M281: A Therapeutic FcRn Blocking Antibody for Rapid Clearance of IgG and IgG Autoantibodies in Immune Cytopenias and other Autoimmune Diseases

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ABSTRACT

INTRODUCTION
IgG antibodies are the primary pathogenic agent in a number of autoimmune diseases. Efficacious therapies which decrease systemic levels of pathogenic antibodies include treatment with IVIG, therapeutic plasmapheresis or immunoabsorption. Here, a novel strategy was evaluated to induce IgG clearance in diseases driven by IgG autoantibodies by blockade of FcRn-mediated IgG recycling.

METHODS
M281 was developed as a high affinity, effectorless IgG1anti-FcRn monoclonal antibody. M281 effect on IgG recycling was evaluated in human endothelial cells *in vitro*. *In vivo* studies in transgenic human FCGRT/mouse FCGRT null mice and cynomolgus monkey were performed to characterize the pharmacokinetics, biodistribution, target occupancy, specificity of M281 and its efficacy in mouse models of thrombocytopenia and arthritis.

RESULTS
M281 demonstrates specific dose-dependent, albumin-sparing IgG clearance in human FCGRT transgenic/mouse FCGRT null mice and in cynomolgus monkeys. M281 inhibits IgG recycling in endothelial cells *in vitro*. Pharmacokinetics, target occupancy, pharmacodynamics and biodistribution indicate typical recombinant antibody biodistribution with rapid, dose dependent target inhibition and systemic clearance. M281 also demonstrated efficacy in mouse idiopathic thrombocytopenia purpura and collagen antibody-induced arthritis models of disease.

CONCLUSIONS
These findings support the evaluation of M281 as a strategy for the rapid and reversible suppression of pathogenic autoantibodies or alloantibodies in the setting of immune cytopenias, acquired inhibitors, thrombotic states and other disorders.

INTRODUCTION

- Decreased levels of pathogenic autoantibodies achieved after treatment with IVIG, therapeutic plasmapheresis or immunoabsorption may contribute to their therapeutic effect in indications such as idiopathic thrombocytopenia purpura, as well as other auto- and alloimmune diseases such as myasthenia gravis, Guillain-Barre syndrome, neuromyelitis optica, and renal antibody-mediated allograft rejection.
- Removal of auto- or alloantibodies may also be addressed by targeting FcRn, the neonatal Fc receptor which recycles IgG and albumin. Both IgG and albumin are internalized in endothelial cells via pinocytosis or Fc-Fcγ receptor-mediated uptake and bind FcRn in the early endosome at acidic pH (~6.0). FcRn-IgG or FcRn–albumin complexes are transported via the endocytic recycling pathway for extracellular release at pH 7.4, whereas free endosomal IgG or albumin are transported to lysosomes and degraded (Figure 1).
- Previous studies demonstrated that the efficacy of mouse anti-FcRn antibodies in several rodent models of autoimmune disease was associated with the induction of IgG catabolism and a decrease in pathogenic autoantibodies. [1-5].
- Here, we describe M281, a fully human aglycosylated IgG1 anti-FcRn antibody, which induces dose-dependent, albumin-sparing IgG catabolism and disease amelioration in animal models.

RESULTS

- M281 is a high affinity human FcRn binding IgG1 which interferes with FcRn binding to IgG **(Table 1)**.
- M281 localizes in early endosomes following uptake by human endothelial cells **(Figure 2a)** and inhibits IgG, but not albumin accumulation in endosomes **(Figure 2b)**.
- M281 interacts with FcRn in or adjacent to the IgG Fc binding site in human FcRn **(Figure 3)**.
- M281 induces dose-dependent IgG catabolism and target occupancy in Tg32 human transgenic FcRn mice **(Figure 4)**.
- M281 induces dose-dependent IgG catabolism and target occupancy in cynomolgus monkey while sparing albumin catabolism **(Figure 5)**.
- M281 distributes to tissues similar to other therapeutic antibodies **(Figure 6)**.
- M281 potently inhibits collagen antibody-induced arthritis in human FcRn transgenic mice when dosed therapeutically **(Figure 7)**.
- M281 dosed therapeutically, rescues platelet levels from chronic anti-platelet antibody-induced thrombocytopenia in human transgenic FcRn mice **(Figure 8)**.

CONCLUSIONS

- M281 induces dose-dependent, albumin-sparing IgG catabolism and target occupancy in both non human primates and in human FcRn transgenic mice.
- M281 exhibits a typical biodistribution profile similar to other IgG1 antibodies.
- Therapeutic dosing of M281 is efficacious in both models inflammatory tissue damage and opsonization-induced cytopenia.
- M281 is under development for first-in-human evaluation.

REFERENCES

1. Challa et al (2013) "Autoantibody depletion ameliorates disease in murine experimental autoimmune encephalomyelitis" *mAbs* 5:1–5.
2. Chen et al (2010) "Animal model of fetal and neonatal immune thrombocytopenia: role of neonatal Fc receptor in the pathogenesis and therapy" Blood 116: 3660-3668
3. Liu et al (2007) "Amelioration of Experimental Autoimmune Myasthenia Gravis in Rats by Neonatal FcR Blockade" *J Immunol* 178:5390-5398.
4. Olaru et al (2013) "Neonatal Fc Receptor Promotes Immune Complex–Mediated Glomerular Disease" *J Am Soc Nephrol* 25: 918–925.
5. Patel et al (2011) "FcRn blockade by Fc engineering ameliorates arthritis in a murine model" *J Immunol. 187*: 1015–1022.

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Figure 1. Mechanism of IgG Recycling and anti-FcRn Antibody Blockade

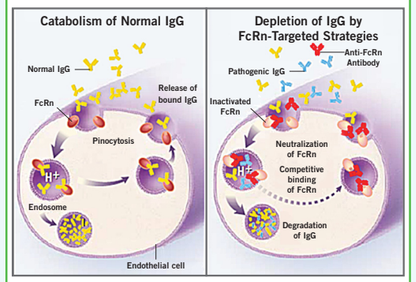


Table 1. M281 Exhibits in Vitro Binding and IgG Competition

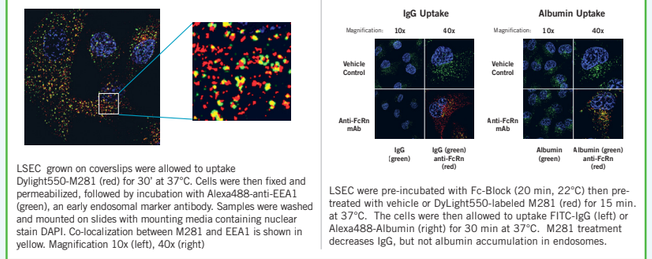
mAbs	Surface Plasmon Resonance						Cell Binding (Human FcRn)	Competitive Inhibiton of IgG Binding	
	ka (1/Ms)	kd (1/s)	K_D (M)	Rmax	Chi2	K_D (pM)	EC_{50} (nM)	Hu FcRn IC_{50} (nM)	Cyno FcRn IC_{50} (nM)
M281	1.19 x 10^6	2.31 x 10^{-5}	1.94 x 10^{-11}	211.33	7.81	19.4	6.33	3.25	4.46

Binding characteristics were determined by surface plasmon resonance (ProteOn). M281 is bound via anti-human Fc antibody on the chip surface and captures soluble human FcRn at pH 7.4. Binding at pH 6.0 for was below 10 pM (not shown). Similar values were obtained for binding to cynomolgus monkey FcRn at pH 7.4 and 6.0.

Binding of fluorescently-labeled human IgG1 to human or cynomolgus monkey FcRn expressed at the cell surface of HEK293 cells was evaluated on ice for 30 minutes at pH 6.0. IgG binding was inhibited by the addition of increasing concentrations of anti-FcRn monoclonal antibody.

Cell binding of antibodies was evaluated on HEK293 cells expressing the extracellular domain of human FcRn at the cell surface for 30 minutes on ice followed by wash and detection by FACS with a labeled secondary anti-human IgG antibody.

Figure 2. Uptake and Localization of IgG, Albumin and M281 in Human Liver Sinusoidal Endothelial Cells (LSEC)

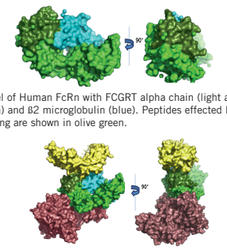


LSEC grown on coverslips were allowed to uptake Dylight550-M281 (red) for 30' at 37°C. Cells were then fixed and permeabilized, followed by incubation with Alexa488-anti-EEA1 (green), an early endosomal marker antibody. Samples were washed and mounted on slides with mounting media containing nuclear stain DAPI. Co-localization between M281 and EEA1 is shown in yellow. Magnification 10x (left), 40x (right)

LSEC were pre-incubated with Fc-Block (20 min, 22°C) then pre-treated with vehicle or DyLight550-labeled M281 (red) for 15 min. at 37°C. The cells were then allowed to uptake FITC-IgG (left) or Alexa488-Albumin (right) for 30 min at 37°C. M281 treatment decreases IgG, but not albumin accumulation in endosomes.

- **M281 localizes in early endosomes following uptake by human endothelial cells and inhibits IgG, but not albumin accumulation in endosomes.**

Figure 3. Localization of N027 Binding to human FcRn by Hydrogen-Deuterium Exchange.

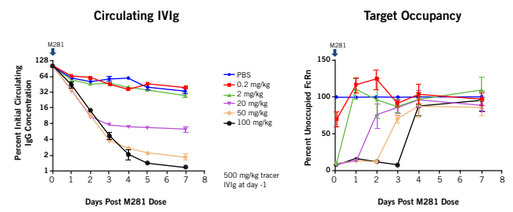


A. Model of Human FcRn with FCGRT alpha chain (light and olive green) and ß2 microglobulin (blue). Peptides effected by M281 binding are shown in olive green.

B. Model of FcRn (FCGRT, light and olive green; ß2 microglobulin, blue) bound to half Fc molecule (yellow) and albumin (purple). M281 binding site (olive green) overlaps with Fc binding site.

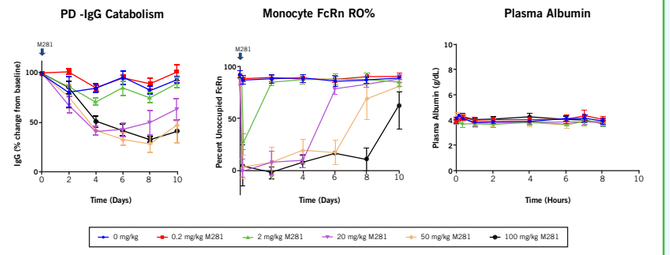
- **M281 interacts with FcRn in or adjacent to the IgG Fc binding site in human FcRn.**

Figure 4. Dose Effect of M281 on IgG Levels and Target Occupancy in Human Transgenic FcRn Mice



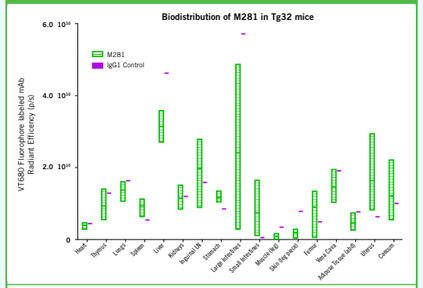
M281 was dosed i.v. 24 hrs after administration of 500 mg/kg IVIG (tracer) to Tg32 human FcRn (hFCGRT) transgenic, mouse FcRn (mFCGRT) knockout mice. Circulating human IgG was detected by ELISA on each day. Target occupancy was measured on each day in monocytes from lysed whole blood by FACS, after incubation of cells with immunophenotyping cell surface markers followed by fixation and permeabilization. Unoccupied FcRn was measured by staining with Dy650-labeled M281. (n = 4 males per group)

Figure 5. Selective Induction of IgG Catabolism and Target Occupancy in Cynomolgus Monkey



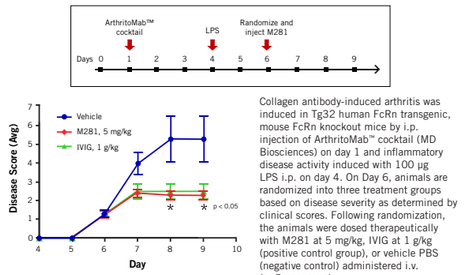
M281 was dosed i.v. at t = 0 in cynomolgus monkeys. Circulating endogenous IgG and albumin was detected by ELISA. Target occupancy was measured in monocytes from lysed whole blood by FACS, after incubation of cells with immunophenotyping cell surface markers followed by fixation and permeabilization. Unoccupied FcRn was measured by staining with Dy650-labeled M281. (n = 3 males per group)

Figure 6. Biodistribution of M281 in Human FcRn Transgenic Mice



M281 or isotype human IgG1 control antibody labeled with fluorophore (VT680) was administered i.v. to Tg32 human FcRn transgenic, mouse FcRn knockout mice at 30 mg/kg. Levels of labeled antibody were measured in individual organs by quantitative ex vivo optical imaging at 72 hours post dosing.

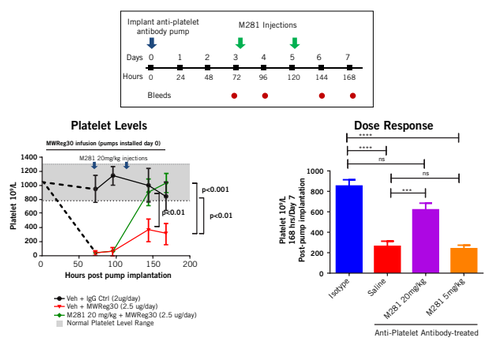
Figure 7. Efficacy of M281 in Mouse Collagen Antibody-Induced Arthritis



Collagen antibody-induced arthritis was induced in Tg32 human FcRn transgenic, mouse FcRn knockout mice by i.p. injection of ArthritoMab™ cocktail (MD Biosciences) on day 1 and inflammatory disease activity induced with 100 µg LPS i.p. on day 4. On Day 6, animals are randomized into three treatment groups based on disease severity as determined by clinical scores. Following randomization, the animals were dosed therapeutically with M281 at 5 mg/kg, IVIG at 1 g/kg (positive control group), or vehicle PBS (negative control) administered i.v. (n=5 per group).

- **M281 potently inhibits collagen antibody-induced arthritis in human transgenic FcRn mice when dosed therapeutically.**

Figure 8. Efficacy of M281 in Mouse Chronic Idiopathic Thrombocytopenia Purpura (ITP)



Thrombocytopenia was induced in Tg32 human FcRn (hFCGRT) transgenic, mouse FcRn (mFCGRT) knockout mice by continuous infusion of anti-platelet antibody (anti-CD41, MWReg30) via s.c. miniosmotic pump. Circulating platelet levels were decreased to 300 x 109/L or less by 72 hrs (Day 3) after pump implantation. M281 was dosed therapeutically i.v. 72 hrs (day 3) and 120 hrs (Day 5) post-pump implantation. (A, n = 4 per group; B, n = 7 per group).