

SUMMARY OF THE INVENTION

The inventors have shown that podocytes may play an important role in complement-mediated kidney disease. For example, the inventors have shown that secreted podocyte complement inhibitors (e.g. CFH) may contribute to local complement regulation. The inventors have also shown that a complement inhibitor rescues the diseased phenotype in a mouse model of podocyte-driven complement-mediated disease (e.g. stx mediated HUS).

Accordingly, the present inventors have developed a viral vector for use in treating complement-mediated kidney diseases, such as C3 glomerulopathy and IgA Nephropathy, which is targeted to podocytes.

The present inventors have shown that use of a podocyte-specific promoter and/or a viral vector which is capable of specifically transducing podocytes may be used to target expression of complement proteins to podocytes. For example, the present inventors have shown that AAV-LK03 vectors can achieve high transduction of close to 100% in human podocytes and can be used to transduce podocytes specifically.

In one aspect, the present ~~invention~~disclosure provides a viral vector comprising a nucleotide sequence encoding a complement protein, wherein the nucleotide sequence is operably linked to a podocyte-specific promoter and/or the viral vector is capable of specifically transducing podocytes. In another aspect, the present disclosure provides a viral vector comprising a nucleotide sequence encoding an inhibitor of the complement system, wherein the nucleotide sequence is operably linked to a podocyte-specific promoter. In yet another aspect, the present disclosure provides a viral vector comprising a nucleotide sequence encoding an inhibitor of the complement system, wherein the viral vector is capable of specifically transducing podocytes, optionally wherein the nucleotide sequence is operably linked to a podocyte-specific promoter.

The viral vector may be an adeno-associated virus (AAV) vector, an adenoviral vector, a herpes simplex viral vector, a retroviral vector, or a lentiviral vector.

Preferably, the viral vector is an AAV vector. The AAV vector may be an AAV vector particle. In some ~~embodiment~~examples the AAV vector particle comprises AAV3B capsid proteins, LK03 capsid proteins, or AAV9 capsid proteins. Preferably, the AAV vector particle comprises AAV3B capsid proteins.

The podocyte-specific promoter may be a promoter selected from a NPHS1 promoter, a NPHS2 promoter, a WT1 promoter, a FOXC2 promoter, a ABCA9 promoter, a ACPD promoter, a ACTN4 promoter, a ADM promoter, a ANGPTL2 promoter, a ANXA1 promoter, a ASB15 promoter, a ATP8B1 promoter, a B3GALT2 promoter, a BB014433 promoter, a BMP7 promoter, a C1QTNF1 promoter, a CAR13 promoter, a CD2AP promoter, a CD55 promoter, a CD59A promoter, a CD59B promoter, a CDC14A promoter, a CDH3 promoter, a CDKN1B promoter, a CDKN1C promoter, a CEP85L promoter, a CLIC3 promoter, a CLIC5 promoter, a COL4A1 promoter, a COL4A2 promoter, a COL4A3 promoter, a COL4A4 promoter, a COL4A5 promoter, a COLEC12 promoter, a CRIM1 promoter, a CST12

clusterin, or fragments or derivatives thereof. Preferably the complement protein is an inhibitor of the complement system. The inhibitor of the complement system may be selected from the list consisting of CFI, CFH, FHL-1, C1INH, C4BP, CD55, CD35, CD46, CD59, vitronectin, and clusterin, or fragments or derivatives thereof. Preferably, the inhibitor of the complement system is CFI, CFH, or FHL-1, or a fragment or derivative thereof.

The nucleotide sequence encoding a complement protein may be operably linked to a Woodchuck hepatitis post-transcriptional regulatory element (WPRE), a polyadenylation signal, and/or a Kozak sequence.

10 In another aspect, the present ~~invention~~disclosure provides an isolated cell comprising the viral vector of the present ~~invention~~disclosure.

In another aspect, the present ~~invention~~disclosure provides a pharmaceutical composition comprising the viral vector or the isolated cell of the present ~~invention~~disclosure, in combination with a pharmaceutically acceptable carrier, diluent or excipient.

15 In another aspect, the present ~~invention~~disclosure provides the viral vector, the isolated cell, or the pharmaceutical composition of the present ~~invention~~disclosure for use as a medicament.

In ~~a related aspect~~one example, the present ~~invention~~disclosure provides for use of the viral vector, the isolated cell, or the pharmaceutical composition of the present ~~invention~~disclosure for the manufacture of a medicament.

20 In ~~a related aspect~~another example, the present ~~invention~~disclosure provides a method comprising administering the viral vector, the isolated cell, or the pharmaceutical composition of the present ~~invention~~disclosure to a subject in need thereof.

In another ~~aspect~~example, the present ~~invention~~disclosure provides the viral vector, the isolated cell, or the pharmaceutical composition of the present ~~invention~~disclosure for use in preventing or treating a complement-mediated kidney disease.

25 In ~~a related one~~ aspect, the present ~~invention~~disclosure provides ~~a~~for use of the viral vector, the isolated cell, or the pharmaceutical composition of the present ~~invention~~disclosure ~~in~~for the manufacture of a medicament for preventing or treating a complement-mediated kidney disease.

30 In ~~a related aspect~~one example, the present ~~invention~~disclosure provides a method of preventing or treating a complement-mediated kidney disease comprising administering the viral vector, the isolated cell, or the pharmaceutical composition of the present ~~invention~~disclosure to a subject in need thereof.

The complement-mediated kidney disease may be IgA nephropathy, C3 glomerulopathy, atypical hemolytic uremic syndrome (aHUS), stx-associated HUS, lupus nephritis, cryoglobulinemia, anti-GBM disease, ANCA-associated vasculitis, bacterial endocarditis, post-infectious glomerulonephritis, antibody-mediated rejection of renal transplant, membranous nephropathy, membranoproliferative glomerulonephritis I, or membranoproliferative glomerulonephritis III. Preferably, the complement-mediated kidney disease is IgA Nephropathy or C3 glomerulopathy, preferably wherein the C3 glomerulopathy is dense deposit disease or C3 glomerulonephritis.

The viral vector, the isolated cell, or the pharmaceutical composition of the present ~~disclosure~~~~invention~~ may be administered to a human subject. The viral vector, the isolated cell, or the pharmaceutical composition of the present ~~invention~~~~disclosure~~ may be administered systemically and/or by intravenous injection. The viral vector, the isolated cell, or the pharmaceutical composition of the present ~~invention~~~~disclosure~~ may be administered by injection into the renal artery or by ureteral or subcapsular injection.

DESCRIPTION OF DRAWINGS

Figure 1 – AAV 2/9 administered by tail vein injection transduces the kidney and expresses HA-tagged podocin in the podocyte.

A) AAV vectors used to express mouse or human podocin or GFP. All vectors contained the Kozak sequence between the promoter and the transgene, as well as WPRE (Woodchuck hepatitis post-transcriptional regulatory element) and the bovine growth hormone (bGH) polyadenylation signal. **B)** Vector or saline was injected via tail vein in iPod NPHS2^{fl/fl} mice at 8 weeks of age, and induction with doxycycline commenced 10-14 days later. **C)** qPCR showing presence of AAV ITRs in mouse kidney cortex in mice injected with the viral vector. **D)** Representative immunofluorescence showing expression of HA tagged podocin with podocyte-specific proteins nephrin and podocin in iPod NPHS2^{fl/fl} mice injected with AAV 2/9. Control (saline) images are of mice without the full iPod NPHS2^{fl/fl} genotype injected and hence did not develop proteinuria or diseased glomeruli, as mice with diseased glomeruli showed loss of podocyte markers.

Figure 2 – tail vein injection of AAV 2/9 expressing wild-type podocin under a podocyte-specific promoter ameliorates proteinuria in the conditional podocin knock-out mouse model (iPod NPHS2^{fl/fl})

A) Urinary albumin:creatinine ratio of mice injected with AAV 2/9 mNPHS1.mpod versus AAV 2/9 hNPHS1.mpod versus saline (n=9 in each group, **p<0.01 ***p<0.001). **B)**

CLAIMS

1. A viral vector comprising a nucleotide sequence encoding an inhibitor of the complement system, wherein the nucleotide sequence is operably linked to a podocyte-specific promoter.
2. A viral vector according to claim 1, wherein the viral vector is capable of specifically transducing podocytes.
3. A viral vector comprising a nucleotide sequence encoding an inhibitor of the complement system, wherein the viral vector is capable of specifically transducing podocytes, optionally wherein the nucleotide sequence is operably linked to a podocyte-specific promoter.
4. A viral vector according to any preceding claim, wherein the viral vector is an adeno-associated virus (AAV) vector, an adenoviral vector, a herpes simplex viral vector, a retroviral vector, or a lentiviral vector.
5. A viral vector according to any preceding claim, wherein the viral vector is an AAV vector.
6. A viral vector according to any preceding claim, wherein the viral vector is an AAV vector particle.
7. A viral vector according to claim 6, wherein the AAV vector particle comprises AAV3B capsid proteins, LK03 capsid proteins, or AAV9 capsid proteins.
8. A viral vector according to claim 6 or claim 7, wherein the AAV vector particle comprises AAV3B capsid proteins.
9. A viral vector according to any one of claims 6-8, wherein the AAV vector particle comprises capsid proteins with at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to one or more of SEQ ID NOs: 1-3, or a fragment or derivative thereof.
10. A viral vector according to any one of claims 6-9, wherein the AAV vector particle comprises capsid proteins with at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to SEQ ID NO: 1, or a fragment or derivative thereof.

11. A viral vector according to any one of claims 6-10, wherein the AAV vector particle comprises capsid proteins comprising or consisting of SEQ ID NO: 1, or a fragment or derivative thereof.

12. A viral vector according to any preceding claim, wherein the podocyte-specific promoter is selected from a NPHS1 promoter, a NPHS2 promoter, a WT1 promoter, a FOXC2 promoter, a ABCA9 promoter, a ACPP promoter, a ACTN4 promoter, a ADM promoter, a ANGPTL2 promoter, a ANXA1 promoter, a ASB15 promoter, a ATP8B1 promoter, a B3GALT2 promoter, a BB014433 promoter, a BMP7 promoter, a C1QTNF1 promoter, a CAR13 promoter, a CD2AP promoter, a CD55 promoter, a CD59A promoter, a CD59B promoter, a CDC14A promoter, a CDH3 promoter, a CDKN1B promoter, a CDKN1C promoter, a CEP85L promoter, a CLIC3 promoter, a CLIC5 promoter, a COL4A1 promoter, a COL4A2 promoter, a COL4A3 promoter, a COL4A4 promoter, a COL4A5 promoter, a COLEC12 promoter, a CRIM1 promoter, a CST12 promoter, a DEGS1 promoter, a DOCK4 promoter, a DOCK5 promoter, a EGF promoter, a ENPEP promoter, a EPHX1 promoter, a FAM81A promoter, a FAT1 promoter, a FGFBP1 promoter, a FOXD1 promoter, a FRYL promoter, a GABRB1 promoter, a GALC promoter, a GM10554 promoter, a H2-D1 promoter, a H2-Q7 promoter, a H2BC4 promoter, a H3C15 promoter, a HS3ST3A1 promoter, a HTRA1 promoter, a IFNGR1 promoter, a IL18 promoter, a ILDR2 promoter, a ITGB5 promoter, a ITGB8 promoter, a KIRREL promoter, a LAMA1 promoter, a LAMA5 promoter, a LAMB1 promoter, a LAMB2 promoter, a LMX1B promoter, a MAFB promoter, a MAGI2 promoter, a MELA promoter, a MERTK promoter, a MGAT4A promoter, a MYO1D promoter, a MYO1E promoter, a MYOM2 promoter, a MYZAP promoter, a NEBL promoter, a NES promoter, a NOD1 promoter, a NPR3 promoter, a NR2F2 promoter, a NUPR1 promoter, a OPTN promoter, a P3H2 promoter, a PAK1 promoter, a PARD3B promoter, a PDPN promoter, a PLAT promoter, a PLCE1 promoter, a PLSCR2 promoter, a PODXL promoter, a PROS1 promoter, a PTPRO promoter, a RAB3B promoter, a RDH1 promoter, a RDH9 promoter, a SDC4 promoter, a SEMA3E promoter, a SERPINB6B promoter, a SH3BGRL2 promoter, a SLC41A2 promoter, a SLCO2A1 promoter, a ST3GAL6 promoter, a SYNPO promoter, a TDRD5 promoter, a THSD7A promoter, a TIMP3 promoter, a TJP1 promoter, a TLR7 promoter, a TM4SF1 promoter, a TMEM108 promoter, a TMEM54 promoter, a TMTC1 promoter, a TOP1MT promoter, a TRAV10 promoter, a TRAV10N promoter, a TRAV5-4 promoter, a TSHB promoter, a UACA promoter, a UBA1Y promoter, a UPRT promoter, a VEGFA promoter, a VTCN1 promoter, a ZBTB20 promoter, and a 5730407107RIK promoter, or a fragment of derivative thereof.

13. A viral vector according to any preceding claim, wherein the podocyte-specific promoter is selected from a NPHS1 promoter, a NPHS2 promoter, a WT1 promoter, a FOXC2 promoter, a ACTN4 promoter, a BMP7 promoter, a CD2AP promoter, a CDH3 promoter, a CDKN1B promoter, a CDKN1C promoter, a COL4A1 promoter, a COL4A2 promoter, a COL4A3 promoter, a COL4A4 promoter, a COL4A5 promoter, a CRIM1 promoter, a FAT1 promoter, a FOXD1 promoter, a KIRREL promoter, a LAMA1 promoter, a LAMA5 promoter, a LAMB1 promoter, a LAMB2 promoter, a LMX1B promoter, a MAFB promoter, a NES promoter, a NR2F2 promoter, a PODXL promoter, a PTPRO promoter, a SYNPO promoter, a TJP1 promoter, and a VEGFA promoter, or a fragment of derivative thereof.
14. A viral vector according to any preceding claim, wherein the podocyte-specific promoter is a NPHS1 promoter, a NPHS2 promoter, a WT1 promoter, or a FOXC2 promoter, or a fragment or derivative thereof.
15. A viral vector according to any preceding claim, wherein the podocyte-specific promoter is a NPHS1 or a NPHS2 promoter, or a fragment or derivative thereof.
16. A viral vector according to any preceding claim, wherein the podocyte-specific promoter is a minimal NPHS1 promoter or a minimal NPHS2 promoter, or a fragment or derivative thereof.
17. A viral vector according to any preceding claim, wherein the podocyte-specific promoter has at least 70% sequence identity, at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 5.
18. A viral vector according to any one of claims 1 to 16, wherein the podocyte-specific promoter consists of the nucleotide sequence of SEQ ID NO: 27.
19. A viral vector according to any preceding claim, wherein the inhibitor of the complement system is selected from the list consisting of CFI, CFH, FHL-1, C1INH, C4BP, CD55, CD35, CD46, CD59, vitronectin, and clusterin, or fragments or derivatives thereof.
20. A viral vector according to any preceding claim, wherein the inhibitor of the complement system is CFI, CFH, or FHL-1, or a fragment or derivative thereof.
21. A viral vector according to any preceding claim, wherein the inhibitor of the complement system has at least 70% sequence identity, at least 80% sequence identity, at

least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to SEQ ID NO: 11, SEQ ID NO: 13, or SEQ ID NO: 15.

22. A viral vector according to any preceding claim, wherein the inhibitor of the complement system comprises or consists of the polypeptide of SEQ ID NO: 11, SEQ ID NO: 13, or SEQ ID NO: 15.

23. A viral vector according to any preceding claim, wherein the nucleotide sequence encoding an inhibitor of the complement system has at least 70% sequence identity, at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to SEQ ID NO: 12, SEQ ID NO: 14, or SEQ ID NO: 16.

24. A viral vector according to any preceding claim, wherein the nucleotide sequence encoding an inhibitor of the complement system comprises or consists of the nucleotide sequence of SEQ ID NO: 12, SEQ ID NO: 14, or SEQ ID NO: 16.

25. A viral vector according to any preceding claim, wherein the nucleotide sequence encoding an inhibitor of the complement system is operably linked to a Woodchuck hepatitis post-transcriptional regulatory element (WPRE).

26. A viral vector according to any preceding claim, wherein the nucleotide sequence encoding an inhibitor of the complement system is operably linked to a polyadenylation signal.

27. A viral vector according to any preceding claim, wherein the nucleotide sequence encoding an inhibitor of the complement system is operably linked to a Kozak sequence.

28. An isolated cell comprising a viral vector according to any one of claims 1-27.

29. A pharmaceutical composition comprising a viral vector according to any one of claims 1-27 or an isolated cell according to claim 28, in combination with a pharmaceutically acceptable carrier, diluent or excipient.

30. A viral vector according to any one of claims 1-27, an isolated cell according to claim 28, or a pharmaceutical composition according to claim 29, for use as a medicament.

~~31. Use of a viral vector according to any one of claims 1-27, an isolated cell according to claim 28, or a pharmaceutical composition according to claim 29, for the manufacture of a medicament.~~

~~32. A method comprising administering a viral vector according to any one of claims 1-27, an isolated cell according to claim 28, or a pharmaceutical composition according to claim 29, to a subject in need thereof.~~

~~33. A viral vector according to any one of claims 1-27, an isolated cell according to claim 28, or a pharmaceutical composition according to claim 29, for use in preventing or treating a complement-mediated kidney disease.~~

3134. Use of a viral vector according to any one of claims 1-27, an isolated cell according to claim 28, or a pharmaceutical composition according to claim 29, ~~for~~ in the manufacture of a medicament for preventing or treating a complement-mediated kidney disease.

~~35. A method of preventing or treating a complement-mediated kidney disease comprising administering a viral vector according to any one of claims 1-27, an isolated cell according to claim 28, or a pharmaceutical composition according to claim 29, to a subject in need thereof.~~

~~3236. A viral vector, an isolated cell, or a pharmaceutical composition for use according to claim 33, The use of a viral vector, an isolated cell, or a pharmaceutical composition according to claim 3134, or a method according to claim 35, wherein the a complement-mediated kidney disease is IgA nephropathy, C3 glomerulopathy, atypical hemolytic uremic syndrome (aHUS), stx-associated HUS, lupus nephritis, cryoglobulinemia, anti-GBM disease, ANCA-associated vasculitis, bacterial endocarditis, post-infectious glomerulonephritis, antibody-mediated rejection of renal transplant, membranous nephropathy, membranoproliferative glomerulonephritis I, or membranoproliferative glomerulonephritis III.~~

~~3337. A viral vector, an isolated cell, or a pharmaceutical composition for use according to claim 33 or claim 36, The use of a viral vector, an isolated cell, or a pharmaceutical composition according to claim 3134 or claim 3236, or a method according to claim 35 or claim 36, wherein the complement-mediated kidney disease is IgA Nephropathy or C3 glomerulopathy, preferably wherein the C3 glomerulopathy is dense deposit disease or C3 glomerulonephritis.~~

3438. A viral vector, an isolated cell, or a pharmaceutical composition for use according to any one of claims 33, 36, 37, The use of a viral vector, an isolated cell, or a pharmaceutical composition according to any one of claims 3134, 3236, and 3337, or a method according to any one of claims 35 to 37, wherein said viral vector, said isolated cell, or said pharmaceutical composition is to be administered to a human subject.

~~3539. A viral vector, an isolated cell, or a pharmaceutical composition for use according to any one of claims 33, 36-38, The use of a viral vector, an isolated cell, or a pharmaceutical composition according to any one of claims 31-34, 34, 36-38, or a method according to any one of claims 35 to 38, wherein said viral vector, said isolated cell, or said pharmaceutical composition is to be administered systemically and/or by intravenous injection.~~

~~3640. A viral vector, an isolated cell, or a pharmaceutical composition for use according to any one of claims 33, 36-39, The use of a viral vector, an isolated cell, or a pharmaceutical composition according to any one of claims 31-35, 34, 36-39, or a method according to any one of claims 35 to 39, wherein said viral vector, said isolated cell, or said pharmaceutical composition is to be administered by injection into the renal artery or by ureteral or subcapsular injection.~~