Therapeutic efficacy of a bi-functional C5 mAb-FH1-5 fusion protein in a mouse model of rapidly progressing lethal C3 glomerulopathy (C3G)

Sayaka Sato[1], Takashi Miwa[1], Damodar Gullipalli[1], Lin Zhou[1], Matthew Palmer[2], Jianjun Zhang[3], Xiaoxia Hu[3], Bingbing Jiang[3], Jingtao Wu[4], Ping Tsui[4], and Wen-Chao Song[1]

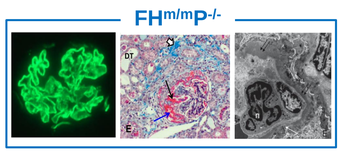
[1]Department of Systems Pharmacology and Translational Therapeutics, [2]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA [3]Kira Pharmaceuticals, Suzhou, China, [4]Kira Pharmaceuticals, Cambridge, MA, USA

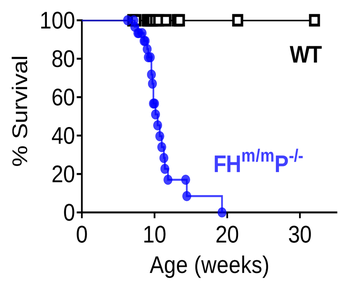
Introduction

C3 glomerulopathy (C3G) is a rare kidney disease caused by dysregulated alternative pathway (AP) complement activation. It is characterized by glomerular C3 deposition, proteinuria, crescentic glomerulonephritis and renal failure. The anti-C5 mAb drug Eculizumab has shown therapeutic effect in some but not all C3G patients and no approved therapy is currently available. We previously characterized a lethal murine model of C3G ($FH^{m/m}P^{-/-}$ mice) and demonstrated an anti-C5 mAb to be completely effective in preventing disease but only partially effective in treating established disease. We have recently humanized factor D (FD) in $FH^{m/m}P^{-/-}$ mice by crossing with a human FD knock-in mouse (hFD). Unexpectedly, hFD-$FH^{m/m}P^{-/-}$ mice not only recapitulated the C3G phenotype but also developed a more aggressive form of C3G, with 100% mortality occurring before 7 (instead of 12-14) weeks of age. In this study, we used this newly developed, rapidly progressing C3G mouse model to test the therapeutic efficacy of an anti-mouse C5 mAb and a bi-functional anti-mouse C5 mAb/mouse FH1-5 fusion protein. hFD-$FH^{m/m}P^{-/-}$ mice aged 3-6 weeks were screened by proteinuria and only those that had developed 3+ proteinuria/hematuria were enrolled in this therapeutic study. The C5 mAb-FH tested in this study is a murine surrogate of KP104, a bi-functional complement inhibitor under clinical development by Kira Pharmaceuticals. Please visit poster/abstract #132, #126 for more details on KP104.

1 $FH^{m/m}P^{-/-}$ mouse: a lethal mouse C3G model, and mixed efficacy of a murine anti-C5 mAb

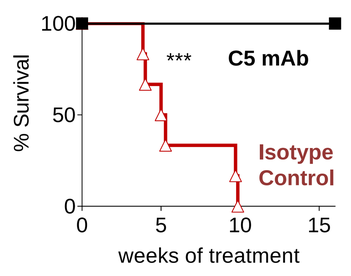
$FH^{m/m}P^{-/-}$ mice die of C3G by 20 weeks of age.





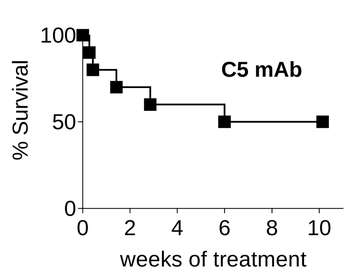
Lesher AM et al, *J Am Soc Nephrol* (2013)

Prophylactic (100% rescue)



***P = 0.0002 (log-rank test)

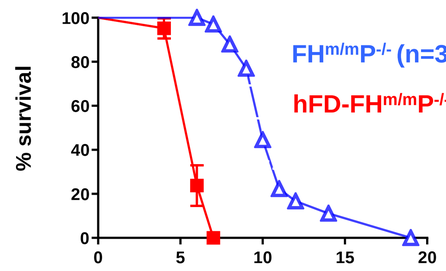
Therapeutic (50% rescue)

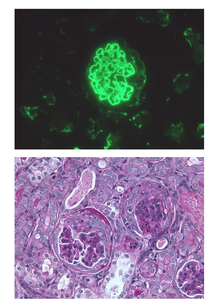


Williams AL et al, *Kidney Int,* 91(6): 1386–1397, 2017

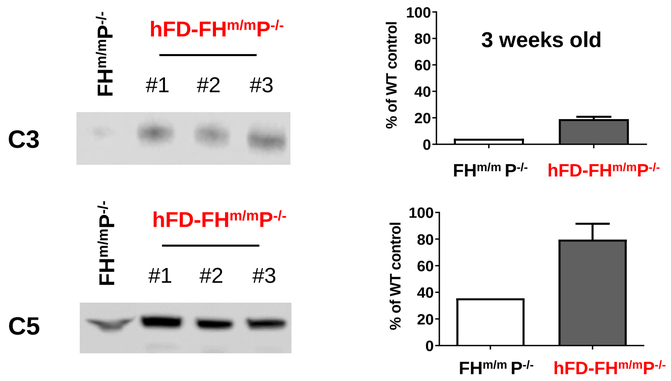
2 Factor D humanization in $FH^{m/m}P^{-/-}$ mice further exacerbates C3G due to higher C3 and C5

Mortality and pathology





Higher C3 and C5 levels in hFD-$FH^{m/m}P^{-/-}$ than in $FH^{m/m}P^{-/-}$ mice



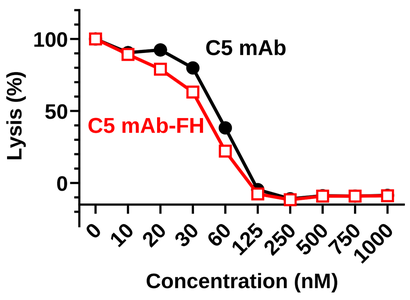
3 Novel bi-functional complement inhibitor: anti-C5 mAb and FH1-5 fusion protein

Anti-C5 mAb (BB5.1 variant) → **TP (C5) regulation**

Mouse FH SCR 1-5 → **AP regulation**

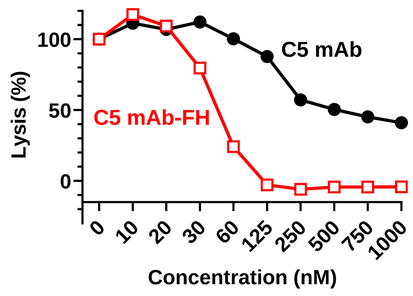
Sheep RBCs lysis assay

C5 mAb-FH and C5 mAb have similar potency in inhibiting terminal pathway complement activation triggered via the classical pathway (sheep RBC lysis test with 50% mouse plasma)



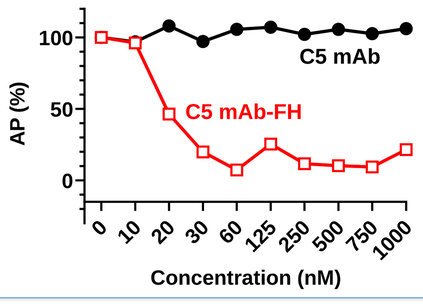
Rabbit RBCs lysis assay

C5 mAb-FH is more potent than C5 mAb in inhibiting terminal pathway complement activation triggered via the alternative pathway (rabbit RBC lysis test with 50% mouse plasma)



LPS-based ELISA assay

C5 mAb-FH inhibits LPS-induced C3b deposition via AP complement activation (modified Wieslab AP assay with 50% mouse plasma) while C5 mAb does not.



Result Summary and Conclusions

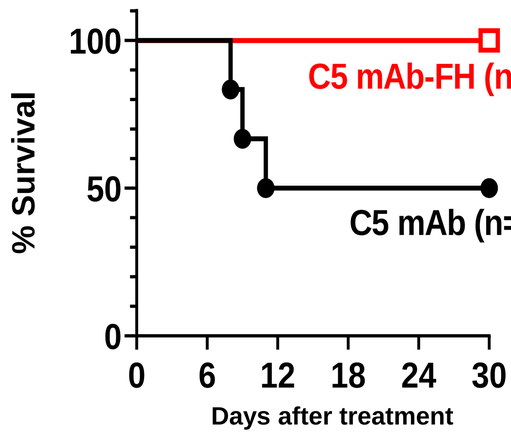
- Humanization of FD in $FH^{m/m}P^{-/-}$ mice exacerbated C3G, likely due to higher plasma C3 and C5 levels in this strain.
- hFD-$FH^{m/m}P^{-/-}$ mice developed severe and rapidly progressing C3G, with 100% mortality by 7 weeks old.
- hFD-$FH^{m/m}P^{-/-}$ mice were screened and those showing 3+ proteinuria/hematuria (age ranging 3-6 weeks) were treated with either a murine anti-C5 mAb or the same mAb fused with mouse FH SCR1-5 for 30 days (n=6)
- All mice treated with C5 mAb-FH survived the 30-day treatment whereas only 50% treated with C5 mAb did.
- Treatment with C5 mAb-FH but not C5 mAb prevented systemic AP activation and C3, FB consumption.
- C5 mAb-FH prevented glomerular C3 and C9 deposition whereas C5 mAb prevented glomerular C9 deposition only.
- Both drugs significantly ameliorated mesangial hypercellularity over baseline, and crescent/fibrin deposition, mesangial hypercellularity over non-treated group, but only C5 mAb-FH significantly reduced proteinuria, hematuria and glomerular endocapillary hypercellularity over baseline.

4 Anti-C5 mAb-FH is therapeutically more efficacious than anti-C5 mAb in hFD-$FH^{m/m}P^{-/-}$ mice

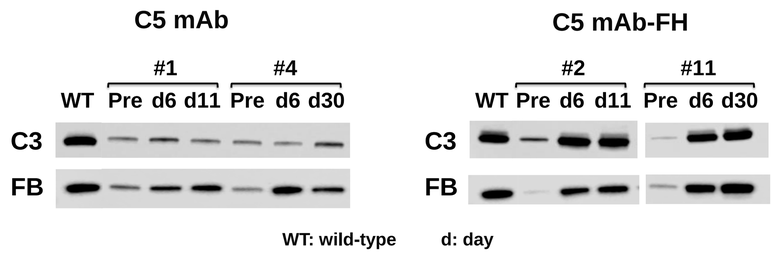
a Study Objective and Experimental Protocol

- Compare C5 mAb and C5 mAb-FH therapeutically
- Use the new and rapidly progressing hFD-$FH^{m/m}P^{-/-}$ model
- Screen mice with 3+ proteinuria/hematuria for enrollment
- C5 mAb (enrolled n=6 male mice, 4-6 weeks old)
- C5 mAb-FH (enrolled n=6 male mice, 3-6 weeks old)
- 40 mg/kg, i.p., every 3 days x 10 doses (30 days treatment)

b Survival curves

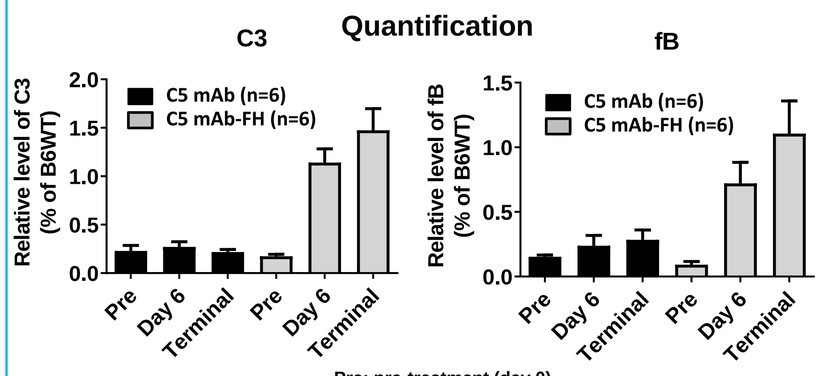


c C5 mAb-FH but not C5 mAb inhibited systemic C3 and Factor B consumption



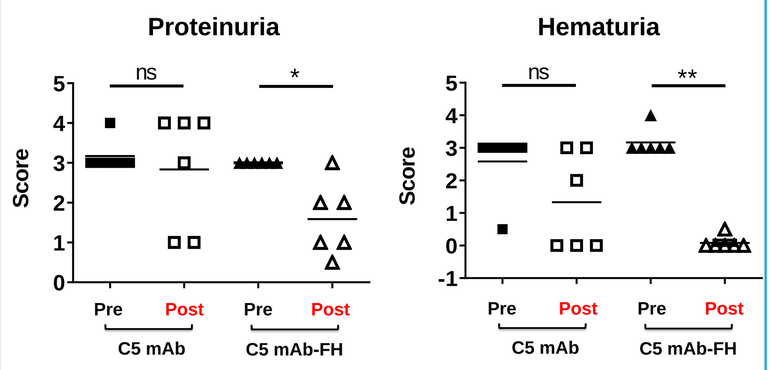
WT: wild-type d: day

Quantification



Pre: pre-treatment (day 0)

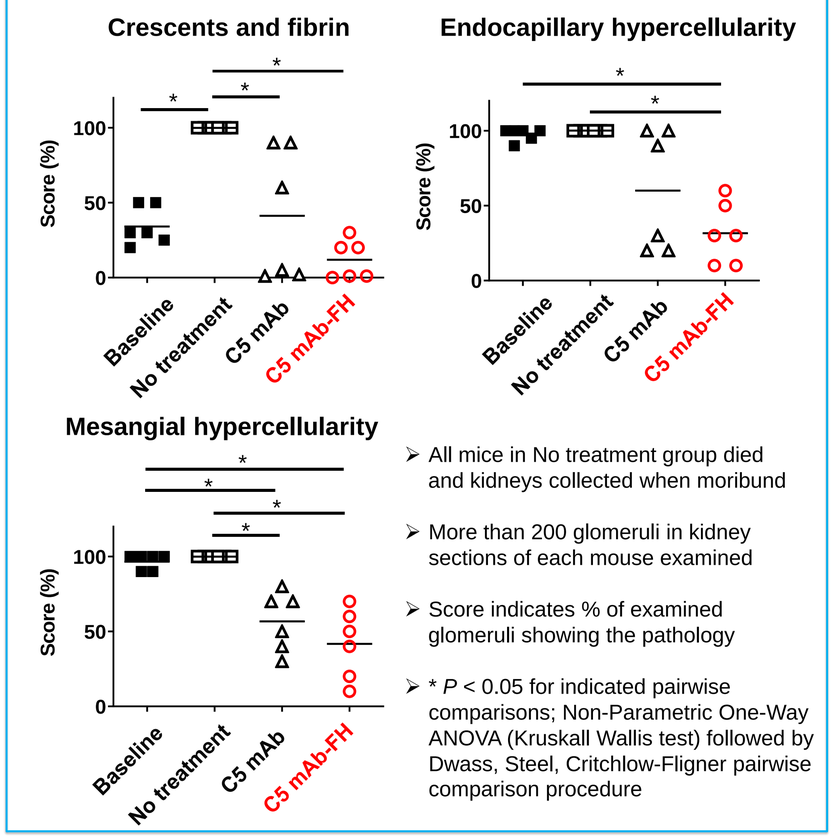
d C5 mAb-FH improved renal function to a greater degree than C5 mAb



Pre: pre-treatment (day 0) Post: post-treatment (moribund or day 30)

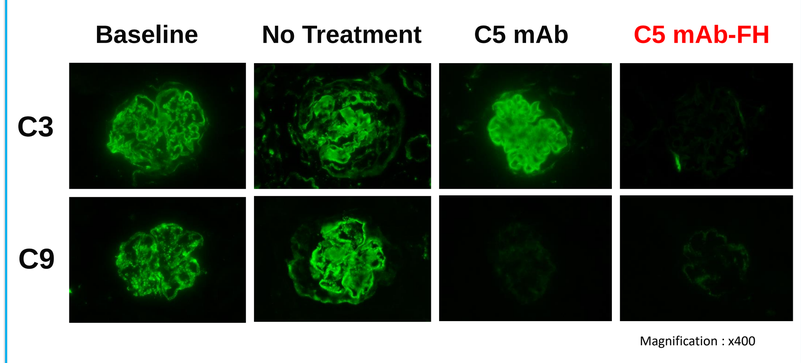
*P < 0.05; **P < 0.01; ns, not significant (Mann Whitney test)

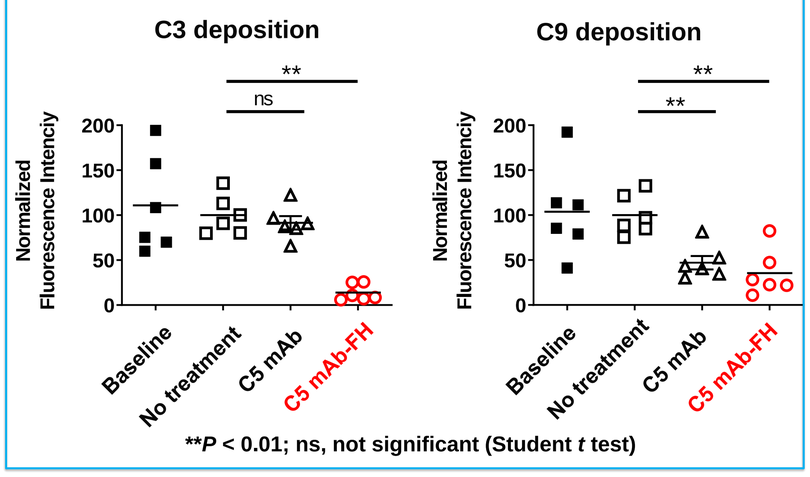
e C5 mAb-FH treatment significantly ameliorated renal pathology



- All mice in No treatment group died and kidneys collected when moribund
- More than 200 glomeruli in kidney sections of each mouse examined
- Score indicates % of examined glomeruli showing the pathology
- * P < 0.05 for indicated pairwise comparisons; Non-Parametric One-Way ANOVA (Kruskall Wallis test) followed by Dwass, Steel, Critchlow-Fligner pairwise comparison procedure

f C5 mAb-FH eliminated both C3 and C9 deposition in glomeruli





**P < 0.01; ns, not significant (Student t test)