

Combination of a HB-EGF inhibitor and a corticosteroid agent for the treatment of kidney diseases associated with activation of the HB-EGF/EGFR pathway

FIELD OF THE INVENTION

The present invention relates to the treatment of kidney diseases associated with activation of the Heparin-Binding Epidermal Growth Factor-like growth factor (HB-EGF)/ Epidermal Growth Factor Receptor (EGFR) pathway and in particular to the use of a HB-EGF inhibitor in combination with a corticosteroid agent.

BACKGROUND OF THE INVENTION

The activation of the HB-EGF/EGFR pathway which is associated with increased expression of pro-HB-EGF and/or the release of HB-EGF leads to harmful cell proliferation responsible for numerous pathological conditions, in particular kidney diseases such as Crescentic glomerulonephritis (CGN) and other related diseases.

Crescentic glomerulonephritis is a rare kidney disease that destroys the kidney's filtration units, the glomeruli. CGN is due to various immuno-inflammatory aggressions, including anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV), anti-glomerular basement membrane (GBM) autoantibodies, systemic lupus erythematosus and immune complexes from infections (Couser et al., *Am. J. Kidney Dis.*, 1988, 11, 449-464; Jennette et al., *Nephrol. Dial. Transplant.*, 2001, 16, 80-82). Treatments are based solely on immunosuppression and up-to-date options combine rituximab (anti-CD20) and corticosteroids (CTC), and complement C5a receptor inhibitor Avacopan for AAV (Chevet et al., *Rheumatology (Oxford)*, 2022, doi:10.1093/rheumatology/keac623). However, CGN remains an unmet medical need. Mortality of CGN patients is up to 20% within one year (Heijl et al., *RMD Open*, 2017, 3, e000435; Wallace et al., *Semin. Arthritis Rheum.*, 2016, 45, 483-489) and 90% of patients have chronic kidney failure with up to 70% risk of end-stage renal disease (Chen et al., *Am. J. Kidney Dis.*, 2016, 67, 376-383).

CGN is characterized by the dedifferentiation and the proliferation of the cells constituting the structure of the glomeruli: the podocytes and the parietal epithelial cells (PECs) (Bollée et al., *Nat. Med.*, 2011, 17, 1242-; Lazareth et al., *Nat. Commun.*, 2019, 10; Shankland et al., *Nature Reviews Nephrology*, 2014, 10, 158-173). The podocytes are wrapped around the

glomerular capillaries and participate in the integrity of the blood/urine filtration barrier. The PECs line the bowman capsule at the circumference of the glomeruli. Upon immune aggressions leading to CGN, podocytes and PECs overexpress the growth factor HB-EGF (Heparin-Binding EGF-like Growth Factor) (Bollée et al., *Nat. Med.*, 2011, 17, 1242-1250; 5 Flamant et al., *Nephrology Dialysis Transplantation*, 2012, 27, 1297-1304). HB-EGF binds to the EGF receptor (EGFR). The EGFR is phosphorylated and activates STAT3 signaling. As a result, podocytes and PECs undergo dedifferentiation and proliferate. This leads to a progressive obliteration of the glomeruli. Mice knocked out for the *Hb-egf* gene or with selective *Egfr* gene or *Stat3* gene targeting in podocytes were shown to be resistant to the 10 development of CGN despite immune aggression (Bollée et al., *Nat. Med.*, 2011, 17, 1242-1250; Dai et al., *Kidney Int.*, 2013, 84, 950-961, Henique et al. *Nat Commun.*, 2017, 8(1):1829). The diagnosis of CGN relies on clinical symptoms (asthenia, weakness, fever, nausea, vomiting, anorexia, arthralgia, abdominal pain and high blood pressure), a nephritic syndrome with hematuria, high proteinuria, hypoalbuminemia, high plasma creatinine and urea nitrogen 15 levels and a progressive kidney failure over days to months (O'Brien, F. Rapidly Progressive Glomerulonephritis (RPGN) (Crescentic Glomerulonephritis). *MSD Manual Professional Version*. 2022). The disease evolves towards severe oliguria. Serology may identify the causative antibodies (ANCA, anti-GBM, anti-DNA histones, anti-streptolysin O...). Histology of kidney biopsies reveal the classical crescentic glomerular lesions. The 20 proliferation of podocytes and PECs produces a crescent-shaped formation that progressively invades the glomeruli. Ultimately, crescentic glomeruli may lead to scarring lesions such as focal and segmental glomerulosclerosis (FSGS) in which collagen deposits replace crescentic cells. Interestingly, upon early diagnosis, a majority of glomeruli are still healthy and functional while their PECs and podocytes are already positive for HB-EGF expression 25 (Bollée et al., *Nat. Med.*, 2011, 17, 1242-1250). The involvement of HB-EGF in PECs and podocytes pathogenic dedifferentiation, migration, and proliferation has been demonstrated (Bollée et al., *Nat. Med.*, 2011, 17, 1242-1250, Lazareth et al. *Nat Commun.* 2019;10(1):3303). This suggests early treatment with HB-EGF inhibitors may rescue and preserve these healthy glomeruli and kidney function (Flamant et al., *Nephrology Dialysis Transplantation*, 2012, 30 27, 1297-1304).

The activation of the HB-EGF/EGFR pathway was also shown to be involved in other kidney diseases such as Diabetic nephropathy (Miyazama et al., *Kidney International*, 2013, 84,

1176-1188), Autosomal dominant polycystic kidney disease, Renal tubulointerstitial fibrosis (Overstreet et al., *FASEB*, 2017, 10, 4407-4421), Chronic kidney disease and Hypertensive nephropathy (Zeng et al., *American Journal of Physiology - Renal Physiology*, 2016, 311, F695-F707).

- 5 Thus, HB-EGF appears as a potential new target for the treatment of CGN (Bollée et al., *Nat. Med.*, 2011, 17, 1242-1250; Flamant et al., *Nephrology Dialysis Transplantation*, 2012, 27, 1297-1304) and other kidney diseases involving the activation of the HB-EGF/EGFR pathway.

There are currently two types of HB-EGF inhibitors that can be used for blocking EGFR stimulation by HB-EGF: antibodies directed against HB-EGF and ligands of HB-EGF derived
10 from diphtheria toxin (DT), a natural ligand of HB-EGF (Gillet & Barbier, Diphtheria toxin. in *The Comprehensive Sourcebook of Bacterial Protein Toxins* 111-132 (Academic Press, 2015). doi:10.1016/B978-0-12-800188-2.00004-5). HB-EGF blocking antibodies are disclosed in WO 2009/040134; WO 2011/21381; WO 2009/72628; EP 2221374; EP 2281001; EP 2093237; EP2078731; EP 2039704; and WO 2008/053270. CRM197 is a natural mutant
15 of DT proposed for ovarian cancer treatment (US 7 700 546; US 2006/0270600). Synthetic mutants of DT cell receptor-binding domain (R or DTR) engineered to have a smaller size, a better affinity for HB-EGF, and reduced antigenicity, immunogenicity and toxicity, compared with CRM197 are disclosed in WO 2013/140335.

Up-to-date treatment of CGN relies mainly on strong immunosuppression with CTC, and anti-
20 CD20 (rituximab) or cyclophosphamide or other antimitotic drugs (O'Brien et al., *MSD Manual Professional Version.*, 2022; Yates et al., *Annals of the Rheumatic Diseases*, 2016, 75, 1583-1594). Immunosuppression aims at reducing circulating auto-antibodies or/and immune complexes responsible for glomerular deposits in the glomeruli. Immunosuppression may also decrease the periglomerular infiltration of immune cells, which participates in
25 immune aggression. However, although a maintenance treatment of prednisone at 7.5-10 mg/day is recommended, the initial treatment of patients upon diagnosis involves methylprednisolone 1 g IV once a day for 3 to 5 days followed by prednisone 1 mg/kg orally once a day with slow decrease over 5 months (O'Brien et al., *MSD Manual Professional Version.*, 2022; Yates et al., *Annals of the Rheumatic Diseases*, 2016, 75, 1583-1594; Walsh
30 et al. *N Engl J Med.* 2020;382(7):622-631; Furuta et al. *Ann Rheum Dis.* 2024;83(1):96-102.) (**Figure 1**). These high starting doses of CTC are recommended for the acute phase of CGN

and relapse. These highly aggressive dosages are responsible for serious adverse effects (Stone et al., *Semin. Arthritis Rheum.*, 2022, 55, 152010) such as infections, myopathy, neuropsychic and skin toxicities, leading to morbidity, low quality of life, and explaining the high rate of mortality of patients in the first years of treatment (10-20 %) (Heijl et al., *RMD*
5 *Open*, 2017, 3, e000435; Wallace et al., *Semin. Arthritis Rheum.*, 2016, 45, 483-489). As a result, current treatments for CGN are unsatisfactory and the disease remains an unmet medical need.

Thus, there remains a need for improved treatment of GCN and related kidney diseases associated with the activation of the HB-EGF/EGFR pathway.

10 BRIEF SUMMARY OF THE INVENTION

Using a pig model of Crescentic glomerulonephritis (CGN), the inventors have demonstrated that an inhibitor of HB-EGF (DTR8) allows to reduce the high starting dose of corticosteroids used in acute phase or relapse and can reduce the progression of CGN glomerular lesions and the degradation of kidney function. These results provide an alternative treatment of GCN and
15 other kidney diseases associated with activation of the HB-EGF/EGFR pathway using a combination of a HB-EGF inhibitor and a corticosteroid agent. The DTR8 inhibitor potentializes the effect of the corticosteroid agent. Therefore, the combination therapy advantageously allows the use of a reduced dose of corticosteroid to avoid the deleterious effects associated with a high corticosteroid dose while at the same time providing an efficient
20 treatment of the disease as evidenced by its ability to limit the progression of the glomerular lesions and the degradation of kidney function.

Therefore, the present invention relates to a combination of a corticosteroid agent and an inhibitor of Heparin-Binding Epidermal Growth Factor-like growth factor (HB-EGF) for use in the treatment of kidney diseases associated with the activation of the HB-EGF/EGF receptor
25 pathway.

In some embodiments, the combination therapy comprises a reduced dose of corticosteroid agent compared to the starting dose of corticosteroid agent used in a reference.

In some embodiments, the combination therapy is for the treatment of an acute phase or relapse of said diseases.

In some embodiments, the combination therapy is for the treatment of Crescentic glomerulonephritis; preferably for the treatment of an acute phase or relapse of Crescentic glomerulonephritis.

In some embodiments, the combination therapy reduces the severity of the glomerular lesions
5 and/or reduces the degradation of kidney function.

In some embodiments, the combination therapy comprises an HB-EGF inhibitor which is a recombinant protein comprising an isolated R domain of the diphtheria toxin comprising at least the substitutions: L390T and Y380K; L390T and Y380E ; Y380K and Q387E; or Y380E and Q387K relative to the amino acid sequence of SEQ ID NO: 1. In some particular
10 embodiments, the recombinant protein further comprises at least one substitution selected from the group consisting of: F389Y, A395T, N399K, T436H V452T, V483Q, H492E, S494K, E497D, G510A and T517E relative to SEQ ID NO: 1. In some more particular embodiments, the recombinant protein comprises the substitutions Y380K, Q387E, L390T, A395T, F389Y, G510A, N399K, V452T, T517E, V483Q, H492E and S494K relative to SEQ ID NO: 1. In
15 some more particular embodiments the recombinant protein comprises an amino acid sequence having at least 70% identity with residues 380 to 535 of SEQ ID NO: 1. In some preferred embodiments, the recombinant protein comprises the amino acid sequence SEQ ID NO: 2.

In some embodiments, the combination therapy comprises a corticosteroid agent which is
20 selected from the group consisting of: prednisolone, betamethasone, prednisone, triamcinolone, methylprednisolone, dexamethasone, and mixtures thereof.

In some particular embodiments, the combination therapy comprises a reduced dose of corticosteroid agent which is of less than 1 mg/kg per day.

In some embodiments, the combination therapy comprises a dose of HB-EGF inhibitor of 0.6
25 mg/kg per day.

In some particular embodiments, the combination therapy comprises the administration of the reduced dose of corticosteroid agent and/or dose of HB-EGF inhibitor every day for at least 3 weeks.

In some particular embodiments, the combination therapy comprises the administration of the reduced dose of corticosteroid agent and/or dose of HB-EGF inhibitor every day for at most 6 months, preferably at most 5 months, preferably at most 4 months, even more preferably at most 3 months.

- 5 In some embodiments, the combination therapy comprises the administration of the HB-EGF inhibitor and corticosteroid agent, simultaneously or separately.

In some embodiments, the combination therapy comprises the administration of the HB-EGF inhibitor and/or corticosteroid agent orally or by injection, in particular intravenous or subcutaneous.

- 10 Another aspect of the invention relates to a product containing a corticosteroid agent and an inhibitor of Heparin-Binding Epidermal Growth Factor-like growth factor (HB-EGF) as a combined preparation for simultaneous, separate or sequential use in the treatment of kidney diseases associated with the activation of the HB-EGF/EGF receptor pathway according to the present disclosure.
- 15 The present invention also relates to a pharmaceutical composition for treating kidney diseases associated with the activation of the HB-EGF/EGF receptor pathway comprising a HB-EGF inhibitor and a reduced dose of corticosteroid agent, according to the present disclosure.

DETAILED DESCRIPTION OF THE INVENTION

- The present invention relates to a combination of a corticosteroid agent and an inhibitor of
- 20 Heparin-Binding Epidermal Growth Factor-like growth factor (HB-EGF) for use in the treatment of kidney diseases associated with the activation of the HB-EGF/EGFR pathway. The invention encompasses a product containing a corticosteroid agent and an inhibitor of Heparin-Binding Epidermal Growth Factor-like growth factor (HB-EGF) as a combined preparation for simultaneous, separate or sequential use in the treatment of kidney diseases
- 25 associated with the activation of the HB-EGF/EGF receptor pathway according to the present disclosure. The invention also encompasses a pharmaceutical composition for treating kidney diseases associated with the activation of the HB-EGF/EGF receptor pathway comprising an HB-EGF inhibitor and a reduced dose of corticosteroid agent, according to the present disclosure. The combination therapy according to the invention is advantageously effective

using a reduced dose of corticosteroid agent compared with the high starting dose used in the acute phase or relapse of said diseases. Therefore, the combination therapy avoids the deleterious effects associated with current corticosteroid therapies for said diseases.

HB-EGF Inhibitors

- 5 Heparin-Binding Epidermal Growth Factor-like growth factor (HB-EGF) is a growth factor expressed at the surface of the cells of the organism in the form of a precursor membrane protein, pro-HB-EGF, which is the natural diphtheria toxin receptor (Gillet & Barbier, Diphtheria toxin. in *The Comprehensive Sourcebook of Bacterial Protein Toxins* 111–132 (Academic Press, 2015). doi:10.1016/B978-0-12-800188-2.00004-5). During inflammatory
- 10 processes involving HB-EGF, the pro-HB-EGF membrane protein is cleaved by a protease and HB-EGF is released from the cell surface, in the form of a secreted protein. It then binds in an autocrine or paracrine manner to the ErbB1 (HER1 or EGFR) and ErbB4 (HER4) subunits of the EGF receptor family (EGFR family or EGFR), expressed in virtually all the tissues of the organism. There are at least ten EGFR ligands in addition to HB-EGF, namely
- 15 EGF, TGF- α , amphiregulin, betacellulin, epigen, epiregulin and neuregulins -1, -2, -3 and -4. The local context determines the stimulation of EGFR by one or the other of these ligands.

An HB-EGF inhibitor refers to an agent able to block EGFR stimulation by HB-EGF, i.e., inhibit the HB-EGF/EGFR pathway. Inhibition of the HB-EGF pathway may be determined by standard assays that are well-known in the art, for example by measuring the inhibition of

20 proliferation activity induced by HB-EGF in a HB-EGF dependent cell line as disclosed in WO 2013/140335. Various HB-EGF inhibitors that are well-known in the art may be used in the combination therapy according to the invention.

“a”, “an”, and “the” include plural referents, unless the context clearly indicates otherwise. As such, the term “a” (or “an”), “one or more” or “at least one” can be used interchangeably

25 herein; unless specified otherwise, “or” means “and/or”.

A first type of HB-EGF inhibitors are antibodies directed against HB-EGF. Such HB-EGF blocking antibodies are disclosed in WO 2009/040134; WO 2011/21381; WO 2009/72628; EP 2221374; EP 2281001; EP 2093237; EP2078731; EP 2039704; and WO 2008/053270. The expression “antibodies” as used herein encompass antigen-binding fragments of

30 antibodies.

Another type of HB-EGF inhibitors are ligands of HB-EGF derived from diphtheria toxin (DT), a natural ligand of HB-EGF. Diphtheria toxin (DT) is an exotoxin of 535 amino acids (SEQ ID NO: 1) composed of a fragment A (N-terminal) and of a fragment B (C-terminal) (Gillet & Barbier, Diphtheria toxin, in *The Comprehensive Sourcebook of Bacterial Protein*
5 *Toxins* 111–132 (Academic Press, 2015). doi:10.1016/B978-0-12-800188-2.00004-5. Fragment A comprises the catalytic domain (C or DTA/DT-A; residues 1 to 193) and fragment B comprises the translocation domain (T; N-terminal; residues 202 to 378), and the cell receptor-binding domain (R or DTR; C-terminal; residues 379-386 to 535). DT binds to the surface of cells via the binding of its R domain to pro-HB-EGF; after activation of DT by
10 proteolytic cleavage between fragments A and B, the T domain allows translocation of fragment A into the cytoplasm. Once in the cytoplasm, the catalytic domain carried by fragment A blocks protein synthesis by inactivating the EF2 elongation factor, thus causing cell death. The study of natural or synthetic mutants of DT has shown that the binding of DT to the pro-HB-EGF receptor is abolished by the substitution of residue S508 (S508F) or of
15 residues of the loop of the region K516-F530 of DTR (K516A, K516E, F530A, F530S and S525F), but not by the deletion of the 4 C-terminal residues (residues E532-S535; Shen et al., *J. Biol. Chem.*, 1994, 269, 29077-29084).

CRM197 is a natural mutant of DT comprising the G52E mutation, that has greatly reduced catalytic activity (US 7 700 546; US 2006/0270600; Giannini et al., *N.A.R.*, 1984, 12, 4063-
20 4069).

Mutants of the isolated DTR domain, devoid of fusion-partner sequences are disclosed in WO 2013/140335. These mutants of the isolated DTR domain are expressed directly and in a large amount in the form of a soluble recombinant DTR protein of small size (approximately 17500 Da) with affinity for pro-HB-EGF and HB-EGF. These DTR mutants have been modified to
25 produce improved recombinant DTR proteins which, surprisingly, have both a reduced antigenicity and immunogenicity and a considerably increased affinity, compared with CRM197.

In some embodiments, the HB-EGF inhibitor for the combination therapy according to the invention is a recombinant protein comprising the substitution of at least one, preferably at
30 least two, of the residues Y380, P382, Q387, P388 and/or L390 of said R domain with another amino acid selected from the group consisting of: S, T, N, C, Y, Q, R, K, H, D and E.

The amino acids are denoted using the one-letter code. The positions are indicated by alignment with wild-type diphtheria toxin of SEQ ID NO: 1.

In the present application, the term "DTR" or "DTR domain" is intended to mean the R domain of diphtheria toxin which corresponds to residues 380-385 to 531-535 of the amino acid sequence of wild-type diphtheria toxin (SEQ ID NO: 1). The expression "DTR protein" denotes a recombinant protein comprising an isolated DTR domain, i.e. which is devoid, at its N- and C-terminal end, of the sequence of the T domain or the C domain and the T domain of diphtheria toxin and of the sequence of a protein or protein domain capable of improving the stability or the purification of said R domain. The recombinant protein which comprises at least one, preferably at least two, substitution(s) as defined above and, is denoted mutant DTR protein, mutated DTR protein, as opposed to the wild-type DTR recombinant protein (DTR_{WT}) which does not comprise this substitution.

The similarity of an amino acid sequence compared with a reference sequence is assessed according to the percentage of amino acid residues which are identical or which differ via conservative substitutions, when the two sequences are aligned so as to obtain the maximum correspondence between them. When only the identical residues are considered and the percentage of identical residues is determined, reference is then made to the identity of said amino acid sequence relative to the reference sequence. For the purpose of the present invention, the expression "conservative substitution in the amino acid sequence of a protein" is intended to mean the substitution of one amino acid with another natural or synthetic amino acid which has similar chemical or physical properties (size, charge or polarity), which do not have a deleterious effect on the biological activity of the protein. Thus, two amino acid sequences of a protein are similar when they differ from one another by the substitution of an amino acid, or the deletion and/or insertion of an amino acid or of a small number of amino acids (generally less than 5) at positions which do not have a deleterious effect on the biological activity of said protein. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways known to a person of skill in the art, for instance using publicly available computer software such as the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin) pileup program, or any of sequence comparison algorithms such as BLAST (Altschul *et al.*, *J. Mol. Biol.*, 1990, 215, 403-410), FASTA or CLUSTALW. When using such software, the default parameters, are preferably used, on a comparison window consisting of residues 380 to 535

of the amino acid sequence SEQ ID NO: 1. The BLASTP program uses as default a word length (W) of 3 and an expectation (E) of 10.

In some more particular embodiments, the inhibitor of HB-EGF for the combination therapy according to the invention is a recombinant protein comprising an amino acid sequence comprising an isolated R domain of the diphtheria toxin comprising at least the substitutions: 5 Y380K and L390T; Y380K and Q387E; Y380E and L390T or Y380E and Q387K relative to the amino acid sequence of SEQ ID NO: 1; preferably comprising at least the substitutions Y380K or Y380E and L390T relative to the amino acid sequence of SEQ ID NO: 1. Preferably, the recombinant protein further comprises at least one substitution selected from the group 10 consisting of: F389Y, A395T, N399K, T436H V452T, V483Q, H492E, S494K, E497D, G510A and T517E relative to SEQ ID NO: 1. Preferably, the recombinant protein comprises an amino acid sequence having at least 70% identity with residues 380 to 535 of SEQ ID NO: 1.

In some preferred embodiments, the inhibitor of HB-EGF for the combination therapy 15 according to the invention is a recombinant protein comprising the substitutions Y380K, Q387E, L390T, A395T, F389Y, G510A, N399K, V452T, T517E, V483Q, H492E and S494K relative to SEQ ID NO: 1. Preferably, the recombinant protein comprises the amino acid sequence SEQ ID NO: 2. This protein is called DTR8. It has a molecular weight of 17327 Da.

These recombinant DTR proteins which are ligand of HB-EGF and of pro-HB-EGF have the 20 following advantages:

- they have greatly increased solubility compared with the wild-type form DTR_{WT}. The substitution of at least one, preferably at least two, of the residues Y380, P382, Q387, P388 and/or L390 with a different hydrophilic, polar or charged amino acid residue makes it possible to considerably increase the solubility of the DTR protein in aqueous solution. Thus, 25 they can be extracted from the host cells and purified without using detergents or chaotropic or denaturing agents. By comparison, the wild-type DTR protein (DTR_{WT}) produced in the same expression system is insoluble and not solubilized using detergents compatible with therapeutic use. The DTR_{WT} protein is solubilized in the presence of 0.5% of sarkosyl or sodium dodecyl sulfate, which are detergents that are incompatible with therapeutic use at 30 these concentrations.

- they are much easier to produce in recombinant form than DTR_{WT}. They are produced directly, without using a fusion partner for improving the stability or the purification of the R domain. They may be produced in *E. coli* according to standard fermentation procedures, in a folded soluble form, and in a large amount after final purification (several tens of mg/l of culture under nonoptimized laboratory conditions).
- they have an affinity for HB-EGF and pro-HB-EGF which is at least 10 times greater than that of CRM197. Surprisingly, although the wild-type DTR protein (DTR_{WT}) has an affinity for HB-EGF and pro-HB-EGF which is 2-fold lower than that of CRM197, the mutant DTR proteins have an affinity for HB-EGF and pro-HB-EGF that is considerably higher than that of CRM197; the protein called DTR8 (SEQ ID NO: 2) has an affinity which is 1400 times higher than that of CRM197. This means that DTR8 could be used at doses much lower than CRM197 for the same therapeutic effect. As a result, the risks of side effects, linked in general to the existence of low-affinity binding with other proteins or ligands of the organism, are therefore considerably reduced by comparison with CRM197. These interactions are eliminated by using low doses, of the order of pM concentrations, which should be the case for DTR8.
- they have a reduced immunogenicity compared with CRM197 and with DTR_{WT}. Ten of the 26 CD4-T cell epitopes identified in DTR_{WT} have been deleted from the DTR8 protein by site-directed mutagenesis, including 7 among 9 immunodominant epitopes.
- they have a significantly reduced antigenicity compared with CRM197 and with DTR_{WT}. The sera of individuals vaccinated against diphtheria toxin preferentially recognize the catalytic domain of said toxin. This domain is present in the CRM197 molecule and absent from the mutated DTR protein according to the invention. Moreover, the mutated DTR protein according to the invention is not as well recognized as DTR_{WT} by the antibodies of vaccinated subjects that recognize DTR_{WT}.
- they block a pathway of EGFR (ErbB1 and ErbB4) activation by HB-EFG much more specifically than the commercial EGFR inhibitors (therapeutic antibodies, nanobodies and small molecules), as a result presenting potentially much less risk of side effects.
- they are small in size; they have a sequence of 157 amino acids and a MW of approximately 17 500 Da, i.e. a size 3.4 times smaller than that of CRM197 and 8.8 times smaller than that

of the anti-HB-EGF antibodies currently used in clinical protocols for blocking the HB-EGF pathway. As a result of its small size, the recombinant protein is more effective in the treatment of kidney diseases associated with the activation of the HB-EGF pathway since it diffuses more readily in the tissues and is capable of crossing the glomerular filtration barrier
5 thereby penetrating into kidney glomeruli.

In some embodiments of the invention, the combination comprises a nucleic acid molecule encoding the inhibitor.

The nucleic acid may be recombinant, synthetic or semi-synthetic nucleic acid which is expressible in a subject cell for protein production *in vivo*. The nucleic acid may be DNA,
10 RNA, or mixed molecule, either single- and/or double-stranded which may further be modified and/or included in any suitable expression vector. The nucleic acid may comprise a coding sequence which is optimized for the cell in which the protein is expressed. As used herein, the terms "vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host
15 and promote expression (e.g. transcription and translation (DNA) or translation (RNA)) of the introduced sequence. An expression vector as defined herein is suitable for expression of proteins in a subject cell, in particular suitable for gene therapy. Such vectors that are well-known in the art include viral and non-viral vectors. Non-viral vectors include plasmids. Viral vectors include for example adenovirus, adeno-associated virus, lentivirus and others.

20 In some more particular embodiments, the combination comprises an expression vector comprising said nucleic acid molecule.

Corticosteroid agent

In the present invention, the terms "corticosteroid agent" or "corticoid agent" are used interchangeably and refer to corticosteroid drugs. Non-limiting examples of corticosteroid
25 agent that can be used in the combination therapy of the invention include: prednisolone, betamethasone, prednisone, triamcinolone, methylprednisolone, dexamethasone, their derivatives and mixtures thereof.

In some embodiments, the corticosteroid agent for the combination therapy according to the invention is selected from the group consisting of: prednisolone, betamethasone, prednisone,
30 triamcinolone, methylprednisolone, dexamethasone, their derivatives and mixtures thereof. In

some particular embodiments, the corticosteroid agent comprised in the combination for use according to the invention is prednisolone or prednisone.

Combination therapy

As used herein, the term "a combination", "therapeutic combination", "combined
5 administration" or "combination therapy" in the context of the invention refers to the administration of a corticosteroid agent and an inhibitor of HB-EGF to a patient for therapeutic benefit.

"Treatment", or "treating" as used herein, is defined as the application or administration of the combination of a corticosteroid agent and an inhibitor of HB-EGF to a patient, or the
10 application or administration of said therapeutic combination to an isolated tissue or cell line from a patient, who has a kidney disease involving the activation of the HB-EGF/EGFR pathway with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect said disease, or any symptom of the disease. The term "treatment" or "treating" is also used in the context of administering the therapeutic agents prophylactically. In particular,
15 the terms "treat" or "treatment" refer to reducing or alleviating at least one adverse clinical symptom associated with a kidney disease involving the activation of the HB-EGF/EGFR pathway. The adverse clinical symptom is in particular nephritic syndrome and kidney failure. The nephritic syndrome comprises in particular hematuria, high proteinuria, hypoalbuminemia, high blood creatinine and urea nitrogen.

20 In some embodiments, the terms "treat" or "treatment" refer to slowing or reversing the progression of glomerular lesions and/or reducing the severity of glomerular lesions. The progression of glomerular lesions or severity of glomerular lesions may be determined by standard assays that are well-known in the art and disclosed in the example of the application such as the percentage of glomeruli obliterated by fibrosis (grade 4 FSGS lesions). Treatment
25 with the combination reduces the percentage of glomeruli obliterated by fibrosis compared with untreated controls or controls treated with corticosteroid agent alone or HB-EGF inhibitor alone (**Figure 2**).

In some embodiments, the terms "treat" or "treatment" refer to slowing or reversing the degradation of kidney function. The progression of glomerular lesions or severity of
30 glomerular lesions may be determined by standard assays that are well-known in the art and

disclosed in the example of the application such as blood markers of renal function, in particular blood chemistry markers of urinary function: creatininemia, blood urea nitrogen, albuminemia, and combination thereof. The percentage of individuals with normal blood markers of renal function is increased by treatment with the combination compared with
5 vehicle controls or controls treated with corticosteroid agent alone or HB-EGF inhibitor alone (**Figure 3**). Similarly, the combination treatment reduces the decrease of albuminemia due to the disease more than the DTR8 or corticosteroid treatment alone, or the vehicle control (**Figure 4**).

As used herein, the term “patient”, “individual” or “subject” includes human and other
10 mammalian subjects that receive either prophylactic or therapeutic treatment. Preferably, a patient, individual or subject according to the invention is a human.

The combination therapy according to the invention may be used for treating various kidney diseases associated with (or involving) the activation of the HB-EGF/EGFR pathway, in particular selected from the group consisting of: Crescentic glomerulonephritis (CGN) and
15 related disease such as focal and segmental glomerulosclerosis (FSGS), Diabetic nephropathy, Autosomal dominant polycystic kidney disease (ADPKD), Renal interstitial fibrosis (IF), chronic kidney disease (CKD), Hypertensive Arteriolar Nephrosclerosis (HTN); more preferably Crescentic glomerulonephritis (CGN) and related disease such as focal and segmental glomerulosclerosis (FSGS).

20 The diseases “Crescentic glomerulonephritis” (CGN) and “Rapidly Progressive Glomerulonephritis” (RPGN) are used indifferently in the present application to refer to the same disease (MSD Manual). CGN is characterized by crescentic glomeruli. When collagen deposits replace crescentic glomeruli, crescents are called “sclerotic”, or the disease is called focal and segmental glomerulosclerosis (FSGS). Therefore, FSGS is an advanced form of
25 CGN (or a CGN-related disease) that is included in the present invention.

The combination therapy may be used for the maintenance treatment, or for treatment of an acute phase or relapse of said diseases such as Crescentic glomerulonephritis (CGN).

In some embodiments, the combination therapy is used for the treatment of an acute phase or relapse of said diseases; preferably for the treatment of an acute phase or relapse of Crescentic
30 glomerulonephritis (CGN).

Preferably, treatment with the combination according to the invention reduces the severity of the glomerular lesions and/or reduces the degradation of kidney function.

The HB-EGF inhibitor and corticosteroid agent may be in the same pharmaceutical composition or in separate compositions. In the various embodiments of the present invention,
5 the pharmaceutical composition or combination of pharmaceutical compositions comprise a therapeutically effective amount of HB-EGF inhibitor and corticosteroid agent. In the context of the invention a therapeutically effective amount refers to a dose sufficient for reversing, alleviating or inhibiting the progress of the disorder or condition to which such term applies, or reversing, alleviating or inhibiting the progress of one or more symptoms of the disorder or
10 condition to which such term applies. The term "effective dose" or "effective dosage" is defined as an amount sufficient to achieve, or at least partially achieve, the desired effect.

The effective dose is determined and adjusted depending on factors such as the composition used, the route of administration, the physical characteristics of the individual under consideration such as sex, age and weight, concurrent medication, and other factors, that those
15 skilled in the medical arts will recognize.

In the various embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier and/or vehicle.

A "pharmaceutically acceptable carrier" refers to a vehicle that does not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as
20 appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

Preferably, the pharmaceutical composition contains vehicles, which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (sodium phosphate, sodium, potassium, calcium or magnesium
25 chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or suspensions. The solution or suspension may comprise additives which are compatible with corticosteroids, proteins, nucleic acids and vectors. In all
30 cases, the form must be sterile and must be fluid to the extent that easy syringe ability exists.

It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. An example of an appropriate solution is a buffer, such as phosphate-buffered saline (PBS) or Ringer lactate.

- 5 The therapeutic combination may comprise at least another therapeutic agent for treating the disease such as anti-inflammatory, immunosuppressor or other therapeutic agents. Particular examples of said other therapeutic agents include cyclophosphamide; methotrexate; mycophenolate mofetil; anti-CD20 such as rituximab; complement C5a receptor inhibitor such as avacopan; and others.
- 10 The therapeutic combination of the present invention is generally administered according to known procedures, at dosages and for periods of time effective to induce a therapeutic effect in the patient. Administration of a combination according to the invention can be effected by any method that enables delivery of the compounds to the site of action. The HB-EGF inhibitor and corticosteroid agent may be administered in either single or multiple doses by
- 15 any of the accepted modes of administration of agents having similar utilities. The pharmaceutical composition may be administered by any convenient route, such as in a non-limiting manner by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.). The administration can be systemic, local or systemic combined with local; systemic includes
- 20 parenteral and oral, and local includes local and loco-regional. Systemic administration is preferably parenteral such as subcutaneous (SC), intramuscular (IM), intravascular such as intravenous (IV) or intraarterial; intraperitoneal (IP); intradermal (ID) or else. In some preferred embodiments, the administration is oral or parenteral; parenteral is preferably intravascular, intramuscular or subcutaneous.
- 25 The use of the term "in combination" does not restrict the order in which the therapies (e.g., a corticosteroid agent and an inhibitor of HB-EGF) are administered to a subject. A therapy can be administered prior to (e.g., 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or
- 30 subsequent to (e.g., 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks,

4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy to a patient which has a disease associated with the activation of the HB-EGF/EGFR pathway. The therapies are administered to a patient in a sequence and within a time interval such that the therapies can act together. In a particular embodiment, the therapies are administered to a
5 subject in a sequence and within a time interval such that they provide an increased benefit than if they were administered otherwise. Any additional therapy can be administered in any order with the other additional therapy.

In some embodiments, the HB-EGF inhibitor and corticosteroid agent are administered simultaneously.

10 In other embodiments, the HB-EGF inhibitor and corticosteroid agent are administered separately.

In some embodiments, the corticosteroid agent and/or the HB-EGF inhibitor are administered orally or by injection, in particular IV, IM or SC. In some particular embodiments, the corticosteroid agent is administered orally or by intravenous injection and the HB-EGF
15 inhibitor is administered via intravenous or subcutaneous injection.

In some embodiments, the therapeutic combination comprises a dose of 0.6 mg/kg of HB-EGF inhibitor per day. The dose of inhibitor administered daily may be in a single administration or two separate administrations; preferably in a single administration. In some particular embodiments, the HB-EGF inhibitor is DTR8, preferably the therapeutic
20 combination comprises a dose of 0.6 mg/kg of DTR8 per day, more preferably in a single administration.

The combined therapy allows the administration of a reduced dose of corticosteroid, in particular at disease onset (acute phase of the disease) or in relapse phase, thereby avoiding undesirable side effects associated with high dose of corticosteroids in current therapies of the
25 disease. In particular, the combined therapy avoids the use of a high starting dose of corticosteroid at disease onset or relapse. The starting dose of corticosteroid administered at disease onset usually corresponds to 1 g/day IV for 3-5 days as shown in Figure 1 illustrating a reference treatment. The reduced dose of corticosteroid according to the combined therapy of the invention is at least 3 times, in particular at least 5, 10, 15, 20, 25, 30, 100 times lower
30 than the starting dose of corticosteroid administered at disease onset in a reference treatment.

The reduced dose of corticosteroid according to the combined therapy of the invention is preferably also lower than the initial dose of corticosteroid administered orally after the starting dose (administered intravenously) in a reference treatment. The initial dose administered after the starting dose is usually 0.5-1 mg/kg (with a maximum of 80 mg) per day
5 oral for 3-4 weeks as shown in Figure 1 illustrating a reference treatment.

In some particular embodiments, the reduced dose of corticosteroid is less than 80 mg per day; typically 30 mg/day administered orally after the starting dose (administered intravenously) and lowered to 7.5 mg/day by 3 months and 5 mg/day by 6 months.

In some particular embodiments, the reduced dose of corticosteroid is less than 1 mg/kg per day, administered at the onset or relapse of the disease, preferably for at least 3 weeks. In
10 some more particular embodiments, the reduced dose of corticosteroid is about 0.5 mg/kg per day, administered at the onset or relapse of the disease, preferably for at least 3 weeks.

In some particular embodiments, the reduced dose of corticosteroid is less than 1 mg/kg per day, administered at the onset or relapse of the disease, preferably for at most 6 months,
15 preferably at most 5 months, preferably at most 4 months, even more preferably at most 3 months. In some more particular embodiments, the reduced dose of corticosteroid is about 0.5 mg/kg per day, administered at the onset or relapse of the disease, preferably for at most 6 months, preferably at most 5 months, preferably at most 4 months, even more preferably at most 3 months.

20 In some more particular embodiments, the reduced dose of corticosteroid is administered IV or orally.

In some more particular embodiments, the corticosteroid is prednisolone, prednisone or a derivative thereof.

In some particular embodiments, the reduced dose of corticosteroid agent and/or the dose of
25 HB-EGF inhibitor are administered every day for at least 3 weeks.

In some particular embodiments, the reduced dose of corticosteroid agent and/or the dose of HB-EGF inhibitor are administered every day for at most 6 months, preferably at most 5 months, preferably at most 4 months, even more preferably at most 3 months.

One example of combined therapy comprises DTR8 and prednisolone administered at disease onset or relapse; preferably DTR8 is administered at 0.6 mg/kg per day SC or IV, and prednisolone or prednisone is administered at up to 1mg/kg per day SC or orally, preferably at up to 0.5 mg/kg per day SC or orally; in particular at 0.37mg/kg per day SC or orally; the
5 combined therapy is preferably administered for at least 3 weeks.

One example of combined therapy comprises DTR8 and prednisolone administered at disease onset or relapse; preferably DTR8 is administered at 0.6 mg/kg per day SC or IV, and prednisolone or prednisone is administered at up to 1mg/kg per day SC or orally, preferably at up to 0.5 mg/kg per day SC or orally; in particular at 0.37mg/kg per day SC or orally; the
10 combined therapy is preferably administered for at most 6 months, preferably at most 5 months, preferably at most 4 months, even more preferably at most 3 months.

The invention provides also a method of treating a kidney disease involving the activation of the HB-EGF/EGFR, comprising : administering to a patient a therapeutically effective amount of the therapeutic combination to a patient according to the present disclosure.

15 The invention provides also the use of the therapeutic combination according to the present disclosure for the treatment of a kidney disease involving the activation of the HB-EGF/EGFR pathway according to the present disclosure.

The invention provides the use of combined drugs comprising an HB-EGF inhibitor and a corticosteroid agent according to the present disclosure in the manufacture of a medicament
20 for treatment of a kidney disease involving the activation of the HB-EGF/EGFR according to the present disclosure.

The invention provides a pharmaceutical composition for treatment of a kidney disease involving the activation of the HB-EGF/EGFR according to the present disclosure, comprising the therapeutic combination comprising an HB-EGF inhibitor and a corticosteroid agent
25 according to the present disclosure as an active component.

The invention provides a pharmaceutical composition comprising the therapeutic combination comprising an HB-EGF inhibitor and a corticosteroid agent according to the present disclosure for treating a kidney disease involving the activation of the HB-EGF/EGFR according to the present disclosure.

In the various therapeutic uses and methods as used herein, the HB-EGF inhibitor and corticosteroid agent of the therapeutic combination may be in the same pharmaceutical composition or in separate pharmaceutical compositions.

Another aspect of the invention relates to a product containing a corticosteroid agent and an
5 inhibitor of Heparin-Binding Epidermal Growth Factor-like growth factor (HB-EGF) as a combined preparation for simultaneous, separate or sequential use in the treatment of kidney diseases associated with the activation of the HB-EGF/EGF receptor pathway according to the present disclosure.

The invention also relates to a pharmaceutical composition for treating kidney diseases
10 associated with the activation of the HB-EGF/EGF receptor pathway comprising a HB-EGF inhibitor and a reduced dose of corticosteroid agent compared to the starting dose used for a reference treatment with the corticosteroid agent according to the present disclosure.

The invention also relates to a pharmaceutical composition comprising the HB-EGF inhibitor DTR8 (SEQ ID NO: 2) and a corticosteroid agent according to the present disclosure.

15 The various embodiments of the present disclosure can be combined with each other and the present disclosure encompasses the various combinations of embodiments of the present disclosure.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques, which are within the skill of the art. Such techniques are explained fully in the
20 literature.

The invention will now be exemplified with the following examples, which are not limitative, with reference to the attached drawings in which:

BRIEF DESCRIPTION OF DRAWINGS

Figure 1. Comparison of corticosteroid dosage for the treatment of CGN (O'Brien et al., *MSD*
25 *Manual Professional Version.*, 2022; Yates et al., *Annals of the Rheumatic Diseases*, 75, 1583–1594, 2016) according to the prior art (reference treatment) and according to the combination therapy of the present invention.

Figure 2 shows the percentage of glomeruli with grade 4 FSGS lesions on kidney sections from experimental animals stained by Masson trichrome and observed by optical microscopy. A grade 4 FSGS lesion is defined as covering 75 % to 100 % of the glomerulus. Data were analyzed with a Kruskal-Wallis test using correction for multiple comparison. * $p < 0.05$.

- 5 **Figure 3** shows the percentage of animals in each experimental group with normal values of blood chemistry markers of urinary function: creatininemia, blood urea nitrogen and albuminemia. Animals were counted when markers were within normal values for all tested samples (day 0, 1, 4, 8, 14 and 21).

- 10 **Figure 4** shows the percentage of variation of albuminemia of animals in each experimental group. The percentage of variation was calculated from values at the end of the experiment (day 21) with respect to values at day 0, prior injection of NTS (nephrotoxic serum triggering the disease).

EXAMPLES

Materials and Methods

15 *Animals*

Female wild-type domestic pigs (*Sus scrofa domesticus*) were used in this study. All animal procedures followed the European Community guidelines (L358–86/609EEC) and were approved by the Ministry for Higher Education and Scientific Research (APAFIS#13259-2017121809512560v3). The animals were housed individually in the Center for Biomedical
20 Research facilities from the National Veterinary School of Maisson-Alfort (France) (authorization E940462).

Nephrotoxic serum (NTS)

25 Sheep nephrotoxic serum containing polyclonal anti-pig glomerular basement membrane (GBM) antibodies was obtained by xeno-immunization. Whole kidneys were obtained from healthy pigs. Cortical tissues were dissected and digested in type I collagenase (17100-017, GIBCO). The tissue samples were then filtered through several sieves. Samples with adequate purification (>80% of glomeruli) were mixed, centrifugated, and resuspended in ultrapure water to induce hypotonic cell swelling and lysis and to eliminate intracellular proteins from

the supernatant. The remaining proteins, essentially from GBM, were dissociated from the cellular debris by ultrasonication (Vibra-Cell, Sonics & Materials, Inc., Newtown, CT, USA) on ice. Six sheep were immunized with the GBM extract by four weekly intradermal injections (1 mg of protein, in complete Freund's adjuvant), followed by one intravenous infusion (1 mg
5 of protein, without Freund's adjuvant) 4 weeks after the last intradermal injection.

Serum was taken from living sheep one week later before being euthanized. Each individual serum was tested by immunofluorescence for GBM specificity on pig's kidney tissue sections. Specific sera were pooled and centrifugated. Finally, the supernatant was heat-inactivated at 56 °C for complement inactivation in a water bath and filtered twice with 0.45 and 0.20 µm
10 filters consecutively. Aliquots of NTS were stored frozen (-80°C) until the experiment.

Induction of crescentic glomerulonephritis

The pig model of CGN was adapted from the accelerated anti-GBM CGN model as described in rodents (Bollée et al., *Nat. med.*, 2011, 17, 1242-1250). Two to three-month-old female pigs (average weight 27 kg) were pre-immunized with a subcutaneous injection of 500 µg of
15 purified sheep IgG (I5131, Sigma) diluted in 1 mL of sterile PBS and 1 mL of incomplete Freund's adjuvant (F5881, Sigma), 7 days before the beginning of the protocol. Each individual received an intravenous injection of NTS on two consecutive days (0.1 mL/kg of body weight on day 0 and 0.2 mL/kg on day 1) to induce an accelerated CGN model.

Pigs received an intramuscular injection of 9 mg of DTR8 twice daily, starting on the
20 afternoon of day 1 or/and an oral dose of 10 mg of prednisolone (Sandoz) inside an apple as a vehicle. The vehicle-treated group (placebo group) received both the intramuscular injections of buffer and an empty apple. The DTR8-treated group received an empty apple as vehicle control for the CTC and the CTC-treated group received the intramuscular injections of buffer as vehicle control for the DTR8 injections. The animals were euthanized on day 21
25 and kidney samples were harvested.

Biochemical measurements in blood and urine

All clinical chemistry measurements were performed at the Medical Biology Laboratory of the CEA Saclay Center on a Cobas 6000/c501 analyzer. Blood samples were drawn on day 0, 1, 4, 8, 14 and 21. Urine samples were drawn on day 0, 8, 14, and 21. Creatininemia was
30 measured using the creatininase/creatinase method. Blood urea was measured using the

kinetic urease glutamate dehydrogenase method. Albuminemia was measured using the bromocresol green method. Urine total protein concentration was quantified by turbidimetry using the EDTA/benzethonium method.

Histology

- 5 Pigs' kidney samples were fixed in 10% formalin, paraffin-embedded, and then 4 μ m-thick sections were processed for Masson's trichrome staining. About 100 to 200 glomerular cross-sections per individual were counted to determine the percentage of healthy glomeruli, crescents and FSGS lesions. The severity of FSGS lesions was evaluated by the surface of the glomerulus section showing pathological features. A grade 4 FSGS lesion is defined as
10 covering 75 % to 100 % of the glomerulus. Data were analyzed with a Kruskal-Wallis test using correction for multiple comparisons with the GraphPad Prism software (GraphPad Software, La Jolla, CA).

Results

Establishing a model of CGN in pigs

- 15 CGN was induced by injecting pigs with a sheep antiserum against purified pig glomerular basement membranes (GBM), as was previously described to induce the disease in mice (Bollée et al., *Nat. Med.*, 2011, 17, 1242-1250). This antiserum named nephrotoxic serum (NTS) mimics the anti-GBM autoantibodies found in patients with the Goodpasture syndrome, one of the four leading causes that trigger CGN. Control animals received serum from sheep
20 drawn before their immunization with GBM instead of NTS. The detection by immunofluorescence (IF) of sheep IgG on kidney sections from NTS-treated pigs shows their deposits at the interface between glomerular capillaries and podocytes that are separated by the GBM. After 4 to 8 days the pigs developed progressively a nephrotic syndrome with proteinuria, hypoalbuminemia, hematuria, subcutaneous oedema and ascites.
- 25 Twenty-one days after disease induction, animals were sacrificed, and kidneys were subjected to histological analyses. Masson trichrome staining revealed the presence of cellular and fibrocellular crescents, destruction of Bowman's capsule together with various degrees of FSGS, tubular injury and interstitial fibrosis. These features are typical of human CGN. Interestingly, healthy glomeruli coexist with altered ones, as observed on patient biopsies at
30 disease onset. Healthy control pigs receiving the pre-immune serum had no lesions at all.

Immunohistochemistry with anti-HB-EGF antibodies confirmed the expression of HB-EGF in crescentic lesions as opposed to normal glomeruli from healthy control animals. However, as expected, healthy glomeruli from CGN animals also expressed HB-EGF (Bollée et al., *Nat. Med.*, 2011, 17, 1242-1250). Eighty-six percent of animals administered with vehicle had
5 altered values of blood markers of urinary function (albuminemia, creatininemia and blood urea nitrogen) (**Figure 3**). Animals developed proteinuria and an at least a 20 % decrease in albuminemia at day 21 as compared to day 0 (**Figure 4**).

Altogether, the data show that pigs receiving NTS develop a set of histopathological features characteristic of CGN. In conclusion, this pig model is well-suited for the evaluation of
10 innovative therapies such as DTR8 combined with a reduced dose of CTC to induce a rapid protection of glomeruli against the cellular and functional consequences of immune injuries in CGN.

Comparative evaluation of DTR8, CTC and their combination as a treatment for CGN

Table 1 summarizes the design of the study. Four groups of animals received NTS and were
15 treated as indicated. The dose of 9 mg of DTR8 (SEQ ID NO: 2) was administered by the intra-muscular (IM) route twice a day for 3 weeks in ~30 kg pigs. The IM route was chosen because the SC route twice a day is impracticable in pigs. This dose was estimated according to pharmacokinetic experiments and simulation in order to achieve a plasmatic concentration $\geq 100 \times K_d$ for 20 hours per day ($2 \text{ pM} < K_d < 4 \text{ pM}$ (WO 2013/140335)). DTR8 is a globular
20 protein of 17.3 kDa. Thus, it is assumed that it would be filtered both by glomeruli with altered and unaltered GBM (Choi et al., *Nat. Biotechnol.*, 2009, 25, 1165-1170). At such concentration, podocytes and PECs located in the filtration chamber and expressing HB-EGF would be well-exposed to the protein. The oral dose of prednisolone of 10 mg/day for 3 weeks would represent about 1/3rd of the oral dose given at disease onset (after the starting dose of
25 1g/day (IV) for 3-5 days) in patients or 3-4 -fold the maintenance dose given after 5 months of treatment (O'Brien et al., *MSD Manual Professional Version.*, 2022). It is in agreement with the recommended dose of corticosteroid for maintenance treatment. This dose was chosen in order to evaluate if the therapeutic effect of DTR8 would compensate for the reduction of corticosteroid at onset.

30 **Table 1: Study design**

Treatment	None	Vehicle, IM, 2x/d	DTR8 9 mg, IM, 2x/d	Prednisolone 10 mg PO, 1x/d	DTR8 + prednisolone
Pre-immune sheep serum	n=5				
NTS		n=12	n=10	n=10	n=14

The combination of DTR8 and CTC reduces the severity of FSGS lesions as compared to CTC or DTR8 alone

Most interestingly, the combination treatment of DTR8 and CTC sharply reduced the severity of FSGS lesions ($p < 0.05$). **Figure 2** shows the percentage of glomeruli with grade 4 FSGS lesions on kidney sections from experimental animals stained by Masson trichrome and observed by optical microscopy. A grade 4 FSGS lesion is defined as covering 75 % to 100 % of the glomerulus. Data were analyzed with a Kruskal-Wallis test using correction for multiple comparisons. On average, vehicle-treated animals had 10 % of their glomeruli showing terminal FSGS lesions (grade 4 FSGS lesions) in which the whole glomerulus is obliterated by fibrosis. This proportion was 8 % for DTR8 animals, 4 % for CTC and 2 % for the combination treatment. These results show that when DTR8 is combined with CTC, the severity of FSGS lesions is strongly reduced. This was obtained for a dose of CTC lower than the dose of CTC usually given at disease onset. Thus, the addition of DTR8 to the treatment of CGN allows to reduce the dose of CTC.

The combination of DTR8 and CTC increases the proportion of animals devoid of urinary blood chemistry anomalies

Blood was drawn from animals on day 0, 1, 4, 8, 14 and 21 during the experiment. Serum creatinine, blood urea nitrogen and albumin levels were monitored. Most vehicle-treated animals experienced a progressive decrease in albuminemia, reaching levels below or well below normal levels. Similarly, anomalies in serum creatinine and blood urea nitrogen were observed in these animals. **Figure 3** shows the percentage of animals in each experimental group that maintained normal values of these three markers of renal function. The combination

treatment protected 43 % of animals from urinary blood chemistry anomalies. In contrast, only 30 % of CTC-treated animals were devoid of these functional anomalies, 20 % of DTR8-treated animals and 14 % of vehicle-treated animals. In summary, combining DTR8 and CTC treatments allowed the renal function of animals subjected to immune aggression towards their glomeruli to be protected better than that of each treatment alone.

The combination of DTR8 and CTC decreases the variation of albuminemia more than DTR8 or CTC alone

The filtration barrier of the glomeruli retains nanoparticles or proteins larger than 6-8 nm or ~70 kDa (Du et al., *Nat. Nanotechnol.*, 2017, 12, 1096-1102; Lawrence and al., *Proc. Natl. Acad. Sci.*, 2017, 114, 2958-2963). Therefore, albumin is retained in the circulation. A decrease in serum albumin levels is associated with an alteration of the filtration barrier. In order to assess the level of such functional alteration of the filtration cut-off of the kidneys, the percentage of variation of serum albumin between day 0 and day 21 was calculated for each animal (**Figure 4**). Vehicle-treated animals had a median decrease of albuminemia of 35 %. In contrast, animals treated with the combination of DTR8 and CTC had a reduction of 24 % only. DTR8 alone did not improve albuminemia (median decrease of 37 %) and CTC alone led to slight improvements (median decrease of 30 %) but less than when combined with DTR8. Altogether, the results show that combining DTR8 and CTC treatments reduced the alteration of albuminemia in CGN animals more than CTC or DTR8 alone, the latter having no effect.

Conclusion

Altogether, the results show that improving kidney histological signs and functional parameters with DTR8 administration reduces the requirement for high doses of CTC during the early treatment of CGN. In particular, the combination treatment with DTR8 and a dose of CTC lower than the high doses used at disease onset led to both a statistically significant reduction of the severity of FSGS lesions ($p < 0.05$) and a higher proportion of animals devoid of urinary blood chemistry anomalies (43 % for the DTR8 + CTC group versus 30 % for the CTC group, 20 % for the DTR8 group and 14 % for the vehicle group). They constitute a proof of concept in a large animal model of the efficacy of DTR8 as an inhibitor of HB-EGF for treating CGN at disease onset and its capacity to allow reduction of CTC dosage during early treatment.

Sequences disclosed in the application**SEQ ID NO: 1 : Wild-type diphtheria toxin**

GADDVVDSSKSFVMENFSSYHGTPGYVDSIQKGIQPKSGTQGNYYDDWKGFYSTDNKY
DAAGYSVDNENPLSGKAGGVVKVTYPGLTKVLALKVDNAETIKKELGLSLTEPLMEQVGT
5 EEFIKRFGDGASRVVLSLPFAEGSSSVEYINNWEQAKALSVELEINFETRGRGQDAMY
YMAQACAGNRVRRSVGSSLSCINLDWDVIRDKTKTKIESLKEHGPIKNKMSESPNKTVSE
EKAKQYLEEFHQTALEHPELSELKTVTGTPVFAGANYAAWAVNVAQVIDSETADNLEKT
TAALSILPGIGSVMGIADGAVHHNTEEIVAQSIALSSLMVAQAIPLVGELVDIGFAAYNF
VESIINLFQVVHNSYNRPAYSPGHKTQPFLLHDGYAVSWNTVEDSIIRTGFQGESGHDIKI
10 TAENTPLPIAGVLLPTIPGKLDVNKSKTHISVNGRKIRMRCRAIDGDVTFCRPKSPVYVG
NGVHANLHVAFHRSSEKIHSNEISSDSIGVLGYQKTVDHDKVNSKLSLFFEIKS

SEQ ID NO: 2 : DTR8

GKSPGHKTEPYTHDGYTVSWKTVEDSIIRTGFQGESGHDIKITAENTPLPIAGVLLPTI
PGKLDVNKSKTHISVNGRKIRMRCRAIDGDVTFCRPKSPVYVGNGQHANLHVAFERKSSE
15 KIHSNEISSDSIAVLGYQKEVDHDKVNSKLSLFFEIKS

CLAIMS

1. A combination of a corticosteroid agent and an inhibitor of Heparin-Binding Epidermal Growth Factor-like growth factor (HB-EGF) chosen from anti-HB-EGF antibodies and diphtheria toxin-derived HB-EGF ligands, for use in the treatment of kidney diseases associated with the activation of the HB-EGF/EGF receptor pathway.
5
2. The combination for use according to claim 1, wherein said disease is selected from the group consisting of: Crescentic glomerulonephritis (CGN) and related disease such as focal and segmental glomerulosclerosis (FSGS), Renal interstitial fibrosis (IF), chronic kidney disease (CKD), and Hypertensive Arteriolar Nephrosclerosis (HTN).
10
3. The combination for use according to claim 1 or claim 2, wherein said treatment with the combination comprises the administration of a reduced dose of corticosteroid agent consisting of less than 1 mg/kg per day, preferably less than 80 mg per day.
4. The combination for use according to any one of claims 1 to 3, for use in the treatment of an acute phase or relapse of said disease.
15
5. The combination for use according to any one of claims 1 to 4, wherein said disease is Crescentic glomerulonephritis (CGN) or focal and segmental glomerulosclerosis (FSGS).
6. The combination for use according to any one of the preceding claims, wherein said treatment reduces the severity of the glomerular lesions and/or reduces the degradation of kidney function.
20
7. The combination for use according to any one of the preceding claims, wherein the HB-EGF inhibitor is a recombinant protein comprising an isolated R domain of the diphtheria toxin comprising at least the substitutions: L390T and Y380K; L390T and Y380E; Y380K and Q387E; or Y380E and Q387K relative to the amino acid sequence of SEQ ID NO: 1.
25
8. The combination for use according to claim 7, wherein the recombinant protein comprises the substitutions Y380K, Q387E, L390T, A395T, F389Y, G510A, N399K, V452T, T517E, V483Q, H492E and S494K relative to SEQ ID NO: 1.

9. The combination for use according to claim 8, wherein the recombinant protein comprises the amino acid sequence SEQ ID NO: 2.
10. The combination for use according to any one of the preceding claims, wherein the corticosteroid agent is selected from the group consisting of: prednisolone, betamethasone, prednisone, triamcinolone, methylprednisolone, dexamethasone, and mixtures thereof.
11. The combination for use according to any one of the preceding claims, which comprises a dose of HB-EGF inhibitor of 0.6 mg/kg per day.
12. The combination for use according to any one of claims 2 to 11, wherein said reduced dose of corticosteroid agent and/or said dose of HB-EGF inhibitor are administered every day for at least 3 weeks and/or at most 6 months.
13. The combination for use according to any one of the preceding claims, wherein said corticosteroid agent and/or HB-EGF inhibitor are administered orally or by injection, in particular via intravenous or subcutaneous injection.
14. A product containing a corticosteroid agent and an inhibitor of Heparin-Binding Epidermal Growth Factor-like growth factor (HB-EGF) chosen from anti-HB-EGF antibodies and diphtheria toxin-derived HB-EGF ligands, as a combined preparation for simultaneous, separate or sequential use in the treatment of kidney diseases associated with the activation of the HB-EGF/EGF receptor pathway, in particular selected from the group consisting of: Crescentic glomerulonephritis (CGN) and related disease such as focal and segmental glomerulosclerosis (FSGS), Renal interstitial fibrosis (IF), chronic kidney disease (CKD), and Hypertensive Arteriolar Nephrosclerosis (HTN).
15. A pharmaceutical composition for treating kidney diseases associated with the activation of the HB-EGF/EGF receptor pathway, comprising a HB-EGF inhibitor and a reduced dose of corticosteroid agent compared to the starting dose of corticosteroid agent used in a reference treatment.