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# ARGX-117, a therapeutic complement inhibiting antibody targeting C2

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**Background:** Activation of the classical and lectin pathway of complement may contribute to tissue damage and organ dysfunction of antibody-mediated diseases and ischemia-reperfusion conditions. Complement factors are being considered as targets for therapeutic intervention.

**Objective:** We sought to characterize ARGX-117, a humanized inhibitory monoclonal antibody against complement C2.

**Methods:** The mode-of-action and binding characteristics of ARGX-117 were investigated in detail. Furthermore, its efficacy was analyzed in *in vitro* complement cytotoxicity assays. Finally, a pharmacokinetic/pharmacodynamic study was conducted in cynomolgus monkeys.

**Results:** Through binding to the Sushi-2 domain of C2, ARGX-117 prevents the formation of the C3 proconvertase and inhibits classical and lectin pathway activation upstream of C3 activation. As ARGX-117 does not inhibit the alternative pathway, it is expected not to affect the antimicrobial activity of this complement pathway. ARGX-117 prevents complement-mediated cytotoxicity in *in vitro* models for autoimmune hemolytic anemia and antibody-mediated rejection of organ transplants. ARGX-117 inhibits pH- and calcium-dependent target binding and is Fc-engineered to increase affinity at acidic pH to the neonatal Fc receptor, and to reduce effector functions. In cynomolgus monkeys, ARGX-117 dose-dependently reduces

free C2 levels and classical pathway activity. A 2-dose regimen of 80 and 20 mg/kg separated by a week, resulted in profound reduction of classical pathway activity lasting for at least 7 weeks. **Conclusions:** ARGX-117 is a promising new complement inhibitor that is uniquely positioned to target both the classical and lectin pathways while leaving the alternative pathway intact. (J Allergy Clin Immunol 2020;■■■■:■■■■-■■■■.)

**Key words:** Complement system, C2, monoclonal antibody, complement inhibitor

The complement system consists of over 20 soluble and membrane-bound proteins. The system is activated via 3 pathways—the classical, lectin, and alternative pathways (CP, LP, and AP, respectively)—which converge at the level of C3 to activate a final common pathway leading to the formation of the membrane attack complex. Whereas its role as an innate defense system against micro-organisms is well established, complement as an inflammatory mediator system contributing to organ dysfunction and tissue injury in human disease has received increasing attention during the last decades.<sup>1,2</sup> Different types of inhibitors, eculizumab (Soliris; Alexion Pharmaceuticals, Boston, Mass), an mAb that blocks C5, a recombinant C1 inhibitor (Ruconest; Pharming, Leiden, The Netherlands) and a plasma-derived C1 inhibitor (Cinryze; Takeda Pharmaceuticals, Tokyo, Japan; and Berinert; CSL/Behring, King of Prussia, Pa), the main inhibitor of CP and LP, are currently approved for clinical application.<sup>3,4</sup> However, there is no “one-size-fits-all” complement inhibitor, and both inhibitors have their limitations. Eculizumab cannot address inflammatory or cytotoxic reactions mediated by C3 activation products, which may explain its failure to prevent extravascular hemolysis in paroxysmal nocturnal hemoglobinuria.<sup>5</sup> The C1 inhibitor is a poor inhibitor of immune complex-bound C1<sup>6</sup> and has a relatively short half-life in plasma, making it less attractive for long-term prophylactic or chronic use.<sup>7</sup> Therefore, several other complement factors are being considered as targets for therapeutic intervention.<sup>8,9</sup>

We hypothesized that inhibition of complement at the level of C2 is an attractive therapeutic approach for several reasons. First, C2 is part of both CP and LP, and both are implicated in diseases driven by auto-antibody formation or ischemia reperfusion. Second, it leaves AP, an important innate defense system, intact. Moreover, C2 deficiencies, compared with C1 or C4 deficiencies, in humans are associated with a lower prevalence of autoimmune diseases, although predisposition to autoimmunity and systemic lupus is increased, particularly in women. C2 deficiency also increases the risk for bacterial pyogenic infections.<sup>10</sup> However,

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**Abbreviations used**

ADA:	Antidrug antibodies
aggIgG:	Aggregated IgG
AP:	Alternative pathway
CH50:	Total hemolytic complement
CP:	Classical pathway
EC <sub>50</sub> :	Half maximal effective concentration
FB:	Factor B
FcRn:	Neonatal Fc receptor
HRP:	Horseshoe peroxidase
LP:	Lectin pathway
PD:	Pharmacodynamic
PK:	Pharmacokinetic
RBC:	Red blood cell
RT:	Room temperature
S2:	Sushi 2
SPR:	Surface plasmon resonance
TBS:	Tris-buffered saline

C2 deficiency is not associated with increased susceptibility to meningococcal infections. Lastly, C2 in plasma is less abundant than other complement factors.<sup>11,12</sup>

It was previously shown that mAb-mediated inhibition of complement proteins in humans is feasible.<sup>3</sup> However, owing to the high concentrations of most complement factors in plasma, in combination with target-mediated clearance, high doses of mAbs are needed to fully block complement. To overcome this, an antibody can be used that takes advantage of pH differences between blood (pH 7.4) and endosome (pH 6.0) to dissociate the antigen from the antibody-antigen complex in the endosome to be degraded into the lysosome, a so-called sweeping antibody. Sweeping properties endow a mAb with a prolonged *in vivo* activity.<sup>13</sup>

Here we describe ARGX-117, an anti-human C2 mAb with pH and calcium-dependent target-binding properties. This mAb was generated in mice, humanized, and formatted as a human IgG<sub>1</sub> antibody with mutations to knock out effector functions<sup>14</sup> and to optimize interaction with neonatal Fc receptor (FcRn).<sup>15</sup> We here report a detailed characterization of ARGX-117.

**METHODS****Serum samples, reagents**

Fresh human serum was obtained from healthy individuals (Mini Donor Service, University Medical Center Utrecht) under approval of the medical ethical committee of the University Medical Center Utrecht. C2-depleted serum was purchased from Sigma-Aldrich (St Louis, Mo), Innovative Research (Plymouth, Minn), or Complement Technology (Tyler, Tex). Recombinant human C2 was obtained from U-Protein Express (Utrecht, The Netherlands). Human plasma-derived C2 and C4b were obtained from Complement Technology. Aggregated human IgG (aggIgG) was prepared by heating purified human IgG (GammaQuin; Sanquin, Amsterdam, The Netherlands) in PBS at 80 mg/mL (20 minutes, 63°C). Leftovers from clinical batches of Soliris (Alexion) were used as inhibitory anti-C5 mAbs.

**Influence of pH and calcium ions on binding of ARGX-117 to C2**

To assess influence of pH on binding of ARGX-117 to human C2, ELISA plates (Meso Scale Discovery; Meso Scale Diagnostics, Gaithersburg, Md) were coated overnight at 4°C with recombinant human C2 (U-Protein Express), followed by a 2-hour incubation at room temperature (RT) with Tris-buffered saline (TBS), pH 7.4 (50 mmol/L Tris-HCl pH 7.4, 150 mmol/L

NaCl), containing 1%, wt/vol, BSA. Plates were washed and incubated for 1 hour with serial dilutions of ARGX-117 in TBS-0.05% Tween20, pH 7.4, or in citrate buffer, pH 6.0 (0.2 mol/L citric acid, 0.2 mol/L sodium citrate, 150 mmol/L NaCl), final volume 25  $\mu$ L. Plates were washed and incubated with Meso Scale Discovery–SulfoTag-labeled goat anti-human IgG antibody in TBS 0.1% BSA, pH 7.4, for 1 hour at RT. Finally, Meso Scale Discovery substrate was applied and the plate was read on a MESO QuickPlex SQ120 system (Meso Scale Diagnostics).

The same ELISA with minor modifications was used to assess influence of Ca<sup>2+</sup> concentration on binding of ARGX-117. Plates were coated with human plasma-derived C2, and serial dilutions of ARGX-117 were incubated in TBS supplemented with 25  $\mu$ mol/L or 1.25 mmol/L CaCl<sub>2</sub>.

**Epitope mapping of ARGX-117**

For mapping the epitope of ARGX-117, we took advantage of the fact that ARGX-117 does not bind the highly homologous protein Factor B (FB). cDNA of domain swap mutants of C2 and FB, each with a C-terminal FLAG tag, were synthesized by GenScript (Piscataway, NJ) and transiently expressed in HEK293T cells. Expression was checked with an anti-FLAG ELISA, in which HEK293T supernatants were coated onto MaxiSorp microplates (Thermo Fisher Scientific, Waltham, Mass). Anti-FLAG Ab (clone M2; Sigma-Aldrich) and horseradish peroxidase (HRP)-labeled goat anti-mouse-IgG (Santa Cruz Biotechnology, Dallas, Tex) were used to detect bound mutants. To map its epitope, ARGX-117 was coated onto MaxiSorp microplates and incubated with the various domain swap mutants. Bound mutants were detected with biotinylated anti-FLAG and streptavidin-peroxidase conjugate (Roche Diagnostics, Indianapolis, Ind).

**Studies in cynomolgus monkeys**

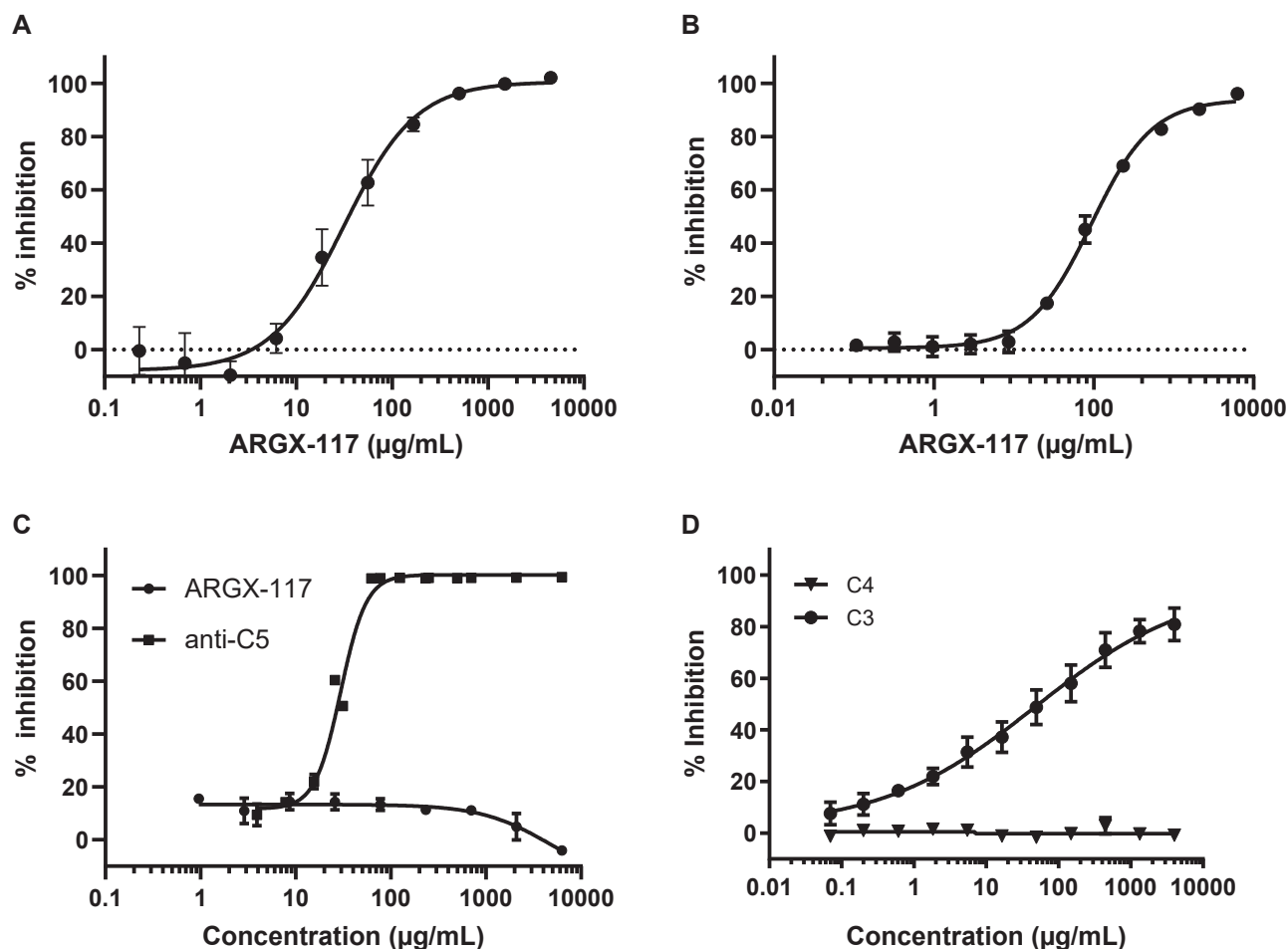
The non-good laboratory practice cynomolgus monkey studies were conducted at an animal facility (Germany) and were compatible with good laboratory practice regulations specified by regulatory authorities throughout the European Community, the United States (Environmental Protection Agency and Food and Drug Administration) and Japan (Ministry of Health, Labor, and Welfare; Ministry of Agriculture, Forestry, and Fisheries; and Ministry of Economy, Trade, and Industry). Single doses of 0.5, 1.5, 5, 10, and 30 mg/kg of ARGX-117 were injected intravenously, each in 2 female and 2 male monkeys. Serum was collected at several time points pre- and postdosing, and day of injection is referred as day 0. Three monkeys were intravenously injected with a loading dose of 80 mg/kg on day 0 followed by a second dose of 20 mg/kg on day 7.

**Total PK assay on cynomolgus monkey study samples**

Microtiter plates coated overnight with 2D11-mFc, recognizing specifically the NHance (argenx, Zwijsnaarde, Belgium) mutations (H433K, N434F), were blocked with PBS 2% BSA for 2 hours at RT. Serum samples diluted in PBS 0.2% BSA, pH 6.0, were pipetted into the wells. After washing, plates were developed with anti-human IgG-HRP (SouthernBiotech, Birmingham, Ala) and 3,3',5,5'-tetramethylbenzidine peroxidase substrate (Calbiochem; Millipore Sigma, Burlington, Mass). The color intensity is proportional to the concentration of ARGX-117 in the sample.

**ELISA for free cynomolgus C2**

MaxiSorp microtiter plates were coated overnight with mouse anti-human C2 mAb32 (Broteio Pharma, Utrecht, The Netherlands) directed against another epitope than ARGX-117 (C2a subunit) and blocked with PBS 2% BSA for 2 hours at RT. Serum samples were diluted in heat-inactivated pooled cynomolgus serum (BioIVT, Westbury, NY) and biotinylated ARGX-117 was added to each sample and transferred to coated plate. After 2-hour incubation at RT, plates were washed and developed with streptavidin-HRP (Jackson ImmunoResearch, West Grove, Pa) and 3,3',5,5'-tetramethylbenzidine. Results were expressed as U/mL with 100 U/mL being the amount in pooled cynomolgus serum. The lower level of quantitation was 2 U/mL.



**FIG 1.** Inhibition of CP (A, D) and LP (B), but not of AP (C) activation by ARGX-117. Human serum was supplemented with ARGX-117 at the indicated concentrations. **A**, Inhibition of CP-mediated complement activation was tested using the Quidel CH50 assay. **B**, Inhibition of LP-mediated complement activation was tested using the Wieslab MP320 assay (Svar, Malmö, Sweden). **C**, No inhibitory effect of ARGX-117 on AP-mediated complement activation was observed using the Wieslab AP330 assay. **D**, Both C4 and C3 fixation were used as read-out in a solid-phase agglgG-mediated complement activation assay, which was developed in-house. A concentration-dependent inhibitory effect of ARGX-117 was observed on C3 fixation, whereas fixation of C4, which acts upstream of C2, was unaffected. Data represent mean and SEM of 3 (A, B) or 2 (C, D) independent experiments. Anti-C5 mAb was tested in the assay for AP as a positive control. For further details, see the Methods section in the Online Repository.

## Statistics

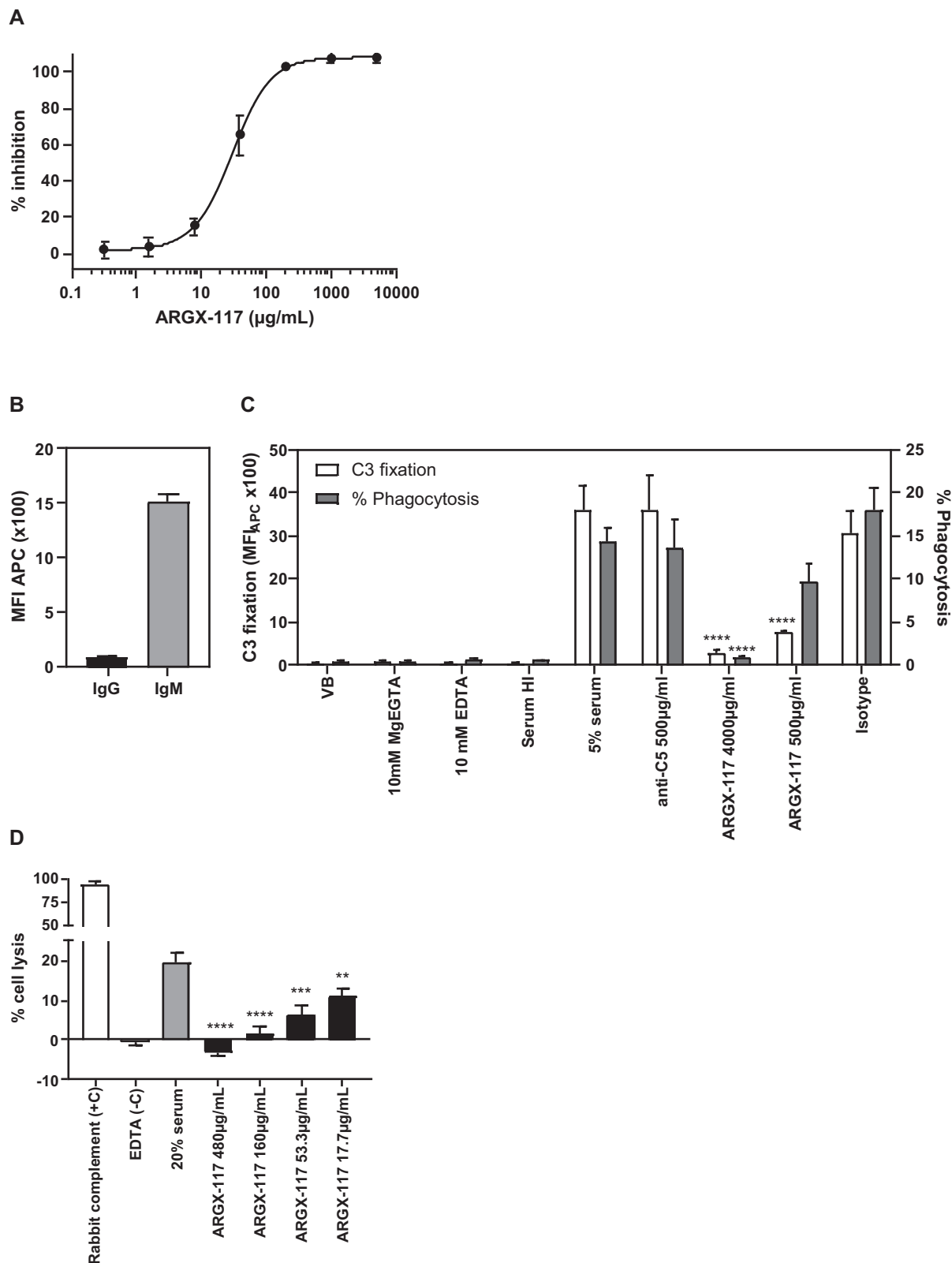
Results of the surface plasmon resonance (SPR) experiments were analyzed using Biacore T100 evaluation software (GE Healthcare, Princeton, NJ). The inhibitory effect of ARGX-117 in the complement-mediated phagocytosis assay and in the complement-dependent cytotoxicity assay was tested via 1-way ANOVA followed by Dunnett multiple comparisons test ( $****P < .0001$ ,  $***P < .001$ ,  $**P < .01$ ). All statistical analyses, including log transformation and non-linear regression analyses, were conducted using GraphPad Prism 8 (GraphPad Software, La Jolla, Calif).

## RESULTS

### Generation of ARGX-117

A panel of 35 hybridomas was generated from splenocytes obtained from mice immunized with human C2 and screened for the production of anti-C2 antibodies with an ELISA using solid-phase human C2. Inhibitory potency of anti-C2 antibodies was evaluated using an in-house ELISA in which fixation of C3 and

C4 from fresh human serum to solid-phase-bound agglgG was measured. Antibodies that inhibited fixation of C3, but not that of C4, were further analyzed. Among the 9 inhibitory mAbs identified, anti-C2 mAb 5F2.4 was selected for humanization using the human composite antibody technique (Abzena Ltd, Cambridge, United Kingdom) as described<sup>16</sup> and was further engineered. ARGX-117 is the humanized antibody formatted as human IgG<sub>1</sub> with L234A and L235A mutations to remove Fc-mediated effector functions<sup>13</sup> and H433K and N434F mutations to improve affinity to the FcRn at acidic pH.<sup>14</sup> Indeed, ARGX-117 demonstrated almost no binding to C1q, CD16 (FcγRIII), or CD32a (FcγRIIA) (see Fig E1, A-C, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)), whereas its affinity to FcRn at acidic pH was increased compared with a control human wild-type IgG<sub>1</sub> (Fig E1, D). Increased FcRn binding at low pH rescues ARGX-117 from lysosomal degradation and enhances recycling into circulation, resulting in a prolonged half-life.



**FIG 2.** ARGX-117 dose-dependently prevents lysis and phagocytosis of antibody-sensitized cells incubated with human serum. **A**, Inhibition of complement-mediated lysis of human RBCs sensitized with rabbit anti-human RBCs. **B**, IgM deposition on RBCs as measured by fluorescence-activated cell sorting. **C**, ARGX-117 inhibits C3 fixation and complement-dependent phagocytosis of human RBCs sensitized with human IgM, by THP-1 cells (compared with 5% serum condition, 1-way ANOVA, followed by Dunnett multiple comparisons test, \*\*\*\* $P < .0001$ ). Phagocytic cells were determined as double positive for carboxyfluorescein

The affinity of the binding of ARGX-117 to C2 was quantitated with SPR (see the Methods in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). The kDa of the binding of Fab ARGX-117 to C2 was about 0.3 nmol/L (see Fig E2 and Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

Assessing cross-reactivity with C2 from several animal species in ELISA revealed that ARGX-117 bound to both cynomolgus C2 and human C2. Moreover, it had a low affinity for rat C2, but did not react with mouse, guinea pig, or rabbit C2 (data not shown).

### ARGX-117 inhibits complement activation *in vitro*

Commercial complement assays (see the Methods in the Online Repository), were used to assess the complement pathways inhibited by ARGX-117 in human serum. ARGX-117 potently inhibited CP and LP (half-maximal effective concentration [EC<sub>50</sub>] = 30.5 ± 4.5 and 93.4 ± 10.4 μg/mL, respectively) in a concentration-dependent manner (Fig 1, A and B). Importantly, AP activation was not affected by ARGX-117 (Fig 1, C). The commercial assays measure complement activation by assessing membrane attack complex fixation to the solid phase. ARGX-117 also inhibited C3 fixation from human serum to solid-phase aggIgG, whereas it had no effect on C4 fixation (Fig 1, D).

The generation of fluid-phase activation products after complement activation can enhance inflammation and infiltration of immune cells, for example, in dense deposit disease and C3 glomerulopathies.<sup>17</sup> To assess inhibition of fluid-phase complement activation products by ARGX-117, aggIgG were added to fresh human serum. Subsequently, the generation of C4b/c and C3b/c was determined using specific ELISAs (see the Methods in the Online Repository). Preincubation of serum with ARGX-117 had no effect on the generation of C4b/c as expected, but dose-dependently, preincubation inhibited C3b/c fluid-phase activation (see Fig E3, A and B, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

Autoimmune hemolytic anemia is characterized by the presence of autoantibodies of both IgG or IgM isotypes against red blood cells (RBCs).<sup>18</sup> It is well established that in this disease complement mediates both autoantibody-initiated direct lysis of human RBCs (intravascular hemolysis) and uptake of opsonized RBCs by myeloid cells (extravascular hemolysis) through C3 receptors. To evaluate the effect of ARGX-117 on the hemolytic activity of complement, sensitized human RBCs were incubated in 10% human pooled serum containing ARGX-117 at increasing concentrations, and hemoglobin release was determined (see the Methods in the Online Repository). ARGX-117 inhibited hemolysis concentration-dependently (half maximal inhibitory concentration = 32 ± 10 μg/mL) (Fig 2, A).

To investigate the effect of ARGX-117 on C3 fixation and complement-mediated phagocytosis, human RBCs were incubated with pooled serum containing IgM anti-RBC antibodies in the presence of different concentrations of ARGX-117.

Incubation resulted in binding of IgM, not of IgG (Fig 2, B), and fixation of C3 (Fig 2, C). Opsonized RBCs were phagocytosed by differentiated THP-1 cells. C3 deposition on RBCs and phagocytosis of the opsonized RBCs was inhibited in the presence of 10 mmol/L EDTA or 10 mmol/L Mg ethyleneglycol-bis-(β-aminoethylether)-N,N,N',N'-tetraacetic acid, as well as when heat-inactivated serum was used, indicating that phagocytosis was complement-dependent. Both C3 fixation and phagocytosis was dose-dependently inhibited by ARGX-117, but not by an anti-C5 mAb or an isotype control for ARGX-117 (Fig 2, C).

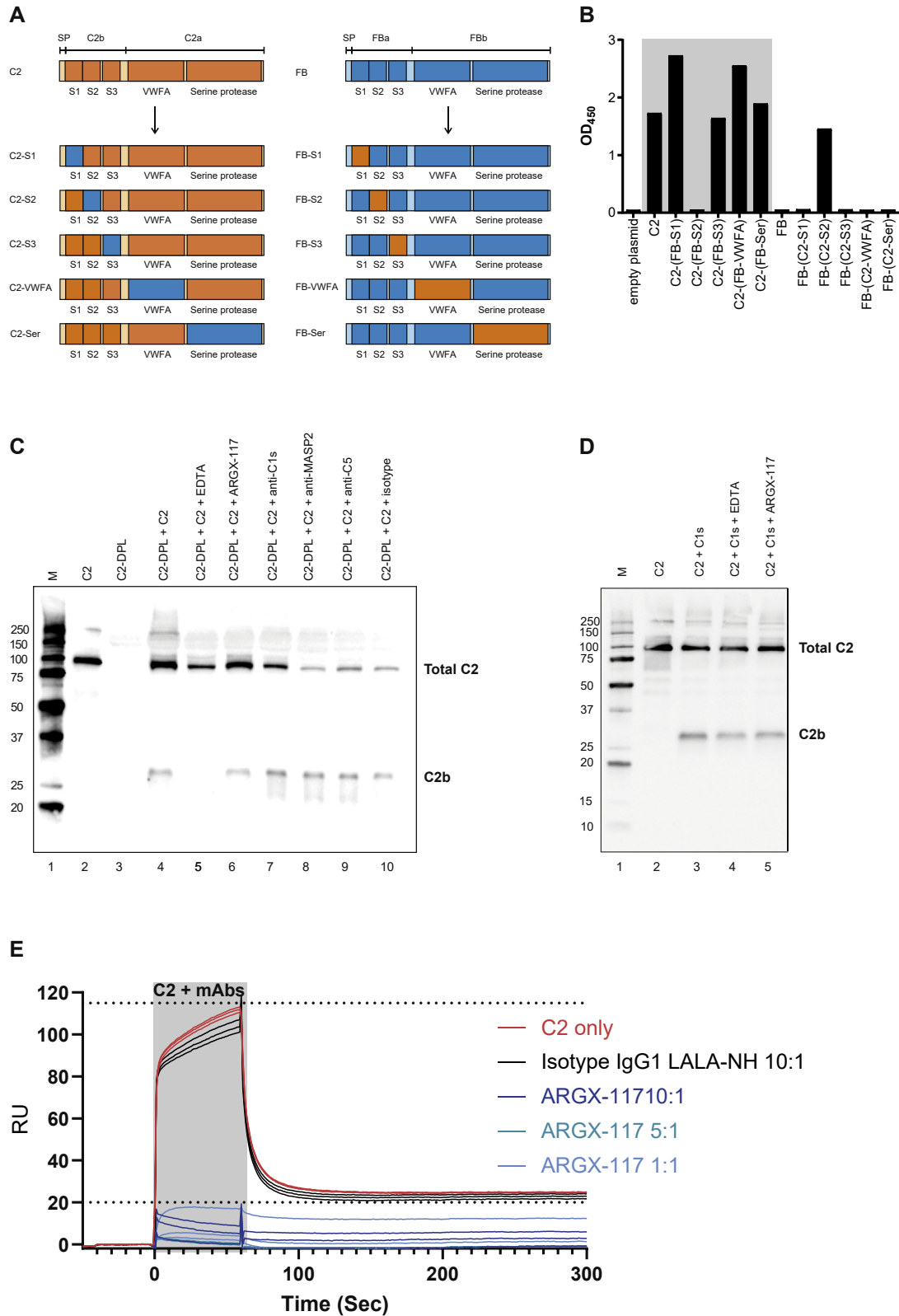
Antibody-mediated rejection is a major concern in solid-organ transplantation, necessitating pretransplantation screening of recipient for complement-activating antibodies against donor HLA antigens with the crossmatch test.<sup>19</sup> We modified this test by using human instead of rabbit complement to evaluate the potency of ARGX-117 to block complement-dependent cytotoxicity by anti-HLA antibodies (see the Methods in the Online Repository). Human PBMCs were sensitized with anti-HLA antibodies from hyperimmunized kidney transplant patients, incubated with fresh human serum with or without ARGX-117, and assessed for cell lysis by microscopy. In the absence of ARGX-117, about 20% of the cells were lysed, which is less than the lysis induced by rabbit complement due to protection of PBMCs against human complement by membrane-bound complement regulatory proteins. ARGX-117 concentration-dependently inhibited lysis of anti-HLA sensitized PBMC (Fig 2, D).

### Mechanism of action of ARGX-117

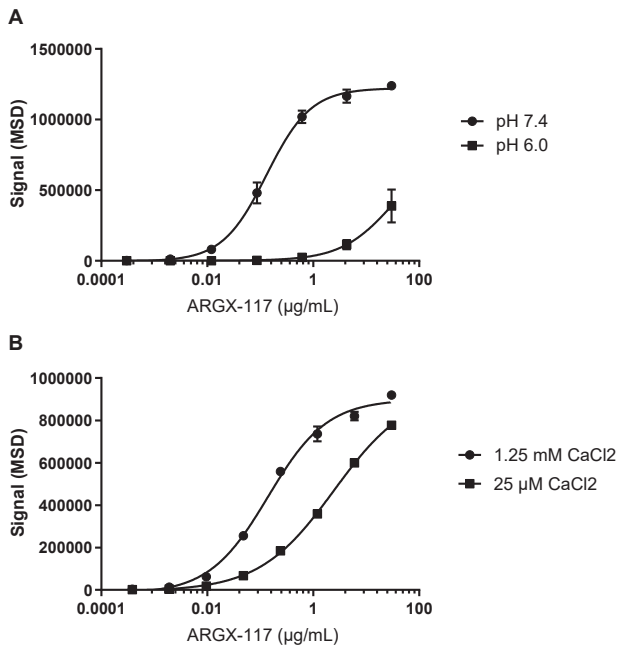
To identify the domain harboring the epitope for ARGX-117, domain swap mutants of C2 and FB were generated. We took advantage of the fact that C2 and FB are homologous proteins and have the same domain structure (Fig 3, A); the small subunits (C2b and FBa) of both proteins harbor 3 Sushi domains (Sushi-1, -2, and -3, also known as the complement control protein or short consensus repeat domain), whereas the large subunits (C2a and FBb) contain a von Willebrand factor-A domain and a serine protease domain. ARGX-117 did not bind to full length FB and to chimeric C2 containing the FB-derived Sushi-2 (S2) domain, whereas the binding was restored to FB containing the S2 domain of C2 (Fig 3, B). Thus, the epitope of ARGX-117 is located in the S2 domain of the C2b subunit of C2.

To investigate the effect of ARGX-117 on C2 cleavage in serum during activation, C2-deficient serum was reconstituted with purified plasma-derived C2, activated with aggIgG, separated on SDS-PAGE, and immunoblotted with biotinylated ARGX-117 as detection antibody (see the Methods in the Online Repository; Fig 3, C). C2 yielded a main band migrating at ~100 kDa (lane 2). No staining of C2-deficient serum was observed indicating specificity of biotinylated ARGX-117 for C2 (lane 3). Analysis of C2-depleted serum reconstituted with purified

succinimidyl ester and CellTrace Violet (Invitrogen, Thermo Fisher Scientific). **D**, Inhibition of complement-dependent lysis of PBMCs sensitized with anti-HLA antibodies. Rabbit complement was used as positive control and 10 mM EDTA as negative control, percentage lysis was calculated to controls. ARGX-117 concentration-dependently inhibits complement-mediated cell lysis (compared with 20% serum condition, 1-way ANOVA, followed by Dunnett multiple comparisons test, \*\*\*\**P* < .0001, \*\*\**P* < .001, \*\**P* < .001). Results represent mean and SEM of 3 (**A**, **B**, **C**) and 2 (**D**) independent experiments. *APC*, Antigen-presenting cell; *HI*, heat-inactivated; *MFI*, mean fluorescence intensity; *MgEGTA*, Mg ethyleneglycol-bis-(β-aminoethylether)-N,N,N',N'-tetraacetic acid; *VB*, veronal buffer.



**FIG 3.** Mode of action of ARGX-117. **A**, Schematic representation of the domain swap mutants of C2 and FB. **B**, Binding of ARGX-117 to C2 domain swap mutants in ELISA. ARGX-117 on the solid phase was incubated with the swap mutants, which were detected with biotinylated anti-FLAG antibody. Note that ARGX-117 binds to S2 domain of C2b. **C**, Western blot analysis of C2-depleted (*C2-DPL*) serum reconstituted with serum-purified C2 and activated with agglgG (800  $\mu$ g/mL). Lane 1: MW markers (*M*); lane 2: purified C2; lane 3: *C2-DPL* serum. Activation of reconstituted *C2-DPL* serum results in a decrease of total C2 and the



**FIG 4.** Characterization of the binding of ARGX-117 to human C2. Influence of pH (A) and Ca<sup>2+</sup> concentration (B) on binding of ARGX-117 to human C2 in ELISA. Data represent mean and SEM of 2 independent experiments. MSD, Meso Scale Discovery.

C2 and activated with aggIgG in absence of ARGX-117, yielded a smaller band of ~30 kDa recognized by biotin-labeled ARGX-117, representing C2b. Also, a decrease in total C2 was observed (lane 4). Preincubation of reconstituted activated serum with EDTA fully inhibited cleavage of total C2 and the formation of C2b (lane 5). Preincubation of reconstituted serum with ARGX-117 inhibited cleavage of total C2 (lane 6) as did an anti-C1s mAb (lane 7). Preincubation with an anti-MASP2 mAb did not affect the increase of C2b and the decrease of total C2 in serum by aggIgG, confirming the observed activation was mediated by the classical pathway. An anti-C5 mAb or an isotype control did not inhibit total C2 cleavage, but did show increased C2b (lanes 8, 9, and 10).

The inhibition of the cleavage of C2 into C2a and C2b during activation by ARGX-117 can either be explained by (1) steric hindrance of C1s cleavage of the peptidyl bond between C2a and C2b or (2) preventing the interaction of C2 with C4b, thereby avoiding exposure of the peptidyl bond. ARGX-117 was not able to inhibit C2 cleavage by C1s in a purified system, suggesting it interferes with the interaction of C2 with C4b. Note that in this

purified system, EDTA does not inhibit C2 cleavage by C1s because the interaction of C1s with C2 is calcium-independent (Fig 3, D).

Next, the effect of ARGX-117 on the formation of the CP C3 proconvertase complex was further studied using SPR. Biotinylated C4b was immobilized onto streptavidin-coated chips, and C2 was added to allow the formation of the C4bC2 complex on the chip surface (Fig 3, E, red line). The binding of C2 to C4b was not influenced by the control mAb (Fig 3, E, black line). However, preincubation of C2 with ARGX-117 in different molar ratios, completely inhibited C2 binding to C4b (Fig 3, E, lines in shades of blue). Taken together, these results argue that ARGX-117 interferes with the binding of C2 via its S2 domain to C4b.

### Unique pH- and Ca<sup>2+</sup>-dependent binding of ARGX-117 to human C2

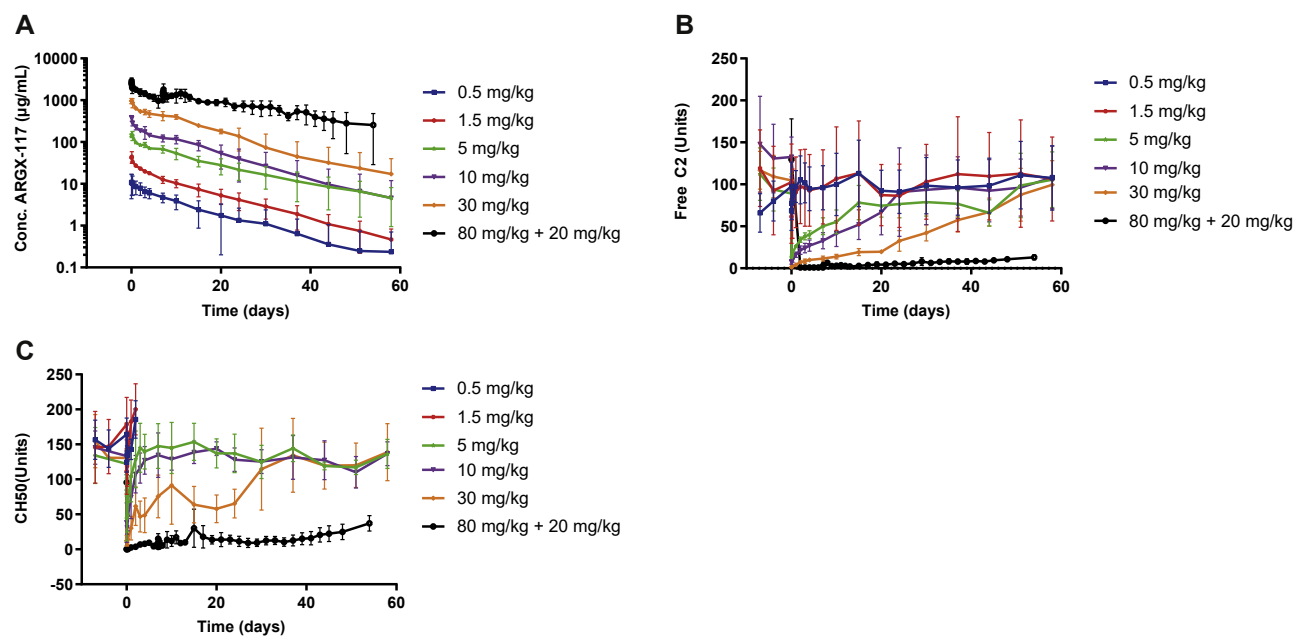
Most anti-complement antibodies suffer of a fast clearance *in vivo* due to target-mediated clearance. Surprisingly, a pilot pharmacokinetic (PK) study in cynomolgus monkeys using a variant of ARGX-117 showed long PK and sustained pharmacodynamic (PD) effect (data not shown). This observation triggered us to investigate the binding properties of ARGX-117 to C2 at different pHs. ARGX-117 binds several hundred-fold better to human C2 at physiological plasma pH (pH 7.4) than at lower pH of endosomes (pH 6.0; EC<sub>50</sub> = 0.1 μg/mL at pH 7.4 vs EC<sub>50</sub> = 32.2 μg/mL at pH 6.0) (Fig 4, A). Additionally, during setup of different hemolysis assays, it was found that ARGX-117 was less potent in EDTA-treated serum reconstituted with suboptimal concentrations of calcium, hinting that ARGX-117 would also be affected by different calcium concentrations. Indeed, the binding to C2 is approximately 25-fold better at physiological (1.25 mmol/L) plasma Ca<sup>2+</sup> concentrations (EC<sub>50</sub> = 0.1 μg/mL) compared with lower (25 μmol/L) Ca<sup>2+</sup> concentrations typically found in endosomes (EC<sub>50</sub> = 2.5 μg/mL) (Fig 4, B). Therefore, it is expected that C2 dissociates from ARGX-117 in the endosome to be degraded into the lysosome, whereas ARGX-117 will be recycled to the circulation enabling binding of a new target.<sup>20</sup> This effect can be optimized by introducing H433K and N434F mutations in the Fc part,<sup>15</sup> which improves the recycling of antibody *in vivo*. The combination of half-life extension by Fc-engineering and pH- and calcium-dependent target binding in ARGX-117 translates into optimal “sweeping” properties.

### PK and sustained PD of ARGX-117

In a single-dose study, groups of 4 animals (2 male, 2 female) were administered with an intravenous dose (bolus injection) of

detection of C2b by biotinylated ARGX-117 (lane 4), which was inhibited in the presence of EDTA (lane 5). Serum preincubation with ARGX-117 (lane 6) or an anti-C1s antibody (lane 7) inhibited C2 cleavage, because no decrease in total C2 was observed. A decrease in total C2 was observed after preincubation with an LP inhibitor (lane 8), an anti-C5 mAb (lane 9), or a control antibody (lane 10). D, Western blot analysis of C2 cleavage by C1s. Lane 1: MW markers, lane 2: purified C2. C1s cleaves C2 resulting in the formation of C2b, which is detected by biotinylated ARGX-117 (lane 3). EDTA (10 mmol/L) does not inhibit C1s-mediated cleavage of C2 because this process is calcium-independent (lane 4). ARGX-117 did not inhibit cleavage of C2 by C1s (lane 5). E, SPR analysis with biotin-C4b immobilized to streptavidin-chip and human C2 preincubated with mAb as eluate. Red, no pre-incubation; black, a control IgG<sub>1</sub> LALA-NH mAb; shades of blue: different molar ratios of ARGX-117; curves were normalized to signal just before the injection of C2 on the C4b chips. The experiment was conducted in triplicate, as indicated by the 3 lines per sample. OD<sub>450</sub>, Oculus dexter 450; RU, response units; VWFA, von Willebrand factor-A.





**FIG 5.** PK/PD of ARGX-117 in cynomolgus monkey. **A**, Course of plasma levels of ARGX-117 in time of the doses indicated in the legend. **B**, PD: Free C2 in serum as a function of time as determined by a free C2 PD ELISA assay. **C**, PD: CH50 equivalent units (U Eq/mL) in function of time for all dose groups.

**TABLE I.** Overview of average half-life, average  $C_{max}$ , and average AUC, per group

Group (mg/kg)	Half-life (days)	$C_{max}$ (µg/mL)	AUC <sub>0-last TD</sub> (day × µg/mL)
0.5	10.7 ± 4.7	11.4 ± 5.8	112.7 ± 66.2*
1.5	10.1 ± 2.7	47.8 ± 11.4	332.7 ± 79.4*
5	12.5 ± 3.6	168.7 ± 18.8	1623.8 ± 476.8*
10	9.3 ± 3.4	397.2 ± 33.9	3153.1 ± 1152.8*
30	12.8 ± 3.2	1016.2 ± 109.4	9981.8 ± 1830*
80 + 20†	21.6 ± 3.2	1952.2 ± 515.5	32002.9 ± 9333.0‡

Overview of average half-life, average  $C_{max}$ , and average area under the curve (AUC), per group of the PK/PD single-dose study in cynomolgus monkeys and in cynomolgus monkeys dosed with 80 mg/kg (day 0) followed by dose of 20 mg/kg (day 7). Average ± SD. On administration of ARGX-117 at doses of ≥1.5 mg/kg, an immediate decrease in free C2 levels was observed, which then gradually returned to predose levels in time (Fig 5, B). In animals that received 80 and 20 mg/kg, free C2 levels stayed low for up to 7 weeks, and only at the latest time points did free C2 levels increased again. Notably, at the latest time points, ADA formation had started in all animals.

\*AUC<sub>0-last TD</sub> last sampling at day 58.

†Half-life and  $C_{max}$  of ARGX-117 were determined after the second dosing.

‡AUC<sub>0-last TD</sub> last sampling at day 54.

0.5, 1.5, 5, 10, or 30 mg/kg of ARGX-117. Dose linearity was observed for all dose groups when the log of the serum concentration of ARGX-117 was plotted against time (Fig 5, A). Using all these data together, an overall half-life of ARGX-117 of approximately 2 weeks was calculated (Table I). Antidrug antibodies (ADAs) were observed in 9 of the 20 animals across all dose groups (see Fig E4 in this article's Online Repository). No relation between apparent half-life and ADA formation was observed.

In a repeat-dose study, 3 cynomolgus monkeys received a loading dose of 80 mg/kg and a second dose of 20 mg/kg 1 week later (Fig 5, A). The apparent half-life of ARGX-117 after the second dose appeared to be longer than the mean half-life in the single-dose group (up to 21.6 days after the second dose), despite measurable ADA formation in all 3 animals.

The course of CP activity in time following administration of ARGX-117 in the animals was measured with the MicroVue

CH50 Eq EIA kit (Quidel, San Diego, Calif) (Fig 5, C). A minor, short-lasting decrease of total hemolytic complement (CH50) activity was observed following administration of 1.5 mg/kg dose group. CH50 activity dose-dependently decreased in the higher dose groups to be not measurable in the animals that received 30 mg/kg. CH50 activity returned gradually to predose levels after 1 day at 5 mg/kg, after 4 days at 10 mg/kg, and after 10 days at 30 mg/kg. In the 80 and 20 mg/kg dose groups, CH50 activity was not measurable during the whole observation period until after 48 days, when it slowly increased. Notably, at this time point, ADA formation was detected.

## DISCUSSION

Complement activation can contribute to tissue damage and organ dysfunction in various human inflammatory diseases.<sup>1</sup> However, therapeutic targeting of complement is a challenge, as

it may increase susceptibility to microbial infections. Indeed, meningococcal vaccination is recommended for patients starting therapy with the anti-C5 mAb eculizumab.<sup>21</sup> Specific targeting of complement factors critically involved in pathologic activation while leaving the rest of the system intact may provide a solution for this challenge. Here we describe ARGX-117, a novel, inhibitory mAb targeting C2. ARGX-117 specifically inhibits CP and LP activation, while leaving AP intact, promising a unique efficacy and safety profile.

Our results show that ARGX-117 inhibits activation at the level of C2 by binding to the S2 domain of C2 and preventing interaction with C4b. On binding to C4b, C2 undergoes conformational changes that lead to exposure of the activating peptidyl bond (R233-K224), and thus enhanced sensitivity of C2 to cleavage by C1s. Therefore, ARGX-117 inhibits CP and LP activation by interfering with the formation of the C3 proconvertase, the C4bC2 complex. Notably, Sushi domains, also termed complement control protein domains, are found in a number of proteins. ARGX-117 is specific for the S2 domain of C2, as it does not bind to the Sushi domains that are most homologous to this S2 domain, which are Sushi-1 and -3 domains of C2 and S2 domain of FB (Fig 3).

ARGX-117 binds C2 with a high, sub-nanomolar affinity translating into effective inhibition of CP and LP in various *in vitro* assays. Intrigued by the long PK and PD effects observed in an exploratory cynomolgus monkey experiment (see Fig E4, A and B), which is not the case for many other complement-targeting antibodies because of target-mediated clearance, we decided to investigate the binding characteristics of ARGX-117. Importantly, this affinity is optimal at physiological pH and free Ca<sup>2+</sup> concentration and decreases by 100- to 1000-fold at pH 6.0 and a calcium concentration of 25 μmol/L; these conditions resemble those in endosomes.<sup>20,22</sup> These unique biochemical characteristics predict that ARGX-117 efficiently captures C2 in the circulation, to release it on endocytosis by endothelial cells in the endosomes where C2 then is sorted to be degraded in the lysosomes (= sweeping antibody).<sup>20</sup> Introducing histidine residues into the complementarity determining regions to increase the pH-dependent antigen binding furthermore was not effective, suggesting that the lead antibody already had maximal sweeping capacity (data not shown). Furthermore, H433K and N434F mutations (NHance) in its Fc region will rescue ARGX-117 from lysosomal degradation as these mutations increase the affinity to FcRn at the acidic pH of the endosomes,<sup>14</sup> thereby favoring recycling of the antibody into circulation where it may capture additional C2 molecules. Indeed, comparison of PK and PD of the C2 antibody with NHance mutations demonstrated a longer serum half-life and longer acting effect as compared to the version without NHance mutations (Fig E5, C and D).

Consistent with the *in vitro* findings, a single administration of ARGX-117 to cynomolgus monkeys at doses ≥1.5 mg/kg induced a temporary dose-dependent inhibition of CP as evidenced by decreasing CH50 activity (Fig 5). A dose-linear PK profile was observed in all ARGX-117 dose groups (0.5 to 30 mg/kg single dose), with an apparent half-life of clearance of 2 weeks. This relatively long half-life strongly suggests the clearance of ARGX-117 is independent of its target C2 and in this respect seems different from anti-C1s sutimlimab<sup>23</sup> or C1q mAbs.<sup>24</sup> After a loading dose of ARGX-117 of 80 mg/kg followed by a maintenance dose of 20 mg/kg at day 8 (80 + 20 mg/kg group), free C2

levels were low to undetectable for up to 7 weeks, and CP was completely inhibited for 7 weeks or longer (Fig 5). The long half-life and the prolonged complement inhibition in cynomolgus monkeys support such a recycling mechanism by ARGX-117. Notably, the *in vivo* potency of ARGX-117 in humans may be underestimated as inhibition of CP in cynomolgus serum using several *in vitro* complement assays requires ~2-fold more ARGX-117 than comparable inhibition of CP in human serum (data not shown).

Various complement effector functions (cell lysis, opsonization, release of fluid phase activation products) may contribute to a different extent to complement-mediated pathologies. Importantly, we show that ARGX-117 can inhibit multiple complement effector functions. It prevents fixation of C3 and the terminal complement complex to solid-phase aggregated IgG (Fig 1), reduces fluid phase activation of C3 (Fig E2) and prevents complement-dependent cytotoxicity (Fig 3). Importantly, ARGX-117 prevents C3 activation via CP or LP. Therefore, ARGX-117 has therapeutic potential in conditions such as C3-mediated-extravascular hemolysis in autoimmune hemolytic anemia (Fig 2). It may also be useful to inhibit CP- or LP-dependent generation of C3a after C3 activation, which is believed to contribute to graft rejection after organ transplantation by enhancing T-cell responses.<sup>25</sup> A similar therapeutic effect on the adaptive immune response may occur in other clinical conditions.

At present it is unknown for most complement factors to what extent pathology is dependent on their concentration. In the various assays we observed that full activation of C3 may require ~1% to >20% of the normal C2 concentration depending on parameters as the density and (sub)class of antibodies on the activator (data not shown). This C2 dependency and the therapeutic target level of complement inhibition will be important considerations for clinical application of ARGX-117. Yet, efficacy of ARGX-117 to prevent complement-mediated cytotoxicity in *in vitro* models for autoimmune hemolytic anemia and antibody-mediated rejection of organ transplants, as well as the overwhelming evidence in literature of a role of CP and LP in ischemia-reperfusion conditions, predict efficacy of ARGX-117 in immune disorders mediated by IgM and IgG, as well as in ischemic diseases. Recently, several studies have demonstrated involvement of both CP and LP in the pathogenesis of acute respiratory distress syndrome caused by coronavirus disease 2019 infection.<sup>26-28</sup> Inhibition of this inflammatory response and complement-associated microvascular injury<sup>29</sup> through pharmacological intervention with ARGX-117 has the potential to improve clinical outcome of coronavirus disease 2019. Future clinical studies should corroborate this promise of ARGX-117.

In conclusion, ARGX-117 is a potent and specific inhibitor of both CP and LP, while it leaves AP, and hence an important part of the anti-microbial function of complement, intact. Studies in cynomolgus monkeys support the feasibility of clinical studies with ARGX-117. Clinical availability of ARGX-117 opens new possibilities in treating complement-mediated immune and inflammatory diseases in humans.

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## Key messages

- Novel therapeutic antibody against complement C2 inhibiting both CP and LP but leaving the AP intact.
- Unique pH- and Ca<sup>2+</sup>-dependent target binding translates into favorable PK and long-lasting PD effects in nonhuman primates.

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## METHODS

### Assays to evaluate Fc-effector functions

To evaluate the binding of C1q to ARGX-117, MaxiSorp microtiter plates (Thermo Fisher Scientific) were coated overnight at 4°C with serial dilutions of purified ARGX-117 in PBS, pH 7.4. As a positive control, a wild-type human IgG<sub>1</sub> antibody (ARGX-18E2), was coated as well. Residual binding sites were blocked by a 2-hour incubation with PBS containing 1%, wt/vol, casein, at RT. Next, plates were incubated with 10%, vol/vol, human serum (Sigma-Aldrich) in PBS 0.1% casein for 1 hour at RT. Binding of C1q was then detected by a 1-hour incubation with biotinylated anti-C1q antibody (Abcam, Cambridge, United Kingdom) in PBS 0.1% casein, followed by visualization with streptavidin-HRP (Jackson ImmunoResearch) and 3,3',5,5'-tetramethylbenzidine (TMB) substrate.

To evaluate affinity of ARGX-117 for CD16, MaxiSorp plates were coated with NeutrAvidin-biotin-binding protein (Thermo Fisher Scientific) diluted in PBS and blocked with casein as described above. Plates were then incubated for 1 hour with biotinylated human CD16 (Sino Biological, North Wales, Pa), and incubated with serial dilutions of ARGX-117 or ARGX-18E2 for 1 hour. Bound antibodies were detected with an anti-human IgG (Fab-specific)-HRP (Sigma-Aldrich).

To evaluate affinity of ARGX-117 for CD32a, MaxiSorp plates were coated with anti-HIS antibody (Novus Biologicals, Centennial, Colo) diluted in PBS and blocked with casein as described above. Plates were then incubated for 1 hour with anti-human CD32a (His Tagged; Sino Biological) and incubated with serial dilutions of ARGX-117 or ARGX-18E2 for 1 hour. Bound antibodies were detected with an anti-human IgG (Fab-specific)-HRP (Sigma-Aldrich).

With a similar assay, the affinity of ARGX-117 for human FcRn at pH 7.4 and at pH 6.0 was determined. NeutrAvidin-coated plates were blocked with 2% BSA in PBS and, subsequently, incubated with biotinylated human FcRn (Immunitrack, Copenhagen, Denmark). Next, plates were incubated with serial dilutions of ARGX-117 or ARGX-18E2 as a positive control, in PBS, pH 7.4, or in citrate, pH 6.0, both containing 0.2% BSA. Detection was done using poly-HRP-labeled goat anti-human IgG antibody (Abcam) followed by TMB.

### Complement assays

**Fluid-phase complement activation model.** One volume of normal human serum, diluted in veronal buffered saline (VBS), was incubated with 1 volume of aggIgG at 1 mg/mL in VBS for 15 minutes at 37°C. Complement activation was then stopped by adding 1 volume of 0.1 mol/L EDTA. The mixture was diluted in PBS-Tween (PBS-T) containing 10 mmol/L EDTA, and tested for activated C3 and C4 with ELISA.<sup>E1</sup> As the assay for activated C3 does not discriminate among C3b, iC3b, or C3c, activated C3 was denoted as C3b/c. Similarly, activated C4 was indicated as C4b/c. The effect of ARGX-117 or other antibodies was tested by preincubating 1 volume of serum with 1 volume of antibody in VBS for 15 minutes on ice. Results were expressed as the percentage of inhibition using serum incubated with buffer only as 100% inhibition, and serum incubated with aggIgG, but without anti-complement antibody, as 0% inhibition.

**Solid-phase complement activation models.** Briefly, plates coated with human aggIgG (10 µg/mL in PBS) were incubated with fresh human serum diluted in VBS, pH 7.4, containing 1 mmol/L MgCl<sub>2</sub>, 0.15 mmol/L CaCl<sub>2</sub>, and 0.1%, wt/vol, Tween-20 (VBS-T) for 30 minutes at 37°C. Bound C4 and C3 were detected with specific biotinylated antibodies (MyBioSource, San Diego, Calif; and biotinylated in-house using an EZ-Link NHS-PEG4-biotin kit; Thermo Fischer Scientific) in PBS containing 0.1%, wt/vol, Tween (PBS-T), followed by streptavidin-HRP 20 (BioLegend, San Diego, Calif) and TMB (Invitrogen, Thermo Fisher Scientific). When appropriate, diluted serum was preincubated with ARGX-117 or controls in VBS-T for 15 minutes at RT and tested. Results are expressed as percentage of inhibition using fixation in absence of anti-complement antibody as 0% and fixation in presence of EDTA, 10 mmol/L final concentration, as 100% inhibition.

**Solid-phase complement activation models.** Commercial ELISAs to assess complement activity were (1) MicroVue CH50 Eq EIA

(A018; Quidel), (2) Wieslab MP320 assay, and (3) Wieslab AP330 assay. These assays measure CP, LP, and AP activity, respectively, and were used according to manufacturers' instructions. An in-house assay was used to measure CP activity as well.

**Hemolytic assay.** Human RBCs (CRI Labo Medische Analyse, Zwijnaarde, Belgium) were washed with VBS containing gelatin, Ca<sup>2+</sup>, and Mg<sup>2+</sup> (GVB<sup>++</sup>; Complement Technology), sensitized for 1 hour with rabbit anti-human RBC antibody (Rockland Immunochemicals, Limerick, Pa) on ice and resuspended in TBS, pH 7.4, supplemented with 1.25 mmol/L CaCl<sub>2</sub> (TBS-Ca<sup>2+</sup>) after washing. ARGX-117 was spiked into human serum, pipetted into a U-bottom plate, and sensitized human erythrocytes were added. Next, the plate was incubated (30 minutes, 37°C), spun down (1500 rpm for 3 minutes, 4°C), and supernatants were transferred to a MaxiSorp flat bottom plate and analyzed for hemoglobin release using a spectrophotometer.

**Complement-dependent cytotoxicity assay.** PBMCs from healthy donors were sensitized with heat-inactivated sera of patients containing anti-HLA antibodies for 30 minutes at RT. Subsequently, cells were incubated with fresh human serum, diluted 5 times in VBS containing 0.25 mmol/L CaCl<sub>2</sub>, for 60 minutes at 37°C. Lysis of the cells was then measured with FluoroQuench (Sanbio, Uden, The Netherlands), quantified with Leica Q WIN program (Leica Microsystems, Buffalo Grove, Ill), and expressed as percentage of lysis setting cells incubated with rabbit complement (Cedarlane Laboratories, Burlington, Canada) at 100% lysis and serum in the presence of 10 mmol/L EDTA final concentration at 0% lysis. The effect of anti-complement antibodies was assessed by preincubating serum with these for 15 minutes at RT.

**Complement-mediated phagocytosis assay.** C3 fixation and complement-mediated phagocytosis were conducted using an adapted protocol from Shi et al.<sup>E2</sup> RBC (Mini Donor Service) were isolated in Alsever solution (Sigma-Aldrich) and stored at 4°C until use. RBCs, labeled with Cell-Trace Violet (Invitrogen) according to manufacturer's instructions, were incubated with pooled normal human serum containing IgM antibodies against AB antigens and active complement for 1 hour at 37°C. In some experiments, pooled serum was preincubated with anti-complement or control antibodies for 15 minutes at RT. Opsonization of RBCs with IgM or C3 was detected with fluorescence-activated cell sorting (FACS) and accompanying FACS Diva software (BD Biosciences, Franklin Lakes, NJ) with goat anti-human IgM biotin (Thermo Fisher Scientific), goat anti-human IgG biotin (eBiosciences, Thermo Fisher Scientific), mouse anti-human C3 biotin (LSBio, Seattle, Wash), and streptavidin antigen-presenting cell (eBiosciences) using standard protocols.

THP-1 cells (TIB-202; ATCC, Manassas, Va), activated with 25 ng/mL phorbol 12-myristate 13-acetate for 48 hours followed by a recovery period of 48 hours in THP-1 culture medium, were labeled with 0.05 µmol/L CFSE. Complement-treated RBCs were coincubated with labeled THP-1 cells for 2 hours at 37°C, while shaking. Phagocytosis, measured by FACS, was determined as percentage of CFSE+/CTV+ THP-1 cells. Prior to analysis, samples were washed with water to lyse nonphagocytosed RBCs.

### Surface plasmon resonance

SPR was used to measure the affinity of ARGX-117 to C2 using Biacore T100 (GE Healthcare) equipment. The running buffer was 20 mmol/L HEPES, 100 mmol/L NaCl, and 2 mmol/L CaCl<sub>2</sub> with 0.05% Tween-20, pH 7.5. The measurements were done at 25°C with a flow rate of 30 µL/min. Plasma-derived human C2 was biotinylated (EZ-Link NHS-PEG4-biotin kit; Thermo Fisher Scientific) and captured on a Series S streptavidin sensor chip (GE Healthcare), yielding ~100 RU. Chips were regenerated with 0.1 mol/L glycine hydrochloride, pH 2.0. Fab of ARGX-117, expressed in HEK293F cells and purified from transfection medium with Protein G affinity chromatography (U-Protein Express), was injected at a range from 100 nmol/L to 0 nmol/L for 60 seconds with a dissociation time of 600 seconds. Regeneration was done for 30 seconds with a stabilization period of 600 seconds. Each sample was tested in triplicate. Results were analyzed with Biacore T100 evaluation software.

SPR was also used to assess the effect of ARGX-117 on the interaction of C2 with C4b (Complement Technology). Measurements were done at 25°C and a flow rate of 30  $\mu\text{L}/\text{min}$ . The same running buffer supplemented with 0.15 mmol/L  $\text{MgCl}_2$  was used. To regenerate the chips, 20 mmol/L HEPES, 1 mol/L NaCl, and 100 mmol/L EDTA, pH 7.5, containing 0.05% Tween was used. Biotinylated C4b (EZ-Link NHS-PEG4-biotin kit) was captured on a streptavidin sensor chip at  $\sim 600$  RU. Plasma-derived human C2 (30  $\mu\text{g}/\text{mL}$ ), either preincubated with antibody or not for 15 minutes RT was injected for 60 seconds with a dissociation time of 600 seconds. Finally, chips were regenerated for 60 seconds with a stabilization period of 600 seconds.

### Immunoblotting of C2

Plasma-derived C2 was incubated with purified activated C1s (Merck, Kenilworth, NJ) at a 100:1 molar ratio for 15 minutes at 37°C while shaking, either in or not in the presence of 10 mmol/L EDTA or ARGX-117 (10:1 molar ratio to C2). The mixture equivalent to 0.4  $\mu\text{g}$  C2 was loaded on gel. Also, C2-depleted serum (Sigma-Aldrich) reconstituted with purified human C2 (30  $\mu\text{g}/\text{mL}$ ) was incubated with ARGX-117 (10:1 molar ratio) or controls for 15 minutes on ice. The serum mixture was then activated with 800  $\mu\text{g}/\text{mL}$  aggIgG for 15 minutes at 37°C. Twenty  $\mu\text{L}$  of a final dilution of 10% serum, (0.06  $\mu\text{g}$  C2) was loaded on gel.

Samples were incubated with 4 $\times$  concentrated Laemmli Sample Buffer (Bio-Rad Laboratories, Hercules, Calif) for 10 minutes at 96°C, and run (20  $\mu\text{L}$ ) on a Mini-Protean TGX gel, AnyKD (Bio-Rad) under nonreducing

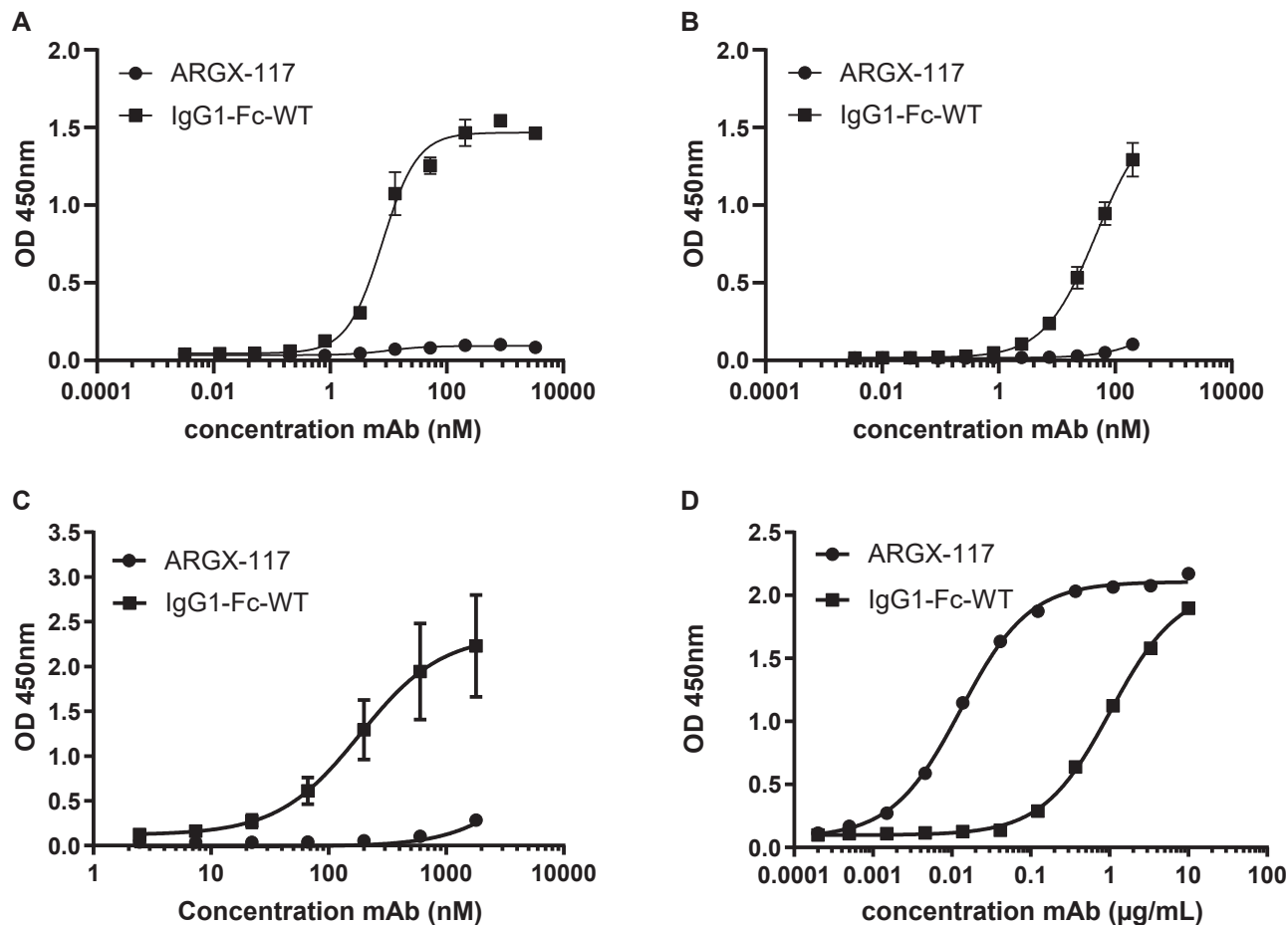
conditions. Proteins were transferred onto a nitrocellulose membrane, which was saturated with 8%, wt/vol, ELK milk powder (Friesland Campina, Amersfoort, The Netherlands), and incubated with 1  $\mu\text{g}/\text{mL}$  biotinylated ARGX-117 mAb in PBS-T containing 2%, wt/vol, ELK overnight at 4°C. Antibody binding was detected using streptavidin-poly-HRP in 2%, wt/vol, ELK PBS-T for 1 hour at RT, visualized with enhanced chemiluminescence (Amersham, Little Chalfont, United Kingdom) and imaged using a ChemiDoc MP imaging system (Bio-Rad) and accompanying software.

### Anti-drug antibody assay

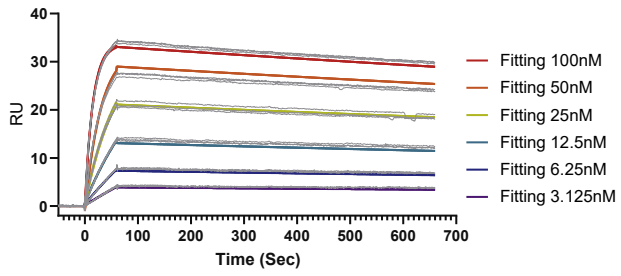
MaxiSorp microtiter plates coated with ARGX-117 overnight at 4°C, were blocked with PBS 1% casein for 2 hours at RT, and subsequently incubated with cynomolgus serum samples diluted in PBS 0.1% casein for 2 hours at RT. Plates were developed with anti-monkey IgG-HRP (SouthernBiotech) and TMB.

### REFERENCES

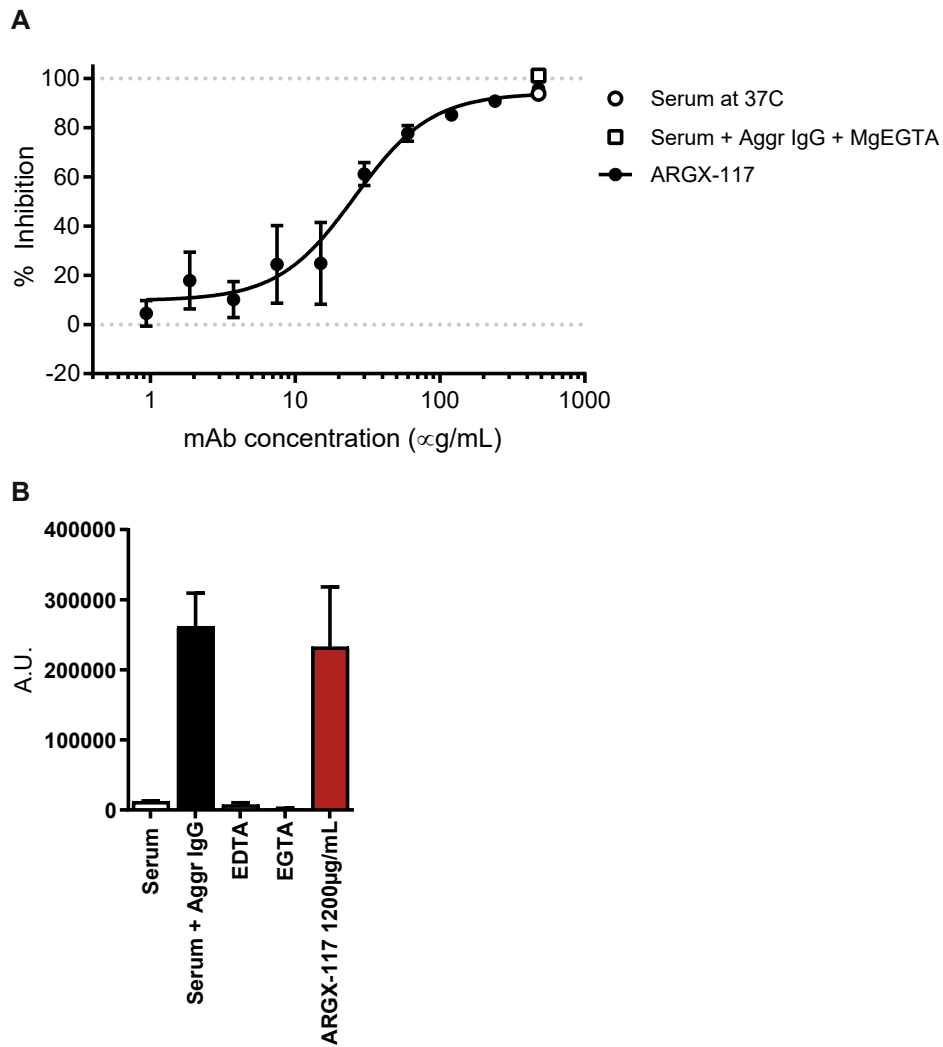
- E1. Wolbink GJ, Bollen J, Baars JW, ten Berge RJ, Swaak AJ, Paardekooper J, et al. Application of a monoclonal antibody against a neopeptide on activated C4 in an ELISA for the quantification of complement activation via the classical pathway. *J Immunol Methods* 1993;163:67-76.
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**FIG E1.** ARGX-117 does not interact with C1q (A), CD16 (B), or CD32a (C), and binds stronger to FcRn than to wild-type (WT) IgG<sub>1</sub> at pH 6.0 (D). A, ELISA plates were coated with ARGX-117, incubated with serum, and C1q was detected with anti-C1q. B-D, Plates were coated with NeutrAvidin and incubated with biotinylated CD16 (B), CD32a (C), or FcRn (D). Subsequently, plates were incubated with ARGX-117. Bound ARGX-117 was then detected with poly-HRP-labeled goat anti-human IgG antibody.

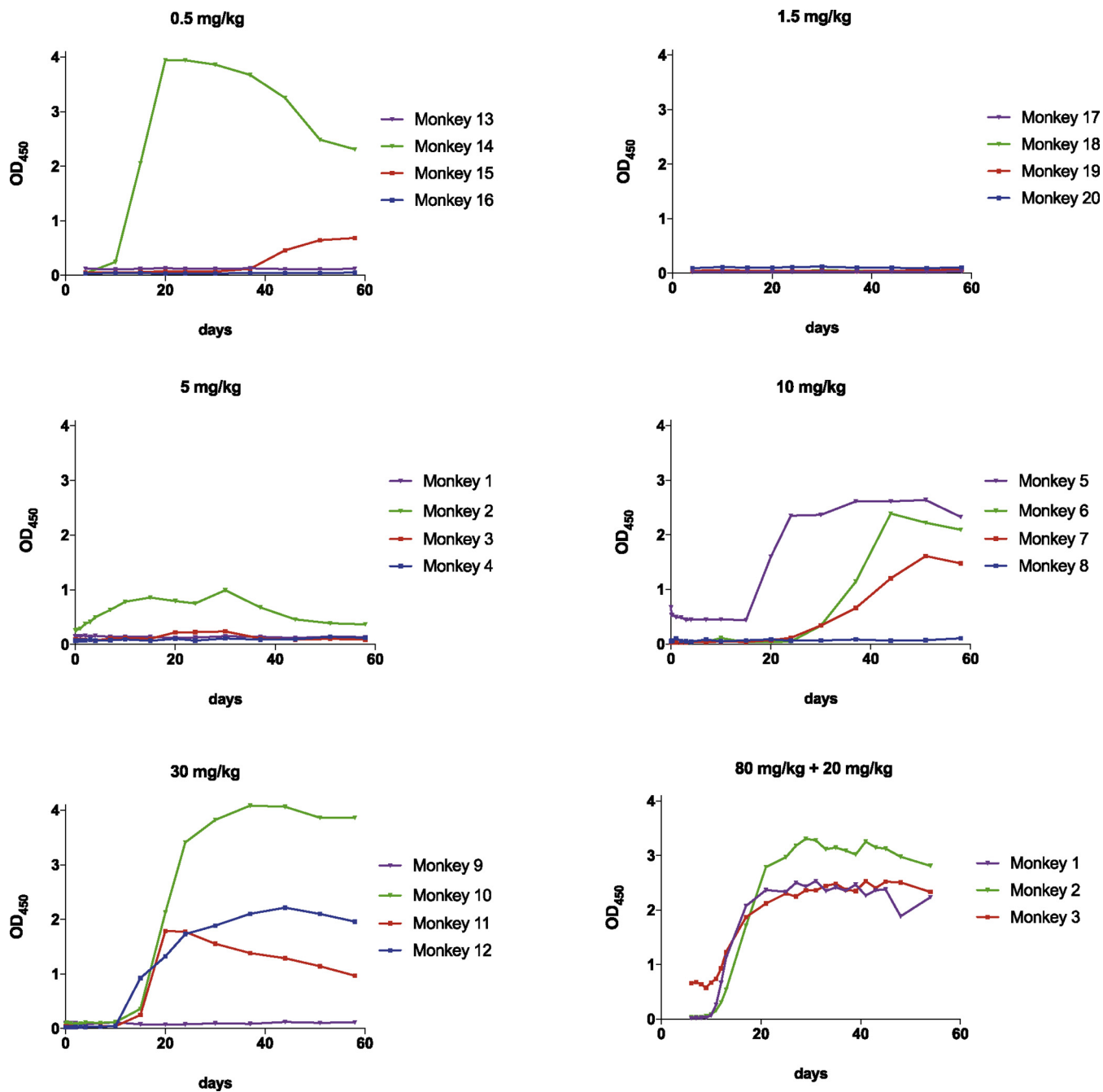


**FIG E2.** Affinity of the binding of ARGX-117 to human C2. SPR analysis of the binding of Fab fragments of ARGX-117 to human C2. *Gray lines* represent actual data; the *colored lines* fitted data at different molarities.



**FIG E3.** ARGX-117 inhibits generation of fluid-phase activated C3 (**A**), but not of activated C4 (**B**), in serum by agglgG. Serum, preincubated with ARGX-117, was incubated with agglgG (1 mg/mL) and analyzed for presence of C3b/c (**A**) and C4b/c (**B**). Serum incubated without ARGX-117 or agglgG was used as 100% inhibition, whereas serum incubated with agglgG in absence of ARGX-117 was taken as 0% inhibition. Results are mean and SEM of 3 experiments. Note that MgEGTA (10 mmol/L, final concentration) and EDTA (10 mmol/L) inhibit the generation of C4b/c and C3b/c, supporting that the complement was activated via the CP.





**FIG E4.** ADA response (OD, 450 nm) in ELISA over time until day 58 for each monkey. ADA samples were collected and 200-fold diluted in 1× PBS/0.1% casein. In the 0.5 mg/kg dose group, ADAs were detectable in monkey 14 from day 15 and in monkey 15 only from day 44 onward. In the 1.5 mg/kg dose group, no ADA response was measured in any of the animals. ADAs were detected in monkey 2 within the 5 mg/kg dose group. In the 10 mg/kg dose group, ADAs were detectable in monkey 5 from day 20 onward and in monkeys 6 and 7 from day 37 onward. In the 30 mg/kg dose group, ADAs were detectable in 3 of 4 monkeys from day 15 onward. In the 80 mg/kg + 20 mg/kg dose group, ADAs were detected in all monkeys from day 10 onward.

**TABLE E1.** Biacore analysis results

Analysis	$k_a$ (1/ms)	$k_d$ (1/s)	$KD$ (M)	$R_{max}$ (RU)	$t_c$	$\chi^2$ (RU <sup>2</sup> )
1	6.56E+5	2.22E-04	3.38E-10	33.84	6.90E+14	0.623
2	5.62E+5	1.72E-04	3.06E-10	33.09	7.29E+14	0.591

$k_a$ , Association rate constant;  $k_d$ , dissociation rate constant;  $KD$ , equilibrium dissociation constant;  $M$ , molar;  $t_c$ , flow rate-independent component.