In order to maximise gene therapy potential, optimal methods to delivery viral vectors to glomerular cells, such as podocytes, are required. However, the kidney is a difficult target for conventional intravenous delivery, since the permselectivity of the glomerulus will prevent entry of most molecular therapies from the blood into the kidney. Moreover, while various direct kidney injection methods have been tested with viral vector, such as adeno-associated viral (AAV) vectors, the efficiency of gene delivery is inconsistent and typically still too small to effectively treat disease (see e.g. Rubin, J.D. and Barry, M.A., 2020. Molecular diagnosis & therapy, 24(4), pp.375-396).

Thus, there is a demand for improved methods to deliver viral vectors to glomerular cells, such as podocytes.

SUMMARY

The present inventors have developed an improved method of delivering viral vectors to the kidney, in particular glomerular cells, such as podocytes, via direct renal artery injection. The method can be performed in a minimally invasive manner and with improved safety compared to prior art direct kidney injection methods.

Further, the present inventors have surprisingly shown that direct renal artery injection of a viral vector can result in a marked increase in transgene expression and viral copy number in the kidney, in particular glomeruli and podocytes, when compared to intravenous injection. Moreover, the direct renal artery injection of a viral vector can result in transgene expression that is localised to the kidney with minimal expression in other tissues (e.g. the liver).

In one aspect, the present disclosure provides a method of delivering a viral vector to a kidney, the method comprising inserting a catheter into the renal artery and delivering the viral vector into the renal artery via the catheter. In another aspect, the present disclosure refers to the use of a viral vector in the manufacture of a medicament for treating a human subject that has or is at risk of a kidney disease, wherein the viral vector is to be delivered to the human subject in a method comprising: (a) inserting a catheter into the renal artery of the kidney; (b) optionally inflating a balloon to occlude the renal artery; and (c) injecting or infusing the viral vector into the renal artery via the catheter, wherein the method is a minimally invasive procedure, wherein the method does not comprise a step of clamping the renal artery of the kidney, the renal vein of the kidney, or the aorta, and wherein the method does not comprise a step of inserting a catheter into the renal vein of the kidney.

The method may be a minimally invasive procedure. Suitably, the method does not comprise a step of occluding the renal vein. Suitably, the method does not comprise a step of inserting

a catheter into the renal vein and/or does not comprise a step of inserting a catheter directly into the aorta. Suitably, the method does not comprise forming a closed circuit through the kidney. Suitably, the method does not comprise a step of clamping the renal artery, the renal vein, or the aorta. Suitably, the total kidney ischemia time is about 60 minutes or less, about 50 minutes or less, about 40 minutes or less, about 30 minutes or less, about 10 minutes to about 30 minutes, about 15 to about 25 minutes, or about 5 minutes.

The catheter may be inserted into the renal artery by any suitable method. In preferred examples, the catheter is inserted into the renal artery via a percutaneous route.

Suitably, the percutaneous route is via the carotid artery or via the femoral artery. Suitably, insertion of the catheter via the percutaneous route is facilitated with a sheath. Suitably, the catheter is inserted into the renal artery via guidewire.

The viral vector may be delivered into the renal artery by any suitable method. Suitably, the viral vector is injected or infused into the renal artery. In some examples, the viral vector is infused into the renal artery under no flow conditions using an infusion pump. Suitably, the viral vector is delivered into the renal artery over about 1 minute to about 30 minutes. In some examples, the viral vector is delivered into the renal artery over about 1 minute to about 5 minutes, optionally about 2 minutes or about 4 minutes. In some examples, the viral vector is delivered into the renal artery over about 15 minutes to about 30 minutes or about 15 minutes to about 20 minutes, optionally about 17 minutes.

In some examples, the catheter is an occlusion balloon catheter. In some examples, the method comprises a step of inflating a balloon to occlude the renal artery. Suitably, the renal artery is occluded for from about 1 minute to about 25 minutes. In some examples, the renal artery is occluded for from about 2 minutes to about 10 minutes, optionally about 5 minutes. In some examples, the renal artery is occluded for from about 15 minutes to about 25 minutes, or from about 15 minutes to about 20 minutes, optionally about 20 minutes.

In one example, the present disclosure provides a method of delivering a viral vector to a subject's kidney, the method comprising:

(a) inserting a catheter into the renal artery of the kidney;

(b) optionally inflating a balloon to occlude the renal artery; and

(c) injecting or infusing the viral vector into the renal artery via the catheter,

wherein the method is a minimally invasive procedure, and wherein the method does not comprise a step of inserting a catheter into the renal vein of the kidney.

In one example, the present disclosure provides a method of delivering a viral vector to a subject's kidney, the method comprising: inserting a catheter into the renal artery of the kidney and injecting or infusing the viral vector into the renal artery via the catheter, wherein the method does not comprise a step of occluding the renal artery of the kidney or the renal vein of the kidney, and wherein the method does not comprise a step of clamping the aorta.

The method of the present disclosure may result in delivery of the viral vector to the kidney. For example, the method may result in delivery of the viral vector to the kidney cortex and/or kidney medulla, in particular the kidney cortex. The method may result in delivery of the viral vector to the kidney glomeruli. The method may result in delivery of the viral vector to the kidney podocytes. The method may result in kidney-specific delivery of the viral vector.

The subject may be any suitable subject. The subject may be a human subject. The human subject may be an adult, an adolescent, or a child. The subject may have or may be at risk of a kidney disease. The subject may have or may be at risk of a glomerular disease. The subject may have or may be at risk of a genetic glomerular disease, optionally wherein the subject has or is at risk of a podocyte-associated genetic glomerular disease.

The viral vector may be delivered in any suitable dose. Suitably, the viral vector is delivered in a dose of from about $1x10^6$ vg/kg to about $1x10^{14}$ vg/kg, or from about $1x10^6$ vg/kg to about $1x10^{13}$ vg/kg. In some examples, the viral vector is delivered in a dose of from about $1x10^9$ vg/kg to about $1x10^{12}$ vg/kg. In some examples, the viral vector is delivered in a dose of from about $3x10^9$ vg/kg to about $3x10^{11}$ vg/kg. Suitably, the viral vector is delivered in a dose of from about $1x10^8$ vg to about $1x10^{15}$ vg, or from about $1x10^8$ vg to about $5x10^{14}$ vg. In some examples, the viral vector is delivered in a dose of from about $1x10^{11}$ vg to about $1x10^{14}$ vg. In some examples, the viral vector is delivered in a dose of from about $2x10^{11}$ vg to about $2x10^{13}$ vg. In some examples, the viral vector is delivered in a dose of from about $5x10^{11}$ vg to about $2x10^{13}$ vg. In some examples, the viral vector is delivered in a dose of from about $1x10^{12}$ vg to about $2x10^{13}$ vg. In some examples, the viral vector is delivered in a dose of about $1x10^{13}$ vg.

The viral vector may be any suitable viral vector. Suitably, the viral vector is capable of transducing kidney cells, optionally wherein the vector is capable of specifically transducing kidney cells. Suitably, the viral vector is capable of transducing glomerular cells, optionally wherein the vector is capable of specifically transducing glomerular cells. Suitably, the viral vector is capable of transducing podocytes, optionally wherein the vector is capable of specifically transducing glomerular podocytes. Suitably the viral vector is selected from an adeno-associated virus (AAV) vector, a lentiviral vector, a retroviral vector, an adenoviral vector, a herpes simplex viral vector, an alphaviral vector, a flaviviral vector, a rhabdoviral vector, a measles viral vector, a Newcastle disease viral vector, a poxviral vector, and a picornaviral vector.

In preferred examples, the viral vector is an adeno-associated virus (AAV) vector particle. In some examples, the viral vector is the form of an AAV vector particle

encapsidated by LK03, AAV3B, or AAV9 capsid proteins. In some examples, the viral vector is the form of an AAV vector particle encapsidated by LK03 capsid proteins.

The viral vector may comprise any suitable protein-coding sequence. Suitably, the protein-coding sequence encodes a therapeutic protein, preferably wherein the protein-coding sequence encodes a polypeptide associated with a genetic glomerular disease, optionally a polypeptide involved in podocyte-associated genetic glomerular disease. In some examples, the protein-coding sequence encodes a COL4A3, COL4A4, COL4A5, NPHS2, CFH, CFL, FHL-1, C1INH, C4BP, MASP2, C3, C5aR1, C5, C5a, CD55, CD35, CD46, CD59, vitronectin, clusterin, ADCK4, ALG1, ARHGAP24, ARGHDIA, CD151, CD2AP, COQ2, COQ6, DGKE, E2F3, EMP2, KANK2, LAGE3, LMNA, LMX1B, MAF B, NUP85, NUP93, NXF5, OSGEP, PAX2, PDSS2, PMM2, PODXL, SCARB2, SGPL1, Smad7, TP53RK, TPRKB, VDR, WDR73, WT1, ZMPSTE24, APOL1, NPHS1, TRPC6, NUP107, NUP133, NUP160, ACTN4, INF2, ANKFY1, ANLN, CRB2, ITGA3, KANK1, KANK4, MAGI2, MYO1E, OCRL, PTPRO, SMARCAL1, SYNPO, TBC1D8B, XPO5, TNS2, NLRP3, or VEGFC polypeptide. In some examples, the protein-coding sequence encodes NPHS2 or a fragment and/or variant thereof; a COL4A3, COL4A4 or COL4A5 polypeptide, or a fragment or derivative thereof; or CFI, CFH or FHL-1, or a fragment and/or variant thereof. In some examples, the protein-coding sequence does not encode a gene-editing agent. In some examples, the protein-coding sequence does not encode a nuclease. In some examples, the protein-coding sequence does not encode a Cas9.

The protein-coding sequence may be operably linked to any promoter. In some examples, the protein-coding sequence is operably linked to a kidney-specific promoter. In some examples, the protein-coding sequence is operably linked to a podocyte-specific promoter. In some examples, the protein-coding sequence is operably linked to a NPHS1 promoter or a NPHS2 promoter. In some examples, the protein-coding sequence is operably linked to a minimal NPHS1 promoter. In some examples, the protein-coding sequence is operably linked to a constitutive promoter. In some examples, the protein-coding sequence is operably linked to a CMV promoter.

The protein-coding sequence may be operably linked to one or more further regulatory elements. Suitably, the protein-coding sequence is operably linked to a post-transcriptional regulatory element and/or a polyadenylation sequence. In some examples, the protein-coding sequence is operably linked to a Woodchuck hepatitis post-transcriptional regulatory element (WPRE). In some examples, the protein-coding sequence is operably linked a polyadenylation signal, for example a bovine growth hormone polyadenylation signal.

The viral vector may be in the form of a viral vector formulation. The viral vector formulation may comprise the viral vector in any suitable amount and may be formulated in any suitable manner. Suitably, the viral vector formulation comprises the viral vector in an amount of from about $1x10^7$ vg/ml to about $1x10^{14}$ vg/ml, or from about $1x10^7$ vg/ml to about $5x10^{13}$ vg/ml. In some examples, the viral vector formulation comprises the viral vector in an amount of from about $1x10^{10}$ vg/ml to about $1x10^{13}$ vg/ml. In some examples, the viral vector formulation comprises the viral vector in an amount of from about $1x10^{10}$ vg/ml to about $1x10^{12}$ vg/ml. Suitably, the viral vector formulation comprises an isotonic buffer, such as phosphate buffered saline (PBS) buffer or plasmalyte. In some examples, the viral vector formulation comprises about 0.001% poloxamer 188. Suitably, the viral vector formulation has a volume of from about 5 ml to about 50 ml, from about 5 ml to about 25 ml, or from about 10 ml to about 25 ml.

The viral vector may be delivered to a single kidney or both kidneys of the subject. In some examples, the method of the present disclosure is carried out once to deliver the viral vector to a single kidney of the subject. In some examples, the method of the present disclosure is carried out twice to deliver the viral vector to both kidneys of the subject.

In one aspect, the present disclosure provides a viral vector for use in therapy, wherein the viral vector is delivered by the method according to the present disclosure.

In one aspect, the present disclosure provides a viral vector for use in treating or preventing a kidney disease, wherein the viral vector is delivered by the method according to the present disclosure.

In one aspect, the present disclosure provides use of a viral vector for the manufacture of a medicament, wherein the medicament is delivered by the method according to the present disclosure.

In one aspect, the present disclosure provides use of a viral vector for the manufacture of a medicament for treating or preventing a kidney disease, wherein the medicament is delivered by the method according to the present disclosure.

DESCRIPTION OF DRAWINGS

Figure 1 – A schematic representation of an AAV vector encoding podocin under control of a hNPHS1 promoter

CLAIMS

1. Use of a viral vector in the manufacture of a medicament for treating a human subject that has or is at risk of a kidney disease, wherein the viral vector is to be delivered to the human subject in a method comprising:

(a) inserting a catheter into the renal artery of the kidney;

(b) optionally inflating a balloon to occlude the renal artery; and

(c) injecting or infusing the viral vector into the renal artery via the catheter,

wherein the method is a minimally invasive procedure, wherein the method does not comprise a step of clamping the renal artery of the kidney, the renal vein of the kidney, or the aorta, and wherein the method does not comprise a step of inserting a catheter into the renal vein of the kidney.

2. The use according to claim 1, wherein the method does not comprise a step of occluding the renal vein of the kidney.

3. The use according to any one of the preceding claims, wherein the method does not comprise a step of inserting a catheter directly into the aorta.

4. The use according to any one of the preceding claims, wherein the method does not comprise forming a closed circuit through the kidney.

5. The use according to any one of the preceding claims, wherein the catheter is an occlusion balloon catheter, and the method further comprises inflating the balloon to occlude the renal artery after inserting the catheter into the renal artery and before the injecting or infusing of the viral vector.

6. The use according to any one of the preceding claims, wherein the renal artery is to be occluded for from 1 minute to 25 minutes; from 2 minutes to 10 minutes, optionally about 5 minutes; from 15 minutes to 25 minutes; or from 15 minutes to 20 minutes, optionally about 20 minutes.

7. The use according to claim 1, wherein the method does not comprise a step of occluding the renal artery of the kidney.

8. The use according to any one of the preceding claims, wherein the catheter is to be inserted into the renal artery via a percutaneous route, optionally wherein the percutaneous route is

via the carotid artery or via the femoral artery and/or wherein insertion of the catheter via the percutaneous route is facilitated with a sheath.

9. The use according to any one of the preceding claims, wherein the viral vector is to be infused into the renal artery under no flow conditions using an infusion pump.

10. The use according to any one of the preceding claims, wherein the viral vector is to be injected or infused into the renal artery over 1 minute to 30 minutes, preferably (a) wherein the viral vector is to be injected or infused into the renal artery over 1 minute to 5 minutes, optionally about 2 minutes or 4 minutes; or (b) wherein the viral vector is to be injected or infused into the renal artery over 5 minutes to 30 minutes or 15 minutes to 20 minutes, optionally about 17 minutes.

11. The use according to any one of the preceding claims, wherein the subject has or is at risk of a glomerular disease, optionally wherein the subject has or is at risk of a podocyte-associated glomerular disease.

12. The use according to any one of the preceding claims, wherein the viral vector is to be delivered in a dose of from $1x10^8$ vg to $1x10^{15}$ vg, or from $1x10^8$ vg to $5x10^{14}$ vg.

13. The use according to any one of the preceding claims, wherein the viral vector is capable of transducing kidney cells, optionally wherein the vector is capable of specifically transducing kidney cells; or wherein the viral vector is capable of transducing glomerular cells, optionally wherein the vector is capable of specifically transducing glomerular cells.

14. The use according to any one of the preceding claims, wherein the viral vector is capable of transducing podocytes, optionally wherein the vector is capable of specifically transducing glomerular podocytes.

15. The use according to any one of the preceding claims, wherein the viral vector is selected from an adeno-associated virus (AAV) vector, a lentiviral vector, a retroviral vector, an adenoviral vector, a herpes simplex viral vector, an alphaviral vector, a flaviviral vector, a rhabdoviral vector, a measles viral vector, a Newcastle disease viral vector, a poxviral vector, and a picornaviral vector.

16. The use according to any one of the preceding claims, wherein the viral vector is an adeno-associated virus (AAV) vector particle.

17. The use according to any one of the preceding claims, wherein the viral vector comprises a protein-coding sequence, optionally wherein the protein-coding sequence is operably linked to a kidney-specific promoter, preferably a podocyte-specific promoter.

18. The use according to any one of the preceding claims, wherein the viral vector is to be injected or infused into the renal artery in the form of a viral vector formulation, optionally wherein the viral vector formulation comprises the viral vector in an amount of from $1x10^{7}$ vg/ml to $1x10^{14}$ vg/ml, or from $1x10^{7}$ vg/ml to $5x10^{13}$ vg/ml.