies[7]**
- **23andMe has developed a high-affinity, first-in-class, anti-CD200R1 monoclonal antibody (23ME-00610) to block CD200R1 signaling and enhance T-cell function (Poster #602)**
- **The safety, pharmacokinetics, and anti-cancer activity of 23ME-00610 is currently being evaluated in patients with advanced solid tumors in a phase 1 clinical trial (ClinicalTrials.gov Identifier: NCT05199272)**

REFERENCES

1. Mihrshahi R, et al. *J Immunol.* 2009;183(8):4879–4886.
2. Timmerman LM, et al. *PLoS One.* 2921;16(3):e0244778.
3. Misstear K, et al. *J Virol.* 2912;86(11):6240–6257.
4. Salek-Ardakani S, et al. *Eur J Immunol.* 2919;49(9):1389–1399.
5. Chouery F, et al. *J Immunother Cancer.* 2928;8:e000910.
6. National Cancer Institute. The Cancer Genome Atlas Program. https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga. Accessed March 3, 2922.
7. Sade-Feldman M, et al. *Cell.* 2918;175(4):998–1913.
8. Markowitz GJ, et al. *JCI Insight.* 2918;3(13):e96836.
9. Thommen DS, et al. *Nat Med.* 2918;24(7):994–1984.
10. Zheng C, et al. *Cell.* 2917;169(7):1342–1356.
11. Cassetta L, et al. *Cancer Cell.* 2919;35(4):588–602.

ACKNOWLEDGMENTS

Medical writing and editorial assistance were provided by Matt McErlean, PhD, of Calio Health Communications/MedErgy, and were financially supported by 23andme, Inc. Pierre Fontanillas assisted with the genetic signal investigation.

SUPPORT

This study was supported by 23andMe, Inc.

An electronic version of the poster can be viewed by scanning the QR code. The QR code is intended to provide scientific information for individual reference. The PDF should not be altered or reproduced in any way. http://tm_aacr.scientificpresentations.org/Fang_TIM2169.pdf