Design and characterization of KP104, a bifunctional anti-C5 mAb and FH1-5 fusion protein that synergistically inhibits alternative and terminal pathways of complement activation

Damodar Gullipalli[1], Takashi Miwa[1] Sayaka Sato[1], Madhu Golla[1], Xihua Zhu[2], Jianjun Zhang[2], Dongqiong Fei[2], Ping Tsui[3], Fengkui Zhang[4], Wen-Chao Song[1]

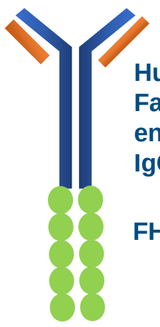
[1]Department of Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, [2]Kira Pharmaceuticals, Suzhou, China, [3]Kira Pharmaceuticals, Cambridge, MA, [4]Institute of Hematology, Chinese Academy of Medical Sciences, Tianjin, China

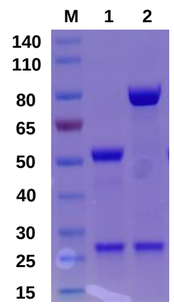
Introduction

Anti-complement drugs hold great promise for treating many human autoimmune and inflammatory diseases. Achieving desirable pharmacokinetics and pharmacodynamics of anti-complement drugs is challenging due to target abundance and target-mediated drug disposition (TMDD), exponential amplification of activation cascade, and multiple effectors implicated in a disease. Currently, drugs targeting C5, C3, C1s and C5aR have been approved in the clinic to treat several orphan diseases. However, there remains significant unmet medical need in these and several other indications, and a vested interest in more efficacious and convenient-to-use complement drugs. We describe here the design and characterization of a novel bifunctional anti-C5 mAb and factor H SCR 1-5 fusion protein that synergistically inhibits both the alternative and terminal complement pathways.

1 KP104 Bifunctional Structure

Sequence of KP104 engineering: murine C5 mAb (2G1-3) → humanization, Fab- and Fc-engineering of IgG4 → fusion with FH SCR1-5 → KP104





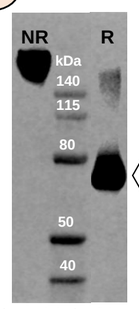
SDS-PAGE

1: Humanized anti-C5 mAb

2: KP104

M: Protein marker

2 2G1-3 binds to a different epitope from Eculizumab



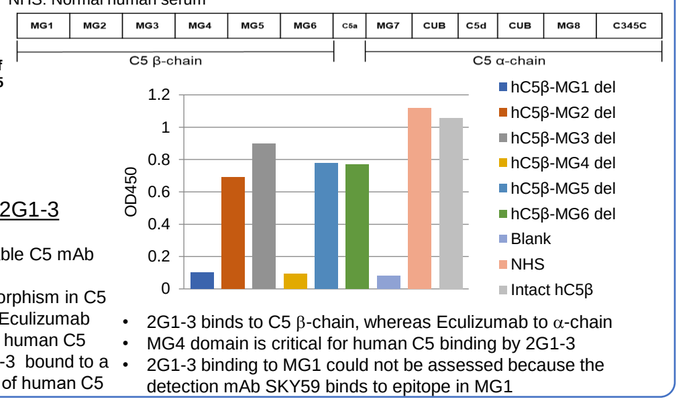
β chain of human C5

Western blot of human C5 by 2G1-3

- Eculizumab, a commercially available C5 mAb binds to the α-chain of C5
- Some PNH patients carry a polymorphism in C5 α-chain and are nonresponsive to Eculizumab
- 2G1-3 bound to non-reduced (NR) human C5
- Under reducing condition (R), 2G1-3 bound to a band corresponding to the β chain of human C5

Detection of human C5 β chain and deletion mutants by Sandwich ELISA

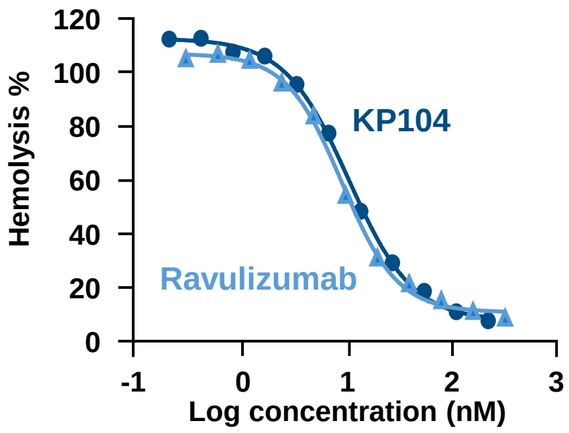
- Capture Ab: 2G1-3 / Detection Ab: SKY59
- Culture supernatants from transfected HEK cells with human C5 β chain deletion mutants lacking individual MG domains
- Blank: Culture supernatants from untransfected HEK cells
- NHS: Normal human serum



- 2G1-3 binds to C5 β-chain, whereas Eculizumab to α-chain
- MG4 domain is critical for human C5 binding by 2G1-3
- 2G1-3 binding to MG1 could not be assessed because the detection mAb SKY59 binds to epitope in MG1

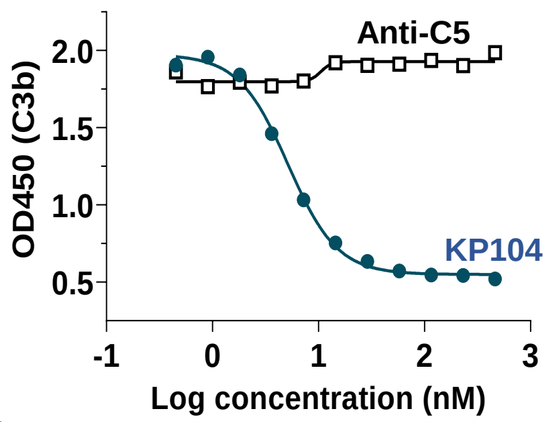
KP104 is as potent as Ravulizumab in inhibiting CP-triggered terminal pathway complement activation (sheep RBC lysis)

3 C5 Inhibition Assay



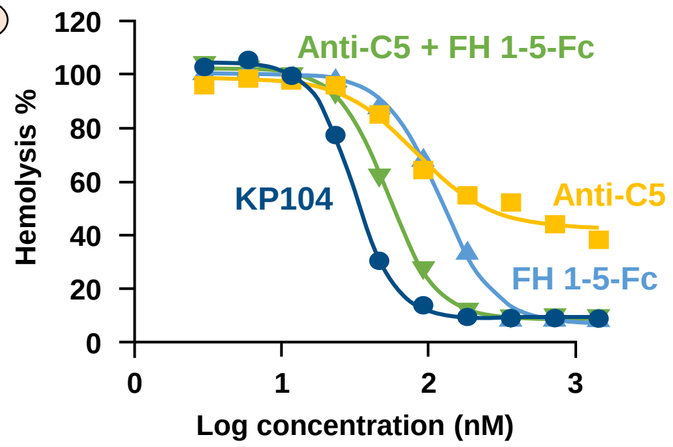
Unlike anti-C5 mAb, KP104 also inhibits AP complement

4 LPS-based ELISA assay



KP104 is more potent than anti-C5 mAb or FH SCR1-5-Fc, alone or combined, in inhibiting AP-triggered terminal pathway complement activation (rabbit RBC lysis)

5



Rabbit RBCs lysis assay

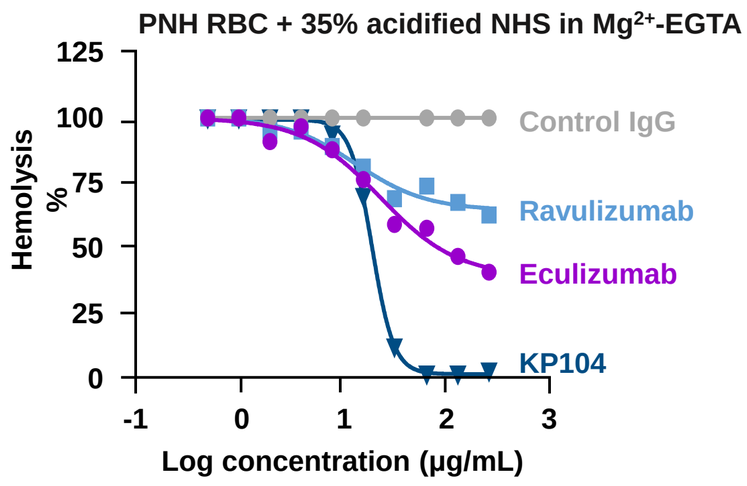
KP104 is more potent than

- Anti-C5 mAb alone
- AP regulator (FH1-5-Fc) alone
- Anti-C5 mAb and AP regulator (FH1-5-Fc) together

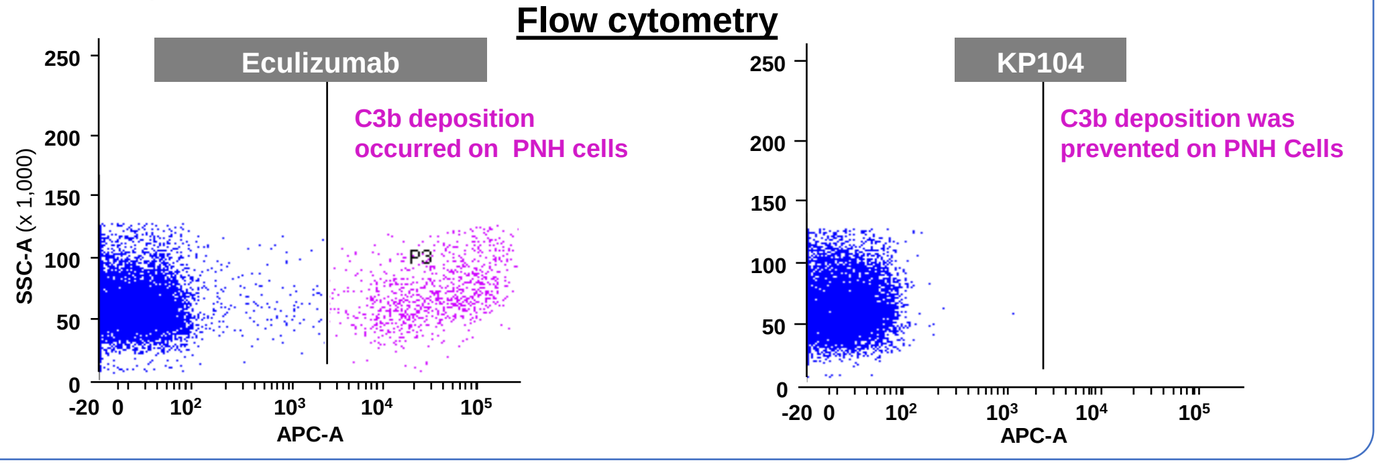
KP104 is more potent than Ecu/Rav mAbs in inhibiting the lysis of human PNH RBCs and it differentiates from Ecu/Rav in inhibiting C3b fragment opsonization of non-lysed PNH RBCs

6 PNH RBCs lysis assay

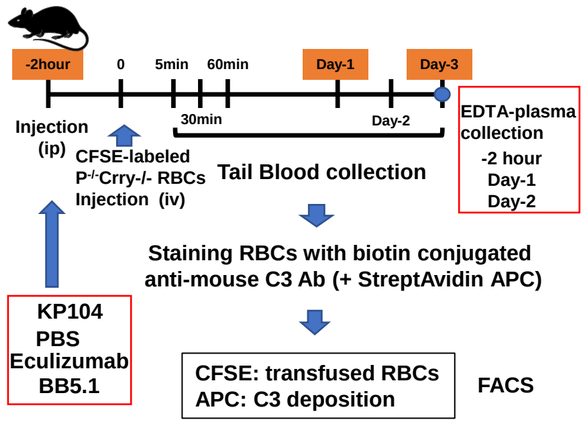
- Anti-C5 mAbs such as Eculizumab and Ravulizumab cannot completely block PNH RBC lysis in modified Ham's test *ex vivo* and significant residual hemolysis remains. In contrast, KP104 can fully block PNH RBC lysis and overcomes the residual hemolysis.
- KP104 effectively blocks activated C3 fragment opsonization of PNH RBCs, whereas Eculizumab had no effect, as expected



Flow cytometry



7 KP104 dose-dependently inhibited extravascular hemolysis (EVH) in a mouse model of EVH

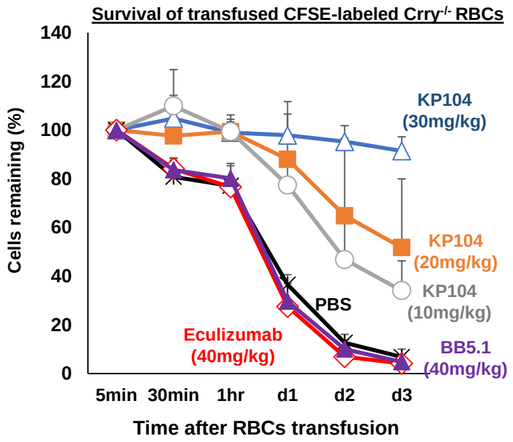


KP104: 30mg/kg, n=4; 20mg/kg, n=3; 10mg/kg, n=4
Eculizumab: 40mg/kg, n=2
BB5.1: 40mg/kg, n=4
PBS: n=4

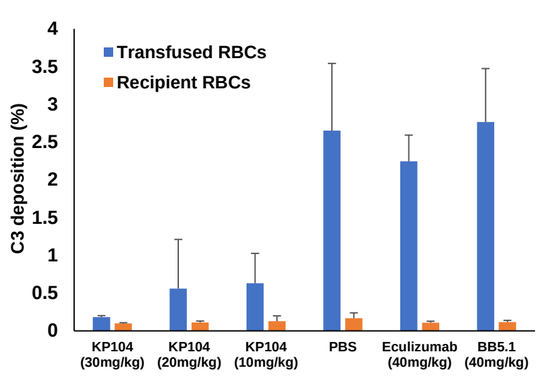
CFSE(+) gated: Transfused P-/- Crry-/- RBCs
CFSE(-) gated: Recipient RBCs

- KP104 prevented EVH dose-dependently, whereas anti-C5 mAbs (Eculizumab and BB5.1) did not prevent EVH.
- Opsonization of the transfused RBCs by activated C3 fragments was inhibited dose-dependently by KP104 but noy by anti-C5 mAbs.

Survival of transfused CFSE-labeled Crry-/- RBCs



FACS analysis of activated C3 fragment deposition on Crry-/- RBCs 30min after their transfusion into Ab treated mice

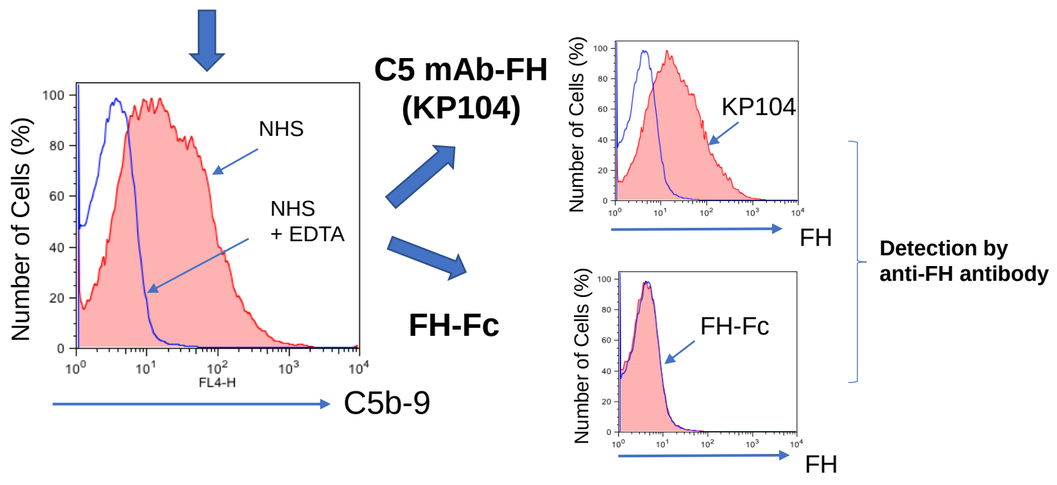


8 KP104 possess tissue targeting property for cells with C5b-9 deposition

Induction of C5b-9 deposition on cell surface

Mouse thymocytes (susceptible to complement activation) are used as a model test

B6 WT mouse thymocytes → Incubate with 40% NHS → **37°C incubation for 45min** → **FACS for C6**



Conclusion

- KP104 is a novel bi-functional complement inhibitor of the alternative and terminal complement pathways
- It is composed of a humanized and extensively engineered (in Fab and Fc) anti-C5 mAb fused to SCR1-5 of factor H
- KP104 is more potent than Eculizumab/Ravulizumab in inhibiting rabbit and PNH RBC lysis and it can block C3b fragment opsonization of PNH RBCs and EVH
- KP104 binds to C5b and has tissue-targeting property for cells/tissues with C5b-9 deposition by virtue of its C5b-binding affinity
- KP104 is potentially more efficacious than either proximal (alternative pathway) or terminal pathway (C5) inhibitors in indications where both pathways are pathogenic