A novel full-length recombinant human complement factor H (CFH; GEM103) for the treatment of age-related macular degeneration shows similar in vitro functional activity to native CFH

Current Eye Research

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/icey20

A Novel Full-Length Recombinant Human Complement Factor H (CFH; GEM103) for the Treatment of Age-Related Macular Degeneration Shows Similar *In Vitro* Functional Activity to Native CFH

Robyn M. Biggs, Elisavet Makou, Scott Lauder, Andrew P. Herbert, Paul N. Barlow & Suresh K. Katti

To cite this article: Robyn M. Biggs, Elisavet Makou, Scott Lauder, Andrew P. Herbert, Paul N. Barlow & Suresh K. Katti (2022): A Novel Full-Length Recombinant Human Complement Factor H (CFH; GEM103) for the Treatment of Age-Related Macular Degeneration Shows Similar *In Vitro* Functional Activity to Native CFH, Current Eye Research, DOI: 10.1080/02713683.2022.2053725

To link to this article: https://doi.org/10.1080/02713683.2022.2053725

CURRENT EYE RESEARCH
https://doi.org/10.1080/02713683.2022.2053725

A Novel Full-Length Recombinant Human Complement Factor H (CFH; GEM103) for the Treatment of Age-Related Macular Degeneration Shows Similar *In Vitro* Functional Activity to Native CFH

Robyn M. Biggs[a*], Elisavet Makou[b*], Scott Lauder[a], Andrew P. Herbert[b], Paul N. Barlow[b,c], and Suresh K. Katti[a]

[a]Gemini Therapeutics Inc., Cambridge, MA, USA; [b]School of Chemistry, University of Edinburgh, Edinburgh, UK; [c]School of Biological Sciences, University of Edinburgh, Edinburgh, UK

ABSTRACT

Purpose: GEM103 is a recombinantly produced full-length version of the human complement factor H (CFH) under clinical investigation for treatment of age-related macular degeneration (AMD) in individuals carrying an AMD risk-associated genetic variant of *CFH*. This study aimed to investigate the complement pathway-related functions of GEM103 in comparison with those of native human CFH.

Methods: Key biological activities of GEM103 and human serum-derived CFH (sdCFH) were compared using four independent functional assays. Assays of C3b binding and C3 convertase decay-accelerating activity (DAA) were performed by surface plasmon resonance (SPR). Cofactor activity (CA) was measured using 8-anilinonaphthalene-1-sulfonic acid as a fluorescent probe of C3b integrity. The abilities of GEM103 and sdCFH to protect sheep erythrocytes from hemolysis by CFH-depleted normal human serum were assessed colorimetrically.

Results: In multiple SPR-based assays of C3b binding and DAA, the performance of GEM103 was consistently comparable to that of sdCFH across a range of matching concentrations. The EC_{50} ± SD in the fluorescence-based fluid-phase CA assay was 0.21 ± 0.06 μM for GEM103 compared to 0.20 ± 0.09 μM for sdCFH. In hemolysis assays, the EC_{50} value of 0.33 ± 0.16 μM for GEM103 versus 0.46 ± 0.06 μM for sdCFH were not significantly different ($p = 0.81$).

Conclusions: GEM103, a recombinant CFH developed by Gemini Therapeutics, shows activity profiles comparable to sdCFH in all complement-related assays employed in this study, suggesting that GEM103 is equivalent to the native glycoprotein in terms of its *in vitro* functional activity. These results support further study of GEM103 as a potential therapy for AMD.

ARTICLE HISTORY
Received 15 December 2021
Accepted 7 March 2022

KEYWORDS
age-related macular degeneration; complement factor H; recombinant protein therapy; GEM103; alternative pathway

Introduction

Age-related macular degeneration (AMD) is a degenerative eye disease of the elderly. Worldwide, AMD caused moderate/severe visual impairment in over six million people aged ≥50 years in 2020.[1] An estimated 280 million could be affected by AMD in 2040.[2] While the neovascularization that characterizes "wet" AMD is therapeutically addressable and Age-Related Eye Disease Study supplements can slow down the progression of AMD to advanced forms in some cases,[3,4] no approved treatment exists to slow the progression of geographic atrophy (GA), the advanced form that degrades vision in "dry" AMD.[5]

Genetics features prominently in AMD development.[6] Amongst AMD-associated genetic loci, the complement factor H (CFH) gene (*CFH*) stands out.[7,8] CFH, a serum glycoprotein consisting of 20 complement control protein (CCP) domains, regulates the alternative pathway (AP) of the complement system.[7,9] In AMD, numerous *CFH* coding variants are strongly associated with enhanced disease risk,[7] suggesting CFH functional insufficiency is a major contributor to pathogenesis.[10] Non-coding AMD-related variants also occur,[11] some of which lead to elevated concentrations of factor-H-related proteins[12] that may function in opposition to CFH, illustrating the complex relationship between genetics and complement regulation.

The AP is a surveillance system, continuously operating at a low level, driven by slow spontaneous hydrolysis of complement component 3 (C3).[13] C3 hydrolysis triggers proteolysis of C3 into C3a and C3b.[14] C3b binds complement factor B (CFB), which in turn is cleaved, yielding C3bBb.[13] In a positive-feedback loop that operates in the absence of regulators like CFH, C3bBb catalyzes the cleavage of additional C3 to C3b.[13] Nascent C3b can covalently attach to nearby surfaces leading to phagocytosis, inflammation and cytolysis resulting from terminal pathway activation.[15]

CONTACT Suresh K. Katti suresh@geminitherapeutics.com Gemini Therapeutics Inc., 300 One Kendall Square, Cambridge, MA 02139, USA
*Co-first authors.

Supplemental data for this article can be accessed here.

Given its spontaneity and potential for amplification, the AP must be regulated everywhere except near invading pathogens. In its canonical role, CFH intervenes at several steps along the AP. It binds C3b, competing with CFB; it is a cofactor for complement factor I (CFI) that cleaves and inactivates C3b; it accelerates dissociation of Bb from C3bBb.[15] Through these multiple mechanisms, CFH prevents excessive C3b formation which otherwise leads to host-tissue damage. Importantly, CFH regulates AP activity both in solution and on host surfaces.[15] CFH regulates the AP on self-surfaces, selectively, by recognizing polyanions typically found only on self-surfaces.[9,16,17]

Given the critical role functional CFH plays in maintaining ocular homeostasis, Gemini Therapeutics Inc. (Cambridge, MA, USA) is clinically investigating a novel full-length non-tagged recombinant human CFH protein (GEM103), administered by intravitreal (IVT) injection, aiming to restore appropriate AP regulation in the eye in AMD. During preclinical development, the activity and potency of GEM103 were assessed in comparison with human endogenous CFH (serum-derived CFH [sdCFH]), for canonical functions including C3b binding, CFI cofactor activity (CA), C3bBb-decay accelerating activity (DAA), and protection of cells from lysis in activated human serum.

Materials and methods

GEM103 (non-GMP [non-good manufacturing practice] preparation; LakePharma, Inc. [Worcester, MA, USA]) was used for this *in vitro* study. Native human sdCFH (purified from pooled human serum), purified human C3b, CFB, complement factor D (CFD) and CFI were supplied by Complement Technology, Inc. (Tyler, TX, USA). C3b-binding affinity and decay-accelerating activity (DAA) assays were performed on the BiaCore T200 (GE Healthcare [Chicago, IL, USA]) surface plasmon resonance (SPR) platform. CA was measured using 8-anilinonaphthalene-1-sulfonic acid (ANS) and a SpectraMax M5e (Molecular Devices [San Jose, CA, USA]) plate reader. For hemolysis assays, human CFH-depleted serum and sheep erythrocytes (SEs) were obtained (Complement Technology, Inc.) and measurements performed in a SpectraMax M5e (Molecular Devices) plate reader. See Supplementary Methods for details.

Results

GEM103 and sdCFH both showed affinities for C3b within the published range

Figure 1(A) (GEM103) and 1(C) (sdCFH) show SPR responses for dilution series of each protein flowed over

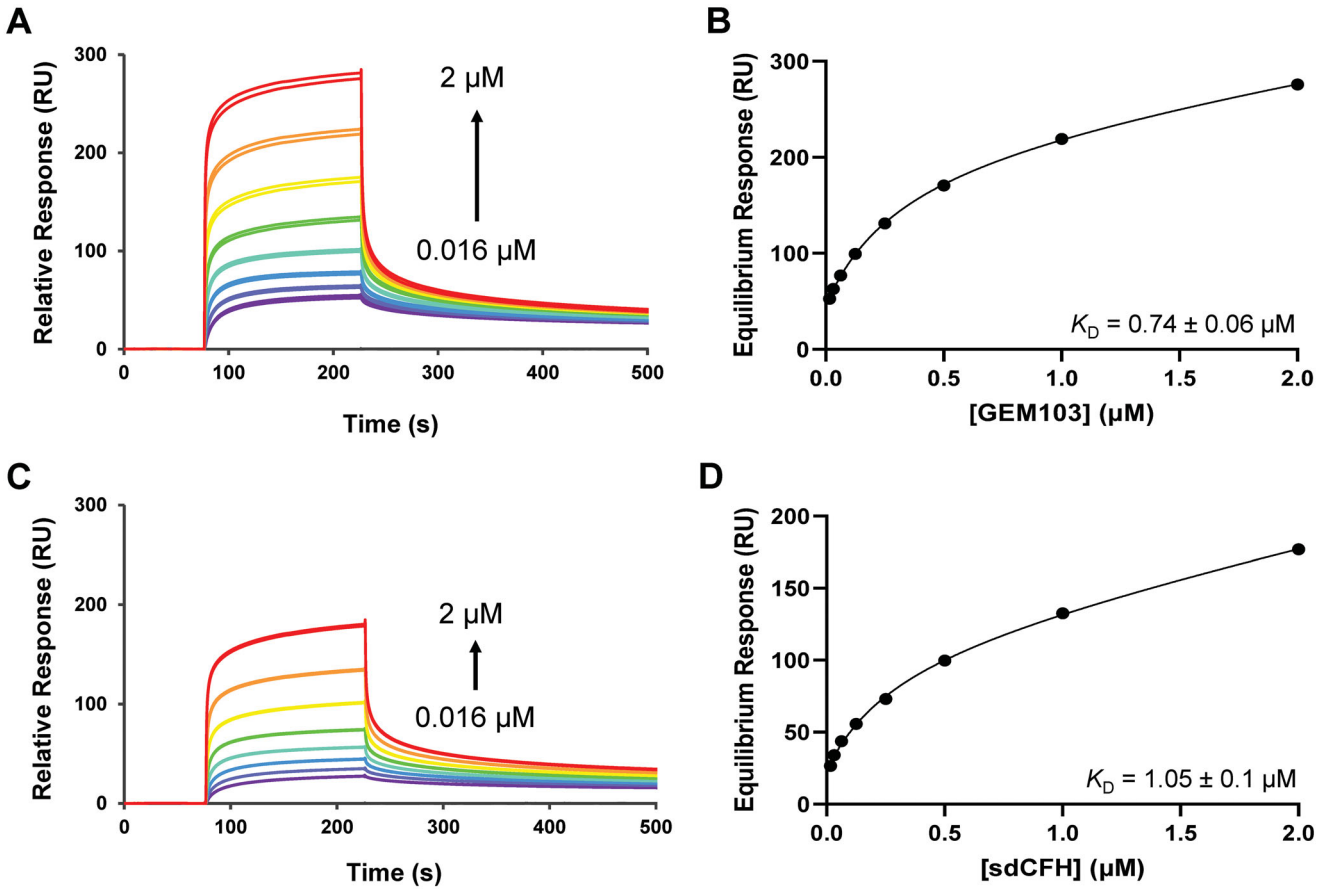

Figure 1. Affinities of GEM103 and sdCFH for C3b measured by SPR. Duplicate measurements using two-fold dilution series of 2 μM to 0.016 μM protein flowed over C3b immobilized on a CM5 chip were performed, and background signals were subtracted. (A) Results for GEM103; (B) equilibrium plot used to calculate a K_D value for GEM103; (C) results for sdCFH; (D) equilibrium plot used to calculate a K_D value for sdCFH. K_D: Equilibrium dissociation constant; RU: response units; sdCFH: serum-derived complement factor H.

C3b amine-coupled to a carboxymethylated dextran-coated ("CM5") sensor chip. Fitting to a 1:1 steady-state binding model (Figure 1(B,D)), yields equilibrium dissociation constants (K_D) for GEM103 ($0.74 \pm 0.06\,\mu M$) and sdCFH ($1.05 \pm 0.1\,\mu M$). The R_{max} values (reflecting total capacity of a chip for binding CFH) for GEM103 (305 response units [RUs]) and sdCFH (226 RUs) were consistent with C3b densities. The χ^2 values for GEM103 and sdCFH were <10% of R_{max} values suggesting acceptable curve fitting. Similar results were obtained when using a chip with a flat carboxymethylated surface ("C1") (Supplementary Table 1).

GEM103 has similar CA to sdCFH

In a fluorescence-based assay of CFI CA that monitored loss of C3b integrity, GEM103 performed similarly to sdCFH (Figure 2). Average EC_{50} and EC_{90} values showed no significant difference between the two proteins ($p = 0.81$ and $p = 0.76$, respectively, Supplementary Table 2), suggesting both are equally able to recruit CFI for C3b cleavage.

GEM103 shows similar DAA to sdCFH

The tested concentrations of GEM103 or sdCFH accelerated, dose-dependently, the decay of C3bBb, previously assembled on a CM5 sensor chip by passing CFB and CFD over immobilized C3b. The data for GEM103 and sdCFH obtained appear similar, with GEM103 performing at least as well as sdCFH at every concentration tested (0.5–20 nM) (Figure 3(A,B)). Comparable results were observed with a different density of immobilized C3bBb (Figure 3(C,D)).

GEM103 protects SEs from hemolysis in a comparable manner to sdCFH

SEs bear human-like sialic acids and so are protected by human CFH from lysis, in normal human serum, mediated *via* the AP by complement's terminal pathway. Some or all of the canonical activities of CFH, including its ability to recognize self-like surfaces, are deployed in this scenario. For each tested concentration of GEM103 or sdCFH, averaged, normalized, percent SE-hemolysis values were plotted (Figure 4), revealing comparable hemolysis-protection activities of the two proteins with no significant differences between their respective EC_{50} and EC_{90} values ($p = 0.81$ and $p = 0.76$, respectively, Supplementary Table 2).

Discussion

Individuals with risk-associated *CFH* variants may have reduced levels of fully functional CFH (although not necessarily a quantitative deficit of CFH) in fluid phase or within tissues like the posterior retina, where CFH is produced locally.[18] The resultant local complement (dys)regulation, of particular relevance to AMD development,[19,20] could potentially be reversed by IVT injection of GEM103. A biodistribution study in non-human primates (cynomolgus monkeys) showed that IVT administration of GEM103 resulted in the distribution of the protein to the inner layers of the eye including the retina, retinal pigment epithelium (RPE) and the choroid, and that GEM103 was retained at the RPE for an extended duration,[21] suggesting that this is an effective method of administration. Subsequently, GEM103 has been employed in Phase 1[22] and 2 studies that

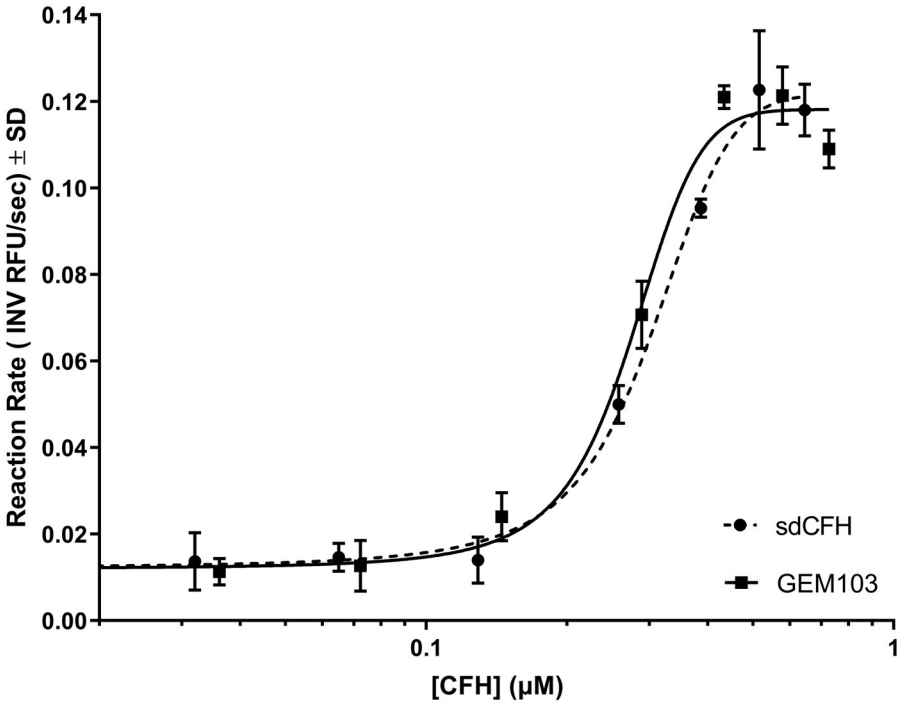

Figure 2. Comparison of cofactor activities of GEM103 and sdCFH. This assay monitors the loss of C3b integrity (and hence affinity for the fluorescent dye, ANS) due to cleavage by CFI that requires CFH as cofactor. Representative plots are shown of the average inverse reaction rates (RFU/second) ± standard deviation plotted against GEM103 (solid line) and sdCFH (dashed line) concentrations. CFH: complement factor H; INV: inverse; RFU: relative fluorescent unit; SD: standard deviation; sdCFH: serum-derived complement factor H.

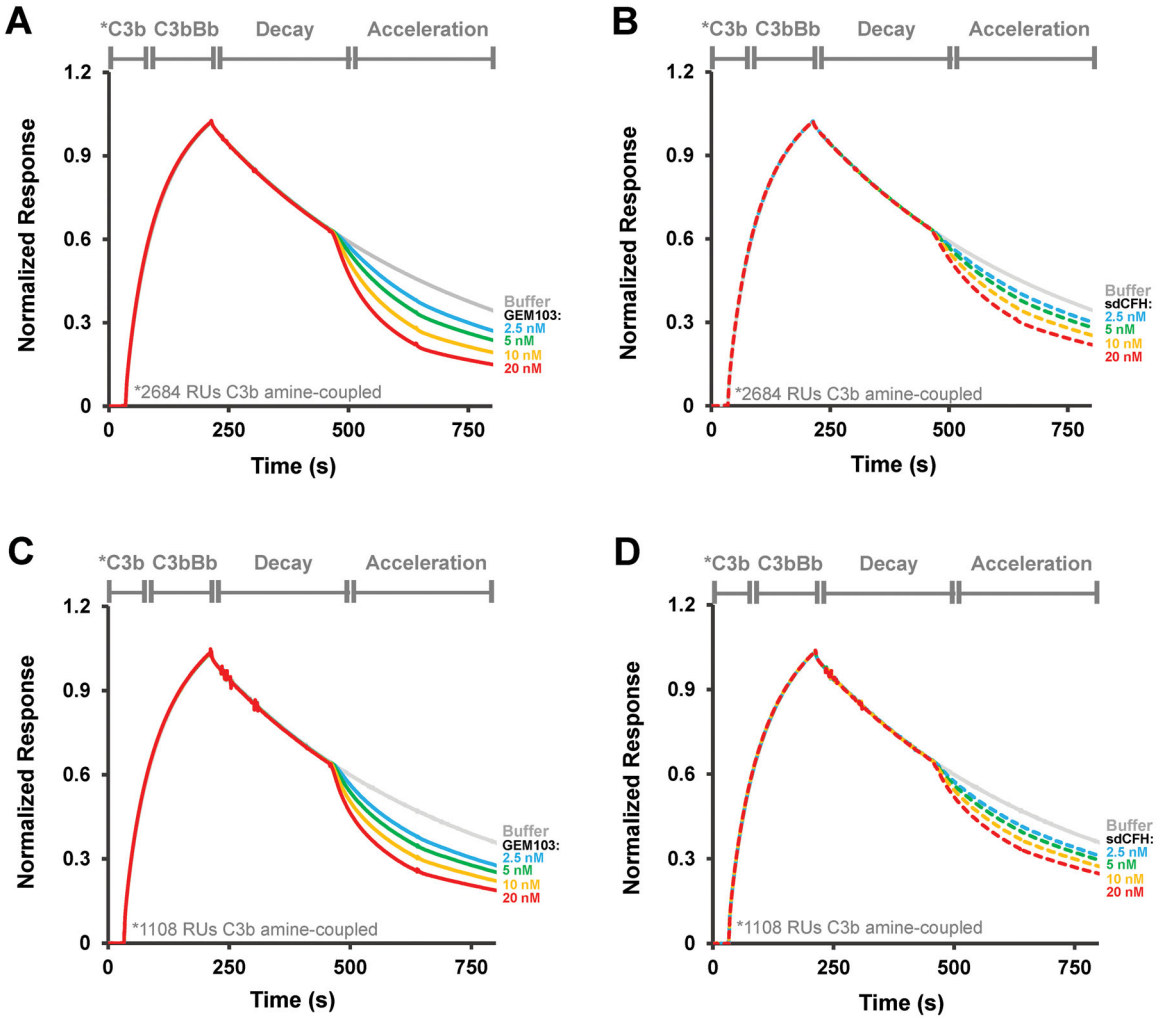

Figure 3. Comparison of C3bBb-DAA of GEM103 and sdCFH. In this assay loss of Bb from immobilized C3bBb is monitored in real time. The curve shows the initial formation of C3bBb on the surface (by flowing CFB and CFD over immobilized C3b) followed by its decay before and after addition of buffer or CFH at a range of concentrations. Results are representative sensorgrams of duplicate measurements at 2684 RUs of immobilized C3b for: (A) GEM103; (B) sdCFH; and at 1108 RUs of immobilized C3b for: (C) GEM103; and (D) sdCFH. C3b: Complement component 3b; C3bBb: C3 convertase; RUs: response units.

enrolled individuals with GA, aiming to restore full CFH functionality (clinicaltrials.gov identifiers: NCT04246866 and NCT04643886). It was, therefore, important to verify that GEM103 has canonical complement-regulating activities that at least match those of the native protein. In this study, native CFH had been derived from pooled human serum. Native CFH preparations were, therefore, likely to be more heterogeneous than the GEM103 preparation, which contains affinity-purified recombinant CFH that has a well-defined sequence and is of known provenance.

Here, the K_D for binding to surface-immobilized C3b, calculated from SPR experiments, for GEM103 was at least as tight as sdCFH, and within the range of previously published values for native CFH and yeast-produced recombinant CFH.[23–26] This suggests that the integrity of GEM103's primary C3b-binding sites (in CCPs 1–4 and CCPs 19–20) is preserved.

The CA of native CFH removes C3b from the amplification loop. This involves CFH recruiting CFI to the CFH:C3b complex.[15] The potencies of GEM103 and sdCFH to support cleavage of C3b by CFI were comparable in a solution assay exploiting the higher affinity of a fluorophore for C3b *versus* its cleavage product, iC3b.[27] CCPs 1–4 of CFH are critical for CA, while CCPs 19 and 20 also participate through their contribution to binding C3b.[24] This supports the presence of intact binding sites for both C3b and CFI in GEM103.

CFH catalyzes decay of C3bBb, which suppresses the C3b-amplifying positive-feedback loop. The potential benefits of restoring normal levels of CFH DAA in patients are highlighted by the decreased DAA of the pathogenic R53C CFH variant, associated with early-onset AMD.[28] DAA involves CFH binding to both C3b and Bb components of C3bBb. GEM103 performed at least as well as sdCFH in SPR-based DAA assays, consistent with the integrity of GEM103 CCPs 1–4, wherein DAA resides.

Native CFH selectively protects self-cell surfaces using primarily its C-terminal domains to recognize sialic acids and glycosaminoglycans characteristic of human glycoproteins and proteoglycans.[29–31] Poor protection of retinal cells from such damage, due to defective CFH, likely contribute

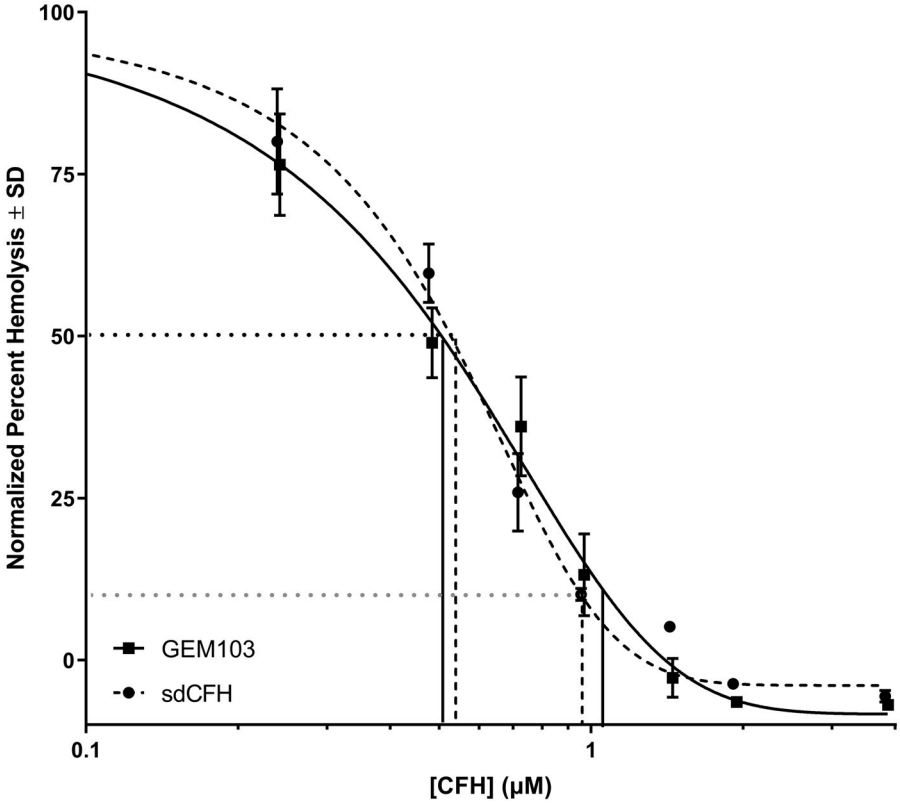

Figure 4. Sheep erythrocyte hemolysis assay, comparing GEM103 with sdCFH. In this assay, the ability of CFH, at a range of concentrations, to prevent the lysis of SEs by CFH-depleted human serum is tested. The average normalized percent hemolysis ± standard deviation was plotted against GEM103 (solid line) or sdCFH (dashed line) concentrations. A representative experiment of three independent experiments is shown. The dotted horizontal black line represents the interpolation at 50% hemolysis and the dotted horizontal grey line represents the interpolation at 10% hemolysis, with the solid vertical lines representing this for GEM103 and the dashed vertical lines representing this for sdCFH. SD: Standard deviation; sdCFH: serum-derived complement factor H.

to AMD pathogenesis. SEs have similar sialic acid residues to human erythrocytes,[32] and are protected by CFH from complement-mediated lysis in human serum. GEM103 is comparable to sdCFH in its ability to protect SEs from hemolysis by CFH-insufficient normal human serum. This suggests that IVT administration of GEM103 in AMD patients with reduced CFH functionality could increase the protection of local surfaces from dysregulated complement.

CFH has multiple non-canonical functions relevant to ocular tissue homeostasis, such as the binding of C-reactive protein, complement receptor 3, and malondialdehyde conjugates.[33–35] The central CCPs of CFH are deemed important for some of these.[15] Although we did not directly address the functional integrity of these domains, the native CFH-like ability of GEM103 to deploy simultaneously both its N- and its C-terminal regions is consistent with integrity of the connecting central segment.

Since it demonstrated equivalent activity and potency to native human CFH in each of its canonical, AP-modulating, functions, locally administered GEM103 has the potential to restore complement regulation in AMD patients lacking adequate levels of fully active, locally produced CFH.[10] Hence the delivery of GEM103 by IVT injection to AMD patients (carriers of AMD-risk variants known to compromise CFH production or activity) is a treatment modality warranting further investigation.

Acknowledgements

Medical writing assistance was provided by Meridian HealthComms Ltd, Plumley, UK.

Disclosure statement

RMB, SL, and SKK were employees of Gemini Therapeutics Inc. at the time of this study. PNB, APH and EM were partially funded by Gemini Therapeutics, Inc. at the time of the study. APH and EM are current employees of Invizius Limited.

Funding

This study was funded by Gemini Therapeutics, Inc.

ORCID

Robyn M. Biggs http://orcid.org/0000-0002-2770-8450

Data availability statement

The data that support the findings of this study are available from Gemini Therapeutics, Inc. but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available.

References

1. GBD 2019 Blindness and Vision Impairment Collaborators on Behalf of the Vision Loss Expert Group of the Global Burden of Disease Study. Causes of blindness and vision impairment in 2020 and trends over 30 years, and prevalence of avoidable blindness in relation to VISION 2020: the Right to Sight: an analysis for the Global Burden of Disease Study. Lancet Glob Health. 2021;9(2):e144–e160.
2. Wong WL, Su X, Li X, Cheung CM, Klein R, Cheng CY, Wong TY. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Glob Health. 2014;2(2):e106-116–e116. doi:10.1016/S2214-109X(13)70145-1.
3. , Chew EY, Clemons TE, Sangiovanni JP, Danis RP, Ferris FL, Elman MJ, Antoszyk AN, Ruby AJ, Orth D, Bressler SB, et al. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report no. 3. JAMA Ophthalmol. 2014;132(2):142–149. doi:10.1001/jamaophthalmol.2013.7376.
4. Chew EY, Clemons TE, Agron E, Sperduto RD, Sangiovanni JP, Kurinij N, Davis MD. Long-term effects of vitamins C and E, beta-carotene, and zinc on age-related macular degeneration: AREDS report no. 35. Ophthalmology. 2013;120(8):1604–1611 e1604. doi:10.1016/j.ophtha.2013.01.021.
5. Bowes Rickman C, Farsiu S, Toth CA, Klingeborn M. Dry age-related macular degeneration: mechanisms, therapeutic targets, and imaging. Invest Ophthalmol Vis Sci. 2013;54(14): ORSF68–80. doi:10.1167/iovs.13-12757.
6. Heesterbeek TJ, Lores-Motta L, Hoyng CB, Lechanteur YTE, den Hollander AI. Risk factors for progression of age-related macular degeneration. Ophthalmic Physiol Opt. 2020;40(2):140–170. doi: 10.1111/opo.12675.
7. Black JR, Clark SJ. Age-related macular degeneration: genome-wide association studies to translation. Genet Med. 2016;18(4): 283–289. doi:10.1038/gim.2015.70.
8. Holliday EG, Smith AV, Cornes BK, Buitendijk GH, Jensen RA, Sim X, Aspelund T, Aung T, Baird PN, Boerwinkle E, et al. Insights into the genetic architecture of early stage age-related macular degeneration: a genome-wide association study meta-analysis. PLoS One. 2013;8(1):e53830. doi:10.1371/journal.pone.0053830.
9. Meri S, Pangburn MK. Regulation of alternative pathway complement activation by glycosaminoglycans: specificity of the polyanion binding site on factor H. Biochem Biophys Res Commun. 1994;198(1):52–59. doi:10.1006/bbrc.1994.1008.
10. Mulfaul K, Mullin NK, Giacalone JC, Voigt AP, DeVore M, Stone EM, Tucker BA, Mullins RF. Local factor H production by human choroidal endothelial cells mitigates complement deposition: implications for macular degeneration. J Pathol. 2022. doi: 10.1002/path.5867.
11. Fritsche LG, Igl W, Bailey JN, Grassmann F, Sengupta S, Bragg-Gresham JL, Burdon KP, Hebbring SJ, Wen C, Gorski M, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. Nat Genet. 2016;48(2):134–143. doi:10.1038/ng.3448.
12. Cipriani V, Tierney A, Griffiths JR, Zuber V, Sergouniotis PI, Yates JRW, Moore AT, Bishop PN, Clark SJ, Unwin RD. Beyond factor H: the impact of genetic-risk variants for age-related macular degeneration on circulating factor-H-like 1 and factor-H-related protein concentrations. Am J Hum Genet. 2021;108(8): 1385–1400. doi:10.1016/j.ajhg.2021.05.015.
13. Lachmann PJ, Lay E, Seilly DJ. Experimental confirmation of the C3 tickover hypothesis by studies with an Ab (S77) that inhibits tickover in whole serum. Faseb J. 2018;32(1):123–129. doi:10.1096/fj.201700734.
14. Whaley K, Ruddy S. Modulation of C3b hemolytic activity by a plasma protein distinct from C3b inactivator. Science. 1976; 193(4257):1011–1013. doi:10.1126/science.948757.
15. Parente R, Clark SJ, Inforzato A, Day AJ. Complement factor H in host defense and immune evasion. Cell Mol Life Sci. 2017; 74(9):1605–1624. doi:10.1007/s00018-016-2418-4.
16. Pangburn MK, Ferreira VP, Cortes C. Discrimination between host and pathogens by the complement system. Vaccine. 2008; 26(Suppl 8):I15–21. doi:10.1016/j.vaccine.2008.11.023.
17. Fearon DT. Regulation by membrane sialic acid of beta1H-dependent decay-dissociation of amplification C3 convertase of the alternative complement pathway. Proc Natl Acad Sci USA. 1978;75(4):1971–1975. doi:10.1073/pnas.75.4.1971.
18. Geerlings MJ, Kremlitzka M, Bakker B, Nilsson SC, Saksens NT, Lechanteur YT, Pauper M, Corominas J, Fauser S, Hoyng CB, et al. The functional effect of rare variants in complement genes on C3b degradation in patients with age-related macular degeneration. JAMA Ophthalmol. 2017;135(1):39–46. doi:10.1001/jamaophthalmol.2016.4604.
19. Khandhadia S, Hakobyan S, Heng LZ, Gibson J, Adams DH, Alexander GJ, Gibson JM, Martin KR, Menon G, Nash K, et al. Age-related macular degeneration and modification of systemic complement factor H production through liver transplantation. Ophthalmology. 2013;120(8):1612–1618. doi:10.1016/j.ophtha.2013.01.004.
20. Schick T, Steinhauer M, Aslanidis A, Altay L, Karlstetter M, Langmann T, Kirschfink M, Fauser S. Local complement activation in aqueous humor in patients with age-related macular degeneration. Eye. 2017;31(5):810–813. doi:10.1038/eye.2016.328.
21. Biggs R, Lauder S, Katti S. Recombinant complement factor H (GEM103) ocular biodistribution and activity following intravitreal administration in cynomolgus monkeys. Poster presented at: 13th International Conference on Complement Therapeutics; September 8-13, 2021, Ioannina, Greece. https://s27.q4cdn.com/518843991/files/doc_downloads/2021/09/2021-ICC-Poster-GEM103-Biodistribution.pdf.
22. Khanani AM, Maturi RK, Bagheri N, Bakall B, Boyer DS, Couvillion SS, Dhoot DS, Holekamp NM, Jamal KN, Marcus DM, et al. 2020. PO389 A phase 1, single ascending dose study of GEM103 (recombinant human CFH) in patients with GA. Poster presented at: American Academy of Ophthalmology Annual Meeting, Virtual; November 13-15, 2020. https://secure.aao.org/aao/meeting-archive.
23. Herbert AP, Makou E, Chen ZA, Kerr H, Richards A, Rappsilber J, Barlow PN. Complement evasion mediated by enhancement of captured factor H: implications for protection of self-surfaces from complement. J Immunol. 2015;195(10):4986–4998. doi:10.4049/jimmunol.1501388.
24. Kerr H, Wong E, Makou E, Yang Y, Marchbank K, Kavanagh D, Richards A, Herbert AP, Barlow PN. Disease-linked mutations in factor H reveal pivotal role of cofactor activity in self-surface-selective regulation of complement activation. J Biol Chem. 2017; 292(32):13345–13360. doi:10.1074/jbc.M117.795088.
25. Schmidt CQ, Bai H, Lin Z, Risitano AM, Barlow PN, Ricklin D, Lambris JD. Rational engineering of a minimized immune inhibitor with unique triple-targeting properties. J Immunol. 2013;190(11):5712–5721. doi:10.4049/jimmunol.1203548.
26. Tortajada A, Montes T, Martinez-Barricarte R, Morgan BP, Harris CL, de Cordoba SR. The disease-protective complement factor H allotypic variant Ile62 shows increased binding affinity for C3b and enhanced cofactor activity. Hum Mol Genet. 2009; 18(18):3452–3461. doi:10.1093/hmg/ddp289.
27. Isenman DE. Conformational changes accompanying proteolytic cleavage of human complement protein C3b by the regulatory enzyme factor I and its cofactor H. Spectroscopic and enzymological studies. J Biol Chem. 1983;258(7):4238–4244.
28. Yu Y, Triebwasser MP, Wong EK, Schramm EC, Thomas B, Reynolds R, Mardis ER, Atkinson JP, Daly M, Raychaudhuri S, et al. Whole-exome sequencing identifies rare, functional CFH variants in families with macular degeneration. Hum Mol Genet. 2014;23(19):5283–5293. doi:10.1093/hmg/ddu226.
29. Ferreira VP, Herbert AP, Hocking HG, Barlow PN, Pangburn MK. Critical role of the C-terminal domains of factor H in

regulating complement activation at cell surfaces. J Immunol. 2006;177(9):6308–6316. doi:10.4049/jimmunol.177.9.6308.

30. Oppermann M, Manuelian T, Jozsi M, Brandt E, Jokiranta TS, Heinen S, Meri S, Skerka C, Gotze O, Zipfel PF. The C-terminus of complement regulator Factor H mediates target recognition: evidence for a compact conformation of the native protein. Clin Exp Immunol. 2006;144(2):342–352. doi:10.1111/j.1365-2249.2006.03071.x.
31. Blaum BS, Hannan JP, Herbert AP, Kavanagh D, Uhrin D, Stehle T. Structural basis for sialic acid-mediated self-recognition by complement factor H. Nat Chem Biol. 2015;11(1):77–82. doi:10.1038/nchembio.1696.
32. Baseman JB, Banai M, Kahane I. Sialic acid residues mediate *Mycoplasma pneumoniae* attachment to human and sheep erythrocytes. Infect Immun. 1982;38(1):389–391. doi:10.1128/iai.38.1.389-391.1982.
33. Losse J, Zipfel PF, Jozsi M. Factor H and factor H-related protein 1 bind to human neutrophils via complement receptor 3, mediate attachment to *Candida albicans*, and enhance neutrophil antimicrobial activity. J Immunol. 2010;184(2):912–921. doi:10.4049/jimmunol.0901702.
34. Molins B, Fuentes-Prior P, Adan A, Anton R, Arostegui JI, Yague J, Dick AD. Complement factor H binding of monomeric C-reactive protein downregulates proinflammatory activity and is impaired with at risk polymorphic CFH variants. Sci Rep. 2016;6:22889. doi:10.1038/srep22889.
35. Weismann D, Hartvigsen K, Lauer N, Bennett KL, Scholl HP, Charbel Issa P, Cano M, Brandstatter H, Tsimikas S, Skerka C, et al. Complement factor H binds malondialdehyde epitopes and protects from oxidative stress. Nature. 2011;478(7367):76–81. doi:10.1038/nature10449.