ARQ-234: A High-Affinity CD200-Fc Fusion Protein for the Treatment of Atopic Dermatitis

Joseph Sheridan,[1] Jonathan Heal,[1] David R. Berk,[2] Patrick Burnett,[2] David J. Blackbourn,[3,4] Philip Huxley,[5] Rebecca Ashfield[2,5]

[1]RxCelerate (previously Prosarix Ltd), Babraham, Cambridge, UK; [2]Arcutis Biotherapeutics, Inc., Westlake Village, CA, USA; [3]School of Biosciences and Medicine, University of Surrey, Guildford, UK; [4]Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, UK; [5]Ducentis BioTherapeutics Ltd., Bristol, UK

Introduction

- Checkpoint receptors are important for maintaining immune homeostasis, and dysregulation of these receptors can contribute to inflammatory disease[1]
- Human CD200 receptor (CD200R, CD200R1; originally OX2R) is an inhibitory immune checkpoint receptor expressed on many myeloid cells and lymphocyte populations, including CD4+ T and type 2 innate lymphoid cells[2,3]
  - Its ligand, CD200, is expressed on the surface of tissues including endothelium, epithelia, and neurons, where it moderates immune activation, particularly at barrier sites[4,5]
  - Signaling occurs via DOK2 and Erk/MAP kinases to inhibit the NFkB pathway and cytokine secretion[6,7]
- Dysregulation of the CD200 axis is associated with allergic, autoimmune, and inflammatory diseases[8,9]
- Furthermore, a CD200R agonist antibody demonstrated favorable safety and efficacy in a 12-week Phase 1 clinical trial in patients with moderate to severe atopic dermatitis[10]

Engineering High-Affinity CD200-Fc Proteins

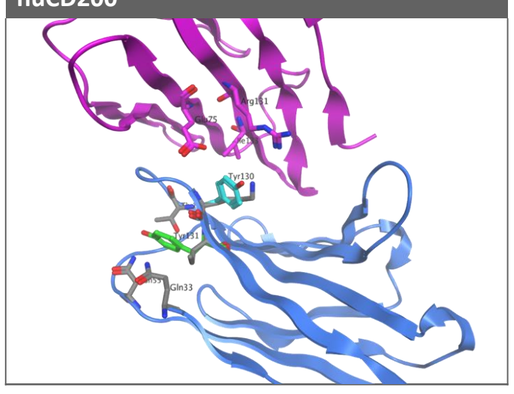
- We engineered high-affinity CD200R agonists, including human CD200-Fc proteins with substantially increased (up to 130-fold) monomeric binding affinity for CD200R, using in silico prediction (**Table 1**; **Figure 1**)

Table 1. CD200-Fc Constructs

Construct	Species	Mutations (CD200)	Fc	Affinity (Monomeric KD [nM]) for Cognate CD200 Receptor
DS-155	Human	wt	IgG4 S228P	179
DS-192	Human	K130Y	IgG4 S228P	13
DS-118	Human	K130Y, I131Y	IgG4 S228P	1
ARQ-234	Human	K130Y, I131Y	IgG4 S228P, M428L, N434S	2
DS-198	Mouse	wt	Murine IgG2a	584
DS-227	Mouse	H82Y, T125I	Murine IgG2a	43
DS-155	Human	wt	IgG4 S228P	179

ID numbers for CD200-Fc variants, showing CD200 mutations, Fc domains, and mean affinity constant (KD) values (to the nearest whole number). Monomeric affinities were measured by SPR at 25°C, mean of 2 experiments. Mutation numbering for CD200 proteins refers to the preprotein, and for the Fc domain the EU antibody numbering system.

Figure 1. Affinity-Enhancing Mutations of huCD200

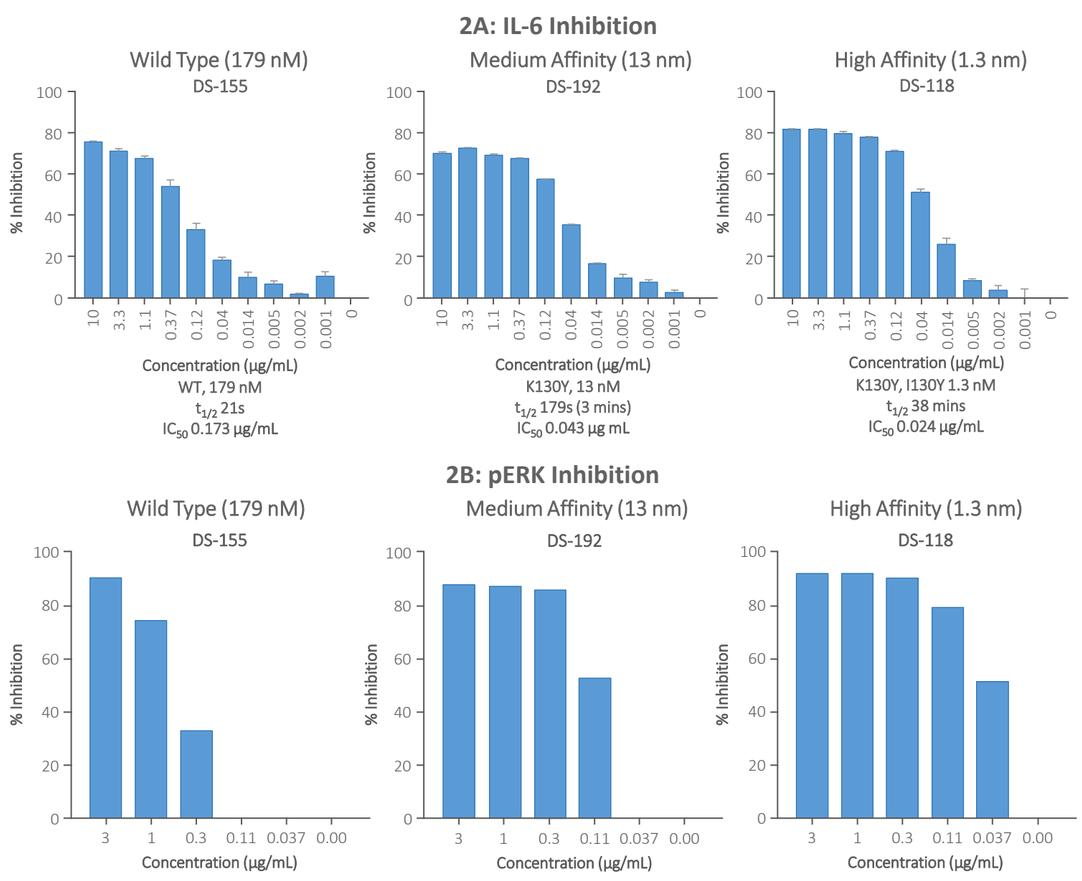


We generated a model for human CD200 (huCD200) bound to its cognate receptor, using the murine crystal structure. In silico design methods were used to identify potential mutations that could enhance affinity between huCD200 and huCD200R. This methodology included the use of affinity prediction protocols scripted within the MOE software (CCG Inc) and the use of Rosetta (Creative Commons).
HuCD200R is shown as magenta ribbons and sticks. HuCD200 is shown as blue ribbons, with wild type residues shown as elemental colored sticks. Mutation I131Y is shown as green sticks and K130Y as cyan sticks. The N-terminus of huCD200 is omitted for clarity. For structural context, local residues Q33, N55, and T125 in huCD200 are shown as elemental colored sticks, and E75, R131, and I133 in huCD200R are shown as magenta/elemental colored sticks.
The higher-affinity murine CD200 fusion protein, DS-227, was designed (directly from the mouse crystal structure) following a similar procedure. The murine modeling work gave rise to a monomeric mutated CD200 domain with 13-fold greater affinity than the wild type protein

High-Affinity Human CD200-Fc Outperforms Wild Type in Inhibiting IL-6 Release From a Cell Line Expressing High Levels of Human CD200R

- Human CD200-Fc proteins were tested for their ability to inhibit cytokine release from lipopolysaccharide (LPS)-activated U937 (pro-monocytic, human myeloid leukemia) cells engineered to express high levels of human CD200R
- High-affinity human CD200-Fc outperforms wild type in inhibiting IL-6 release (**Figure 2A**)
- The same trend in activity (ie, high-affinity > medium affinity > wild type) is observed with inhibition of ERK phosphorylation (**Figure 2B**)

Figure 2. IL-6 and pERK Inhibition in Response to Dose Titrations of Human CD200-Fc Proteins With Varying Affinity

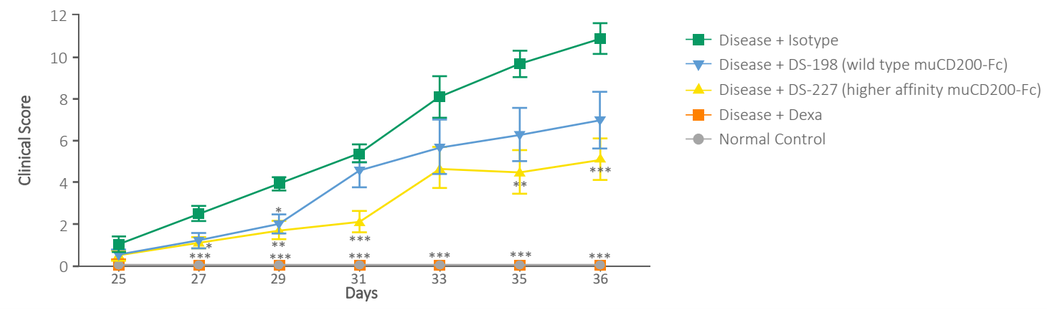


U937 cells were transduced with a lentiviral vector expressing human CD200R, and polyclonal cells over-expressing the receptor were sorted by flow cytometry.
2A: Cells were differentiated with PMA and activated with 100 ng/mL LPS in the presence of huCD200-Fc proteins; each dilution was carried out in triplicate. Cytokine levels in supernatant were measured at 24 hours by ELISA. 2B: In the presence of huCD200-Fc fusions, U937-CD200R cells were treated with PMA for 20 mins, and inhibition of ERK-phosphorylation measured by flow cytometry in permeabilized cells with an anti-pERK antibody.
LPS: lipopolysaccharide; PMA: phorbol myristate acetate; $t_{1/2}$: half-life.

In Vivo Proof of Concept for Inhibiting Inflammation With Higher-Affinity CD200-Fc

- We compared wild type (DS-198) and higher-affinity (DS-227) murine CD200-Fc proteins in the in vivo collagen-induced arthritis (CIA) model, initiating dosing just prior to symptom onset
- Higher-affinity murine CD200-Fc (DS-227) is significantly more potent in reducing clinical score than wild type (DS-198; **Figure 3**)

Figure 3. Higher-Affinity muCD200-Fc Is More Potent Than Wild Type in a Mouse CIA Model



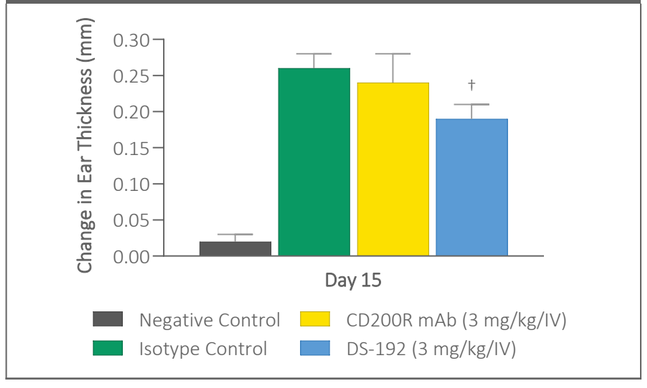
Arthritis was induced in male DBA/1J mice by intradermal injection of bovine type 2 collagen in CFA (complete Freund's adjuvant) on Day 1, followed by a booster injection in incomplete Freund's adjuvant on Day 21. On Day 22, animals were randomized based on body weight, and injected IV once every 3 days until Day 36 with 3 mg/kg murine IgG2a isotype control antibody, DS-198 (wild type muCD200-Fc) or DS-227 (high-affinity 43 nM muCD200-Fc); the positive control group received oral 0.5 mg/kg dexamethasone dosed daily. N=10 for treatment groups, and n=6 for the non-diseased control group. Clinical scores of paw arthritis (blinded assessment) were measured from Days 25–36 on alternate days. Data are represented as mean ± SEM. *P<0.05; **P<0.01;***P<0.001 versus disease + dexamethasone, disease + DS-198, and disease + DS-227, two-way RM ANOVA followed by Tukey's multiple comparisons test.

In Vivo Proof of Concept for High-Affinity Human CD200-Fc Proteins

High-affinity human CD200-Fc has superior potency to a CD200R agonist antibody in a humanized mouse model of contact hypersensitivity (**Figure 4**)

- huNOG-EXL mice were engrafted with human lymphocytes and myeloid cells to allow testing of our human CD200-Fc constructs
- Oxazolone-induced contact hypersensitivity mimics many of the features of human atopic dermatitis, including erythema, excoriation, and increased levels of IgE and type 2 cytokines
- Mice were treated with DS-192 (huCD200-Fc, 13 nM) or a CD200R agonist antibody (CD200R mAb) on the same day as repeat oxazolone challenges to the ear
- Change in ear thickness was significantly reduced by DS-192 on the day after the final challenge compared with isotype control; CD200R mAb did not result in a significant decrease (**Figure 4**)
- We also observed significant decreases in GM-CSF, and IL-13 in ear tissue at the end of the study in DS-192–treated mice

Figure 4. Oxazolone Contact Hypersensitivity Model in Humanized Mice: Change in Ear Thickness at End of Study

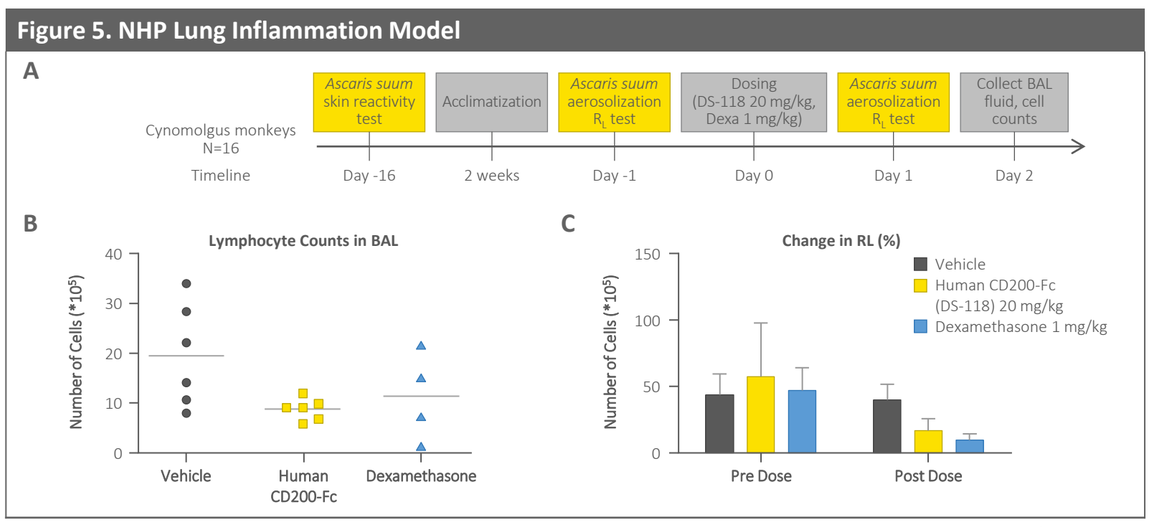


The CD200R agonist antibody was constructed from heavy- and light-chain sequences in patent application US2020/0087395.
Female NOG-EXL mice were engrafted with human cells and randomized on the basis of %CD45+ cells aged Weeks 20–21 (Day -1). On Day 0, mice were sensitized with abdominal application of oxazolone (100 µL of 3% w/v oxazolone in acetone:alcohol 1:4), and challenged on Days 5, 10, and 14 with topical application of 20 µL 2% w/v oxazolone (acetone:alcohol 1:4) to each ear (10 µL/side). Isotype control antibody, CD200R agonist antibody, and high-affinity huCD200-Fc (DS-192) were dosed IV at 3 mg/kg on Days 5, 10, and 14, 4 hours before oxazolone challenge (N=8 for all treatment groups; n=5 for non-diseased controls). Ear thickness was measured just before challenge and 24 hours after each challenge, and on Day 15, punch biopsies were taken for cytokine analysis by multiplex.
Change in ear thickness from Day 0, combined values for the right and left ear. Data are represented as mean ± SEM.
†P<0.05 vs isotype control, unpaired Student's t-test.
IV: intravenous.

The highest affinity huCD200-Fc, DS-118, shows activity in a model of *Ascaris suum* (roundworm)- induced lung inflammation in nonhuman primates (NHPs; **Figure 5**)

- This model is Th2-driven, and is widely used to assess the efficacy of drugs for asthma
- High-affinity human CD200-Fc significantly reduces cell infiltrate in bronchoalveolar lavage fluid 24 hours post challenge (**Figure 5B**)
- Although a reduction in airway resistance occurred post sensitization, this did not reach statistical significance (**Figure 5C**)

Figure 5. NHP Lung Inflammation Model

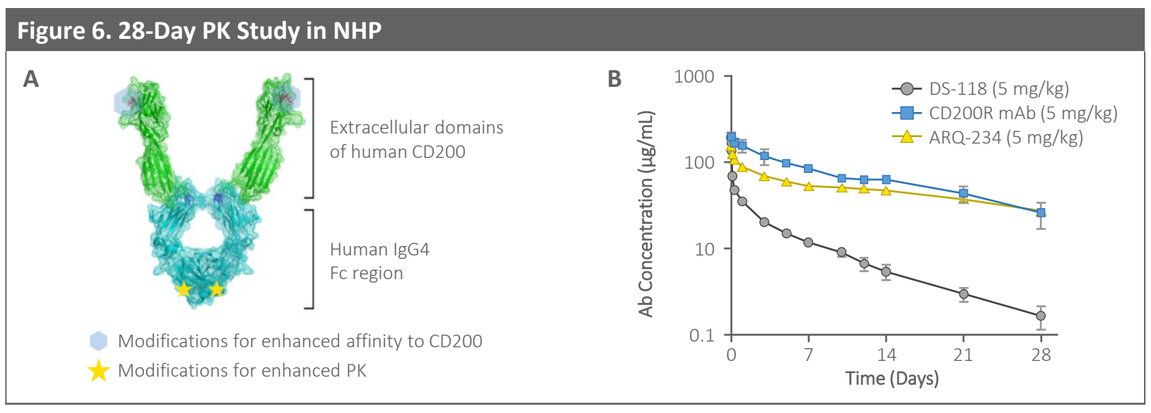


(A) Cynomolgus monkeys were screened for pre-exiting sensitivity to *Ascaris suum* antigen, and on Day 0 were dosed with high-affinity huCD200-Fc (DS-118) at 20 mg/kg IV (n=6), vehicle control (n=6), and dexamethasone at 1 mg/kg IV (n=4). All animals were challenged on Day +1 with 5000 µg/mL intrabronchial *A. suum* antigen. (B) Lymphocyte levels in BAL fluid measured on Day +2 (24 hours post challenge, 48 hours post drug treatment) by flow cytometry. (C) Change in airway resistance immediately following *A. suum* antigen challenge, compared with immediately prior to challenge. Pre-dose measurements were taken on Day -1 (relative to huCD200-Fc dosing), and post dose on Day +1.
BAL: bronchoalveolar lavage; IV: intravenous; R_L: lung resistance.

Engineering ARQ-234, a High-Affinity CD200-Fc Therapeutic With Long Serum Half-Life

- To optimize high-affinity CD200-Fc for development as a human therapeutic, we introduced two mutations ("LS") into the Fc domain, which increase binding to FcRn at low pH[11]
  - A diagram representing the resulting Fc fusion, ARQ-234, is shown in **Figure 6A**
- The PK profiles of DS-118 and ARQ-234 were compared with that of the CD200R agonist antibody in cynomolgus monkeys (**Figure 6B**)
  - Although serum levels of ARQ-234 initially decreased faster than CD200R mAb, the clearance rate was slower later in the study and the calculated half-life is longer (**Table 2**)
  - The volume of distribution for ARQ-234 is over double that for the antibody (148 vs 65 mL/kg)

Figure 6. 28-Day PK Study in NHP



Cynomolgus monkeys (*Macaca fascicularis*) were injected with an IV bolus of huCD200-Fc or CD200R antibody, at 5 mg/kg (n=2, 1 male, 1 female per group). At least 0.8 mL blood was collected from a cephalic or saphenous vein at pre dose; 0.25, 0.5, 1 ,4, 8, and 24 hours; Days 3, 5, 7, 10, 12, 14, 21, and 28 from each animal. Protein concentrations were determined by ELISA. The serum concentration was subjected to a noncompartmental pharmacokinetic (PK) analysis by Phoenix WinNonlin software (version 8.1, Pharsight, Mountain View, CA).

Table 2. Calculated Mean Half-Life Values

Construct	Fc Domain	Calculated Half-Life (Mean of n=2), Days
ARQ-234	IgG4 S228P, M428L, N434S	15.5
DS-118	IgG4 S228P	4.4
DS-155	IgG4 S228P	8.3

CONCLUSIONS

- Targeted intervention of the CD200-CD200R axis could resolve chronic inflammation and restore immune homeostasis
- ARQ-234 is a potent, high-affinity CD200-Fc protein with antibody-like serum half-life in NHP
- ARQ-234 is currently in preclinical development as a therapy for atopic dermatitis
- ARQ-234 has potential to treat other inflammatory diseases, particularly those that are Th2-driven

REFERENCES

1. Paluch C, et al. *Front Immunol* 2018;9:2306.
2. Gorczynski RM. *ISRN Immunol* 2012;2012:1–18.
3. Shafiei-Jahani P, et al. *Nat Commun* 2021;12:2526.
4. Wright GJ, et al. *Immunology* 2001;102:173–179.
5. Snelgrove RJ, et al. *Nature Immunol* 2008;9:1074–1083.
6. Jenmalm MC, et al. *J Immunol* 2006;176:191-199.
7. Mihrshahi R, et al. *J Immunol* 2009;183:4879–4886.
8. Ferreira MAR, et al. *Plos Genet* 2020;16:e1008725.
9. Mucha S, et al. *J Allergy Clin Immunol* 2020;145:1208–1218.
10. Alonso D, et al. 31st European Academy Of Dermatology And Venereology Congress 2022.
11. Zalevsky J, et al. *Nat Biotechnol* 2010;28:157–159.

DISCLOSURES

RA and **PH** were previously employed by Ducentis BioTherapeutics Ltd., acquired by Arcutis in 2022. **DJB** was previously employed as a consultant by Ducentis. **RA, PH, JS,** and **JH** are inventors on patents filed by Ducentis BioTherapeutics Ltd. **DRB, PB,** and **RA** are employees of Arcutis Biotherapeutics, Inc. The authors have no additional financial interests.

ACKNOWLEDGMENTS

We are grateful to the scientists at the following companies who carried out protein manufacture, PK modeling, and in vitro and in vivo experiments: Aragen, Syngene, Wu'Xi, and Physiomics. We also thank Mark Edwards for providing the diagram of ARQ-234, and Michelle Linterman for advice on planning in vitro experiments. Writing support was provided by Christina McManus, PhD, Alligent Biopharm Consulting LLC, and funded by Arcutis Biotherapeutics, Inc.